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CONTENTS

1913-1914

No. 1. JULY, 1913

C. M. Jackson. Postnatal growth and variability of the body and of the various organs in the albino rat. Seven figures 1

J. A. Badertscher. Muscle degeneration and its relation to the origin of eosinophile leucocytes in amphibia (Salamandra atra). Seven figures 69

Shinkishi Hatai. On the weights of the abdominal and the thoracic vicera, the sex glands, ductless glands and the eyeballs of the albino rat (Mus norvegicus albinus) according to body weight. Twelve charts 87

Edward F. Malone. The nucleus cardiacus nervi vagi and the three distinct types of nerve cells which innervate the three different types of muscle. Three figures 121

No. 2. SEPTEMBER

Adam M. Miller. Histogenesis and morphogenesis of the thoracic duct in the chick; development of blood cells and their passage to the blood stream via the thoracic duct. Twenty-eight figures (seventeen plates) 131

William H. F. Addison and Harold W. How. On the prenatal and neonatal lung. Eight figures 199

Chester H. Heuser. The development of the cerebral ventricles in the pig. Twenty-six figures 215

No. 3. NOVEMBER

Charles R. Stockard. An experimental study of the position of the optic anlage in Amblystoma punctatum, with a discussion of certain eye defects. Nine figures 253

W. B. Kirkham and H. S. Burr. The breeding habits, maturation of eggs and ovulation of the albino rat. Eighteen figures 291

Ivan E. Wallin. A human embryo of thirteen somites. Seven figures 319

Walter E. Dandy. The nerve supply to the pituitary body. Three figures 333

B. F. Kingsbury. The morphogenesis of the mammalian ovary: Felis domestica. Thirty-two figures 345

No. 4. JANUARY, 1914

E. V. Cowdry. The development of the cytoplasmic constituents of the nerve cells of the chick. Five plates.... 389

J. F. Gudernatsch. Feeding experiments on tadpoles. II. A further contribution to the knowledge of organs with internal secretion. Two double plates.... 431

Charles H. Swift. Origin and early history of the primordial germ-cells in the chick. Fifteen figures.... 483

POSTNATAL GROWTH AND VARIABILITY OF THE BODY AND OF THE VARIOUS ORGANS IN THE ALBINO RAT

C. M. JACKSON

The Anatomical Laboratory of the University of Missouri

SEVEN FIGURES

CONTENTS

Introduction.......... 2
Material and methods.......... 3
Growth and variability of the body.......... 8
1. Growth in body weight.......... 8
2. Variability in body weight.......... 11
3. Fraternal variability.......... 16
Growth and variability of the individual organs.......... 20
1. Head.......... 20
2. Central nervous system.......... 21
3. Eyeballs.......... 23
4. Thyroid gland.......... 25
5. Thymus.......... 26
6. Heart.......... 27
7. Lungs.......... 31
8. Liver.......... 33
9. Spleen.......... 34
10. Stomach and intestines.......... 35
11. Suprarenal glands.......... 36
12. Kidneys.......... 38
13. Gonads.......... 39
a. Testes.......... 39
b. Ovaries.......... 40
14. General considerations.......... 41
Summary.......... 48
Bibliography.......... 50
Tables.......... 53

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JULY, 1913

INTRODUCTION

The present paper includes statistical data upon certain phases of the growth of the body as a whole and more especially the relative growth and variability of the individual organs in the albino rat. A study of this kind may be of value in two respects. In the first place, it should give a better insight into the nature of growth, a fundamental biological phenomenon worthy of more study for its own sake. In the second place, a more complete knowledge of the process of growth, including the limits of normal variation, should be of value for reference in experimental work of various kinds, for which the rat is often used.

For both these purposes there is great need of a series of complete growth norms, comparable to Keibel's morphological 'Normentafeln.' A complete growth norm for any given species would involve adequate data upon the prenatal and postnatal growth of the body as a whole, and of its component parts, organs, tissues and cells. It would include a determination of the extent of variability due to intrinsic or hereditary factors and to extrinsic or environmental factors. It is evident that a single fixed or absolute growth norm for a given species does not exist. We can, however, discover the norm and limits of variation within a group of animals homogeneous in constitution and in an environment as constant as possible. Furthermore, it is possible to determine to what extent the norm and variability are changed by varying the different factors involved.

Such a complete growth norm has not as yet been even approximately determined for any species, although numerous observations have been made upon different phases of growth in various animals. For the rat, extensive data on the growth of the body and of the central nervous system are available in a series of papers by Donaldson and Hatai. The relative growth of the principal parts and systems of the rat has recently been studied by Jackson and Lowrey ('12); and formulas for the growth of the individual viscera have been determined by Hatai ('13) and are published in the present number of this journal. Further data upon the growth and especially the variability of the body and of the various organs are included in the present paper.

MATERIAL AND METHODS

The following data are utilized in the present paper. For the weight of the whole body of the albino rat (Mus norvegicus albinus) at various ages a series of 570 original observations is given in table 1. For the individual organs, 344 animals were killed and dissected at various ages, and the weights of the organs were observed (tables 3 to 15).

Of data available for comparison, there should be mentioned first the extensive series of observations by Donaldson on the growth of the whole body ('06) and of the brain and spinal cord ('08) of the albino rat. I am furthermore indebted to Professor Donaldson and Dr. Hatai, of The Wistar Institute of Anatomy, for unpublished observations upon the weight of the principal viscera in about 200 albino rats. With the exception of the central nervous system, however, these data are not included with my own, but were utilized merely for comparison.

The albino rats used by me (table 1) were reared from stock obtained partly from M. Cattell, Garrison-on-Hudson, New York, and partly from B. F. McCurdy, a dealer in Chicago. The litters from each source were kept separate, but no constant difference in growth and variability was noticeable between them. The 68 litters included in table 1 are for the most part not closely related (although in a few cases observations upon the body weight of the same litter are repeated at successive ages).

The albino rats were kept in stationary cages in an animal house and were well cared for. A supply of chopped corn was kept constantly in the cages. A liberal amount of wheat bread soaked in whole milk was supplied daily, and fresh meat (beef) once a week. Water in abundance was provided.

From the age of about six weeks onward, the sexes of each litter were kept separate, and (with the exception of a part of the females at one year) therefore represent unmated animals. Watson ('05) has shown that weights of the body and of the central nervous system are somewhat increased in the female through bearing young. It is probable that other organs, also, may be thereby more or less affected (especially the reproductive

system), although data are wanting to determine this point. The external characters whereby the sexes may be distinguished in young rats have been described by Jackson ('12).

In general, the albino rat remains healthy and thrives in captivity. The chief exception noted is the frequent tendency to lung disease, especially in the older rats and more rarely in the younger. Two forms of disease were observed. The first is an acute pneumonitis, which is usually fatal. This form appeared but once in a period of three years. It occurred as an epidemic which in a short time destroyed nearly the entire colony. This epidemic was caused by a specific bacterium, bacillus muris, as determined by Mitchell ('12). The second form of lung disease noted is a chronic disturbance which is common in the wild Norway rat at Columbia as well as in the domesticated albino. It appears to develop first in the form of multiple small clear spots scattered over the surface of the lung. Later these spots may form small abscesses, or lead to the consolidation of one or more lobes. An associated catarrhal condition of the respiratory mucosa causes an audible snuffling or wheezing, through which the disease may usually be recognized when well developed. It usually is not fatal, or at least not immediately so, and in some cases does not appreciably affect the growth or the general state of nutrition. Generally, however, it tends to produce more or less emaciation. This is probably the same disease as the pneumonia mentioned by King ('11) and by Currie ('10). Mitchell ('12) failed in repeated attempts to find in this disease the specific bacterium causing the first form, and its etiology appears uncertain.

Animals affected with lung disease were excluded from the present data except in some cases in which the lesions were slight and it appeared necessary to exclude only the lungs. This was the case in 3 of the 43 albino rats dissected at the age of ten weeks, 11 of 41 at five months, and 20 of 25 at one year. Even though the lesions were slight, however, it is probable that the average body weight at five months, and especially at one year, is somewhat below the normal.

Since the number of observations was somewhat limited, it was thought better, especially for the study of variation with age, to restrict them to certain definite ages, rather than to scatter them over the entire period. Seven ages were chosen for this purpose, namely, newborn (one day or less), seven days, twenty days, six weeks, ten weeks, five months, and one year. These ages were selected for the following reasons. At seven days, the body weight at birth has about doubled. At twenty days, the weight has approximately doubled again, and at this age the albino is usually weaned. At six weeks, the body weight has somewhat more than doubled again, and the animal is well established upon the new diet. At ten weeks, the rat has again about doubled its weight, and sexual maturity is reached. At one year, the weight has again approximately doubled, and at this age, according to the observations of Slonaker ('12), the albino rat (in stationary cages) has nearly reached its maximum body weight. Five months was arbitrarily selected as a point intermediate between ten weeks and one year.

A few observations upon the body weight at fourteen days and at thirty days are also recorded in table 1. The continuous growth curves for the absolute weight of the various organs, in terms of body weight, are given by Hatai ('13). It is hoped that the age periods selected for the present paper are sufficiently close together so that no material change in variability, correlation, and so forth, will be overlooked.

In some cases, as previously mentioned, only the gross body weight was observed. This weight was always taken in the forenoon, before feeding. In the cases where the animal was to be dissected, it was killed by chloroform, and the gross body weight, and lengths of trunk and tail recorded. The head was then removed on a plane just posterior to the cranium and anterior to the larynx, and was weighed; while the trunk was suspended by the tail, allowing the blood (unmeasured) to escape. The eyeballs and in some cases the brain were then removed and placed in a closed jar upon moist filter paper. Next the trunk was dissected and the following organs successively removed: thyroid gland; thymus (dissected out of surrounding fat); heart

(cavities opened and blood-clots removed); lungs (right and left separately); liver; spleen; stomach and intestines, including contents, mesentery and pancreas; same, without contents; suprarenal glands; kidneys; ovaries or testes (including epididymis); spinal cord (in a few cases). The extremities, skin, skeleton and musculature were also weighed in some cases, as described in a separate paper by Jackson and Lowrey ('12).

The organs were weighed in closed containers, and loss by evaporation was avoided so far as possible. The organs werè usually weighed to 0.1 mgm. (0.0001 gm.) excepting some of the larger organs in the older rats, which were weighed to 1 mgm. The observations were recorded on printed cards, and any unusual conditions carefully noted. The calculations were made by the aid of a Burroughs adding machine, Crelle's Rechentafeln, and the tables in Davenport's 'Statistical Methods.' The calculations were carefully checked independently to eliminate errors.

I am greatly indebted to Dr. S. Hatai of The Wistar Institute, for valuable aid and criticism, especially on the mathematical phases of the work.

In calculating the various statistical constants—mean, standard deviation, coefficients of variation and of correlation and probable errors—the usual formulas (Pearson's) were employed as given by Davenport ('04). The ungrouped data were used in all cases.

Since the present paper is concerned largely with variability as measured by the coefficient of variation, a brief discussion of this coefficient may be desirable. The coefficient of variation is one hundred times the ratio which the standard deviation bears to the mean; or the percentage of the mean which the standard deviation forms, as expressed by the formula:

$$C = \frac{\sigma}{A} 100 \, (\%)$$

For the standard deviation, the formula is as follows:

$$\sigma = \frac{\sqrt{\Sigma d^2}}{n}$$

where d represents the deviation from the mean, and n the number of variates. The standard deviation is therefore a concrete number which serves to measure the dispersion from the mean. If the variates tend to diverge greatly from the mean, the standard deviation will of course be large; while if they are concentrated closely around the mean, the standard deviation will be small. It is further clear that the standard deviation is not dependent upon the number of cases, but merely upon the manner of their distribution.

Since the standard deviation is a concrete number, however, it will in general vary with the absolute size of the variate. It is therefore often difficult to judge the relative variability of different objects by comparing their standard deviations, and where different units of measurement are used the comparison is meaningless. On this account the coefficient of variation is preferable for measuring the relative magnitude of variations, since it is independent of the size or character of the unit of measurement.

It is of course obvious that from the statistical point of view the number of observations is small and the results cannot be considered final. Even although the probable errors are relatively large, however, it will be found that certain conclusions of importance may be drawn with a fair degree of certainty; and others, more or less strongly indicated, may point the way to further investigation with more adequate data.

To economize space, the individual data are not included in the tables, but only the averages and ranges are given. The cards containing the original individual data are, however, deposited in The Wistar Institute of Anatomy in Philadelphia whence they may be obtained if desired by anyone interested.

GROWTH AND VARIABILITY OF THE BODY

1. Growth in body weight

The data presented in this paper are inadequate for a complete discussion of the growth of the body as a whole. Certain phases, however, may be noted. Donaldson ('06) has made numerous observations upon the postnatal growth of the albino rat, on the basis of which he has drawn certain conclusions, with which the present data may be compared. Table A shows the average

TABLE A

Average gross body weight of albino rat at various ages, sexes separated[1]

AGE		JACKSON'S LARGER SERIES		JACKSON'S SMALLER SERIES		DONALDSON'S SERIES (1906)	
			grams		*grams*		*grams*
newborn		(63 m.)	5.13	(44 m.)	5.06	(40 m.)	5.4
		(66 f.)	4.89	(43 f.)	4.82	(17 f.)	5.2
7 days		(56 m.)	10.53	(30 m.)	10.61	(11 m.)	9.2
		(64 f.)	10.29	(27 f.)	10.48	(8 f.)	8.7
3 weeks		(53 m.)	23.99	(24 m.)	22.20	(19 m.)	21.2
		(59 f.)	21.50	(25 f.)	17.01	(17 f.)	22.6
6 weeks		(45 m.)	63.72	(22 m.)	52.89	(19 m.)	46.3
		(50 f.)	64.25	(20 f.)	54.86	(17 f.)	47.9
10 weeks		(23 m.)	130.4	(20 m.)	121.9	(19 m.)	106.6
		(25 f.)	108.9	(23 f.)	103.3	(11 f.)	99.8
5 months				(20 m.)	167.5	(19 m.)	225.4
				(21 f.)	142.1	(11 f.)	184.6
1 year				(5 m.)	213.0	(6 m.)	279.0
				(20 f.)	163.7	(7 f.)	226.4

[1] Jackson's observations listed under '3 weeks' were nearly all at 20 days, and those of Donaldson under '6 weeks' were at 43 days. The females were unmated, excepting all of Donaldson's series at 1 year, and a part of Jackson's series at 1 year.

gross body weight in each sex at various ages in my larger series (recorded in table 1), in my smaller series (dissected for the organs), and in those observed by Donaldson at corresponding ages.

From table A it is evident that the rats used in the present study averaged somewhat smaller at birth for both sexes than those of Donaldson, but show a more rapid growth during the first week, so that at seven days their average weight is greater. At three weeks the males in Donaldson's series average somewhat

smaller than in mine but the females are heavier. At six weeks and ten weeks both sexes average smaller in Donaldson's series, but at five months and one year they are decidedly larger.

In general, therefore, it is evident that Donaldson's rats remained smaller than mine up to the age of six weeks, but surpassed them in the later stages. The probable errors were not calculated for the *gross* body weights (except at six weeks, smaller series); but as will be seen later the variation for the gross weight is not materially different from that of the *net* body weight, the probable errors of which, in the smaller series, are given in table 2. The differences between Donaldson's results and mine are greater than the probable errors and therefore appear significant. The explanation of these differences will be considered later under variability.[1]

The relative growth of the sexes of the albino rat was noted by Donaldson ('06), who found that, beginning about the end of the first week, the female grows more vigorously, overtaking and usually exceeding the male in body weight from the fifteenth to the fifty-fifth day of age. My data (table 1) show that in 16 newborn litters containing both sexes the males averaged greater in every case; at seven days, the males exceed in 11 litters, the females in 5; at fourteen days, the males exceed in 3, the females in 1; at twenty days, the males exceed in 10, the females in 3; at thirty days; the males exceed in 2, females in none; at six weeks, the males exceed in 10, the females in 2; at ten weeks, the males exceed in 6, the females in 1; at five months the males exceed in 4, females in 1; at one year, the males exceed in 2, the females in 1. According to litters, therefore, the excess of average weight was invariably in favor of the male at birth, and also in the majority of cases at all succeeding ages.

The ratio of the average gross body weight in the male to that in the female at corresponding ages in my data is given in table B.

[1] It may be pointed out in this connection that whereas my rats represent for the most part a 'random sample' of the general population at each age, those of Dr. Donaldson were taken while young, and used throughout the period of observation.

According to table B, it is seen that the average body weight of the male was greater at every age noted, except at six weeks, when the female was slightly larger. At seven days, however, the male was but slightly the greater.

The extent of the variation in the ratio of body weights between males and females of the same litter may be noted from data in table 1. Further information of interest is afforded by table

TABLE B

AGE	NUMBER OF EACH SEX		AVERAGE GROSS BODY WEIGHT	AVERAGE GROSS BODY WEIGHT	RATIO OF MALE TO FEMALE
	Males	Females	Male	Female	
			grams	*grams*	
newborn	63	66	5.13	4.89	1.05
7 days	56	64	10.53	10.29	1.02
20 days	53	59	23.99	21.50	1.12
6 weeks	45	50	63.72	64.25	0.99
10 weeks	23	25	130.40	108.90	1.20
5 months	20	21	167.50	142.10	1.18
1 year	5	20	213.00	163.70	1.30

TABLE C

Ratio of average male to female gross body weight in individual litters at successive ages

LITTER AND NUMBER OF EACH SEX	NEWBORN	7 DAYS	14 DAYS	20 DAYS	30 DAYS	6 WEEKS
A 32 (3 m. 5 f.)	1.03	1.03				
M 9 (5 m. 3 f.)	1.06	1.06[1]				
M 9 (3 m. 3 f.)		1.06	1.04	1.05		
M 6 (5 m. 3 f.)		1.04	1.08	1.03		
M 10 (2 m. 4 f.)	1.05	1.05	0.99	1.00		1.11
M 8 (3 m. 5 f.)	1.12	1.06	1.06	1.05	1.09	1.22[2]
M 7 (2 m. 4 f.)		0.98		0.86[3]		
M 7 (1 m. 4 f.)					1.11[4]	1.00[4]
M 1 (4 m. 1 f.)				0.95		1.04
M 2 (4 m. 1 f.)				1.08		1.10
A 29 (2 m. 4 f.)	1.02	1.16		1.04		1.08
A 28 (4 m. 2 f.)	1.08	0.99		1.01		0.90

[1] Two males of this litter were killed at 7 days.

[2] In this litter, the ratio at 10 weeks was 1.39.

[3] One male, a 'dwarf,' died before the age of 30 days.

[4] One female a 'dwarf' at these ages.

C showing the ratios in a few cases where the gross body weight was observed in the same litters at successive ages.

It is clear that there is considerable variation among individual litters, and evidently observations upon a much larger number of litters would be necessary to determine the typical growth relations by sexes. The available data seem in general to confirm the conclusion of Donaldson ('06) that during the first two months of postnatal life the growth of the body of the albino rat is more vigorous in the female. In the majority of litters, however, the average body weight of the females does not appear to reach that of the males, at least at the ages observed.

2. Variability in body weight

It is evident from the foregoing that there is in some respects considerable difference between my data and those of Donaldson on the growth of the body of the albino rat. The question naturally arises as to the significance of this difference, as both series are presumably normal (excepting the possible effect of lung disease in the older rats). This brings up the general question as to the nature and extent of normal variation in the growth of the body. It is known that this variation is greatly influenced by external factors, such as the quantity and quality of food, temperature and so forth. Slonaker ('12) has recently demonstrated that the growth of the albino rat in body weight may be greatly retarded by exercise, and that it is also much less with vegetarian than with mixed diet.

These factors, however, were nearly alike in the case of Donaldson's rats and mine, though unavoidable differences in environment may account in part for their difference in growth. But even when the environment is kept constant, there still remains the variability due to intrinsic factors, which probably varies somewhat according to the ancestral strain from which the animals were derived, as well as according to litter, sex and individual. Pearson ('00, p. 473) states that "The individual contains in itself, owing to a bathmic (i.e., intrinsic) law of growth, a variability which is quite sensible, being 80 to 90 per cent of the variability of the race." As before mentioned, it is therefore

evident that in general there is no such thing as a single 'normal' course of growth and variation for a given species. We may, however, determine the process under a given set of conditions, and also learn the effect produced by varying the individual factors. It is probable that some variation in the intrinsic growth factors is largely responsible for the differences noted between Donaldson's data and mine. The possibility of unrecognized pathological factors must also be kept in mind.

We may now consider the variability in body weight found in the present series.[2] The variability is best measured by the coefficient of variation, which expresses the percentage of the mean formed by the standard deviation. For the *net* body weight (table 2), it is seen that the coefficient of variation, taking both sexes together, increases from 12.3 at birth to 15.6 at seven days and to a maximum of 28.4 at twenty days. Thereafter it decreases, being 21.3 at six weeks, 19.9 at ten weeks, and 19.1 at five months. On account of the small number of observations and probable abnormalities at one year, no calculations were made for the total at this age; but the coefficient of variation in body weight for the 20 females at this age was slightly greater than for those at five months. Even when allowance is made for accidental variations due to the small number of observations (as indicated by the relatively large probable errors), the approximate extent of variation, and the general trend according to age are clearly evident. Hatai ('08) in mature albino rats (over 150 days old) found the coefficient of variation for the gross body weight in 53 males, 25.076 ± 2.675 and in 51 females, 12.235 ± 0.974.

The difference in variability between the sexes of the rats used in the present study is indicated in table 2. It is seen that the coefficient of variation appears greater in the males at every age, except at twenty days. No great stress can be laid upon

[2] The variability in body length was not calculated. According to Donaldson ('09), however, the body length of the albino rat is less variable than the body weight; but the two are closely correlated, the coefficient of correlation being 0.90. In the human species, the body length (height) is only about one-third as variable as the body weight (measured by the coefficient of variation).

this indication, however, as it must be remembered that the number of observations is comparatively small, and the differences are usually within the limits of probable error. It is noteworthy, however, that Hatai ('08) likewise found for the body weight and for various skull measurements of the albino rat a tendency to greater variability in the male.

At the ages of twenty days and six weeks, the standard deviation and coefficient of variation were also calculated for the corresponding *gross* body weights. At twenty days, the coefficient of variation was, for the males 28.7 (as compared with 28.4 for the net body), and for the female 18.4 (17.5 for the net); at six weeks, for the male 17.7 (17.4 for the net), and for the female 24.6 (24.7 for the net). It is therefore evident that there is no material difference between the coefficients of variation for the gross and for the corresponding net body weights.

For a larger series of 56 males and 36 females newborn, corresponding only in part to those given for net body weight, the coefficient of variation for the gross body weight of the male was 14.0 (13.6 for the net), and of the female 9.2 (9.9 for the net). A larger series was also calculated for the gross body weight at twenty days and six weeks, including all those in table 1. In this larger series (which was about twice as large as the smaller) the coefficient of variation at twenty days for the male was 24.4 (28.7 in the smaller series) and for the female, 29.4 (18.4 in the smaller series). At six weeks, the coefficient of variation in the larger series for the male was 20.8 (17.7 in the smaller series) and for the female, 24.2 (24.6 in the smaller series). This would seem to indicate that the great difference in the coefficient of variation between the sexes of the smaller series at twenty days and at six weeks is due to an abnormally small variation among the females at twenty days and among the males at six weeks. This should be remembered in considering the variability of the organs, which correspond to the smaller series and are, as will be shown later, all more or less closely correlated with the body weight. The necessity for caution in drawing conclusions as to variability from a comparatively small number of observations is thus emphasized.

The foregoing data indicate clearly that the coefficient of variation in body weight is smaller in the younger rats (newborn and seven days) and larger in the older (twenty days, six weeks, ten weeks and five months). Less conclusive is the evidence that the maximum variation, for the ages observed, occurs at twenty days, and that the variation is in general greater in the male than in the female. The changes in variation cannot be ascribed to a selective death rate, as there were in the litters observed very few deaths before the age of five months.

For the variation in the weight of the human body at different ages, fairly complete data are available for comparison. For the English newborn, Pearson ('99) finds the coefficient of variation for the male to be about 15.7, and for the female 14.2. For Cambridge University students, nineteen to twenty-five years of age, he finds the coefficient for 1000 males to be about 10.8, and for 160 females 11.2. For Oxford students (male) of eighteen to twenty-three years, Schuster ('11) similarly finds the average coefficient of variation in body weight to be 10.8, varying from 10.2 to 11.1 for different years of age.

For the period from birth to the age of six years, no data are available. But from six years onward we have the extensive observations of Porter ('95) on St. Louis school children. Porter uses the 'probable deviation' (which equals approximately 0.6745 times the standard deviation) as a measure of dispersion, but for convenience of comparison this has been reduced to the standard deviation for calculating the coefficient of variation in table D.

Porter concludes that variation is correlated with rapidity of growth, a conclusion previously reached by Thoma ('82). Boas ('97, '04) reaches similar conclusions. This relation will not hold good for the human newborn, however; for although the growth rate is then far more rapid than at any subsequent period, the coefficient of variation is slightly less than at the age of puberty. In the rat, as we have seen, the coefficient of variation is smaller in the younger animals although the growth rate is far more rapid then than later.

We may therefore conclude that for the human (British and American) the coefficient of variation for the body weight in the newborn male is about 15.7. At six years, it has diminished to a little below 11, and fluctuates in this neighborhood until the twelfth year. During the acceleration of growth from thirteen to sixteen years, the coefficient of variation increases to 16 or 17, thereafter diminishing rapidly to about 11 in the young adult. For the female newborn the coefficient of variation (14.2) is somewhat below that of the male. From six years onward, the coefficient of variation appears to be correlated with the relative rapidity of body growth, being somewhat greater in the female up to the age of fourteen, after which the male exceeds it.

In comparison with the human species, the coefficient of variation in the body weight of the rat at birth appears somewhat smaller. Although in all subsequent stages (except at the age of puberty) the variation in the human body is smaller than at birth, in the rat it appears constantly greater, and shows no evident correlation with the rate of growth of the body.

TABLE D

Body weight of St. Louis school children (from Porter's data)

AGE AT NEAREST BIRTHDAY	NUMBER OF OBSERVATIONS		COEFFICIENT OF VARIATION		RELATIVE ANNUAL INCREASE IN AVERAGE BODY WEIGHT	
	Male	Female	Male	Female	Male	Female
					per cent	*per cent*
6	707	798	10.7	11.3		
7	1814	1714	11.6	13.3	9.7	10.0
8	2188	2147	12.2	12.6	9.7	9.9
9	2188	2055	11.9	13.2	9.6	9.6
10	2064	1947	11.7	12.4	8.7	9.6
11	1743	1708	12.4	14.2	9.5	9.7
12	1644	1676	10.8	14.5	8.1	11.6
13	1242	1343	15.7	16.3	9.3	14.3
14	946	1082	16.7	16.3	10.5	9.9
15	498	690	16.3	12.9	14.3	10.4
16	203	420	17.8	12.4	11.6	7.6
17	71	230	11.7	10.4	7.9	4.7
18		155		10.2		
19		81		10.7		
20		66		10.4		

3. Fraternal variability

It is a matter of common knowledge that variation within fraternities is always less than that of the general population. In the various litters of rats used for the present study, it is in most cases evident from mere inspection that variation in the body weights within the litter is much less than the variation in the total population of the same age. To measure the variation within the litter, three methods were used and the results are summarized in table E.

TABLE E

Coefficient of variation in body weight for total population by ordinary method, and on litter basis (fraternal variation) estimated by various methods

	SEX	NEW-BORN	7 DAYS	20 DAYS	6 WEEKS	10 WEEKS	5 MONTHS
Total population (ordinary method)	male	13.6[1]	16.9[1]	24.4[2]	20.8[2]	18.8[1]	18.5[1]
	female	9.9[1]	13.7[1]	29.4[2]	24.2[2]	16.8[1]	15.3[1]
Litter basis (average of litters, calculated by ordinary method	male	7.0	6.1	5.7	6.6	5.8	7.4
	female	4.4	5.4	4.0	5.9	12.0	10.4
Litter basis (calculated from Yule's formula)	male	6.8	7.6	6.8	7.1	6.1	8.1
	female	5.2	4.4	4.5	7.9	12.2	9.3
Litter basis (from Kellogg's formula)	male	7.3	8.4	6.0	7.2	6.7	8.5
	female	5.2	4.5	4.1	8.5	12.0	9.0

[1] For net body weight.
[2] For gross body weight, larger series.

The first method of determining fraternal variation consists simply in calculating the standard deviation and coefficient of variation for each separate litter by the usual formula. The sexes were calculated separately, only those litters with four or more of either sex being used. The average coefficient for each sex at each age is given in the preceding table. On account of the small number of individuals in each litter, the results can of course be considered only as grossly approximate.

The second method of calculating the fraternal variability is based upon a formula by Yule ('11) who demonstrates (p. 142) that "if a series of (N) observations consists of r component series with standard deviations $\sigma_1, \sigma_2, \ldots \sigma_r$, and means diverging from the general mean of the whole series by d_1,

d_2, ... d_r, the standard deviation σ of the whole series is given (using m to denote any subscript) by the equation: $N . \sigma^2 = \Sigma (N_m . \sigma_m^2) + \Sigma (N_m . d_m^2)$." If we let d_m represent the mean standard deviation of the component series (which may be assumed to be fairly constant) then

$$\Sigma (N_m \sigma_m^2)$$

becomes

$$N \sigma_m^2$$

and

$$\sigma_m^2 = \frac{N \sigma^2 - \Sigma (N_m d_m^2)}{N}$$

whence the value of σ_m is obtained.[3] In applying this method to the rat data N represents the total number of rats at a given age; σ, the standard deviation of the body weight of all the rats; d_m, the deviation of any litter mean from the total mean, N_m, the number of individuals in the corresponding litter; and σ_m, the average standard deviation within a litter. The sexes are considered separately. The results, as shown by the preceding table, do not differ materially from those by the first and third methods.

In the third method[4] of determining fraternal variation, all the litters at each age (sexes separately) are reduced to a common litter basis. This is accomplished by multiplying the body weight of each member of a given litter by the factor

$$\frac{\text{Mean of total population (at given age)}}{\text{Mean of given litter}}$$

[3] Since the formula may be written in the form:

$$\sigma_m^2 = \sigma^2 - \frac{\Sigma (N_m d_m^2)}{N}$$

and since $\frac{\Sigma (N_m d_m^2)}{N}$ is necessarily a positive quantity, it is evident that according to this formula the standard deviation of the individual fraternity is usually (and on the average necessarily) less than that of the total population. The formula gives no indication as to the magnitude of the difference, however.

[4] For this method, and for aid otherwise, I am indebted to my colleague Prof. O. D. Kellogg, of the Department of Mathematics. He adds the caution that under some circumstances the method might tend to give a spurious reduction in the standard deviation and coefficient of variation, but this does not appear to be the case with the present data.

Since the coefficient of variation for each litter and also the mean of the total population remains unchanged by this procedure, the products may be considered as forming a single large litter, whose coefficient of variation may be calculated in the usual way. The results by this method are seen to correspond closely to those by the preceding methods. In fact all three methods agree much more closely than might have been anticipated, considering the relatively small number of litters and of individuals.

Comparing the coefficients of fraternal variation, calculated on the litter basis, with those of the total population calculated by the ordinary method at corresponding ages, it is evident that (excepting only the unusually large fraternal variation for the female at ten weeks) the litter variation is always very much smaller, being usually only from one-third to one-half as large as the total variation. It also appears that the increased variability in the body weight of the total population from twenty days onward is due to an increased variability *between litters* more than to increased variability among the individuals within the litter. Dunn ('12) states (p. 137) that if conditions of growth are favorable the rats of unlike initial weight within a litter tend to approach each other in weight as growth goes on. If this be true, the fraternal (intra-litter) variation should decrease with age.

Table F includes my observations upon the variation in individual litters at successive ages. The sexes are here taken together. It is evident that there is considerable variation, both among different litters at the same age and in the same litter at different ages. The data suggest that there is a tendency for intra-litter variation to decrease up to the age of about twenty days, followed by a tendency to increase. This is supported to certain extent by table E, showing the coefficients of variation on the litter basis.

It may be concluded from all the evidence available that in general the variation in body weight within a given litter of albino rats is *probably* less than half that of the general population of the same age under similar environment. Nevertheless, even within the litter, variations are sometimes so great that it

is never safe to draw conclusions from a single litter, and there is some risk even with several litters. These conclusions are of practical importance in selecting animals for experimental work, and in interpreting the results of such work. Although fraternal variability was not directly calculated for the individual organs, the same principle doubtless holds good with them; for, as will be shown later, all the organs are more or less closely correlated with the body weight.

In the human species, fraternal variation appears to be relatively greater than in the rat. For example, Galton ('94) found the mid-stature for the human adult (male) population to be 68.2 inches. The quartile (or probable) deviation for the whole adult population he found to be about 1.70 inches, and that for brothers (average of four methods) about 1.06 inches. Thus for human stature by this method the fraternal variability appears to be only about 62 per cent of the racial variability. On the basis

TABLE F

Coefficient of variation in individual litters at different ages

LITTER NO.	NUMBER OF EACH SEX		NEWBORN	7 DAYS	14 DAYS	20 DAYS	30 DAYS	6 WEEKS
	Males	Females						
A 32	3	5	5.81	3.82				
M 9	5	3	5.44	4.85				
M 9[1]	3	3		4.19	2.92	4.45		
M 6	5	3		10.20[2]	4.11	5.61		
M 10	2	4	8.18	7.61	7.47	7.64		11.8
M 8	3	5	8.78	4.82	3.94	2.42	6.76	12.4[3]
A 33	0	7		12.00		6.67		
A 33[1]	0	5				7.34	4.70	6.94
M 7	2	4		3.95		18.00		
M 7[1]	1	4					21.30	30.60
M 1	4	1				2.83		7.91
M 2	4	1				4.11		6.57
A 29	2	4				4.47		4.95
A 28	4	2				5.06		6.86

[1] Two males killed in litter M 9 at 7 days, and 2 females in A 33 at 20 days; one (a 'dwarf') in litter M 7 died between 20 and 30 days.

[2] The large variation here was due to a 'dwarf' which caught up with the others in body weight before the age of 14 days.

[3] At 10 weeks the coefficient of variation in this litter was 17.0, the increase being due chiefly to sexual differentiation in body weights.

of more recent biometric investigations in various lines by Pearson and others, it appears (cf. Encyclopaedia Britannica, 13th edition, vol. 27, p. 911) that in general the fraternal S. D. = racial S. D. $\times \frac{\sqrt{3}}{2}$, reckoning the fraternal correlation at 0.50. According to this formula, the fraternal variability would amount to about 87 per cent of the racial variability. Compared with these figures the fraternal variability of the rat, which is less than half of the racial, appears relatively small. This, however, is *intra-litter* variability, which may be somewhat less than fraternal variability in general. The latter could be determined for the rat only by comparing numerous litters born from the *same pair* of rats at different times.

GROWTH AND VARIABILITY OF THE INDIVIDUAL ORGANS

1. Head

In a previous paper by Jackson and Lowrey ('12), the relative growth of the head was considered. It was pointed out, as may be observed in table 3, that the head (sexes together) of the albino rat increases in relative size from an average of about 23 per cent of the body at birth to a maximum of nearly 26 per cent at seven days, after which it decreases gradually to about 10 per cent in the adult. The figures in the table for the percentage at one year are probably somewhat high, as they include some slightly emaciated individuals, in which the head is always relatively large. In very large rats (above 300 grams in body weight) the head continues to decrease in relative weight and may reach 8 per cent or less.

As is furthermore evident from table 3, the variability of the head is less than that of the body as a whole, the coefficient increasing from about 9 in the newborn to about 13 in the adult. The variation also appears slightly greater in the male than in the female (except at six weeks), as is also the case with the whole body.

The coefficient of correlation between the head and the total body weight varies at different ages from 0.76 to 0.95 (sexes together). These figures are somewhat too high, however, the

true correlation being augmented by a 'spurious correlation,' as will be explained later. There appear no constant differences according to age and sex. If such exist, as is probably the case, they are obscured by accidental variations in the comparatively small number of observations.

The close correlation between the weights of the head and of the whole body is also evident from the slight variability in the *percentage* weight which the head forms of the whole body (table 3). If the head at each age formed a constant per cent of the body, the variability of the percentage weight would be zero. The coefficient of variation of the percentage weight of the head is, however, usually not very much smaller than that of the absolute weight, and in a few cases even appears slightly larger. That is to say, at any given age the absolute weight of the head can be predicted almost as accurately as the percentage weight. This is not the case with most of the individual organs, as will be seen later.

Whether reckoned upon the absolute or upon the percentage basis, it is evident the head of the rat exhibits slight variability when compared with most other organs as well as with the body as a whole. This agrees with the observations of Hatai ('08) upon the variability of the skull bones of the rat, as well as with what has been found in the human body. Quetelet ('71) and other anthropometrists have observed that the head is the least variable portion of the human body, a conclusion supported by extensive and careful measurements on the skeleton of different parts of the body by various observers.

2. Central nervous system

Since the growth of the brain and spinal cord has been carefully worked out by Donaldson ('08) from extensive data, it was thought unnecessary to include these organs in the present investigation. Incidentally, however, some observations were made upon the brain, and a few upon the spinal cord. From these, together with those published by Donaldson ('08) and some unpublished data from The Wistar Institute, a few additional conclusions may be drawn.

In regard to the brain, it may be noted that the maximum postnatal relative size occurs, not at birth, but a short time later. Using the data derived from Donaldson's formula expressing the brain weight in terms of (gross) body weight ('08, table 1), calculations show that with a body weight of 5 grams (approximately that at birth) the brain weight forms 4.6 per cent of the body weight. This increases to 6.7 per cent at a body weight of 15 grams. From this (apparent) maximum, the relative size of the brain decreases to 5.0 per cent at 25 grams, 2.7 per per cent at 55 grams, 1.5 per cent at 115 grams, 1.2 per cent at 155 grams, 0.9 per cent at 205 grams, and 0.62 per cent at 315 grams. In this case, the sexes are not separated, although the male brain is relatively slightly heavier than that of the female.

The foregoing figures for the relative size of the brain, as derived from Donaldson's formula, agree fairly well with the present data grouped according to age. We find (combining Donaldson's data with my own) that in 92 newborn albino rats (56 males, 36 females) the brain averaged 4.8 per cent of the (gross) body weight; in 22 (10 males, 12 females) at seven days, 6.4 per cent; in 31 (28 males, 3 females) at three weeks, 5.6 per cent; in 30 (27 males, 3 females) at six weeks, 3.1 per cent. Only scattering observations are available at the later stages. It therefore appears probable that the brain reaches its maximum relative size about the second or third week, when the body weight is between 10 and 15 grams.

The combined data for the brain are sufficient for estimation of variability and correlation with body weight only at birth, three weeks, and six weeks. The coefficient of variation for the brain was found to be at birth, male, 13.9; female, 9.1, total (sexes together) 12.2. The coefficient of variation for the corresponding (gross) body weights was 12.8. At three weeks the coefficient of variation of the brain, for males only, was 6.8, which, excepting the eyeballs (table 4) was the lowest variability found for any organ at any age. The corresponding coefficient of variation for total body weight was 14.8. At six weeks, the coefficient of variation for the brain, males only, was 12.2, the coefficient for the corresponding total body weights being 36.2.

For the same data, the coefficient of correlation between brain weight and (gross) body weights was, at birth, male, 0.736; female, 0.566, total, 0.690±0.037; at three weeks, male only, 0.783±0.049; at six weeks, male only, 0.883±0.020. These high figures are due in part to 'spurious correlation'(as will be explained later). In general, Donaldson ('08) found (in 680 records at various ages) the coefficient of correlation between brain weight and body weight to be 0.7639±0.0108.

Hatai ('08) found for the *cranial capacity* of adult albino rats a coefficient of variation of about 6.7 in the male, and 7.2 in the female. The coefficient of correlation between cranial capacity and (gross) body weight was 0.516±0.074 in the male and 0.692±0.058 in the female.

For the spinal cord, the relative weight (according to Donaldson's formula) increases from about 0.66 per cent of the body at 5 grams to a maximum of 0.77 per cent at 10 to 15 grams, declining to 0.28 per cent at 205 grams and 0.22 per cent at 315 grams. From the combined data the coefficient of variation was calculated for the newborn only (48 males, 29 females) and was found to be as follows: male, 17.8 (for total body, 13.1); female 12.6 (total body, 9.4); total (sexes combined) 16.0 (for total body 12.5). The corresponding coefficient of correlation with the body weight was 0.666±0.043, which is somewhat lower than that found by Donaldson ('08) for the spinal cord of the rat in general (0.8564±0.0071).

In comparing these results with those for other organs, it must be remembered that the rats from which the brain and spinal cord were derived correspond only in part to those for all the other organs.

3. *Eyeballs*

The relative or percentage weight of the eyeballs for various body weights, when calculated from Hatai's ('13) formula, is shown by the curve in figure 1 b. According to this curve, it is seen that the eyeballs increase in relative weight from about 0.59 per cent of the body weight at 5 grams to 0.61 per cent at 10 grams, thereafter decreasing steadily, reaching 0.48 per cent

at 20 grams, 0.29 per cent at 50 grams, 0.18 per cent at 120 grams, 0.15 per cent at 170 grams, 14 per cent at 200 grams, and 0.11 per cent at 300 grams.

In my data grouped according to age periods (table 4, fig. 1 b) the average weight of the eyeballs at birth forms about 0.52 per cent of the (net) body weight in the male, and 0.54 per cent in the female. The relative weight increases to a maximum at seven days of about 0.60 per cent in the male, and 0.64 per cent in the female.[5] Thereafter the eyeballs decrease steadily in relative weight, excepting a slight rise at one year (which, like that for the head, is probably abnormal). It will be noted that the percentage weight of the eyeballs is larger in the female at every age except at six weeks. Whether this expresses a true difference according to sex is uncertain, however, as the difference is small and the data perhaps inadequate to determine this point.

In connection with this difference in relative weight according to sex, it is noteworthy (table 4) that the *absolute* weight of the eyeballs is nearly the same in both sexes at every age. Since the average body weight of the female is smaller at each of the ages noted (except six weeks), it follows that the *relative* weight of the eyeballs must be correspondingly larger in the females.

The similarity in the absolute weight of the eyeballs in both cases at various ages suggests the possibility that the growth of these organs may be somewhat independent of influences affecting the growth of the body as a whole. This idea is to a certain extent confirmed by the coefficient of variation in the absolute weight of the eyeballs (table 4). Although at birth this coefficient (15.5) appears larger than that of the whole body (12.3), after the age of seven days the variation in the absolute weight of the eyeballs is exceedingly low (7.45 to 13.3), only the brain approaching it in this respect.

[5] In comparing the observed with the calculated values in this and the other organs, it should be pointed out that even slight fluctuations in absolute weight which may lie within the experimental error, may produce a much greater percentage deviation in the youngest animals, owing (1) to the small absolute size of the organ, and (2) to the relatively greater weight of the organ in the earlier periods.

Furthermore, the idea of an independent growth of the eyeballs is supported by the variation in the percentage weight relations (table 4). While in the newborn the coefficient of variation in the percentage weight is less than that for the absolute weight (the usual relation in other organs at all ages), it is a remarkable fact that after seven days the variation in the eyeballs is *decidedly greater for the percentage weight.* Finally, the coefficient of correlation between body weight and weight of the eyeballs, as might be expected from the foregoing, is comparatively low and very irregular (0.21 to 0.67).

4. Thyroid gland

When the calculations are made according to Hatai's formula, the relative weight of the thyroid (fig. 3 a) is greatest in the earliest stages, forming about 0.029 per cent of the body weight at 5 grams. The relative weight decreases slowly to 0.028 per cent of the body at 10 grams, 0.026 per cent at 20 grams, 0.022 per cent at 50 grams, 0.019 per cent at 100 grams, 0.016 per cent at 200 grams, and 0.015 per cent at 300 grams.

On account of certain difficulties, the number of observations upon the thyroid gland in my own series is somewhat limited. The gland is small and difficult to separate accurately from the adjacent muscles, especially in the younger stages. It was also sometimes injured in the process of decapitation. The observations recorded, arranged according to age (table 5, fig. 3 a), indicate that the thyroid is relatively largest in the newborn, decreasing from about 0.04 per cent of the body weight to 0.018 per cent at one year. In comparison with Hatai's theoretical curve of growth, there is in my data a lagging behind during the first week. The thyroid apparently increases but slightly during this period while the body weight doubles. This causes a considerable drop in the percentage weight of the thyroid at seven days. No difference according to sex is apparent. On account of the limited data, the coefficients of variation and correlation were not calculated.

Watson ('10) finds the thyroid (and parathyroid) glands usually enlarged in rats fed upon meat, and especially oatmeal, diet.

They were also found relatively much larger in wild rats, due probably to difference in exercise and diet.

5. *Thymus*

On account of inadequate data, Dr. Hatai was unable to construct a satisfactory formula for the growth in absolute weight of the thymus in the albino rat.

In my own data grouped according to age periods (table 6, fig. 2 b), it appears that the thymus increases, in the male, from about 0.15 per cent of the body weight at birth to 0.24 per cent at seven days, and to a maximum of 0.38 per cent at twenty days. Thereafter it decreases, and at one year forms an average of only 0.02 per cent of the body. The relative weight undergoes a similar change in the female with no significant difference according to sex.

A unique feature of the thymus is its decrease in absolute as well as in relative weight. As shown in table 6, the average absolute weight at five months is slightly smaller than at ten weeks, and at one year it has undergone a very striking decrease. This is of course in connection with the process of involution, following the age of puberty, whereby the thymus is largely transformed into a mass of adipose tissue.

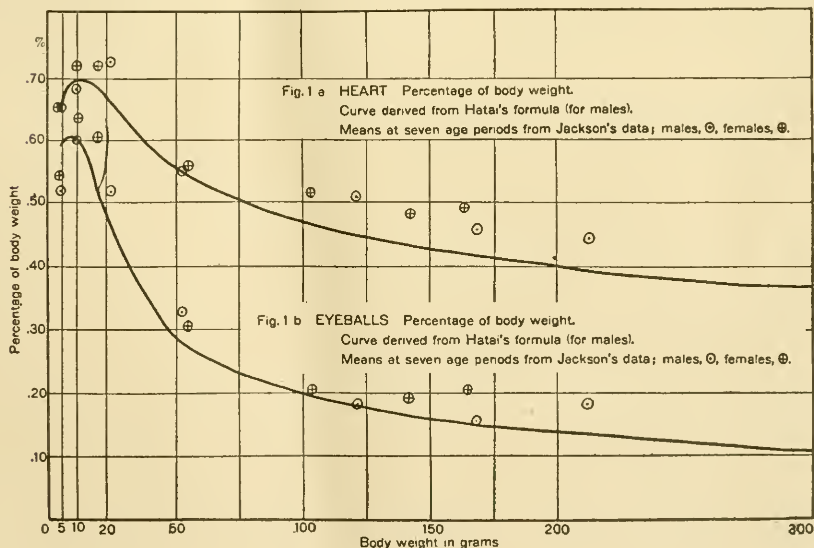
As would necessarily follow from the decrease in the absolute weight of the thymus during the process of involution, there is actually a *negative* correlation at five months between the thymus and body weights. During the earlier life, on the other hand, there is a well marked positive correlation, the coefficient increasing from 0.668 at birth to 0.904 at six weeks (the high figures being partly due to 'spurious correlation').

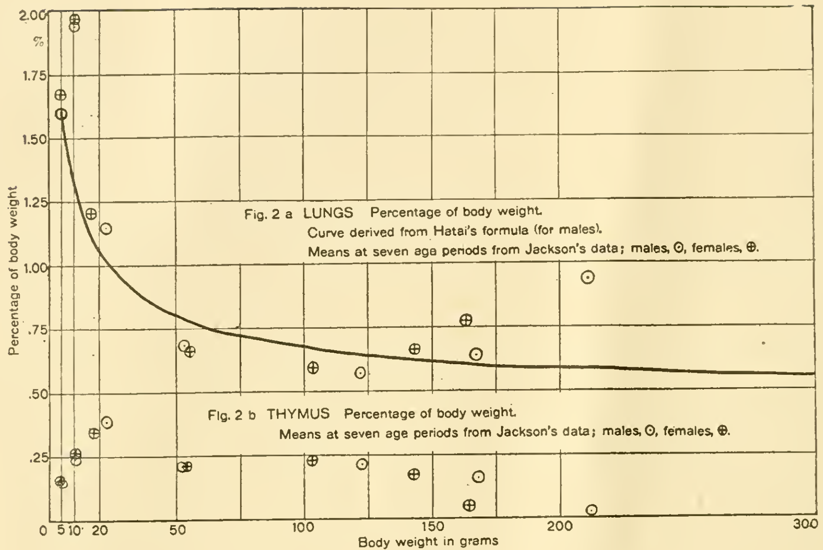
The coefficient of variation in absolute weight is high, increasing from 30.9 at birth to 50.4 at six weeks. Later, however, when a greater variation might naturally be expected during the involution process, it is actually less, the coefficient being 25.3 and 22.2. For the percentage weight, the coefficient of variation, though still high, is considerably smaller than that for the absolute weight, excepting the periods at ten weeks and five months.

6. Heart

According to the curve of relative (percentage) weight (fig. 1 a) constructed from Hatai's formula, the heart increases from 0.64 per cent of the body weight at 5 grams to a maximum of 0.70 per cent at 10 grams. Thereafter it gradually decreases to 0.67 per cent at 20 grams, 0.56 per cent at 50 grams, 0.45 per cent at 120 grams, 0.42 per cent at 170 grams, and 0.40 per cent at 200 grams, 0.37 per cent at 300 grams and 0.35 per cent at 400 grams.

When grouped according to age periods, my own data (table 7, fig. 1 a) show an increase from an average in the male of 0.65 per cent at birth to 0.68 per cent at seven days and to a maximum of nearly 0.73 per cent at twenty days; thereafter it decreases to about 0.45 per cent at one year. In the female the relations are similar, although the average relative weight is greater at every age (except twenty days). This apparently greater relative weight of the heart in the female, however, is probably without significance. In the first place, the relative weight of the heart in the female at any given age beyond six weeks should be slightly greater on account of the smaller body weight. More-





over, the differences between the sexes in percentage weight shown in the table are comparatively slight, usually well within the limits of probable error. It is noteworthy that my data give percentage weights which are slightly, but almost constantly, higher than those of the curve derived from Hatai's formula (fig. 1 a).

The heart shows a considerable degree of variability in absolute weight (table 7), the coefficient increasing from 18 at birth to 33.7 at twenty days and 29.7 at six weeks. At ten weeks and five months the coefficient decreases (18.4 and 21.3) to near that at birth. For the *relative* weight, however, the relations are quite different. At birth and seven days, the coefficient of variation for the percentage weight of the heart is rather high (15 to 18), though still somewhat less than that for the absolute weight. From twenty days onward, however, there is remarkably little variation in the percentage weight of the heart, the coefficient being usually less than 10. This corresponds to the high coefficient of correlation between the weight of the heart and the body weight after twenty days (0.840 to 0.968), which

at birth and seven days is much lower (0.583 and 0.504). However, the high figures for the older rats are partly due to a 'spurious correlation,' as will be explained later.

7. *Lungs*

According to calculations made from Hatai's ('13) formula, the lungs, as shown by the curve in figure 2 a, decrease in relative (percentage) weight from a maximum of 1.60 per cent of the entire body weight at 5 grams to 1.32 per cent at 10 grams, 1.06 per cent at 20 grams, 0.80 per cent at 50 grams, 0.64 per cent at 120 grams, 0.59 per cent at 200 grams, 0.55 per cent at 300 grams, and 0.54 per cent at 400 grams.

The relative weight of the lungs in the male according to my data increases from about 1.59 per cent of the body weight at birth to 1.93 per cent at seven days. From this maximum it decreases to 0.68 per cent at six weeks, and 0.57 per cent at ten weeks. The apparent increase at five months and one year is probably due to the inclusion of unrecognized pathological cases. Except at twenty days, the relative weight of the lungs appears slightly higher in the female than in the male. This is chiefly because the body weight is usually greater in the male. Any sexual difference aside from this is very doubtful. It will be observed that the curve derived from Hatai's formula does not show the preliminary increase in percentage weight which is indicated by my data, and which is found so characteristic of the viscera in general.

The variability of the lungs in absolute weight at birth (23.3) is much greater than that of the whole body, but is later approximately equal to it in most cases (16.6 to 23.9). The variation in percentage weight is usually much less than in absolute weight; and the correlation with the body weight is correspondingly high (73.6 to 94.3 except at ten weeks, 62.3). The high figures are partly due to a 'spurious correlation.'

The weights of the right and the left lungs were also taken separately, although not recorded in the table. The right lung is always much larger than the left, the ratio being approxi-

mately 2 : 1, which does not change appreciably according to age. The variability in absolute weight of right and left lungs taken separately, is about the same as for both together, as shown by table G.

TABLE G

Coefficient of variation for the lungs (sexes together)

	NEWBORN	7 DAYS	20 DAYS	6 WEEKS
	44 m. 43 f.	30 m. 27 f.	24 m. 25 f.	22 m. 17 f.
Both lungs..................	23.3±1.3	16.6±1.1	23.9±1.7	19.4±1.5
Right lung..................	24.2	17.0	24.9	19.7
Left lung....................	22.9	17.8	22.3	19.4

The coefficient of correlation between the body weight and weight of lungs, including right and left lung separately, is given in table H. While the coefficients are too high (due to 'spurious correlation') those at any given age may be safely compared with each other.

TABLE H

Coefficient of correlation between the lungs and the net body weight (sexes together)

	NEWBORN	7 DAYS	20 DAYS	6 WEEKS
	44 m. 43 f.	30 m. 27 f.	24 m. 25 f.	22 m. 17 f.
Both lungs......	0.736±0.033	0.799±0.032	0.868±0.024	0.943±0.012
Right lung......	0.739±0.033	0.813±0.030	0.854±0.026	0.949±0.011
Left lung........	0.693±0.038	0.687±0.047	0.876±0.022	0.902±0.020

From this table it is evident that the correlation between body weight and right lung is practically the same as between body weight and both lungs; and that the correlation between body weight and left lung is usually but slightly less. These figures show no evidence of the existence between the right and left lungs of any compensatory regulation in size, for deviations within the limits of normal variation. This question may be complicated by other factors, however, so that it is unsafe to draw any definite conclusion regarding the matter.

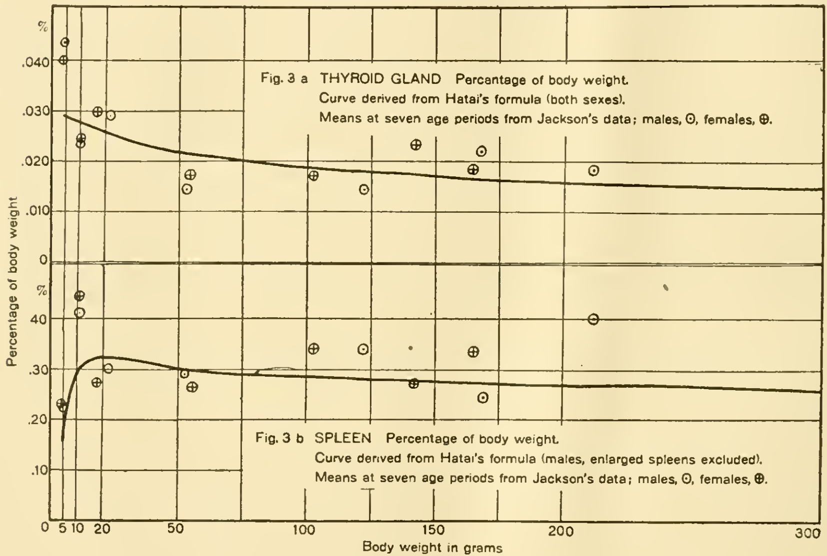
8. *Liver*

The curve representing the relative (percentage) weight of the liver, according to calculations from Hatai's formula, is shown in figure 6 a. It is seen that the relative weight of the liver increases from 4.87 per cent of the body weight at 10 grams to a maximum of 7.80 per cent at 25 grams, after which it gradually decreases to 7.17 per cent at 50 grams, 5.75 per cent at 120 grams, 5.02 per cent at 200 grams, 4.55 per cent at 300 grams, and 4.27 per cent at 400 grams.

When grouped according to age periods, my own data (table 9, fig. 6 a) show that in the male the relative weight of the liver forms an average of 4.74 per cent of the body weight at birth. Unlike the organs previously considered, it apparently *decreases* at seven days, to 3.39 per cent, increasing to 4.64 per cent at twenty days and reaching a maximum of 6.78 per cent at six weeks. Thereafter it decreases to an average of 4.42 per cent at one year. The female exhibits a similar course of growth; but is slightly larger relatively than the male at birth and seven days, and smaller at all later periods. My data therefore indicate a sexual difference, although none was found by Dr. Hatai in his data.

It will also be noted that the liver in my data is at all periods relatively small, when compared with the formula derived from Hatai's data. It is possible that the discrepancy may be due to a slight difference in the diet. Chalmers Watson ('10) finds a marked decrease in the relative size of the liver in captured wild rats, and ascribes the decrease to a diminution in the protein of the bread and milk diet during captivity. If the lower figures in my data were due to this cause, however, we should expect to find a similar condition in the kidneys, which Watson finds also to be larger with rich protein diet. As will be seen later, however, my data usually show for the kidneys a higher relative weight than that according to the formula calculated from Hatai's data. The difference, therefore, can hardly be explained on this basis.

The large but irregular variability of the liver in the present data is shown in table 9. The coefficient of variation in absolute weight appears at different periods from 18.6 to 40.8, but with no definite change according to age or sex. The variation in the percentage weight is seen to be, as a rule, very much less. Accordingly, the coefficient of correlation between liver and body weight is high, from 73.6 to 96.8 (high figures, however, partly due to 'spurious correlation').



9. *Spleen*

The relative (percentage) weight of the spleen, calculated from Hatai's ('13) formula, is represented by the curve in figure 3 b. It is seen that the spleen increases in relative size from 0.16 per cent of the body weight at 5 grams to 0.30 per cent at 10 grams, and a maximum of 0.32 per cent at 20 grams, thereafter decreasing slowly to 0.30 per cent at 50 grams, 0.28 per cent at 120 grams, 0.27 per cent at 200 grams, and 0.26 per cent at 300 to 500 grams.

When grouped according to age periods (table 10, fig. 3 b) my own data show an increase in the relative size of the (male) spleen from 0.22 per cent of the body weight at birth to a maximum of 0.41 per cent at seven days. For succeeding ages, the percentage weight agrees fairly with that derived from the formula, excepting the unusually high figure (0.396 per cent) at one year. There is no distinct difference between the sexes, or change in relative size according to age (after the first week).

It may be noted that my data for the spleen tend to run somewhat higher than the curve derived from Hatai's formula. This may be because Hatai's formula is based upon data from which the 'enlarged' spleens had been excluded. His figures for *all* spleens average very much higher than mine.

The spleen is known to be an unusually variable organ and this is certainly true for the rat. As shown in table 10, the coefficient of variation in absolute weight is from 25 to 51 (excepting at five months, 18.9) The coefficient of variation in percentage weight is usually but slightly less than that in absolute weight. The coefficient of correlation between spleen and body weight is accordingly somewhat low, varying from 0.406 to 0.542 with an exceptionally high correlation (partly 'spurious') of 0.967 at twenty days.

10. Stomach and intestines

From the curve of relative (percentage) weight constructed from Hatai's ('13) formula (fig. 5), it appears that the empty alimentary canal increases from about 3.0 per cent of the body at 10 grams, to 7.5 per cent at 20 grams, and a maximum of 8.0 per cent at about 35 grams, after which it decreases to 6.3 per cent at 100 grams, 5.0 per cent at 200 grams, and 4.5 per cent at 300 grams.

Observations were made upon the empty intestinal canal in only a part of my own series. These observations, grouped according to ages (table 11, fig. 5) indicate that the relative weight of the empty tract increases from an average of about 2.4 per cent of the body weight in the newborn to a maximum of about 8 per cent at six weeks, decreasing to 5 per cent at one year.

They therefore agree fairly well with the curve from Hatai's formula. The data are too few for calculation of the coefficients of variation and correlation at the various ages.

A complete series of observations is available for the stomach and intestines *plus contents,* however, a summary of which is given in table 12 and figure 5. The increase in relative weight runs somewhat parallel to that for the empty tract, increasing from an average of about 6 per cent of the body weight in the newborn to a maximum of about 16 per cent at six weeks, decreasing thereafter to about 7 per cent at one year.

The coefficient of variation for the canal *with contents* (calculated only to six weeks) is somewhat high, 28.6 to 42.1, but not so high as was anticipated. It is, as might be expected, highest in the newborn (only a part of which had suckled) and at twenty days (weaning period). The coefficient of correlation between body weight and canal with contents is low in the newborn (0.29) but comparatively high later (0.59 to 0.84), partly due to 'spurious correlation.' Although one naturally thinks of the alimentary canal with contents as exceedingly variable in size, it is interesting to note that it is actually less variable than some other viscera. In general, after the newborn age, it is usually less variable and more closely correlated with the body weight than are the ovaries, spleen and suprarenals. It is on account of this degree of correlation between body weight and alimentary canal with contents that in the rat the gross weight serves almost as well as the net body weight as a basis for estimating the relative weight of the various organs.

11. Suprarenal glands

From the curves of relative (percentage) weight (fig. 4) calculated according to Hatai's formulas, it is seen that the relative weight of the suprarenals in the male increases from 0.031 per cent of the body at 5 grams to a maximum of about 0.040 per cent at 10 to 15 grams, thereafter decreasing to 0.038 per cent at 20 grams, 0.030 per cent at 50 grams, 0.021 per cent at 120 grams, 0.018 per cent at 160 grams, and 0.014 per cent at 300 grams. In

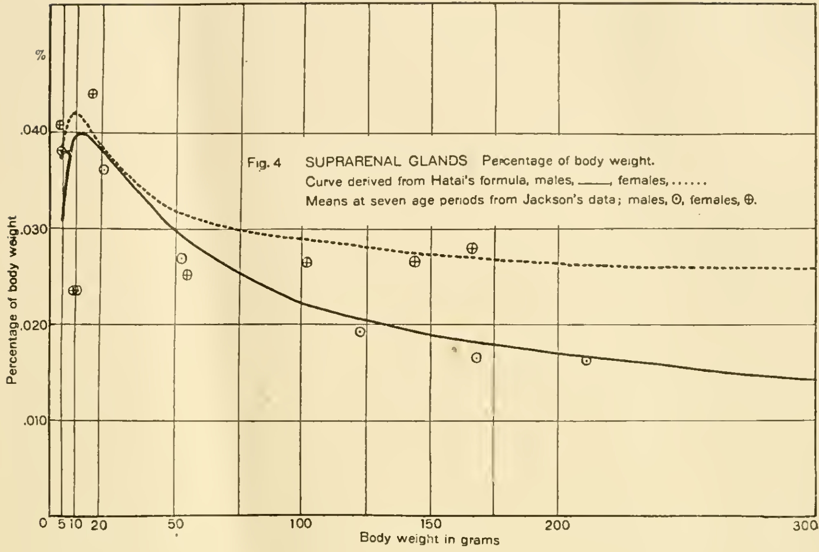


Fig. 4 SUPRARENAL GLANDS Percentage of body weight.
Curve derived from Hatai's formula, males, ——, females, ...
Means at seven age periods from Jackson's data; males, ⊙, females, ⊕.

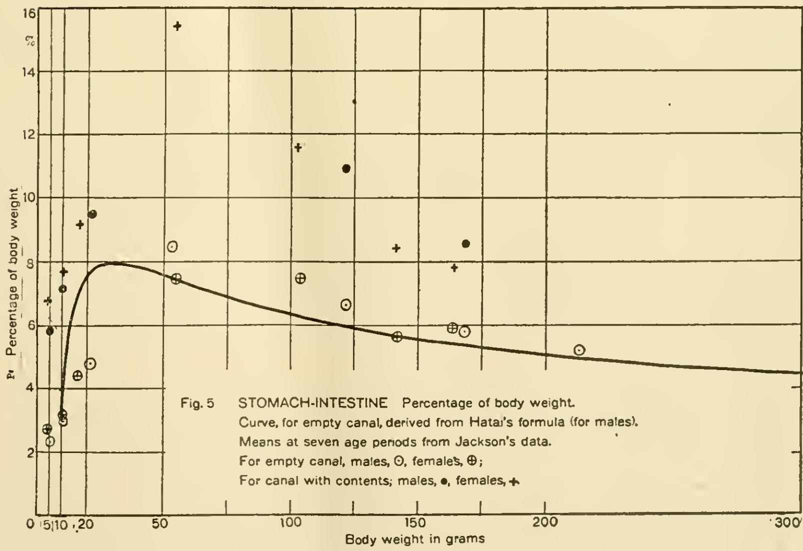


Fig. 5 STOMACH-INTESTINE Percentage of body weight.
Curve, for empty canal, derived from Hatai's formula (for males).
Means at seven age periods from Jackson's data.
For empty canal, males, ⊙, females, ⊕;
For canal with contents; males, •, females, +.

the female the relative weight increases from 0.037 per cent of the body at 5 grams to a maximum of about 0.042 per cent at 10 grams, decreasing to 0.038 per cent at 20 grams, 0.032 per cent at 50 grams, 0.029 per cent at 100 grams, 0.027 per cent at 160 grams, and 0.026 at 300 grams. Thus the suprarenal bodies appear relatively much larger in the female, especially for a body weight above 100 grams.

Grouped according to age periods, my own data (table 13, fig. 4) likewise show the relative growth of the suprarenals to be quite different in the two sexes. In the male, the maximum relative size, 0.038 per cent of the body weight, apparently occurs in the newborn, decreasing to 0.023 per cent at seven days, but increasing again to 0.036 per cent at twenty days, which is nearly as large as the maximum. Thereafter the relative weight decreases to 0.027 per cent at six weeks, 0.018 per cent at ten weeks, and 0.016 per cent at five months and one year. The course of relative growth is somewhat similar in the female, but the maximum (0.043 per cent) occurs at twenty days instead of at birth (0.041 per cent). After six weeks, the relative size is much greater in the female, being 0.026 per cent to 0.028 per cent of the body weight. Even the absolute weight of the suprarenals (after six weeks) is considerably greater in the female, although the body weight is much less.

When compared with the theoretical curve of growth, a marked discrepancy may be noted in the present data (table 13) at seven days. For some unaccountable reason, the suprarenals in my series appear to lag behind during the first week, increasing in weight only about 20 per cent, while the body weight is doubled. There is also a corresponding irregularity in correlation with the body weight at seven days, which is very low, especially in the male.

The coefficient of variation in absolute weight is somewhat high and irregular (20.4 to 38.7) with variations showing no definite relation to age or sex. The coefficient of variation in percentage weight is usually but little, if any, lower. The coefficient of correlation between suprarenals and body weight is cor-

respondingly low, 0.347 to 0.583 (except at seven days) for the sexes combined. In the sexes, separately, however, the correlation is usually somewhat higher, as might be expected, since the sexes show a characteristic difference in the relative weight of the suprarenals.

12. Kidneys

The relative (percentage) weight of the kidneys, reckoned according to Hatai's formula, is shown by the curve in figure 6 b. The kidneys appear in the newborn male to form 0.90 per cent of the body weight at 5 grams, which increases to a maximum of 1.53 per cent at 10 grams, thereafter decreasing to 1.38 per cent at 20 grams, 1.10 per cent at 50 grams, 0.91 per cent at 130 grams, 0.85 per cent at 200 grams, 0.82 per cent at 300 grams, and 0.80 per cent at 400 grams.

When grouped according to age periods, my own data (table 14, fig. 6 b) show the relative (percentage) weight of the kidneys to increase in the male from 0.96 per cent of the body weight at birth to 1.29 per cent at seven days and to a maximum of 1.44 per cent at twenty days. Thereafter it decreases to 1.27 per cent at six weeks, 1.03 per cent at ten weeks, 0.93 per cent at five months, and 0.95 per cent at one year. In the females, the average percentage weight of the kidneys is usually slightly higher than in the male of the same age, but nearly the same as that of a male, with the same body weight. The agreement of my data with the curve derived from Hatai's formula is remarkably close (fig. 6 b).

The coefficient of variation of the kidneys in absolute weight (table 14) is greater than that of the body in the earlier periods, reaching a maximum of 33.7 at twenty days. Later, however, the kidneys become less variable than the body weight, the coefficient being 15 to 19. The variation in the percentage weight is low, especially from seven days onward. The coefficient of correlation between kidneys and body weight is correspondingly high, being 0.703 at birth, and 0.788 to 0.955 at later age periods, (partly due to 'spurious correlation'). No constant difference in variation is noticeable according to age or sex.

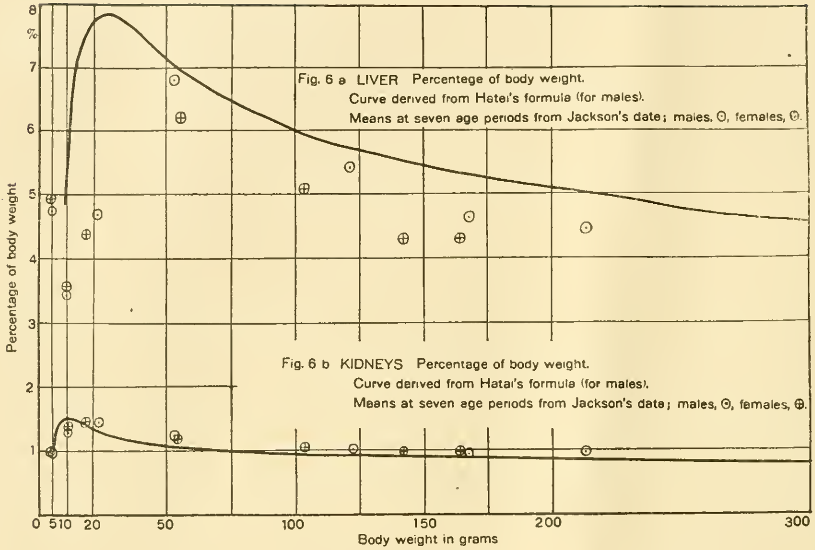


Fig. 6 a LIVER Percentege of body weight.
Curve derived from Hatai's formula (for males).
Means at seven age periods from Jackson's date; males, ⊙, females, ⊕.
Fig. 6 b KIDNEYS Percentage of body weight.
Curve derived from Hatai's formula (for males).
Means at seven age periods from Jackson's data; males, ⊙, females, ⊕.
Percentage of body weight
Body weight in grams
8
%
7
6
5
4
3
2
1
0 5 10 20 50 100 150 200 300

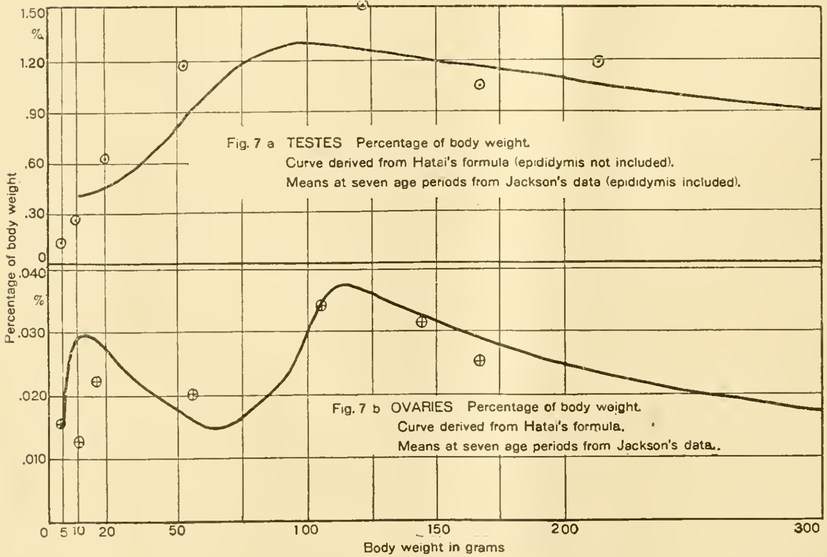


Fig. 7 a TESTES Percentage of body weight.
Curve derived from Hatai's formula (epididymis not included).
Means at seven age periods from Jackson's data (epididymis included).
Fig. 7 b OVARIES Percentage of body weight.
Curve derived from Hatai's formula.
Means at seven age periods from Jackson's data.
Percentage of body weight
Body weight in grams
1.50
%
1.20
.90
.60
.30
0
.040
%
.030
.020
.010
0 5 10 20 50 100 150 200 300

13. Gonads

a. Testes. The relative (percentage) weight of the testes, calculated from Hatai's (13) formulas, is represented by the curve in figure 7 a. This gives a relative weight of 0.40 per cent of the body weight at 10 grams and about the same at 20 grams. Unlike that of other organs, the relative weight of the testes continues to increase for a long time (up to age of puberty), being 0.80 per cent at 50 grams and reaching a maximum of 1.30 per cent from 90 to 100 grams. Thereafter it decreases to 1.28 per cent at 120 grams, 1.16 per cent at 170 grams, 1.09 per cent at 200 grams, 0.90 per cent at 300 grams, and 0.76 per cent at 400 grams.

Unfortunately, in my own data the epididymis is included with the testes and the results are therefore not strictly comparable with those of the formula above, which do not include the epididymis. A few special measurements indicate that the epididymis forms one-fifth or one-sixth of the weight of the testes proper in the younger rats, which however increases (with irregular variations) to one-third after the age of puberty, at about ten weeks. In extreme cases, the weight of the epididymis is one-half that of the testis proper. The weight of the testes proper, as well as of accessory sexual apparatus (epididymis, seminal vesicles, et cetera), probably undergoes considerable change during cycles of sexual activity. This has been noted by Disselhorst ('98, '08) in various mammals and especially in birds, for the gonads in both sexes.

My own data (table 15, fig. 7 a) indicate that the testes (including the epididymis) form an average of about 0.13 per cent of the body at birth, increasing gradually to a maximum of 1.50 per cent at ten weeks, and decreasing somewhat at later periods. The variability in absolute weight is quite high, the coefficient varying from 25.3 at birth to 40.7 at five months (excepting at seven days, when it is only 17.7). The coefficient of variation in percentage weight is much lower (10.3 to 32.8) and the correlation with the body weight higher than might be expected (0.67 to 0.95, except at ten weeks, 0.48). These figures are somewhat too high, however, due to 'spurious correlation.'

b. Ovaries The growth of the ovaries is quite complex. In the relative (percentage) growth curve (fig. 7 b) constructed from data according to Hatai's formulas, two distinct phases are noticeable. In the first phase, beginning at birth, the ovaries increase in relative size from about 0.017 per cent to a maximum of about 0.030 per cent at a body weight of 10 to 15 grams. Thereafter the ovaries decrease to about 0.015 per cent of the body weight at 60 grams. Then begins the second period of acceleration, corresponding to the advent of puberty, during which the ovaries increase to a second maximum of about 0.037 per cent of the body weight at 110 to 120 grams. Thereafter the ovaries lag behind steadily in relative growth, and form only about 0.017 per cent of the body weight at 300 grams.

When grouped according to age periods, my data (table 15, fig. 7 b), show considerable irregularity in the relative size of the ovaries. From an average of about 0.017 per cent of the body at birth they increase (after an apparent drop at seven days) to a first maximum of about 0.022 per cent at twenty days. Then they decrease to about 0.020 per cent at six weeks, increasing to a second maximum of 0.034 per cent at ten weeks, the age of puberty. Thereafter they decrease, averaging 0.025 per cent of the body weight at one year.

The extremes recorded in the size of the ovaries (table 15) show a remarkable range. This is due partly to difficulty during the earlier stages in dissecting out the ovaries accurately, and partly to fluctuations in size in the later stages, probably on account of cycles of ovulation. Coefficients of variation were not calculated for the earliest stages, as the data were considered inadequate. For periods from twenty days onward, the highest coefficient of variation in absolute weight, 50.9, was found at ten weeks (the age of puberty), and the lowest, 32.7, at five months. The coefficients of variation in percentage weight are somewhat lower (25 to 39); and the coefficient of correlation between ovaries and body weight varies from 0.64 to 0.82 (somewhat too high, due to 'spurious correlation').

14. General considerations

We may now review briefly certain phases of growth and variability of the viscera. First should be noted the growth of the viscera, taking the group as a whole (including brain), in comparison with the remainder of the body. As found by Jackson and Lowrey ('12), the visceral group of the rat at birth forms about 18 per cent of the body weight. It increases in relative weight to an average of about 19.2 per cent at seven days, and to a maximum of about 21.3 per cent of the body weight at three weeks. At six weeks, it has decreased slightly, to 20.4 per cent, but continues to decrease in relative weight to about 16 per cent at ten weeks, 14.8 per cent at five months, and 13.3 per cent at one year.

Data for comparison of the relative size in other animals are cited by Jackson and Lowrey ('12), who point out that in general the smaller mammals have a relatively larger visceral apparatus, probably correlated with a more intense metabolism. The rat occupies a somewhat intermediate position, the relative weight of the viscera being less than that of most of the smaller mammals, but greater than that of the larger mammals.

Since the visceral group forms a comparatively small part of the entire body, its *relative* size will evidently be influenced greatly by the rate of growth of the other parts of the body. Jackson and Lowrey ('12) have shown that in the rat the relative increase in the weight of the viscera during the first week is accompanied by a similar slight increase in the skeleton and a very marked increase in the relative weight of the integument. The relative increase in these three systems is apparently balanced by a small decrease in the relative weight of the musculature, and by a remarkable decrease in the 'remainder,' due chiefly to a disappearance of excess body liquids. The slight further increase in the relative weight of the viscera at three weeks is accompanied by an increase in the musculature, and is balanced by decreases in the skin, skeleton and remainder. Thereafter the slow gradual decrease in the relative weight of the visceral group is accompanied by a similar decrease in the skin and skeleton. This is

balanced by a corresponding increase in the musculature, which by its large size virtually dominates the further growth of the body as a whole.

It is further evident that the growth of the body as a whole is the resultant of unlike growths of its various component systems. This principle applies likewise to the growth of the visceral group, the various organs having each its own characteristic mode of growth. As a matter of fact, only three individual organs, thymus, heart, and kidneys (and perhaps also the suprarenals) have their apparent maximum relative weight at the age of three weeks, when the visceral group as a whole appears relatively largest. The stomach and intestines and the liver apparently reach their maximum relative weight at the latter period of six weeks, and the gonads at ten weeks. Of those reaching their maximum at an earlier period, the brain, spinal cord, eyeballs, lungs, and spleen appear to be relatively heaviest in the second week while the thyroid gland appears relatively heaviest in the newborn.

Somewhat similar relations as to the relative growth of the various organs were found by Kellicott ('08) in the dogfish. Of the various organs observed, only the brain and rectal gland appear relatively largest at birth. The heart, pancreas, spleen and liver increase rapidly so as to reach their maximum relative size a short time after birth, and thereafter decline steadily (with a secondary increase in the liver, due to accumulation of fat). The gonads reach their maximum relative size at sexual maturity in the dog-fish.

Scattering data for the growth of the human viscera are recorded by various authors (for references, see paper by Jackson '09), but they are scarcely adequate to determine the question as to the course of postnatal relative growth for the individual organs. In general, so far as may be judged from the data available, the human visceral group appears relatively larger at birth than at any subsequent age. The lungs, however, appear to increase somewhat in relative weight so as to reach a maximum after birth. This is perhaps also true of the heart, kidneys and gonads, and it is quite possible that more extensive data would

show this to be true also for other organs. Some difference between man and rat might naturally be expected, however, since the rat is born in a more immature condition.

The variability in the weight of the body must also depend ultimately upon the variability of its component parts. As to the variability of the component parts of the rat, we have data for the head and viscera only. A brief summary of the coefficients of variation in round numbers (from tables 2 to 15; sexes together, excepting gonads and brain) is given in table I.

The difference in variation between the sexes is usually not very marked (as may be noted in tables 2 to 15); although there appears to be a tendency to greater variability in the male, both in body weight and in weight of the individual viscera. In the table above it may be noted that the head and head organs (brain, eyeballs) form a group[6] of small variability (average coefficient 10 to 12) which is usually far below that for the body as a whole (average 19). The other organs are more variable than the body. The lungs, kidneys, heart, liver, and suprarenals form a moderately variable group (average 21 to 26) while the gonads, thymus, spleen and intestinal canal (with contents) are exceedingly variable (average 29 to 43).

The average coefficient of variation for the viscera examined is 25. It is noteworthy, however, that the average coefficient of variation is lowest at birth and one week, and highest at three weeks. This agrees with what is found also for the body as a whole.

A brief summary of the coefficients of correlation between the body weight and the weight of the individual viscera (from tables 3 to 15, sexes together, excepting brain and gonads) is given in round numbers in table J.

In this table likewise the differences according to sex are disregarded, but even when these are taken into account (cf. tables 3 to 15) the general relations are not materially changed. In about two-thirds of the cases, however, the coefficient of correlation is higher in the males than in the corresponding females;

[6] The blood, the *percentage* weight of which in adult rats, according to Chisolm ('11), has a coefficient of variation of 10.7, possibly also belongs in this group.

TABLE I

Coefficients of variation in weight: albino rat

	NEWBORN	1 WEEK	3 WEEKS	6 WEEKS	10 WEEKS	5 MONTHS	AVERAGE
Brain[1]........	12		7	12			10
Eyeballs......	16	15	13	8	11	9	12
Head.........	10	11	15	10	14	13	12
Total body...	12	16	28	21	20	19	19
Lungs........	23	17	24	19	21		21
Kidneys......	24	22	34	15	17	19	22
Heart........	18	20	34	30	18	21	24
Liver.........	22	19	41	19	33	25	26
Suprarenals..	24	20	33	22	21	39	26
Testes........	25	18	30	27	35	41	29
Thymus......	31	32	43	50	25	22	34
Spleen......	39	34	51	26	38	19	34
Intestinal canal (plus contents)..	38	29	42	30			35
Ovaries.......			42	47	51	33	43
Average of viscera...	23	22	31	24	26	24	25

[1] From combined data as explained under Central nervous system.

TABLE J

Coefficients of correlation with the body weight: albino rat

	NEWBORN	1 WEEK	3 WEEKS	6 WEEKS	10 WEEKS	5 MONTHS	AVERAGE
Head.........	0.76	0.89	0.93	0.95	0.75	0.85	0.86
Kidneys......	0.70	0.79	0.96	0.92	0.90	0.91	0.86
Liver.........	0.76	0.76	0.97	0.84	0.74	0.87	0.83
Lungs........	0.74	0.80	0.87	0.94	0.62		0.80
Brain.........	0.69		0.78	0.88			0.78
Heart........	0.58	0.50	0.91	0.97	0.86	0.84	0.78
Testes........	0.67	0.75	0.95	0.75	0.48	0.88	0.75
Ovaries.......			0.73	0.64	0.82	0.81	0.75
Intestinal canal (plus contents)..	0.29	0.59	0.84	0.76			0.62
Thymus......	0.67	0.74	0.89	0.90	0.51	−0.09	0.60
Spleen........	0.54	0.44	0.97	0.50	0.41	0.46	0.55
Eyeballs......	0.67	0.52	0.67	0.31	0.22	0.32	0.45
Suprarenals..	0.51	0.13	0.58	0.41	0.41	0.35	0.40
Average....	0.63	0.63	0.85	0.75	0.62	0.70	0.70

so there appears to be a tendency to closer correlation, as well as to greater variability, in the males. It will be observed, on comparing this table with the preceding, that (excepting the head) the organs most closely correlated with the body weight are not those of least variability in weight, but a group which is somewhat more variable than the body as a whole (kidneys, liver, lungs). In these organs, the average coefficient of correlation varies from 0.80 to 0.86.

Next come the brain and heart (0.78). The gonads are more closely correlated (0.75) with the body weight than might be anticipated from their variability in weight; while the eyeballs (0.45) and suprarenals (0.40) are lowest in the scale.

The average coefficient of correlation for the organs studied is 0.70. It will be noted that there seems to be a direct relation between the variability of the viscera and their correlation with the body weight. In both it is evident that the average coefficient is lowest at birth and seven days, and highest at three weeks.

As a matter of fact, however, a higher coefficient of correlation is to be expected during growth at times when the body weight and organ weight are most variable. This is due to a '*spurious correlation*,' which has been referred to repeatedly in connection with the discussion of correlation of the individual organs with the body weight at the definite age periods. When all the rats at a given age are grouped together there is naturally, on account of their unequal growth, a considerable scattering of the body weights and corresponding organ weights. This causes an augmentation of the real coefficient of correlation, in accordance with the general principle of "correlation due to heterogeneity of material" (Yule '11, p. 214). To determine the true correlation between the body weight and organ weight at any given age, it would be necessary to have a sufficient number of animals so that they could be separated into groups of approximately the same body weight or organ weight. The present data are unfortunately inadequate for this purpose. It is evident, however, that all the coefficients of correlation above given are somewhat too high, and that no conclusions can be drawn from them as

to change in correlation with age. Coefficients of correlation in different organs *at the same age* may be compared with each other, however, since the body weights and the 'spurious correlation' factor would be the same for all.

The coefficient of variation of the *relative* or *percentage weight* is usually much lower than that of the absolute weight of the various organs.[7] The difference is inconstant, however, and the eyeballs form a marked exception to the rule. In most cases, therefore, the percentage weight of an organ can be predicted much more accurately than the absolute weight of the organ at any given age. The data agree in general with the theory that the growth of the individual organs is correlated with the growth of the whole body more closely than with age, as Donaldson ('08) has found for the brain of the rat.

For comparison of the variability in the human viscera, a few data are available. Pearson ('97) from data of Reid and Peacock calculates the coefficients of variation for the human heart (absolute weight), male, 19.8, female, 20.7; liver, male, 14.5, female, 22.2; kidneys, male, 20.5, female, 22.5. Greenwood ('04) from more extensive data finds the coefficients of variation for healthy organs (males only) approximately as follows: heart, 17.7; liver, 14.8; kidneys, 16.8; spleen, 38. From the foregoing it would therefore appear that the heart, liver and kidneys are less variable in man than in the rat. The spleen is more nearly alike in the two forms, and in both is by far the most variable of the organs compared. Pearl ('05) classifies the human bodily characters with reference to variability in three groups: (1) those with coefficient of variation above 10, viscera in general, whose weight depends largely upon the general metabolic condition of the body and in which natural selection is concerned with functional ability rather than with size: (2) those below 7, chiefly skeletal dimensions, in which the conditions of (1) are reversed; and (3) those with coefficient from 7 to 10, including brain and skull capacity, in which intermediate conditions are found.

[7] Chisolm ('11) finds the coefficient of variation for the *percentage* weights in adult rabbits, for the spleen, 46.7; kidneys, 16.5; liver, 32.5 This would indicate that these organs are more variable in the rabbit than in the albino rat.

For various skull measurements, Hatai ('08) finds the variability in the adult albino rat slightly less than in man.

Finally, the question may be raised as to the significance of the relative size of an organ. Why does it vary so greatly in different species and even in individuals of the same species, especially at different stages of development? While a satisfactory answer to this question is impossible in the present state of our knowledge, certain features of interest in this connection may be noted. There are several conditions according to which a priori differences in the relative size of organs might be expected; even though, to a certain extent, a change in the relative size of any part tends, through physiological correlation, to produce a corresponding change in other parts of the body.

1. The relative size of an organ may vary according to the size of the whole body. On account of mechanical principles, with changes in the ratio of dimensions to surface and mass of the body, and so forth, corresponding changes in the relative size of skin, skeleton, musculature, et cetera, might be expected, as has been pointed out by Welcker and Brandt ('03).

2. Unequal growth of any (especially of a large) part of the body necessarily involves an inverse change in the *relative* size of the remaining parts of the body. For example, the relative increase in the musculature during the later periods of growth involves a corresponding decrease in the relative size of other organs. Fluctuations in the relative amounts of intestinal contents, body fat, hair, and so forth, may likewise produce changes in the relative size of the various organs, as has been emphasized by E. Voit ('05).

3. Differentiation in histological structure may result in more efficient physiological activity, so that a relatively smaller organ may suffice to perform a given function. For example, it is doubtless partly on this account that the embryonic heart and other organs are relatively larger than later.

4. Changes in functional activity, as is well known, occasion an atrophy or hypertrophy of the corresponding organs, with resultant changes in their relative size. Thus an increase of protein in the diet throws more work upon the liver and kidneys,

causing them to become relatively larger. This principle of 'use and disuse' is widespread in its application as an explanation of changes in the relative size of organs. Joseph ('08) suggests that the relatively large heart in the smaller mammals may be due to a 'physiological hypertrophy,' correlated with the more rapid rate of pulsation.

5. Within certain limits, however, an organ is normally somewhat larger than is necessary to perform the functional activity usually demanded. Fluctuations in the amount of this excess of structure (which Melzer has termed the 'factor of safety') may account for a part of the individual variations in relative size found to a greater or less extent in every organ.

6. Various pathological conditions may be associated, either directly or indirectly, with changes in the relative size of individual organs or parts of the body.

The foregoing illustrate conditions associated with changes in the relative size of organs, and representing for the most part teleological explanations, rather than actual causes of varying growth rates in the different organs and parts. For any given organ the immediate cause of its characteristic rate of growth is, like that for the whole body, a complex of factors. These may be divided into (a) intrinsic structure and chemical composition of the organ and (b) its environment, especially the conditions affecting the vascular supply, which transmits the respiratory, nutritive and excretory materials, as well as specific hormones or substances which may stimulate the organ to growth and activity.

SUMMARY

Although the extensive data presented cannot satisfactorily be summarized, some of the more important general conclusions concerning growth and variability of the albino rat are included in the following:

1. At birth the males invariably exceed the females of the same litter in average body weight. Although growth is in general more vigorous in the females during the first six weeks, they fail to overtake the males in the majority of litters (at observed ages). At six weeks, however, the total average body weight of

the females approaches or slightly exceeds that of the males. After six weeks, growth is more vigorous in the males and the females lag behind.

2. Variability in body weight is lowest at birth (the coefficient being about 12) and is not much higher at seven days (16). It appears highest at three weeks (28), and at later periods varies from 19 to 21. The average coefficient, taking all ages together, is 19. There is in the rat no evident correlation between variability and rapidity of growth. The coefficient of variation is practically the same for the gross as for the net body weight. The coefficient usually appears higher in the male, but the difference is slight and of doubtful significance.

3. Fraternal variability (within the litter) in body weight is very low, usually less than half that of the total population of the same age.

4. As the growth rate of the whole body is a resultant of the varying growth rates of the component systems (musculature, skeleton, viscera, and so forth) so the growth rate of the visceral group is a resultant of the different growth rates of the individual organs. While the visceral group as a whole reaches its maximum *relative* (percentage) weight at about the age of three weeks, the thyroid gland apparently is relatively largest at birth, the brain, spinal cord, eyeballs, lungs and spleen about the second week, the thymus, heart, suprarenals (?) and kidneys at three weeks, intestinal canal and liver at six weeks, and the gonads at ten weeks. Differences according to sex, aside from the gonads, are most marked in the eyeballs (?) and suprarenal glands.

5. Similarly the variability in the weight of the body as a whole depends upon the variability of its component parts. In this respect the individual organs may be classified in three groups: (1) the head and head organs (brain, eyeballs) form a group of comparatively slight variability (average coefficient 10 to 12); (2) the heart, lungs, liver, suprarenals and kidneys form a group of moderate variability (average coefficient 21 to 26); (3) the thymus, spleen, gonads and intestinal canal (with contents) form an extremely variable group (average coefficient above 29).

The average coefficient of variation for the viscera, taking all ages together, is about 25. The average however is lowest at birth and one week (22 or 23) and highest at three weeks (31), resembling in this respect the variability in the weight of the body as a whole.

6. The coefficient of variation for the relative or percentage weight is usually much lower than that for the absolute weight of the various organs, as the growth of the organs is correlated with that of the whole body more closely than with age. The eyeballs, however, form a conspicuous exception to this rule.

7. The highest degree of correlation with the body weight (average coefficient 0.80 to 0.86) is found, not among the organs of least variability (excepting the head), but among those of moderate variability (kidneys, liver, lungs). Next come the brain, heart and gonads (0.75 to 0.78). The remaining organs are much less closely correlated with the body weight, the lowest being the eyeballs and suprarenals (0.40 to 0.45). The average coefficient of correlation, all ages together, is about 0.70. The figures for coefficient of correlation are somewhat too high, being augmented by a 'spurious correlation' due to the heterogeneity of the weights when grouped at the age periods. Like the variability, the correlation appears lowest at birth and one week (0.63) and highest at three weeks (0.85). Similarly the coefficient of correlation appears usually higher in the male, as is also the case with the coefficient of variation.

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TABLE 1

Litters of albino rats used

LITTER NO.	AGE OF LITTER	MALES No.	MALES Average gross weight (and range)	MALES Average net weight (and range)	FEMALES No.	FEMALES Average gross weight (and range)	FEMALES Average net weight (and range)
			grams	*grams*		*grams*	*grams*
20	newborn	2	4.12 (3.67–4.57)	4.04 (3.59–4.48)	2	3.71 (3.56–3.86)	3.65 (3.50–3.79)
22	newborn	5	4.78 (3.48–5.56)	4.69 (3.43–5.17)	3	4.43 (4.03–4.70)	4.24 (4.01–4.62)
23	newborn	1	4.44	4.30	0		
24	newborn	5	5.00 (4.68–5.25)	4.90 (4.56–5.17)	4	4.74 (4.62–4.91)	4.63 (4.50–4.84)
25	newborn	1	5.19	4.98	1	4.28	4.07
47	newborn	3	6.50 (6.40–6.62)	6.03 (5.88–6.25)	0		
48	newborn	3	5.73 (5.53–5.97)	5.42 (5.24–5.59)	3	5.47 (5.29–5.59)	5.05 (4.90–5.20)
52	newborn	4	3.96 (3.41–4.75)	3.82 (3.30–4.60)	1	3.88	3.72
53a	newborn	1	5.27	5.09	5	4.93 (4.67–5.14)	4.77 (4.52–4.98)
58	newborn	4	5.48 (5.30–5.62)	5.32 (5.16–5.45)	3	4.95 (4.23–5.41)	4.82 (4.12–5.26)
61	newborn	4	5.49 (5.23–5.66)	5.35 (5.10–5.52)	5	5.27 (5.00–5.53)	5.13 (4.91–5.44)
64	newborn	8	4.77 (4.35–5.09)	4.46 (4.06–4.74)	3	4.37 (4.24–4.55)	4.07 (3.96–4.22)
M9	newborn	5	5.42 (5.1–5.8)		3	5.13 (4.9–5.4)	
M10	newborn	2	5.85 (5.7–6.0)		4	5.58 (4.8–6.3)	
A28	newborn	4	4.71		2	4.37	
A29	newborn	2	4.70		4	4.63	
A32	newborn	3	5.11 (4.95–5.38)		5	4.73 (4.50–4.91)	
M8	1 day	3	6.08 (5.75–6.35)		5	5.43 (4.70–6.05)	
62	1 day	3	5.41 (5.30–5.56)	5.13 (4.97–5.33)	8	4.96 (4.45–5.19)	4.74 (4.27–4.98)
63	1 day	0			5	4.90 (4.68–5.07)	4.53 (4.15–4.78)
18	7 days	5	9.92 (8.54–10.46)	9.56 (8.29–10.02)	3	9.13 (8.88–9.57)	8.78 (8.58–9.11)
19	7 days	6	9.42 (9.11–9.58)	9.15 (8.85–9.33)	3	8.04 (7.56–8.72)	7.71 (7.19–8.32)
42	7 days	2	11.62 (10.80–12.43)	10.59 (9.84–11.33)	4	12.07 (11.32–12.70)	11.02 (10.04–11.77)
46	7 days	1	15.56	14.95	1	14.13	13.23
49	7 days	7	12.36 (10.58–13.06)	11.77 (9.94–12.41)	3	11.60 (10.80–12.17)	11.07 (10.33 11–68)
51	7 days	2	11.95 (11.89–12.00)	11.16 (11.04–11.27)	3	11.67 (11.50–11.77)	10.93 (10.71–11.07)
56a	7 days	0			3	10.46 (9.87–10.96)	10.01 (9.48–10.43)
59	7 days	4	9.39 (8.07–10.13)	8.78 (7.40–9.55)	3	10.12 (9.78–10.42)	9.44 (9.08–9.85)
60	7 days	3	8.48 (6.46–10.46)	8.24 (6.32–10.15)	4	9.40 (9.05–9.78)	9.10 (8.70–9.63)
M6	7 days	5	12.01 (9.10–12.95)		3	11.55 (11.15–12.15)	
M7	7 days	2	10.75 (10.20–11.30)		4	10.96 (10.55–11.40)	
M8	7 days	3	12.22 (12.05–12.55)		5	11.52 (10.55–12.25)	
M9	7 days	5	9.26 (8.8–9.7)		3	8.7 (8.4–9.1)	
M10	7 days	2	11.3 (11.1–11.5)		4	10.75 (9.2–11.8)	
A28	7 days	4	7.52		2	7.56	
A29	7 days	2	12.42		4	10.42	
A32	7 days	3	9.83 (9.44–10.14)		5	9.50 (9.04–10.08)	
A33	7 days	0			7	9.44 (6.70–10.12)	
M6	14 days	5	21.0 (20.4–21.9)		3	19.5 (19.1–19.9)	
M8	14 days	3	18.8 18.3–19.2)		5	17.8 (16.9–18.7)	
M9	14 days	3	18.7 (18.5–19.0)		3	17.9 (17.5–18.6)	
M10	14 days	2	17.7 (17.0–18.4)		4	17.9 (15.3–19.3)	
26	20 days	5	16.66 (13.92–18.52)	16.12 (13.82–17.74)	3	16.98 (15.39–17.82)	16.20 (14.99–17.03)
43	20 days	3	22.68 (21.90–23.15)	21.67 (20.98–22.04)	3	22.58 (22.45–22.65)	21 59 (21.52–21.71)
45	20 days	3	14.02 (13.49–14.56)	13.55 (13.00–14.09)	8	13.31 (12.71–13.76)	12.85 (12.34–13.23)
53b	20 days	5	29.42 (27.25–30.94)	28.31 (25.80–29.79)	0		
56b	20 days	2	33.84 (29.70–37.97)	32.05 (28.20–35.90)	0		
57	20 days	4	22.11 (21.16–22.43)	20.50 (19.64–21.90)	3	19.46 (18.30–20.22)	18.05 (17.34–18.60)
M1	20 days	4	23.7 (22.9–24.1)		1	25.0	
M2	20 days	4	26.9 (25.8–27.9)		1	24.8	
M6	20 days	5	27.2 (23.7–28.8)		3	26.3 (25.8–26.8)	
M7	20 days	2	21.0 (15.7–26.4)		4	24.4 (19.7–27.0)	

TABLE 1 (Continued)

LITTER NO.	AGE OF LITTER	MALES			FEMALES		
		No.	Average gross weight (and range)	Average net weight (and range)	No.	Average gross weight (and range)	Average net weight (and range)
			grams	*grams*		*grams*	*grams*
M8	20 days	3	26.2 (25.8–26.5)		5	25.1 (24.7–25.5)	
M9	20 days	3	26.9 (25.6–27.9)		3	25.8 (24.3–26.6)	
M10	20 days	2	24.7 (22.6–26.8)		4	24.7 (21.8–26.5)	
A28	20 days	4	25.4 (23.5–27.0)		2	25.2 (24.3–26.2)	
A29	20 days	2	24.1 (23.4–24.8)		4	23.2 (21.5–24.1)	
A33	20 days	0			7	24.6 (21.0–26.1)	
15	21 days	2	18.22 (17.71–18.72)	17.77 (17.45–18.08)	8	17.72 (15.27–19.19)	16.95 (14.77–17.89)
M7	30 days	1	45.2 (1 died)		4	40.8 (24.1–48.7)	
M8	30 days	3	47.9 (44.8–50.2)		5	44.0 (41.1–48.1)	
M9	30 days		(killed)		3	49.9 (47.2–51.4)	
29	41 days	2	54.55 (52.9–56.2)	49.65 (48.9–50.4)	6	49.65 (42.8–53.7)	46.0 (40.5–48.6)
30	41 days	4	56.58 (55.1–58.4)	50.78 (49.6–52.2)	3	52.67 (40.8–58.7)	48.43 (39.4–53.9)
M2	41 days	4	73.5 (70.2–80.9)		1	66.7	
M9	42 days		(killed)		3	82.5 (75.2–86.6)	
M10	42 days	2	89.0 (83.0–95.0)		4	80.0 (66.0–92.0)	
A28	42 days	4	60.8 (57.3–63.5)		2	65.3 (59.8–70.7)	
A29	42 days	2	66.7 (66.1–67.4)		4	61.1 (59.6–63.6)	
A33	42 days	0			5	62.1 (58.4–69.8)	(2 killed)
31	42 days	10	48.02 (39.4–53.4)	44.63 (38.1–49.6)	1	42.4	39.5
32	42 days	5	51.78 (43.1–57.4)	49.24 (41.3–54.9)	4	46.27 (42.9–48.2)	44.05 (39.6–46.3)
50a	42 days	1	89.0	82.5	3	84.57 (83.5–85.6)	78.07 (76.8–78.8)
M6	42 days	3	62.7 (55.0–69.0)		1	66.0	
33	43 days	0			3	49.83 (42.9–60.8)	45.4 (40.1–54.5)
M1	43 days	4	73.1 (64.4–81.6)		1	70.0	
M7	44 days	1	57.1		4	56.8 (23.5–71.8)	
M8	48 days	3	105.9 (93.5–114.2)		5	86.5 (81.6–95.0)	
4	69 days	3	117.1 (107.4–125.8)	112.5 (103.6–120.8)	0		
5	70 days	0			3	100.7 (86.1–127.3)	97.2 (83.2–122.2)
12	70 days	3	89.9 (77.5–97.9)	86.2 (74.1–93.7)	2	100.2 (98.2–102.1)	89.2 (87.2–91.1)
40	70 days	1	151.1	145.3	1	101.9	97.6
41	70 days	4	117.8 (110.1–124.8)	112.7 (105.4–119.1)	4	91.5 (82.2–106.9)	88.1 (78.2–102.3)
50b	70 days	1	182.2	175.2	2	129.6 (123.9–135.4)	125.3 (120.3–130.2)
1	71 days	2	149.9 (140.8–159.)	144.1 (134.1–154.)	7	110.7 (85.4–126.9)	107.1 (82.7–123.5)
44	71 days	6	119.0 (111.8–134.1)	114.0 (108.4–128.6)	4	93.0 (75.5–100.)	88.2 (71.1–94.8)
M8	70 days	3	187.3 (177.–193.)		5	135.0 (124.–145.)	
10	149 days	0			2	137.8 (126.–149.6)	134.4 (122.5–146.3)
6	150 days	5	158.1 (142.5–167.8)	154.7 (139.4–164.6)	4	145.3 (120.6–159.9)	142.1 (115.9–156.7)
11	150 days	4	157.2 (150.7–166.2)	151.7 (145.9–157.1)	3	160.0 (150.–164.0)	151.9 (140.1–158.1)
36	150 days	3	172.6 (163.3–189.8)	168.0 (156.3–186.5)	3	127.9 (111.8–136.3)	125.2 (110.3–133.)
38	150 days	6	152.0 (120.6–176.8)	149.7 (118.8–176.)	4	114.9 (97.3–130.3)	111.9 (94.6–126.2)
28	151 days	2	247.2 (241.8–252.5)	243.1 (239.4–246.8)	5	160.8 (133.5–174.1)	158.2 (131.8–171.5)
2	1 year	0			2	184.3 (183.5–185.5)	181.9 (181.–182.7)
3	1 year	0			3	192.0 (156.2–209.7)	189.1 (154.–207.3)
4	1 year	0			2	195.8 (188.7–202.8)	190.6 (182.6–198.5)
7	1 year	1	290.5	287.3	2	120.9 (107.3–134.5)	119.5 (105.8–133.1)
8	1 year	1	142.8	139.6	1	188.3	186.0
13	1 year	0			2	174.8 (142.–207.5)	172.5 (141.–204.)
34	1 year	0			1	115.8	114.2
37	1 year	1	281.1	276.4	0		
39	1 year	2	175.3 (118.4–232.1)	172.4 (115.6–229.2)	3	113.8 (86.1–165.0)	110.8 (83.4–161.1)
35	13 mo.	0			4	175.0 (167.2–181.3)	170.7 (161.9–178.6)

TABLE 2

Body weight of albino rats

A. Gross body weight (grams)

		NEWBORN 44 m. 43 f.	7 DAYS 30 m. 27 f.	20 DAYS 24 m. 25 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 20 m. 23 f.	5 MONTHS 20 m. 21 f.	1 YEAR 5 m. 20 f.
Mean	male	5.06	10.61	22.20	52.89 (±1.35)	121.9	167.5	213.0
	female	4.82	10.48	17.01	51.86 (±2.04)	103.3	142.1	163.7
	total	4.94	10.55	19.55	53.59	112.0	154.5	
Range	male	3.41–6.62	6.46–15.6	13.5–38.0	39.4–89.0	77.5–182.2	120.6–252.5	118.4–290.5
	female	3.56–5.59	7.56–14.1	12.7–22.7	40.8–85.6	75.5–135.4	97.3–174.1	86.1–209.7

B. Net body weight (grams)

		NEWBORN 44 m. 43 f.	7 DAYS 30 m. 27 f.	20 DAYS 24 m. 25 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 20 m. 23 f.	5 MONTHS 20 m. 21 f.	1 YEAR 5 m. 20 f.
Mean	male	4.857±.068	10.11±.21	21.23±.83	48.97±1.22	117.0±3.3	163.4±4.6	209.6
	female	4.610±.047	9.91±.18	16.24±.38	50.37±1.88	98.8±2.3	138.4±3.1	160.6
	total	4.735±0.42	10.01±.14	18.68±.52	49.64±1.10	107.2±2.2	150.6±3.0	
Range	male	3.30–6.25	6.32–14.95	13.0–35.9	38.1–82.5	74.1–175.2	118.8–246.8	115.6–287.3
	female	3.50–5.44	7.19–13.23	12.3–21.7	39.4–78.8	71.1–130.2	94.6–171.5	83.4–207.3
Standard deviation	male	.662±.048	1.71±.15	6.02±.59	8.50±.86	22.0±2.3	30.2±3.2	
	female	.458±.033	1.36±.12	2.84±.27	12.45±1.33	16.6±1.7	21.1±2.2	
	total	.583±.030	1.56±.099	5.31±.36	10.58±.78	21.3±1.5	28.8±2.1	
Coefficient of variation	male	13.6±.99	16.9±1.5	28.4±3.0	17.4±1.8	18.8±2.1	18.5±2.0	
	female	9.93±.72	13.7±1.3	17.5±1.7	24.7±2.8	16.8±1.7	15.3±1.6	
	total	12.3±.64	15.6±1.0	28.4±2.1	21.3±1.2	19.9±1.5	19.1±1.5	
Skewness[1]	male	−.41	+.55	+.43	+.02	+.54	+.69	
	female	−.46	+.13	−.57	+.94	+.69	−.23	
	total	−.44	+.32	+.62	+.53	−.16	−.06	

[1] Calculated according to formula: $\text{Skewness} = \frac{3\ (\text{mean} - \text{median})}{\text{Standard deviation}}$.

TABLE 3

Head of albino rats

A. Absolute weight (grams)

		NEWBORN 44 m. 43 f.	7 DAYS 30 m. 27 f.	20 DAYS 24 m. 25 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 20 m. 23 f.	5 MONTHS 20 m. 21 f.	1 YEAR 5 m. 20 f.
Mean	male	1.095±.011	2.552±.036	4.60±.089	7.33±.086	12.22±.24	15.83±.31	19.5
	female	1.051±.0097	2.543±.037	4.09±.064	7.44±.13	11.45±.21	15.31±.29	16.9
	total	1.073±.0075	2.548±.026	4.34±.061	7.386±.077	11.80±.16	15.57±.21	
Range	male	.889–1.335	1.76–3.28	3.44–5.85	6.3–9.3	10.3–15.9	12.7–21.9	17.0–25.4
	female	.854–1.260	2.00–3.17	3.42–4.86	6.5–9.4	8.9–14.1	11.5–18.0	11.7–20.4
Standard deviation	male	.1092±.0078	.296±.026	.642±.063	.600±.061	1.61±.17	2.06±.22	
	female	.0937±.0068	.283±.026	.473±.045	.861±.092	1.48±.15	1.93±.20	
	total	.1042±.0053	.290±.018	.628±.042	.740±.054	1.59±.12	2.01±.15	
Coefficient of variation	male	9.97±.73	11.6±1.0	14.0±1.4	8.19±.84	13.2±1.4	13.0±1.4	
	female	8.93±.65	11.2±1.0	11.6±1.1	11.6±1.3	13.0±1.3	12.6±1.3	
	total	9.71±.50	11.4±.73	14.5±1.0	10.0±.74	13.5±.99	12.9±.98	
Coeffic. of correlation	male	.798±.037	.896±.024	.970±.082	.879±.033	.698±.077	.891±.031	
	female	.655±.059	.882±.029	.950±.013	.936±.019	.786±.054	.909±.026	
	total	.763±.030	.885±.019	.934±.012	.946±.011	.748±.045	.854±.028	

B. Percentage of net body weight

		NEWBORN 44 m. 43 f.	7 DAYS 30 m. 27 f.	20 DAYS 24 m. 25 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 20 m. 23 f.	5 MONTHS 20 m. 21 f.	1 YEAR 5 m. 20 f.
Mean	male	22.75±.21	25.49±.25	22.51±.43	15.17±.19	10.64±.23	9.79±.12	10.03
	female	22.86±.18	25.81±.23	25.41±.49	15.17±.25	11.70±.17	11.13±.095	10.93
Range	male	19.92–28.91	21.92–30.68	16.3–27.29	11.25–17.85	8.56–13.9	8.35–11.04	6.68–13.03
	female	20.11–26.46	23.51–29.65	22.27–28.11	11.82–18.00	9.98–13.8	10.0–12.51	8.92–14.4
Standard deviation	male	2.10±.15	2.05±.18	3.15±.31	1.31±.13	1.54±.16	.767±.082	
	female	1.77±.13	1.76±.16	3.60±.34	1.67±.18	1.20±.12	.645±.067	
Coeffic. of var.	male	9.25±.67	8.04±.70	14.0±1.4	8.63±.89	14.6±1.6	7.83±.84	
	female	7.73±.56	6.82±.63	14.2±1.4	11.0±1.2	10.3±1.0	5.81±.60	

TABLE 4

Eyeballs of albino rats

A. Absolute weight (grams)

		NEWBORN 44 m. 43 f.	7 DAYS 29 m. 26 f.	20 DAYS 24 m. 24 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 19 m. 23 f.	5 MONTHS 17 m. 21 f.	1 YEAR 5 m. 20 f.
Mean	male	.0252 ± .00044	.0597 ± .0010	.1052 ± .0021	.1572 ± .0014	.2032 ± .0038	.2588 ± .0046	.331
	female	.0248 ± .00035	.0630 ± .0013	.0963 ± .0014	.1500 ± .0018	.1996 ± .0024	.2600 ± .0022	.320
	total	.0250 ± .00028	.0613 ± .00081	.1008 ± .0013	.1534 ± .0012	.2012 ± .0023	.2595 ± .0024	
Range	male	.018–.040	.046–.083	.073–125	.136–.175	.138–.249	.199–.316	.296–.356
	female	.017–.032	.046–.082	.072–.113	.125–.172	.169–.223	.232–.287	.204–.403
Standard deviation	male	.00427 ± .00031	.00798 ± .00071	.0150 ± .0015	.00962 ± .00098	.0253 ± .0027	.0283 ± .0033	
	female	.00340 ± .00025	.00945 ± .00088	.00996 ± .00097	.0117 ± .00125	.0173 ± .0017	.0148 ± .0015	
	total	.00387 ± .00020	.00886 ± .00057	.0134 ± .00092	.0114 ± .00083	.0217 ± .0016	.0219 ± .0017	
Coefficient of variation	male	17.0 ± 1.3	13.4 ± 1.2	14.3 ± 1.4	6.13 ± .63	12.5 ± 1.4	10.9 ± 1.3	
	female	13.7 ± 1.0	15.0 ± 1.5	10.4 ± 1.0	7.80 ± .83	8.69 ± .86	5.69 ± .59	
	total	15.5 ± .82	14.5 ± .95	13.3 ± .93	7.45 ± .55	10.8 ± .80	8.45 ± .66	
Coeffic. of correlation	male	.783 ± .039	.451 ± .099	.721 ± .066	.517 ± .105	.181 ± .146	.520 ± .120	
	female	.495 ± .079	.597 ± .085	.242 ± .130	.330 ± .134	.230 ± .133	.336 ± .130	
	total	.666 ± .040	.518 ± .067	.666 ± .054	.306 ± .094	.216 ± .099	.319 ± .098	

B. Percentage of net body weight

		NEWBORN 44 m. 43 f.	7 DAYS 29 m. 26 f.	20 DAYS 24 m. 24 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 19 m. 23 f.	5 MONTHS 17 m. 21 f.	1 YEAR 5 m. 20 f.
Mean	male	.5186 ± .0058	.6034 ± .012	.5192 ± .014	.3264 ± .0055	.1795 ± .0059	.1629 ± .0045	.180
	female	.5392 ± .0070	.6365 ± .011	.6088 ± .016	.3125 ± .0092	.2056 ± .0048	.1914 ± .0043	.211
Range	male	.42–.67	.42–.81	.31–.73	.21–.39	.12–.26	.11–.22	.10–.28
	female	.40–.65	.44–.77	.41–.80	.18–.40	.13–.26	.14–.26	.14–.38
Standard deviation	male	.0564 ± .0041	.0926 ± .0082	.100 ± .0097	.038 ± .0039	.0380 ± .0041	.0273 ± .0032	
	female	.0670 ± .0049	.0823 ± .0078	.119 ± .012	.061 ± .0065	.0339 ± .0034	.0290 ± .0030	
Coeffic. of var.	male	10.9 ± .79	15.4 ± 1.4	19.3 ± 2.0	11.7 ± 1.2	21.2 ± 2.4	16.8 ± 2.0	
	female	12.4 ± .93	12.8 ± 1.3	19.5 ± 2.0	19.5 ± 2.2	16.5 ± 1.7	15.2 ± 1.6	

TABLE 5

Thyroid gland of albino rats

A. Absolute weight (grams)

		NEWBORN 10 m. 19 f.	7 DAYS 14 m. 11 f.	20 DAYS 17 m. 9 f.	6 WEEKS 6 m. 13 f.	10 WEEKS 13 m. 11 f.	5 MONTHS 11 m. 13 f.	1 YEAR 5 m. 12 f.
Mean	male	.00225	.0025	.0066	.0081	.017	.035	.035
	female	.0019	.0025	.0053	.0093	.016	.030	.029
Range	male	.0012–.0036	.0013–.0043	.0040–.0104	.003–.0164	.0106–.030	.017–.061	.020–.049
	female	.0012–.0030	.0019–.0038	.0034–.0080	.005–.0172	.010–.026	.022–.036	.018–.047

B. Percentage of net body weight

		NEWBORN 10 m. 19 f.	7 DAYS 14 m. 11 f.	20 DAYS 17 m. 9 f.	6 WEEKS 6 m. 13 f.	10 WEEKS 13 m. 11 f.	5 MONTHS 11 m. 13 f.	1 YEAR 5 m. 12 f.
Mean	male	.043	.023	.029	.014	.014	.022	.018
	female	.040	.024	.030	.017	.017	.023	.018
Range	male	.022–.071	.014–.036	.018–.041	.006–.020	.008–.021	.011–.039	.012–.030
	female	.022–.075	.019–.029	.026–.039	.009–.02[illegible]	.013–.022	.017–.029	.014–.024

TABLE 6

Thymus of albino rats

A. Absolute weight (grams)

		NEWBORN 44 m. 43 f.	7 DAYS 30 m. 27 f.	20 DAYS 24 m. 25 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 19 m. 23 f.	5 MONTHS 20 m. 21 f.	1 YEAR 3 m. 11 f.
Mean	male....	.00766±.00027	.0243±.0010	.0845±.0047	.1027±.0055	.2566±.0107	.2340±.0073	.042
	female...	.00757±.00021	.0273±.0010	.0574±.0024	.1134±.0072	.2321±.0073	.2162±.0073	.075
	total....	.00762±.00017	0257±.00073	.0707±.0029	.1078±.0056	.2434±.0064	.2249±.0052	
Range	male....	.0031-.0141	.012-.043	.034-.135	.052-.250	.172-.442	.141-.317	.023-.080
	female...	0036-.0136	.015-.044	.037-.093	.055-.284	124-.315	.111-.289	.032-.096
Standard devia-tion	male....	.00262±.00019	.00821±.00072	.0341±.0033	.0379±.0039	.0693±.0076	.0485±.0052	
	female...	.00205±.00015	.00769±.00071	.0181±.0017	.0677±.0102	.0519±.0052	.0495±.0051	
	total....	.00235±.00012	.00821±.00052	.0303±.0021	.0544±.0040	.0617±.0045	.0499±.0037	
Coeffi-cient of variation	male....	34.2±2.7	33.8±3.3	40.4±4.5	36.8±4.5	26.9±3.1	20.7±2.3	
	female...	27.1±2.1	28.2±2.8	31.5±3.3	59.6±8.3	22.3±2.3	22.9±2.5	
	total....	30.9±1.7	32.0±2.2	42.9±3.4	50.4±4.6	25.3±2.0	22.2±1.7	
Coeffic. of cor-relation	male....	.753±.044	.737±.056	.862±.035	.824±.046	.593±.100	-.194±.145	
	female...	.562±.070	.834±.040	.839±.040	.958±.012	.290±.129	-.310±.133	
	total....	.668±.040	.740±.040	.889±.020	.904±.019	.508±.077	-.088±.100	

B. Percentage of net body weight

		NEWBORN 44 m. 43 f.	7 DAYS 30 m. 27 f.	20 DAYS 24 m. 25 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 19 m. 23 f.	5 MONTHS 20 m. 21 f.	1 YEAR 3 m. 11 f.
Mean	male....	.1547±.0040	.2380±.0070	.385±.012	.2068±.0066	.2195±.0091	.1475±.0055	.020
	female...	.1637±.0039	.2711±.0064	.348±.0085	.2120±.0095	.2372±.0080	.1586±.0070	.043
Range	male....	.088-.240	.17-.44	.23-.55	.14-.30	.13-.30	.067-.19	.01-.029
	female...	.097-.270	.19-.37	.28-.51	.14-.36	.15-.35	.065-.25	.019-.063
Standard deviation	male...	.0392±.)028	.0569±.0050	.0899±.0088	.046±.0047	.0588±.0064	.0363±.0039	
	female.	.0375±.0027	0492±.0045	.0632±.0060	.063±.0067	.0565±.0056	.0477±.0050	
Coeffic. of var.	male...	25.3±1.9	23.9±2.2	23.3±2.4	22.2±2.4	26.9±3.1	24.6±2.8	
	female.	22.9±1.7	18.2±1.7	18.2±1.8	29.7±3.4	23.9±2.5	30.0±3.4	

TABLE 7

Heart of albino rats

A. Absolute weight (grams)

		NEWBORN 44 m. 43 f.	7 DAYS 29 m. 27 f.	20 DAYS 24 m. 25 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 20 m. 23 f.	5 MONTHS 20 m. 21 f.	1 YEAR 5 m. 20 f.
Mean	male...	.03150±.00062	.0676±.0016	.1563±.0075	.2696±.0095	.597±.016	.748±.022	.909
	female.	.02992±.00048	.0710±.0020	.1154±.0026	.286±.015	.516±.011	.663±.020	.759
	total...	.03072±.00040	.0693±.0013	.1354±.0044	.2772±.0085	.554±.011	.704±.016	
Range	male...	.0199–.0432	.046–.104	.084–.251	.183–.513	.496–.951	.563–1.189	.602–1.308
	female .	.0195–.0408	.051–.109	.082–.150	.190–.535	.375–722	.465–990	.493–1.135
Standard deviation	male...	.00611±.00044	.0125±.0011	.0547±.0049	.0656±.0066	.108±.012	.147±.016	
	female.	.00470±.00034	.0150±.0014	.0191±.0018	.096±.010	.0975±.0079	.137±.014	
	total...	.00552±.00028	.01405±.00089	.0455±.0031	.0820±.0059	.1019±.0074	.150±.011	
Coefficient of variation	male...	19.4±1.4	18.5±1.7	35.1±3.8	24.3±2.6	18.1±2.0	19.7±2.2	
	female.	15.7±1.2	21.1±2.0	16.6±1.6	33.6±4.0	15.4±1.6	20.7±2.2	
	total...	18.0±9.5	20.3±1.3	33.7±2.6	29.7±2.4	18.4±1.4	21.3±1.7	
Coeffic. of correlation	male...	.670±.056	.399±.105	.893±.028	.941±.016	.891±.031	.867±.037	
	female.	.390±.087	.665±.073	.871±.033	.979±.0063	.780±.055	.762±.062	
	total...	.583±.048	.504±.067	.908±.017	.968±.0066	.860±.027	.840±.031	

B. Percentage of net body weight

		NEWBORN 44 m. 43 f.	7 DAYS 29 m. 27 f.	20 DAYS 24 m. 25 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 20 m. 23 f.	5 MONTHS 20 m. 21 f.	1 YEAR 5 m. 20 f.
Mean	male...	.650±.010	.681±.015	.728±.013	.5445±.0069	.5135±.0076	.4550±.0060	.450
	female.	.652±.010	.717±.014	.717±.0072	.5580±.0080	.5248±.0081	.4762±.0078	.492
Range	male...	.50–1.00	.50–1.00	.56–.93	.44–.63	.45–.68	.37–.52	.38–.52
	female.	.43–.92	.54–.94	.60–.83	.48–.68	.44–.71	.39–.58	.35–.91
Standard deviation	male...	.100±.0072	.121±.011	.0930±.0091	.048±.0049	.0506±.0054	.0400±.0043	
	female.	.101±.0073	.105±.0096	.0531±.0051	.053±.0056	.0571±.0057	.0546±.0058	
Coeffic. of var.	male...	15.4±1.1	17.8±1.6	12.8±1.3	8.81±.90	9.87±1.1	8.79±.94	
	female .	15.5±1.2	14.7±1.4	7.41±.71	9.50±1.0	10.9±1.1	11.5±1.2	

TABLE 8

Lungs of albino rats

A. Absolute weight (grams)

		NEWBORN 44 m 43 f.	7 DAYS 30 m. 27 f.	20 DAYS 24 m. 25 f.	6 WEEKS 22 m. 17 f.	10 WEEKS 20 m. 20 f.	5 MONTHS 17 m. 14 f.	1 YEAR 2 m. 3 f.
Mean	male	0783±.0021	.1938±.0038	.2377±.0074	.3319±.0077	.662±.023	1.049	2.41
	female	0776±.0016	.1939±.0043	.1960±.0055	.3345±.0130	.599±.016	.911	1.46
	total...	.0779±.0013	.1938±.0029	.2164±.0050	.3331±.0070	.630±.014		
Range	male...	.0402-.1268	.110-.254	.156-.354	.271-.547	.39-.90	.62-2.01	1.76-3.07
	female .	.0403-.1202	.154-.256	.151-.302	.244-.515	.44-.81	.64-1.22	1.17-1.65
Standard devi- ation	male...	.0207±.0015	.0311±.0027	.0535±.0052	.0537±.0055	.151±.016		
	female.	.0153±.0011	.0333±.0031	.0405±.0039	.0764±.0089	.103±.011		
	total...	.0182±.00093	.0322±.0020	0517±.0035	.0646±.0049	.133±.010		
Coeffi- cient of variation	male...	26.4±2.0	16.0±1.4	22.5±2.3	16.2±1.7	22.8±2.6		
	female.	19.7±1.5	17.2±1.6	20.7±2.1	22.8±2.8	17.2±1.9		
	total...	23.3±1.3	16.6±1.1	23.9±1.7	19.4±1.5	21.1±1.7		
Coeffic. of cor- relation	male...	.811±.035	.807±.043	.858±.036	.837±.030	.718±.073		
	female .	.624±.063	.815±.046	.855±.036	.955±.014	.348±.133		
	total...	.736±.033	.799±.032	.868±.024	.943±.012	.623±.065		

B. Percentage of net body weight

		NEWBORN	7 DAYS	20 DAYS	6 WEEKS	10 WEEKS	5 MONTHS	1 YEAR
Mean	male...	1.593±.028	1.926±.023	1.144±.019	.683±.011	.567±.014	.641	.93
	female.	1.679±.026	1.956±.024	1.204±.016	.653±.0055	.600±.018	.661	.77
Range	male...	1.13-2.36	1.46-2.39	.86-1.39	.59-.94	.42-.78	.36-.94	.77-1.11
	female.	1.15-2.45	1.62-2.33	.98-1.46	.58-.71	.46-.99	.42-.90	.63-.89
Standard deviation	male...	.273±.020	.186±.016	.139±.014	.0731±.0074	.0930±.0099		
	female.	.256±.019	.187±.017	.120±.011	.0335±.0039	.122±.013		
Coeffic. of var.	male...	17.2±1.3	9.64±.84	12.2±1.2	10.7 ±1.1	16.4±1.8		
	female.	15.2±1.1	9.55±.88	10.0±.95	5.13±.60	20.3±2.3		

TABLE 9

Liver of albino rats

A. Absolute weight (grams)

		NEWBORN 44 m. 43 f.	7 DAYS 30 m. 27 f.	20 DAYS 24 m. 25 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 20 m. 23 f.	5 MONTHS 20 m. 21 f.	1 YEAR 5 m. 20 f.
Mean	male...	.2323±.0056	.3418±.0078	1.016±.056	3.276±.065	6.28±.29	7.54±.25	9.08
	female.	.2283±.0046	.3446±.0090	.722±.028	3.096±.107	5.01±.21	5.87±.17	6.77
	total...	.2303±.0036	.3431±.0059	.866±.034	3.190±.062	5.60±.19	6.69±.17	
Range	male...	.116–.341	.213–.504	.418–2.088	2.64–4.76	3.66–10.18	5.68–12.60	5.68–13.12
	female.	.145–.317	.261–.476	.426–1.045	2.18–4.71	3.20–9.90	4.25–8.88	4.27–9.55
Standard deviation	male...	.0547±.0039	.0637±.0055	.407±.040	.451±.046	1.94±.21	1.66±.18	
	female.	.0448±.0033	.0695±.0064	.206±.020	.708±.075	1.51±.15	1.16±.12	
	total...	.0501±.0026	.0665±.0042	.353±.034	.593±.043	1.83±.13	1.66±.12	
Coefficient of variation	male...	23.5±1.8	18.6±1.7	40.1±4.5	13.8±1.4	30.9±3.6	22.0±2.5	
	female .	19.6±1.5	20.1±1.9	28.5±2.9	22.9±2.6	30.1±3.3	19.8±2.1	
	total...	21.8±1.2	19.4±1.3	40.8±3.2	18.6±1.4	32.7±2.6	24.8±2.0	
Coeffic. of correlation	male...	.861±.026	.751±.054	.981±.0052	.704±.073	.819±.050	.873±.036	
	female .	.610±.065	.807±.045	.928±.019	.951±.014	.488±.107	.791±.055	
	total...	.756±.032	.762±.037	.968±.0061	.836±.031	.736±.047	.874±.026	

B. Percentage of net body weight

		NEWBORN 44 m. 43 f.	7 DAYS 30 m. 27 f.	20 DAYS 24 m. 25 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 20 m. 23 f.	5 MONTHS 20 m. 21 f.	1 YEAR 5 m. 20 f.
Mean	male...	4.736±.066	3.393±.052	4.64±.10	6.78±.12	5.30±.16	4.61±.078	4.42
	female.	4.937±.078	3.504±.052	4.37±.092	6.19±.085	5.08±.19	4.24±.072	4.24
Range	male...	3.51–6.06	2.96–5.12	3.21–5.82	5.40–8.46	3.23–7.17	3.90–5.81	3.51–4.92
	female.	3.51–6.36	2.91–4.20	3.42–5.63	5.39–7.33	3.72–10.68	3.31–5.18	3.31–5.40
Standard deviation	male...	.647±.047	.426±.037	.725±.071	.81±.082	1.04±.11	.518±.055	
	female.	.760±.055	.402±.037	.665±.065	.56±.060	1.38±.14	.491±.051	
Coeffic. of var.	male...	13.7±1.0	12.6±1.1	15.6±1.6	11.9±1.2	19.6±2.2	11.2±1.2	
	female.	15.4±1.1	11.6±1.1	15.2±1.5	9.05±.98	27.1±2.9	11.6±1.2	

TABLE 10

Spleen of albino rats

A. Absolute weight (grams)

		NEWBORN 43 m. 43 f.	7 DAYS 29 m. 27 f.	20 DAYS 24 m. 25 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 20 m. 23 f.	5 MONTHS 20 m. 20 f.	1 YEAR 5 m. 20 f.
Mean	male...	.01090±.00040	.0407±.0014	.0665±.0046	.1406±.0052	.396±.025	.384±.010	.785
	female.	.01080±.00047	.0444±.0022	.0447±.0024	.1292±.0049	.329±.014	.368±.012	.533
	total...	.01085±.00031	.0425±.0013	.0554±.0027	.1352±.0036	.361±.014	.376±.0076	
Range	male...	.0050–.0223	.027–.065	.023–.145	.099–.203	.211–.767	.294–.511	.269–1.51
	female.	.0040–.0230	.022–.089	.019–.073	.086–.204	.200–.522	.233–.521	.092–1.27
Standard deviation	male...	.00386±.00028	.0108±.00096	.0327±.0032	.0359±.0037	.164±.018	.0669±.0072	
	female.	.00458±.00033	.0168±.0015	.0177±.0017	.0323±.0034	.102±.010	.0791±.0085	
	total...	.00424±.00022	.0141±.00090	.0283±.0019	.0348±.0025	.138±.010	.0710±.0054	
Coeffic. of variation	male...	35.4±2.9	26.5±2.5	49.2±5.8	25.5±2.8	41.3±5.1	17.4±1.9	
	female.	42.4±3.6	37.8±3.9	39.6±4.3	25.0±2.8	30.9±3.4	21.5±2.4	
	total...	39.1±2.3	33.6±2.4	51.1±4.3	25.7±2.0	38.3±3.1	18.9±1.5	
Coeffic. of correlation	male...	.669±.057	.381±.107	.981±.0052	.317±.129	.486±.115	.384±.129	
	female.	.437±.083	.539±.092	.929±.018	.714±.074	.100±.140	.517±.111	
	total...	.542±.051	.438±.073	.967±.0063	.498±.078	.406±.086	.459±.084	

B. Percentage of net body weight

		NEWBORN 43 m. 43 f.	7 DAYS 29 m. 27 f.	20 DAYS 24 m. 25 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 20 m. 23 f.	5 MONTHS 20 m. 20 f.	1 YEAR 5 m. 20 f.
Mean	male...	.2193±.0063	.408±.012	.296±.0098	.291±.012	.337±.018	.237±.0068	.396
	female.	.2330±.0095	.441±.018	.266±.0094	.2615±0077	.340±.017	.266±.0089	.327
Range	male...	.14–.38	.24–.66	.18–.42	.20–.47	.20–.57	.18–.33	.16–.66
	female.	.13–.48	.29–.76	.15–.42	.19–.40	.21–.63	.21–.47	.11–.77
Standard deviation	male...	.0608±.0044	.0975±.0086	.0713±.0069	.080±.0080	.121±.013	.0448±.0048	
	female.	.0921±.0067	.137±.013	.0695±.0066	.051±.0054	.120±.012	.0591±.0063	
Coeffic. of var.	male...	27.8±2.2	23.9±2.2	24.1±2.5	27.5±3.0	35.9±4.3	18.9±2.1	
	female.	39.5±3.3	31.1±3.1	26.1±2.7	19.5±2.2	35.3±3.9	22.2±2.5	

TABLE 11

Empty stomach and intestines of albino rats

A. Absolute weight (grams)

		NEWBORN 7 m. 9 f.	7 DAYS 13 m. 12 f.	20 DAYS 6 m. 10 f.	6 WEEKS 5 m. 4 f.	10 WEEKS 3 m. 6 f.	5 MONTHS 6 m. 9 f.	1 YEAR 5 m. 15 f.
Mean	male..	.121	.296	1.21	4.64	9.35	9.13	10.40
	female.	.114	.297	.715	5.00	7.84	8.06	9.29
Range	male..	.098–.179	.183–.481	.91–1.61	2.88–6.87	8.40–10.06	7.12–10.73	6.44–15.23
	female.	.049–.180	.146–.431	.367–1.014	3.13–5.90	6.22–9.28	6.70–8.78	5.82–12.92

B. Percentage of net body weight

		NEWBORN 7 m. 9 f.	7 DAYS 13 m. 12 f.	20 DAYS 6 m. 10 f.	6 WEEKS 5 m. 4 f.	10 WEEKS 3 m. 6 f.	5 MONTHS 6 m. 9 f.	1 YEAR 5 m. 15 f.
Mean	male..	2.25	2.96	4.69	8.49	6.6	5.8	5.1
	female.	2.46	3.06	4.35	7.36	7.4	5.6	5.9
Range	male..	1.64–3.46	1.54–4.17	2.89–5.58	6.85–9.56	4.8–8.5	4.5–6.6	4.2–5.6
	female.	1.19–4.42	1.41–4.50	2.97–6.11	6.74–7.81	5.2–9.9	5.0–6.7	4.2–7.6

TABLE 12

Stomach and intestines with contents

A. Absolute weight (grams)

		NEWBORN 44 m. 43 f.	7 DAYS 30 m. 27 f.	20 DAYS 24 m. 25 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 20 m. 23 f.	5 MONTHS 20 m. 21 f.	1 YEAR 5 m. 20 f.
Mean	male	.288	.706	2.05	8.04	12.83	13.75	13.76
	female	.307	.764	1.53	8.06	11.18	11.48	12.24
	total	.297±.0073	.733±.019	1.78±.072	8.05±.25			
Range	male	.124-.559	.357-1.109	.78-3.08	4.48-13.4	8.08-16.99	9.10-19.58	9.25-18.4
	female	.143-.648	.394-1.331	.74-2.63	5.08-12.91	7.24-18.75	8.26-17.93	7.80-17.65
Standard deviation	male				2.24±.23			
	female				2.57±.27			
	total	.1125±.0058	.210±.013	.749±.051	2.40±.18			
Coeffic. of variation	male				27.9			
	female				31.9			
	total	37.9	28.6	42.1	29.8			
Coeffic. of correlation	male							
	female							
	total	.289±.066	.588±.058	.844±.028	.758±.043			

B. Percentage of net body weight

		NEWBORN	7 DAYS	20 DAYS	6 WEEKS	10 WEEKS	5 MONTHS	1 YEAR
Mean	male	5.90	7.02	9.47	16.4	10.9	8.5	6.8
	female	6.68	7.63	9.17	15.4	11.5	8.4	7.8
Range	male	3.62-10.08	4.83-12.0	5.51-13.9	11.2-23.4	8.5-14.3	6.0-12.8	5.9-8.0
	female	3.97-15.6	5.15-10.5	5.71-15.5	10.6-22.0	8.1-20.6	6.0-12.8	6.3-10.7

TABLE 13

Suprarenal glands of albino rats

A. Absolute weight (grams)

		NEWBORN 42 m. 43 f.	7 DAYS 30 m. 27 f.	20 DAYS 24 m. 25 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 20 m. 23 f.	5 MONTHS 20 m. 21 f.	1 YEAR 5 m. 20 f.
Mean	male...	.00186±.000042	.00231±.000062	.0076±.00033	.01323±.00034	.0220±.00072	.0258±.00087	.0304
	female.	.00190±.000050	.00220±.000053	.0069±.00033	.01225±.00047	.0253±.00064	.0369±.0021	.0430
	total...	.00188±.000033	.00226±.000042	.0072±.00023	.01276±.00029	.0238±.00051	.0315±.0013	
Range	male...	.0010-.0028	.0013-.0033	.004-.014	.008-.016	.015-.0336	.017-.042	.027-.035
	female.	.0010-.0031	.0014-.0031	.004-.012	.007-.019	.018-.0329	.020-.074	.028-.052
Standard dev.	male...	.000406±.000030	.000507±.000044	.00240±.00023	.00233±.00024	.00477±.00051	.00575±.00062	
	female.	.000490±.000036	.000408±.000038	.00243±.00023	.00309±.00033	.00457±.00045	.0141±.0015	
	total...	.000446±.000023	.000468±.000030	.00242±.00016	.00276±.00020	.00495±.00036	.0122±.00091	
Coeffic. of variation	male...	21.8±1.7	21.9±2.0	31.6±3.4	17.1±1.8	21.7±2.4	22.3±2.5	
	female.	25.8±2.0	18.5±1.7	35.3±3.8	25.2±2.9	18.1±1.9	38.2±4.5	
	total...	23.7±1.3	20.4±1.3	33.4±2.5	21.7±1.7	20.8±1.6	38.7±3.3	
Coeffic. of correlation	male...	.714±.051	.014±.123	.743±.062	.150±.140	.602±.096	.795±.056	
	female.	.588±.067	.384±.111	.449±.108	.692±.079	.659±.080	.780±.058	
	total...	.510±.054	.130±.088	.583±.064	.410±.087	.410±.086	.347±.092	

B. Percentage of net body weight

		NEWBORN	7 DAYS	20 DAYS	6 WEEKS	10 WEEKS	5 MONTHS	1 YEAR
Mean	male...	.0382±.00068	.0233±.00062	.0360±.00089	.0273±.00074	.0185±.00055	.0158±.00036	.0160
	female.	.0412±.00091	.0225±.00056	.0430±.0017	.02485±.00092	.0257±.00049	.0260±.0010	.0278
Range	male...	.018-.051	.011-.033	.023-.052	.016-.039	.011-.025	.011-.019	.010-.025
	female.	.022-.061	.015-.034	.028-.074	.015-.035	.017-.033	.018-.043	.018-.048
Stand. dev.	male...	.00658±.00048	.00507±.00044	.00644±.00063	.0060±.00061	.00363±.00039	.00237±.00025	
	female.	.00885±.00064	.00433±.00040	.0128±.0012	.0061±.00065	.00354±.00035	.00705±.00073	
Coeffic. of var	male...	17.2±1.3	21.8±2.0	17.9±1.8	22.0±2.3	19.6±2.2	15.0±1.6	
	female.	21.5±1.6	19.2±1.8	29.8±3.1	24.5±2.8	13.8±1.4	27.1±3.0	

TABLE 14

Kidneys of albino rats

A. Absolute weight (grams)

		NEWBORN 44 m. 43 f.	7 DAYS 30 m. 27 f.	20 DAYS 24 m. 25 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 20 m. 23 f.	5 MONTHS 20 m. 21 f.	1 YEAR 5 m. 20 f.
Mean	male...	.04707±.0013	.1305±.0033	.3090±.015	.615±.011	1.195±.030	1.509±.040	1.899
	female.	.04530±.00090	.1383±.0042	.2348±.0062	.617±.016	1.054±.021	1.311±.035	1.538
	total...	.04620±.00079	.1342±.0027	.2712±.0088	.616±.0096	1.120±.019	1.408±.029	
Range	male...	.0240–.0842	.083–.211	.177–.531	.546–.943	.862–1.763	1.164–2.304	1.38–2.33
	female.	.0250–.0269	.094–.214	.169–.322	.500–.859	.858–1.333	.856–1.649	1.00–2.078
Standard deviation	male...	.0127±.00091	.0268±.0023	.109±.011	.0760±.0077	.198±.021	.267±.029	
	female.	.00874±.00064	.0325±.0030	.0458±.0044	.108±.012	.150±.015	.239±.025	
	total...	01093±.00056	.0299±.0019	.0912±.0062	.0927±.0068	1.88±.014	.272±.020	
Coeffic. of variation	male...	27.0±2.1	20.5±1.9	35.3±3.9	12.4±1.3	16.7±1.8	17.7±2.0	
	female.	19.3±1.5	23.5±2.3	19.5±1.9	17.5±1.9	14.3±1.5	18.2±2.0	
	total...	23.7±1.3	22.3±1.5	33.7±2.5	15.0±1.1	16.8±1.3	19.3±1.5	
Coeffic. of correlation	male...	.758±.043	.870±.029	.962±.010	.875±.034	.922±.023	.942±.017	
	female.	.577±.069	.788±.049	.943±.015	.946±.016	.825±.045	.855±.040	
	total...	.703±.037	.788±.034	.955±.0085	.915±.017	.897±.020	.911±.018	

B. Percentage of net body weight

		NEWBORN	7 DAYS	20 DAYS	6 WEEKS	10 WEEKS	5 MONTHS	1 YEAR
Mean	male...	.961±.018	1.290±.015	1.436±.022	1.269±.017	1.029±.011	.927±.0086	.954
	female.	.982±.016	1.385±.026	1.444±.014	1.230±.019	1.077±.015	.945±.014	.980
Range	male...	.66–1.41	1.01–1.59	1.19–1.87	1.00–1.55	.90–1.16	.85–1.06	.78–1.23
	female.	.60–1.40	1.14–1.99	1.27–1.72	1.05–1.50	.92–1.35	.70–1.14	.83–1.21
Standard deviation	male...	.176±.013	.126±.011	.160±.016	.120±.012	.0755±.0081	.0571±.0061	
	female.	.160±.012	.202±.019	.107±.010	.124±.013	.107±.011	.0952±.0099	
Coeffic. of var.	male...	18.3±1.4	9.77±.85	11.1±1.1	9.46±.97	7.33±.78	6.16±.66	
	female.	16.3±1.2	14.6±1.4	7.43±.71	10.1±1.1	9.91±.99	10.1±1.1	

TABLE 15

Gonads of albino rats

A. Absolute weight (grams)

		NEWBORN 42 m. 20 f.	7 DAYS 30 m. 16 f.	20 DAYS 24 m. 24 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 20 m. 23 f.	5 MONTHS 20 m. 20 f.	1 YEAR 5 m. 19 f.
Mean	male...	.00650±.00017	.0273±.00059	.1335±.0055	.592±.023	1.747±.092	1.885±.12	2.506
	female .	.00078	.00121	.00363±.00021	.0106±.00074	.0349±.0025	.0450±.0022	.0399
Range	male...	.0034–.0103	.015–.041	.076–.224	.368–.958	.678–2.622	.787–3.789	1.279–3.322
	female .	.0003–.0012	.0005–.0025	.0015–.0067	.0050–.0278	.0100–.0698	.015–.068	.0128–.070
Standard deviation	male...	.00164±.00012	.00483±.00042	.0397±.0039	.158±.018	.607±.065	.770±.082	
	female .			.00154±.00015	.00493±.00053	.0177±.0018	.0147±.0016	
Coeffic. of var.	male...	25.3±2.0	17.7±1.7	29.6±3.1	26.7±2.9	34.7±4.2	40.7±5.0	
	female .			42.4±4.8	47.0±6.0	50.9±6.2	32.7±3.4	
Coeffic. of cor.	male...	.670±.057	.752±.053	.950±.013	.747±.063	.483±.116	.880±.034	
	female .			.727±.065	.642±.062	.824±.045	.806±.052	

B. Percentage of net body weight

		NEWBORN 42 m. 20 f.	7 DAYS 30 m. 16 f.	20 DAYS 24 m. 24 f.	6 WEEKS 22 m. 20 f.	10 WEEKS 20 m. 23 f.	5 MONTHS 20 m. 20 f.	1 YEAR 5 m. 19 f.
Mean	male...	.1340±.0027	.2720±.0040	.6329±.0090	1.194±.032	1.505±.074	1.125±.044	1.184
	female .	.0167	.0124	.0219±.00095	.0206±.00074	.0339±.0019	.0315±.0012	.0254
Range	male...	.077–.19	.19–.34	.53–.78	.88–1.72	.63–2.41	.66–1.56	1.07–1.45
	female .	.006–.026	.005–.030	.011–.039	.012–.035	.013–.055	.016–.046	.012–.053
Standard deviation	male...	.0261±.0019	.0322±.0028	.0651±.0063	.225±.023	.492±.053	.289±.031	
	female .			.00689±.00067	.00723±.00077	.0133±.0013	.00784±.00084	
Coeffic. of var.	male...	19.5±1.5	11.9±1.1	10.3±1.0	18.9±2.0	32.8±3.9	25.6±2.9	
	female .			31.5±3.3	35.3±4.2	39.2±4.5	25.0±2.8	

MUSCLE DEGENERATION AND ITS RELATION TO THE ORIGIN OF EOSINOPHILE LEUCOCYTES IN AMPHIBIA (SALAMANDRA ATRA)

J. A. BADERTSCHER

Department of Histology and Embryology, Cornell University, Ithaca

SEVEN FIGURES

The investigation presented in this paper has a twofold object: Primarily, to offer an explanation for the source of large numbers of free eosin-staining granules and eosinophile leucocytes found in connection with degenerating muscle and with the absorption of the gills taking place during the period of transformation of Salamandra atra; and, secondarily, to present briefly the manner of muscle degeneration as it occurs in this form. In Salamandra atra an intimate relation exists between the origin of the eosinophile leucocytes and degenerating muscle tissue so that the one cannot be studied without considering the other.[1]

An investigation into the origin of the eosinophile leucocytes seemed proper for the reason that the source and nature of the granules in the granular leucocytes is still a much debated question. Also, as far as I was able to determine, no work has yet

[1] The material used was prepared by Dr. B. F. Kingsbury to whom it was kindly furnished by Professor Mollier of the Histological-Embryological Institute, Munich. I wish to express my thanks to Professor Kingsbury for the use of the material and for the valuable suggestions given me on this work. Twenty-seven series were examined, of which six were of animals still in the larval condition, ranging in length from 12.5 to 38 mm.; thirteen in the period of transformation, ranging in length from 44 to 48 mm.; and seven in the adult condition. Different approved fixers (Helly's, Zenker's, Tri-chlor-acetic) were used in fixing the material. Only the head and anterior part of the body, including the heart, were sectioned. The sections were cut from 8 to 15 micra in thickness. The greater number of the series were stained with iron or copper (Weigert's) hematoxylin, and eosin or orange-G was used as a counter stain.

been done on amphibians with a view of tracing out the origin of eosinophile leucocytes.

Brown ('98) is of the opinion that the products of degenerated muscle tissue are a source of eosinophile granules. In cases of trichinosis in man, he observed that the muscle fibers invaded by Trichina spiralis undergo a granulation in the immediate neighborhood of the parasite. All through the affected parts of a muscle are found large numbers of leucocytes, many of which are of the eosinophile type. The latter are very numerous in areas of marked degeneration. Though there were no evidences that the leucocytes ingested bodily fragments of muscle tissue, he thinks that possibly the degenerated substance is taken up in a soluble form and transformed into eosinophile granules.

Weidenreich ('08, '11), who worked with mammals, is the chief exponent of the theory that the eosinophile granules are hemoglobin, containing products of degenerated erythrocytes. According to him, leucocytes may ingest entire erythrocytes which then undergo disintegration, resulting in the formation of eosinophile granules, or the disintegration of the red cells may take place extracellularly and the eosinophile granules thus formed may be taken up, in their fully formed condition, by the leucocytes. In referring to the work of Brown he says ('11, p. 635): ". . . . auch die Möglichkeit ist nicht ausgeschlossen, dass das Hämoglobin der Muskelfasern, ... eine Rolle bei der Bildung der Granula spielt."

The amphibians furnish most suitable material to test the correctness of this theory, for during the period of metamorphosis marked disintegration of both erythrocytes and muscle tissue takes place. Also the degeneration of muscle tissue in this class of animals, in contrast to the pathological degeneration in cases of trichinosis, is a normal process that regularly occurs while they are adapting themselves to a different mode of life. While Brown ('98) pointed out a possible relation of the origin of eosinophile cells to pathological degenerating muscle, no work apparently has yet been done on any form to show a relation of the origin of those cells to normally degenerating muscle.

Among some of the muscles that undergo degeneration in this salamander are the gill muscles, some of the muscles of the lower jaw (fig. 1) and some of the dorsal muscles along the spinal column (fig. 4). In the specimens studied, muscle degeneration was most pronounced in those far along in their period of transformation or in the young adult, thus indicating that even after the external signs of metamorphosis have been obliterated, there are still morphological changes taking place internally. In the degenerating

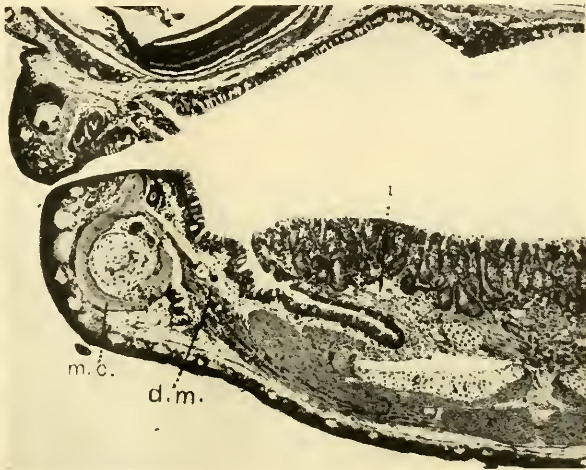
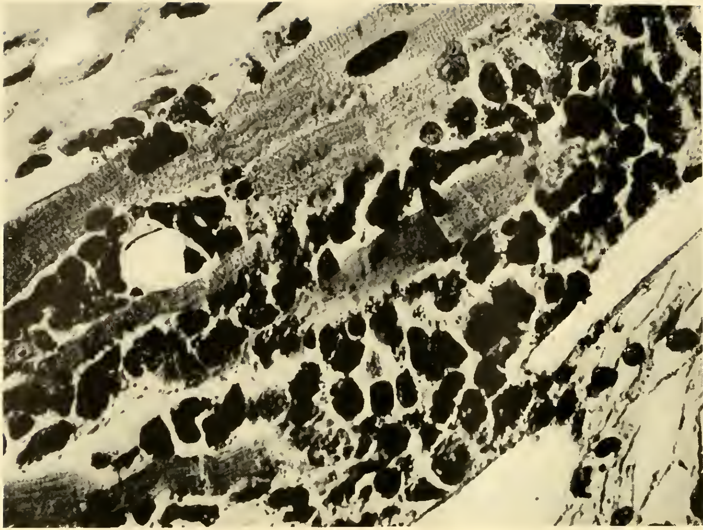


Fig. 1 Part of a transverse section through the head of a 51 mm. young adult, showing the position in the lower jaw of a muscle well advanced in degeneration. *M.c.*, Meckel's cartilage; *d.m.*, degenerating muscle; *t.*, tongue. Photo. × 30.

muscles and gills there is also a degeneration of blood capillaries. As the capillaries are broken down there is a marked destruction of erythrocytes which can be satisfactorily demonstrated especially in the degenerating gills. If the eosinophile granules are derived from degenerating red cells and degenerating muscle tissue, both granules and eosinophile leucocytes should be found in connection with those tissues. On examining the different series from the larval to the adult condition the evidence is conclusive that the free eosinophile granules are products of the degenerating



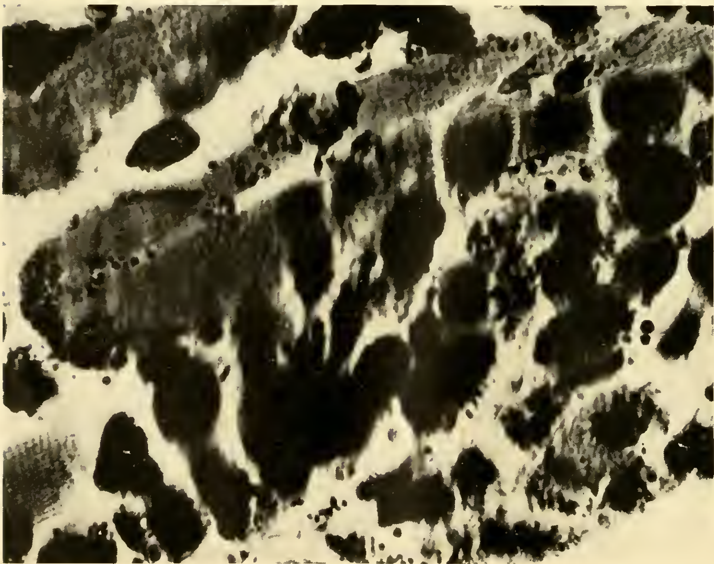


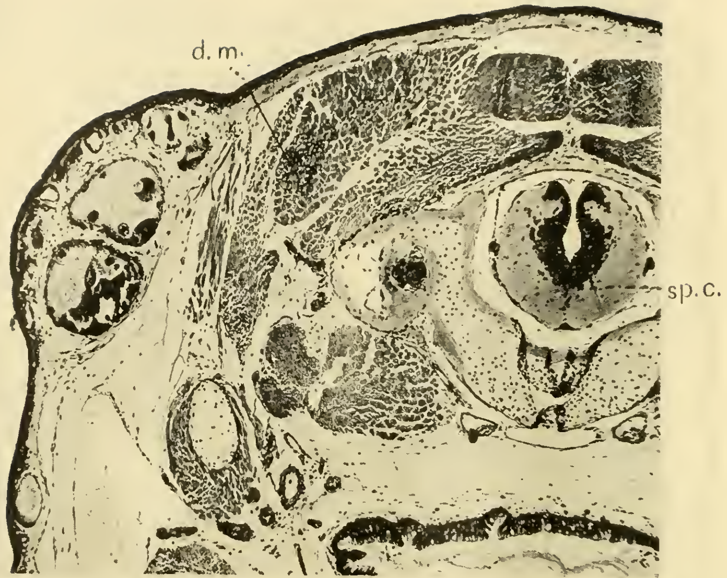
Fig. 2 Section of a part of the degenerating muscle represented in Fig. 1, showing a large accumulation of eosinophile leucocytes in connection with the degenerating muscle fibers. The section being rather thick the granules in the eosinophile cells could not be photographed sharply. Photo. × 370.

Fig. 3 Degenerating muscle taken a few sections posterior to that represented in figure 1. Free eosinophile granules, represented by black dots about the size of an ordinary pin head; some a little smaller, can be seen scattered promiscuously along two degenerating muscle fibers and in the spaces not occupied by cells, most of which are eosinophile leucocytes. On account of the section being rather thick not all parts could be focused sharply. Photo. × 700.

tissues above named and are taken up by the leucocytes, transforming them into the eosinophile type. These observations extend the scope of Weidenreich's views to the amphibia.

During the larval period only an occasional eosinophile leucocyte can be found in the blood stream. A few cells containing eosinophile granules can also be demonstrated in the connective tissue. The number of eosinophile cells at this stage of development is so small that only after considerable searching could one be found. During the period of transformation, eosinophile cells can invariably be demonstrated in connection with degenerating muscle, the number of those cells corresponding approximately with the advancement of degeneration. Where degeneration is at its greatest height a large accumulation of eosinophile cells is present (fig. 2). Also more eosinophile cells can be demonstrated in the blood stream and especially in the connective tissue in the neighborhood of the degenerating parts during the period of transformation than before or after this period. No differential counts were made of the leucocytes in the blood stream. The greater number of eosinophile cells during the perod of metamorphosis was evident by the great frequency in their occurrence when searching for them in the sections.

In the section from which figure 3 was photographed can be seen free eosinophile granules lying among the debris of degenerating muscle. That these granules lie free in the regions mentioned is beyond doubt for when the sections are followed along in serial order they are not found to lie in the bodies of leucocytes but lie loose in the intercellular spaces. These granules, apparently formed from the degenerating muscle and degerating erythrocytes, vary somewhat in size, but the majority closely approximate in size and hue those found in the eosinophile leucocytes. They have various positions in respect to the degenerating muscles fibers. They may be found among lymphocytes or eosinophile leucocytes some distance away from a muscle fiber, scattered promiscuously among them, strung along in a row between two adjacent fibers, or often in small recesses of a muscle fiber. Some muscle fibers can be seen, the central portions of which are completely degenerated, the gap between the two remaining ends being filled in



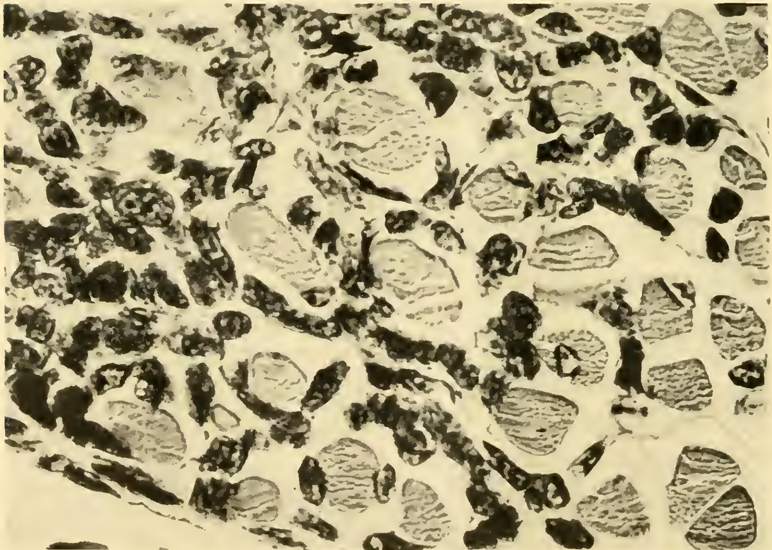


Fig 4 Transverse section through the neck of a 47 mm. adult, showing the position of a muscle in which degeneration has just begun; *sp.c.*, spinal cord; *d.m.*, degenerating muscle. Photo. × 30.

Fig. 5 Portion of the degenerating muscle represented in figure 4, showing the accumulation of lymphocytes among the muscle fibers in the first stage of muscle degeneration. Photo. × 450.

with numerous eosinophile granules and while blood cells, thus suggesting the origin of the granules from the degenerating muscle fiber. Disintegrated erythrocytes can also be seen in connection with degenerating muscle tissue. However, from the material studied the majority of eosinophile granules found in degenerating muscle are products of muscle degeneration while the smaller portion is contributed by degenerating red cells. Eosinophile leucocytes can often be seen in connective tissue in the neighborhood of degenerating muscle. These through their amoeboid movements have perhaps wandered away from their source of origin.

In the degeneration of the gills numerous blood vessels are necessarily broken down, producing in some cases stasis in the small vessels resulting in the disintegration of the erythrocytes in those parts. Degenerating red cells can be demonstrated especially where the degeneration of the gills is well advanced. They can also be found isolated or in smaller and larger groups outside of the walls of blood vessels. The degeneration of some is evident, as can be seen from the appearance of their nuclei which stain intensely, lose their oval shape and become irregular in outline and a few were seen apparently about to be extruded from the cell body. The cell envelope also becomes very irregular in outline and in some cases even ruptured. In some the cytoplasmic material is coarsely granular, losing its seemingly homogeneous nature. In others globular masses of varying sizes are apparently about to be detached from the cell. Many of these large masses or coarse granules together with smaller granules can often be seen lying free in close approximation to degenerating erythrocytes. These larger masses and granules stain with eosin, the smaller granules staining more deeply than the coarse granules. A difference readily observed between the eosinophile granules formed from the degenerating erythrocytes and degenerating muscle tissue is that the granules formed from the former vary much in size as stated above while those formed from the latter, while varying somewhat in size, approximate more nearly the dimensions of those found in the eosinophile cells. That these eosinophile granules are taken up by cells of the character of leucocytes is indicated by the presence of such cells, sometimes in large numbers, in the immediate

neighborhood or in contact with them (fig. 6). I have been unable to find leucocytes ingesting entire or large fragments of erythrocytes as was observed by Lewis ('04) who worked with the ungulates and by Weidenreich ('08) who worked with guinea-pigs. A possible reason for amphibian leucocytes not ingesting entire erythrocytes is found in the large size of the red blood cells in this class of animals.

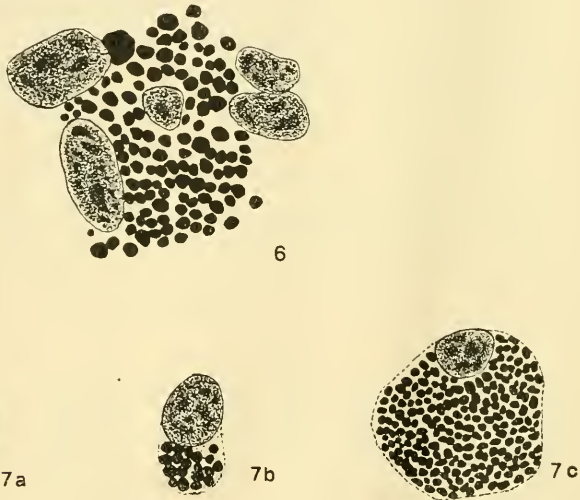


Fig. 6 Lymphocytes among free eosinophile granules found in degenerating muscle. Camera lucida drawing.

Fig. 7 Series of diagrams, *a*, *b*, *c*, to show the formation of eosinophile leucocytes from lymphocytes. The limit of the cytoplasm is marked by a dotted line. Camera lucida drawings.

The first evidences of muscle degeneration are marked by a collection of leucocytes among the muscle fibers. The number of leucocytes that accumulate between the fibers is so great that the width of the intercellular spaces is increased to several times their normal extent (fig. 5). In this accumulation of white blood cells the great majority are the smallest of the leucocytes. Their nuclei are large, taking up almost the entire cell body. The outline of the nuclei which vary, may be round, slightly elongated, gently dented on one side or irregularly crescent-shaped, indicat-

ing amoeboid movement. The rim of cytoplasm around the nucleus is very narrow, in some cells so scanty in its amount that it is difficult to see. The minority of cells in this accumulation are larger than the ones described. They have large nuclei which may vary somewhat in outline as those of the smallest white cells. They have a wide rim of cytoplasm around the nuclei to which the large size of the cell is due. According to Friedsohn's work ('10) on the amphibian blood, the smaller cells appear to be the small lymphocytes and the larger ones the large lymphocytes. An occasional eosinophile leucocyte can also be seen, while partially and completely degenerated erythrocytes are thinly scattered throughout the accumulation of leucocytes. No perceptible change in the muscle fiber itself has yet taken place in this stage of degeneration.

After the degeneration of the muscle tissue has advanced to the stage in which the degeneration of the fibers is readily perceptible the number of white blood cells is as numerous as at the time when the degeneration was first evident, but the lymphocytes have greatly decreased, while the eosinophile leucocytes have increased in number. In this stage of degeneration numerous eosinophile granules can be seen lying free in the intercellular spaces and often in contact with lymphocytes and eosinophile leucocytes. The number of eosinophile granules varies greatly in the eosinophile cells, from only a few granules to the gorged condition of some of the cells (fig. 7), thus suggesting again that the lymphocytes take up eosinophile granules. The presence of the large number of lymphocytes during the first stages of degeneration and a subsequent reduction of their number and replacement by large numbers of eosinophile leucocytes as degeneration advances, clearly indicates that the eosinophile granules are taken up by the lymphocytes. The point in case is that the lymphocytes are the main cells that take up the granules and are thus converted into eosinophile cells.

The majority of the eosinophile leucocytes found in connection with degenerating muscle and erythrocytes have round or nearly round nuclei. In some the nuclei are of the transitional type

while in others they are of the polymorphic type. In studying the eosinophile leucocytes with the various types of nuclei, one can find sufficient evidence—as can be indicated by a series of gradual transitions from one type of nucleus to another—to confirm the conclusion that all are derived from the round nucleated type of eosinophile cells. This transformation takes place by a gradual change in the shape of their nuclei. They are thus genetically related, the round nucleated type being the forerunner of the transitional and polymorphic types.

No attempt was made to determine the chemical nature of the eosinophile granules. The material was not prepared for that purpose. However, the hemaglobin nature of the eosinophile granules as held by Weidenreich and others, is indicated by circumstantial evidence in that the free eosinophile granules and groupings of eosinophile leucocytes are found only in connection with tissue containing hemoglobin, namely, muscle tissue and erythrocytes. The hemoglobin-containing material of degenerating muscle and erythrocytes apparently does not break up directly into the eosinophile granules, that is, this compound is altered in some way before it is taken up by the lymphocytes. This is indicated by the fact stated above that the very coarse granules or masses are stained more faintly than the smaller granules, which are often stained as deeply as the eosinophile granules in the cells.

Although the evidences are strongly in favor of a hemoglobin-nature of the granules, yet when their complex chemical source (erythrocytes and muscle tissue) is considered, it would not be unreasonable to assume that other elements besides hemoglobin also enter into their composition. In considering the chemical nature of the granules Weidenreich ('11, p. 622) says: "Der von Sherrington angeblich festgestellte Phosphorgehalt der Granulation braucht nun keineswegs, wie dieser Autor annimmt, für eine Nucleinnatur zu sprechen; denn wir wissen heute, dass die roten Blutkörperchen auch aus dem phosphorhaltigen Lecithin aufgebaut werden, so dass also unter der Voraussetzung, die Granula sind Zerfallsprodukte der Erythrocyten, auch Phosphor in ihnen enthalten sein könnte." If the granules do contain both phosphorous and hemoglobin then the term 'phosphorous-nature' would

represent the chemical nature of the granules as well as 'hemoglobin-nature,' for both muscle tissue and erythocytes contain phosphorous-containing compounds. The term 'hemoglobin-nature' does not seem to be comprehensive enought to express their chemical nature. The simple phrase "Zerfallsprodukte der Erythrocyten" to which I add 'products of degenerating muscle tissue,' would express a greater probability of the granules containing other elements beside hemoglobin, than does the term 'Hämoglobinnatur.'

To give a detailed account of the different theories relative to the degeneration of muscle tissue in different classes of animals is beyond the scope of this paper. Observations by different investigators of various animal groups indicate that the processes involved in the degeneration of muscle tissue vary considerably. Briefly stated, the interpretations of different workers of the manner by which a muscle degenerates and the part played by the leucocytes in this phenomenon has resulted in the establishment of three general views: (1) a purely aphagocytic process by which the muscle fibers undergo liquefaction, either through the activity of internal conditions of the fiber or through the action of the fluids surrounding them; (2) a purely phagocytic process by which the phagocytes break up the muscle fibers into fragments which are taken up and removed by them; (3) by a combination of the aphagocytic and phagocytic processes. The views enumerated above are stated only in a general way and the theories as worked out by different investigators have detailed modifications.

Barfurth ('87), in his work on the frog larvae, came to the conclusion that a muscle degenerates independently of the action of phagocytes. The myofibrillae break up into comparatively long fragments, bundles of which make up the sarcolytes. The cross-striations are present in the sarcolytes when they are first formed but gradually disappear as the muscle fragments undergo dissolution. The liquefaction of the fragments takes place within the sarcolemma, which is comparatively resistent to the action of the liquefacient, and disappears only after the sarcolytes have been reduced to granules—the débris of degeneration. The initiatory causes of degeneration are unknown to him but he thinks

that they are extrinsic in their nature and is of the opinion that a lack of nutrition or atrophy through disuse after the appearance of the forelegs, are factors to be considered.

According to Katz ('00), who worked with transforming toads, the initiatory causes of degeneration appear to be intrinsic in the muscle itself. Sarcolytes are formed only in some fibers. In the course of degeneration a homogeneous material is produced, which disappears by liquefaction in situ.

Looss ('92) arrived at practically the same conclusions as those of Barfurth, with the exception that a small portion (less than 10 per cent) of the sarcolytes are taken up and destroyed by the phagocytes. The remaining large proportion undergo dissolution in place through the activity of the surrounding fluids.

Metchnikoff ('84), in his work on larval frogs, held that muscle degeneration was effected entirely through the action of phagocytes which collected in large numbers among the degenerating fibers. The source of the numerous phagocytes he then ascribed to the blood. Further investigations ('92) led him to modify the phagocytosis theory of muscle degeneration. In his later work he holds that the phagocytes, which play the important part in muscle degeneration, are formed in the muscle fiber. The first perceptible changes in degeneration are a proliferation of muscle nuclei and an increase in the amount of sarcoplasm. The muscle nuclei and protoplasm then differentiate into cells, the muscle phagocytes, which find their way among the fibrillae, breaking them up into fragments. These fragments (sarcolytes) are ingested and removed by them. The usual type of phagocytes takes no part in muscle degneration, that work being entirely limited to the muscle phagocytes.

Muscle degeneration in Salamandra atra is, to all appearances, a purely aphagocytic process. However, on comparing the degenerating jaw muscles in this class of animals with those in the tails of larval toads, a marked point of difference is recognized in that the formation of sarcolytes does not occur in the former. On examining a series of toads from the larval to the adult condition, sarcolytes in the degenerating tail muscles were found as described by the investigators mentioned above. In the Sala-

mander the myofibrillae, instead of forming sarcolytes, break up into fragments at short intervals in their course, thus obliterating the cross-striations and giving the affected part of a muscle fiber a granular appearance. These fragments or granules fade away as if by liquefaction. Their dissolution does not take place uniformly along the course of a fiber, so that in the extent of a well advanced degenerating fiber indefinitely outlined groups of dismembered fibrillae can be seen. Coincidently with the liquefaction of the fibrillae appear numerous spherical granules that stain intensely with eosin, the eosinophile granules, which are taken up by the leucocytes in the manner described above. According to Barfurth, in the frog larvae degeneration begins at one end and gradually advances to the opposite end of the fiber. While this is the case in some of the degenerating fibers in Salamandra atra, it is not so with all, for in some fibers can be seen a large gap, produced by degeneration, and containing numerous eosinophile granules and leucocytes, while the ends of the fibers appear quite normal. The sarcolemma degenerates simultaneously with the fibrillae. There are no evidences indicating that it spans the gap of a degenerated portion of a fiber, which would be the case if it were more resistant to the agencies bringing about the liquefaction of the fibrillae. The muscle nuclei and sarcoplasm are not transformed into phagocytes as is the case, according to Metchnikoff, in larval frogs. No perceptible increase in the amount of protoplasm or in the number of nuclei was noticeable. They are, however, very resistant to the agencies that cause the liquefaction of the fibrillae. During the last stages of degeneration they can be seen lying free among the numerous leucocytes and débris of degenerated muscle. On account of their large size they cannot be mistaken for lymphocytes or for connective tissue cells. Their form varies. Some retain their natural rod shape, some are crescent in outline, some are bent double so that the two ends are in close approximation, while others are bent and twisted. A considerable number show marked signs of degeneration by the massing of their chromatin. Their fate is unknown to me but from the appearance of some I am of the opinion that they undergo degeneration.

The origin of the lymphocytes found in a muscle during its first stages of degeneration can be attributed to two sources, namely, connective tissue cells and the blood. The connective tissue cells in the affected part of a muscle lose their stellate form, become rounded, their nuclei grow denser, thus acquiring all the appearances of nongranular leucocytes. This process is much like the transformation into lymphocytes of the mesenchymal cells which surround the epithelial anlage of the thymus in Axolotol as described by Maximow ('12). Metchnikoff ('84) also holds that in Triton larvae transformed connective tissue cells assume phagocytic functions. It is difficult to determine to just what extent the formation of lymphocytes takes place in muscle. However, I am confident that the connective tissue cells do not transform rapidly enough to account for the large numbers of lymphocytes present. No mitotic figures were seen and only an occasional dumbbell-shaped nucleus suggested amitosis. It is, therefore, necessary to assume that the blood also is a source of a part of the lymphocytes. According to an hypothesis of Metchnikoff ('84) the endothelial walls of the blood capillaries are acted upon chemically, rendering them more permeable to leucocytes and permitting of a 'passive diapedesis' of the red blood cells. Although this is only an hypothesis, it seems to be a very reasonable way to account for the presence of such large numbers of leucocytes and also of red blood cells which are scattered in small numbers among them, as was stated above. Since muscle degeneration in Salamandra atra is an aphagocytic process it seems probable that the cells of the delicate endothelial walls of capillaries may be among the first elements to be affected. Another view, however, may be taken. Dantschakoff ('08), in her work on the development of the blood in birds, gives evidence that the cells of the vascular endothelium have the power of transforming into lymphocytes. This genetic relationship of the leucocytes to the vascular endothelium has also been held by other investigators. If this view be correct, is it not probable that the vascular endothelial cells are transformed into leucocytes? This process also would permit leucocytes and erythrocytes to escape into the intercellular spaces.

The only apparent rôle of the leucocytes in the degenerating muscles of Salamandra atra is the taking up and removal of the products of degeneration. They take no perceptible active part in breaking down the muscle tissue. According to a theory of Anglas ('00), the leucocytes produce an enzyme which acts upon the tissues, causing their dissolution. The only indication of the correctness of this view is the large accumulation of leucocytes in the first stages of degeneration, even before any changes of the fibers are apparent under the microscope.

CONCLUSIONS

The conclusions of this work are summarized as follows:

1. The eosinophile granules are exogenous in their nature, that is, they are derived from material outside of the cell containing them and not directly a product of the activity of the leucocyte itself. The presence of free eosinophile granules found to be so plentiful among degenerating erythrocytes and muscle tissue and later taken up by lymphocytes, support this conclusion.

2. The eosinophile granules are products of degenerated muscle tissue and degenerated erythrocytes. This is supported by the fact that they are found only in connection with the above named degenerating tissues. The fact also that more eosinophile leucocytes are found in the blood during the period of metamorphosis than in the larval or in the adult condition, supports this conclusion.

3. As they are found only in connection with tissue containing a relatively large amount of hemoglobin, it is believed that hemoglobin is a part of their chemical composition. This conclusion by no means excludes the probability that the eosinophile granules contain chemical compounds besides hemoglobin.

4. The eosinophile granules are taken up by the lymphocytes (large and small) which are thus converted into eosinophile cells. The eosinophile leucocytes are, therefore, the white blood cells ingested with the products of degenerated erythrocytes and degenerated muscle tissue.

5. The eosinophile cells with different types of nuclei are genetically related to one another.

6. Muscle degeneration in Salamandra atra is a process of liquefaction (a purely aphagocytic process), and is apparently brought about through the activity of the surrounding fluids.

7. The only apparent function of the leucocytes in degenerating muscle is the removal of the products of degenerated muscle tissue and erythrocytes. They apparently play no part in the processes bringing about the degeneration of those tissues.

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ON THE WEIGHTS OF THE ABDOMINAL AND THE THORACIC VISCERA, THE SEX GLANDS, DUCTLESS GLANDS AND THE EYEBALLS OF THE ALBINO RAT (MUS NORVEGICUS ALBINUS) ACCORDING TO BODY WEIGHT

SHINKISHI HATAI

The Wistar Institute of Anatomy and Biology

TWELVE CHARTS

Complete quantitative data on the various anatomical components of the body are important not only for the study of growth, but also for cross reference. A lack of such reference data interferes in many cases with a clear recognition of alterations which are taking place in the animal body under various experimental conditions. Fortunately the following data on the growth of the albino rat are already available:

Growth of body in weight in respect to age (Donaldson '06) and body length (Donaldson '09); weight of the brain and spinal cord (Donaldson '08); weight of total amount of blood and hemoglobin content (Chisolm '11), the growth of the head, trunk, extremities, skin, skeleton, musculature and viscera in weight according to age (Jackson and Lowrey '12); and finally the growth of the dry substance in the albino rat (Lowrey '13). Besides these we have several other series of data on the albino rat, though not so comprehensive as those just cited.

The addition of the present data on the weight of individual organs to the records above cited will make available observations on nearly all the important anatomical components of the albino rat in a form useful either for the study of growth or for cross reference. Indeed in no other mammal are such adequate data available at the present moment.

Since the object of the present paper is to report the objective findings, mainly for reference purposes, many interesting points

relating to growth will not be discussed at length, but merely mentioned as they come, in connection with the presentation of the formulas and charts.

The data used were secured mainly from two colonies of albino rats: (1) a colony kept at The Wistar Institute (during 1911 and 1912), and (2) a colony kept at the University of Missouri (during 1910 and 1911). For the latter, the present writer is under great obligations to Professor Jackson, who not only supplied numerous rats for the purpose of control examination, but has also granted the free use of his entire collection of data on the weight of the viscera.

I take this opportunity therefore to acknowledge my indebtedness to Professor Jackson for his courtesy.

In addition, I have secured some rats from both New Haven and Chicago for the purpose of determining the range of variability under different climatic and nutritional conditions. For these rats I am indebted to Professor Mendel of New Haven and Professor Carr of Chicago, and I desire to thank both of these gentlemen for their generous assistance.

For the interpretation of the results which follow, it is of the greatest importance to bear in mind that the animals examined were nearly all less than one year old—a very few having attained this age. If we take the normal span of life of the albino rat as three years, then it is plain that we are dealing with rats in the first third of their life and that the end of the records gives the conditions at the close of the growing period only, leaving untouched the changes which may be expected to occur during the next two years. For the discussion of the span of life in the albino rat, the reader is referred to Donaldson ('08, p. 368), and to Slonaker ('12).

TECHNIQUE

Although most of the organs are clearly marked off from the surrounding tissues, and thus may be readily removed in an exact manner, nevertheless I shall describe my method of dissection in detail so that others may be fully informed concerning it.

Method of dissection

The animal was removed from the colony in the morning before feeding and killed by chloroform. The gross body weight (to one-tenth of a gram) and the linear measurements were next taken. For the latter determinations, the animal was placed with the abdominal side up and the following measurements made with the calipers: (1) Total length, the distance between the tip of the nose and the tip of the tail; (2) body length, distance between the tip of the nose and anal opening; (3) tail length, obtained by the difference between measurements (1) and (2). The linear measurements were made to the nearest millimeter.

The animal was then at once opened with the scissors along the abdominal line from the anus to the level of the diaphragm. Thus the thorax was left closed to prevent evaporation from the thoracic viscera while the abdominal organs were being removed.

All the dissections were made beneath a glass hood designed to protect the operator against draughts and so prevent a loss of moisture from the organs during their removal.

The details of the procedure for each organ will be given in the order of removal. The following entries give the names of the organs in this order, unless otherwise stated. Each organ was prepared rapidly and then put alone or with others into a closed weighing bottle. Only the special treatment (if any) of an organ is noted in each case:

1. Liver: The blood which fills the main vessels was gently squeezed out before the liver was placed in the weighing bottle.

2. Spleen: The blood vessels were cut close to the hilum.

3. Testes: Accessory organs such as the epididymis, are not included.

4. The alimentary tract was removed from the level of the diaphragm to the anus, together with the pancreas and mesentery, and any other adherent structures, such as fat, were left intact. The oesophagus was therefore not included. The stomach was cut open and the contents removed. The contents of the small intestine were removed by gentle pressure along its entire course

from above downward. In the case of the rectum and large intestine, it was usually necessary to cut them open in order to remove the contents. The entire group of the structures above named but minus contents, is here called 'alimentary tract.' The removal of contents can be accomplished easily and uniformly after short practice. The body weight was *not* corrected for the contents of the alimentary tract.

5. The suprarenal glands were carefully separated from the surrounding structures. These glands are usually imbedded within some fatty tissue but a little familiarity will enable one to dissect them out without difficulty.

6. The ovaries were carefully dissected out of the capsule. The fallopian tube was not included. On account of its minuteness, the ovary is often difficult to remove when the animal is young. A dissecting microscope is sometimes necessary.

7. The kidneys: All blood vessels were cut close to the hilum and any masses of fat carefully removed.

All these organs having been removed and placed in weighing bottles, the thoracic cavity was next opened along the median ventral line by a cut extending as far as the upper end of the neck. Care was taken not to injure the thyroid gland, which lies close to the trachea.

8. Thymus gland: Large lymphatic glands together with fat lie close to this organ but were not included. Because the weight of this gland is so closely correlated with age, the data already obtained will not be presented at this time but reserved until the study of an extended series of animals of known age has been completed.

9. The heart was removed by cutting all the vessels close to their proximal ends. The heart was next cut open by longitudinal slits and any blood clots carefully removed.

10. The lungs were severed from the trachea. The esophagus which lies near to the lungs must be removed. The infected lungs of rats suffering from so-called 'pneumonia' are more or less filled with pus. Such lungs were weighed as removed but these data were not used in computing the formula for lung weight.

Associated with the infected lung a pathological condition of the alimentary tract is usually found. The tract is diminished in weight.

11. Thyroid gland: The removal of the thyroid is rather difficult on account of the minute muscles, similar in coloration to the gland, which adhere very closely to it. A little experience enables one to avoid this difficulty.

This completes the list of viscera removed by the ventral incisions.

The animal was now turned with the dorsal side up, an incision made along median dorsal line, from the root of the tail to the tip of the nose, and the skin laid back on each side.

12. The eyeballs were removed free from all the muscles.

13. The vertebral canal was then opened and the spinal cord removed, free from the spinal nerves, which were severed at the point of their emergence from the cord. The cord was separated from the brain at the tip of calamus scriptorius, equivalent to the level of the first spinal nerve.

14. The brain: Care was taken not to injure the olfactory bulbs or the flocculus; the latter is embedded in a bony capsule.

15. The hypophysis was removed last. Removal of this organ is very simple in the rat as it can be lifted from the floor of the cranium with a fine forceps without further dissection. Both the glandular and infundibular portions were included.

For weighing, these fifteen organs were placed as removed in four closed bottles. Bottle 1 contained liver, spleen, kidneys, testes, heart and lungs. Bottle 2 contained alimentary tract, ovaries, suprarenals, thymus, thyroid, eyeballs and hypophysis. Bottle 3 contained brain. Bottle 4 contained spinal cord.

In each instance the bottle with all the organs in it was first weighed, then the organs were successively removed, one at a time, and the bottle reweighed after each removal. The emptied bottle with such small quantities of fluid as drained from the organs was finally weighed, and from these data the weight of each viscus was computed. It is seen from this that each organ was weighed with its contained blood. The weighings were all made on delicate balances to one-tenth of a milligram.

In making these weighings I had the help of Miss Wolfe and Miss Conrow and desire to acknowledge here my indebtedness to both of them for their accurate work.

The formulas and their application

By means of a mathematical formula the relations between the weight of the body and that of the organ have been expressed in each case. Such formulas have an evident use for the purpose of interpolation, while for the study of various growth phenomena, such as relative rate of growth, form of graph during various stages of life, and so forth, they give a proper basis for discussion.

In expressing the weight relations mentioned above, I have employed a type of formula (Hatai '11) which may be written as follows:

$$Y = aX + b \log X + c$$

or in some cases a simpler form was used

$$Y = b \log X + c$$

where Y = weight of organ; X = body weight; a, b, c = constants; to be determined from the observed data.

In the case of the sex glands it was necessary to use the two formulas in order to express the several phases of growth, that is

$Y = a + bX + cX^2$ for one phase and
$Y = b \log X + c$ for the other phase.

In determining the constants of the formulas, I have used as far as possible the data obtained from the rats reared in The Wistar Institute. This procedure was important in order to avoid the mixture of data.

In some instances however, I have made use of the data obtained by Dr. Jackson, when my own observations were too few for an adequate treatment. Dr. Jackson's data were used, as follows:

1. For studying the eyeballs and ovaries I have used only the data obtained by Dr. Jackson. None of my own observations were employed.

2. For studying the suprarenals, thyroid and alimentary tract, I have used the data obtained by Dr. Jackson to fill the interval

between 5 and 50 grams in body weight. I have however, tested with my own limited data the weights of the organs belonging to the rats of these body weights (5 to 50 grams) and found that the two sets of data agree perfectly.

In fact, in this period all the organs determined by both of us agree closely, though I have not used the data obtained by Dr. Jackson, except in the cases named. Thus in the cases of the three organs mentioned above, the observed data are those furnished by Dr. Jackson and none of my own have been added, while after 50 grams in body weight, all the data are from my own observations made on the rats from the colony at The Wistar Institute. In the case of all of the other organs, the formulas are based on my own data alone.

The total number of The Wistar Institute rats examined by me for the present purpose was 271 (220 males and 51 females) while Dr. Jackson examined 344 (165 males and 179 females) at the University of Missouri. The actual number of rats used in the case of each organ will be stated when the formula is presented.

It should be added that when a rat with infected lungs was found—the rest of the organs except the alimentary tract being apparently normal—the records for the lungs and alimentary tract were discarded, while the data on the remaining organs were employed. Furthermore, in order to simplify the matter, the determinations of the constants were based on the male data alone, as in the case of most of the viscera, there is no clear evidence of a sex difference.

When however the sex differences are evident, the constants were determined separately for each sex. As will be seen later, there are no distinct differences between the two sexes, except in the case of some of the ductless glands. The formula deduced for the males can therefore also be used for the females unless the contrary is explicitly stated.

It should be noted also that in determining the form of the graphs, especially at the upper end where both my data and those of Dr. Jackson were scanty, I have been much helped by

making cross references to the data on a series of inbred albino rats which have been grown here by Dr. King for several years past. There are already on hand data for over 800 inbred rats of various body weights—mostly very large. In addition to these, the records for several hundred Norway and hybrid rats were also employed for cross reference. Therefore, although our data on the stock albino rats are not very extensive, nevertheless, by the aid of the various cross references, it has been possible to produce graphs which are believed by the present writer to be nearly correct in their essential features.

In table 1 the calculated weights of the organs for various values of the body weight are given. This table has been used in making the charts and for the purpose of graphic interpolation. Complete tables giving the calculated weights of all the individual organs for every millimeter of the body length will be published later for the purpose of laboratory reference. Table 2 gives the mean values of the observed weights for all the organs examined.

We now pass to a brief description of the formulas, the graphs based on them and the relations between the observed and computed values.

In charts 1 to 12 the growth of organs in weight for increasing body weight is illustrated. Since I have not taken the age into consideration, any given body weight may be represented by individuals of various ages. In general, the weight of the organs follows closely the body weight, and therefore in rats growing normally, the weight of the organs may be considered as a function of the body weight. We notice in all cases that the greater fluctuations occur among the larger individuals. This is due, in part at least, to the fact that the relation between body weight and body length varies much more in the larger than in the smaller animals, owing either to abnormal fat deposition or emaciation. Such fluctuations may be reduced to a minimum by computing the normal body weight from the observed body length (Donaldson '09) and substituting the computed value of the body weight. However, I have not adopted this method except in the case of a few observations where the emaciation was very considerable.

TABLE 1

Showing the calculated weights of the organs for various values of the body weight. The weights are given in grams

BODY WEIGHT	HEART	KIDNEYS	LIVER	LUNGS	SPLEEN	ALIMENTARY TRACT	TESTES	OVARIES	SUPRA RENALS ♂	SUPRA RENALS ♀	HYPOPHYSIS ♂	HYPOPHYSIS ♀	THYROID	EYEBALLS
5	0.042	0.045	0.271	0.080	0.008	0.120[1]	0.042	0.0008	0.0016	0.0016	0.0005	0.0005	0.0014	0.030
10	0.070	0.153	0.487	0.132	0.030	0.300	0.045	0.0029	0.0044	0.0044	0.0009	0.0009	0.0028	0.061
15	0.104	0.220	1.055	0.174	0.047	0.951	0.065	0.0043	0.0064	0.0064	0.0013	0.0013	0.0040	0.081
20	0.134	0.275	1.531	0.211	0.063	1.493	0.090	0.0054	0.0081	0.0081	0.0015	0.0015	0.0052	0.095
30	0.188	0.374	2.322	0.278	0.093	2.384	0.161	0.0070	0.0107	0.0107	0.0020	0.0020	0.0074	0.117
40	0.236	0.464	2.989	0.340	0.121	3.119	0.265	0.0081	0.0129	0.0134	0.0024	0.0024	0.0093	0.133
50	0.280	0.549	3.583	0.398	0.149	3.760	0.402	0.0090	0.0147	0.0161	0.0028	0.0028	0.0111	0.147
70	0.362	0.713	4.639	0.510	0.202	4.866	0.774	0.0107	0.0180	0.0213	0.0035	0.0041	0.0145	0.170
100	0.473	0.950	6.036	0.669	0.280	6.276	1.300	0.0295	0.0222	0.0288	0.0045	0.0062	0.0190	0.198
130	0.576	1.181	7.310	0.824	0.357	7.517	1.631	0.0450	0.0261	0.0361	0.0054	0.0084	0.0232	0.222
170	0.706	1.484	8.898	1.027	0.459	9.023	1.971	0.0479	0.0308	0.0458	0.0066	0.0115	0.0285	0.250
200	0.801	1.708	10.037	1.177	0.534	10.079	2.176	0.0490	0.0342	0.0529	0.0075	0.0137	0.0322	0.270
230	0.893	1.932	11.144	1.325	0.610	11.091	2.353	0.0500	0.0374	0.0601	0.0083	0.0161	0.0359	0.289
270	1.013	2.228	12.584	1.522	0.710	12.391	2.555	0.0507	0.0415	0.0695	0.0095	0.0172	0.0407	0.313
300	1.102	2.450	13.643	1.669	0.785	13.336	2.688	0.0512	0.0446	0.0766	0.0103	0.0215	0.0442	0.330
350	1.248	2.818	15.378	1.913	0.909	14.871	2.883		0.0497		0.0117		0.0499	0.358
400	1.392	3.185	17.085	2.155	1.033	16.364	3.052		0.0546		0.0130		0.0555	0.385
450	1.534	3.550	18.768	2.397	1.157	17.827	3.201		0.0594		0.0144		0.0610	0.412

[1] From observation.

TABLE 2

Showing the mean values of the observed weights for all the organs examined. The weights are given in grams

BODY WEIGHT	HEART ♂	NO.	BODY WEIGHT	KIDNEYS ♂	NO.	BODY WEIGHT	LIVER ♂	NO.	BODY WEIGHT	LUNGS ♂	NO.	BODY WEIGHT	SPLEEN ♂	NO.
5.3	0.039	2	5.3	0.044	2	5.3	0.271	2	5.3	0.081	2	5.3	0.012	2
14.3	0.103	4	14.3	0.199	4	14.3	0.711	4	14.3	0.156	4	14.3	0.036	4
26.7	0.140	1	26.7	0.370	1	26.7	1.782	1	26.7	0.243	1	26.7	0.081	1
37 6	0.241	6	37.6	0.431	6	37.6	2.600	6	37.6	0.329	6	37.0	0.134	4
44 2	0.252	5	44.2	0.472	5	44.2	3.066	5	44.2	0.386	5	46.9	0.139	2
54 8	0.286	7	54.8	0.558	7	54.8	3.385	7	54.8	0.439	7	54.5	0.208	5
65.1	0.339	6	65.1	0.673	6	65.1	4.739	6	65.1	0.478	6	66.0	0.186	3
76.3	0.387	7	76.3	0.775	7	76.3	5.111	7	76.3	0.563	7	77.6	0.200	4
83.0	0.432	4	83.0	0.955	4	83.0	5.999	4	83.0	0.620	4	80.6	0.218	2
93.4	0.487	2	93.4	1.005	2	93.4	5.980	2	94.9	0.584	1	94.9	0.229	1
107.7	0.493	4	107.7	1.090	4	107.7	6.325	4	107.1	0.614	3	107.1	0.294	2
115.9	0.571	2	115.9	1.119	2	115.9	7.784	2	113.2	0.680	1	115.9	0.368	2
124.4	0.575	11	124.4	1.357	11	124.4	7.611	11	124.4	0.805	7	124.0	0.350	7
138.1	0.587	3	138.1	1.232	3	138.1	7.165	3	138.4	0.983	2	138.4	0.432	2
145.2	0.616	2	145.2	1.292	2	145.2	8.005	2	145.2	1.141	2	141.9	0.288	1
156.8	0.669	3	156.8	1.496	3	156.8	10.206	3	155.2	0.944	2	157.1	0.487	2
164.9	0.706	6	164.9	1.496	6	164.9	9.666	6	164.9	0.982	6	165.4	0.484	2
173.6	0.705	8	173.6	1.425	8	173.6	9.025	8	175.4	1.040	2	173.9	0.508	7
185.1	0.757	4	185.1	1.599	4	185.1	10.385	4	180.5	1.198	1	185.1	0.499	4
195.0	0.786	4	195.0	1.624	4	195.0	9.955	4	199.5	1.137	1	197.3	0.496	1
204.9	0.853	4	204.9	1.547	4	204.9	8.967	4	209.4	1.202	1	205 1	0.591	3
215.7	0.834	1	215.7	1.647	1	215.7	11.174	1	226.4	1.200	4	224.0	0.438	2
226 0	0.840	5	226.0	1.649	5	226.0	10.916	5	237.6	1.356	2	236.4	0.571	5
236.4	0.884	5	236.4	2.035	5	236.4	9.717	5	240.6	1.175	1	254.7	0.804	3
240 6	0.929	1	240.6	1.982	1	240.6	13.699	1	260.5	1.507	3	260.5	0.598	2
254.2	0.938	5	254.2	2.166	5	254.2	11.698	5	279.7	1.760	1	273.2	0.429	1
263.5	0.988	3	263.5	2.264	3	263.5	12.246	3	296.3	1.104	1	294.6	0.758	3
274.4	1.016	3	274.4	2.098	3	274.4	12.871	3	314.7	1.685	2	315.4	0.933	4
294.6	1.011	3	294.6	2.200	3	294.6	12.159	3	324.4	1.713	2	323.8	0.612	3
314.6	1.171	5	314.6	2.550	5	314.6	12.580	5	331.4	1.820	1	358.7	0.729	1
323.8	1.150	3	323.8	2.855	3	323.8	12.794	3	397.0	1.697	1	373.4	1.042	1
332.0	1.132	2	332.0	2.722	2	332.0	15.276	2	454.0	2.376	1	454.0	1.286	1
343.7	1 299	1	343.7	2.867	1	343.7	13.628	1			90			87
358.7	1.194	1	358.7	2.805	1	358.7	16.175	1						
			373.4	3.007	1	373.4	16.152	1						
397.0	1.439	1	397.0	3.179	1	397.0	16.964	1						
454.0	1.504	1	454.0	3.954	1	454.0	21.786	1						
		135			136			136						

BODY WEIGHT	ALIMENTARY TRACT ♂	NO.	BODY WEIGHT	TESTES	NO.	BODY WEIGHT	OVARIES ♀	NO.	BODY WEIGHT	EYEBALLS ♂	NO.	BODY WEIGHT	THYROID ♀	NO.
5.0	0.120	37	5.3	0.004	2	5.2	0.0008	13	5.4	0.026	34	5.2	0.0019	12
10.4	0.310	25	14.3	0.095	4	9.8	0.0009	3	7.2	0.045	7	9.8	0.0021	3
15.9	0.734	12	26.7	0.096	1	14.2	0.0025	30	9.5	0.057	8	13.2	0.0033	12
23.5	1.640	13	37.6	0.272	6	22.0	0.0054	4	11.4	0.061	17	22.2	0.0063	5
36.1	2.261	3	44.2	0.311	5	45.2	0.0093	11	15.9	0.087	12	45.2	0.0090	4
46.7	3.800	8	55.3	0.451	6	55.0	0.0088	5	25.2	0.116	12	66.1	0.0162	2
54.3	4.253	14	65.1	0.691	6	60.8	0.0080	1	36.1	0.124	3	95.5	0.0236	3

TABLE 2—Continued

BODY WEIGHT	ALIMENTARY TRACT ♂	NO.	BODY WEIGHT	TESTES	NO.	BODY WEIGHT	OVARIES ♀	NO.	BODY WEIGHT	EYEBALLS ♂	NO.	BODY WEIGHT	THYROID ♀	NO.
64.0	4.779	5	76.3	0.886	7	75.5	0.0140	1	45.8	0.153	6	120.4	0.0196	3
75.8	5.146	7	82.1	0.822	3	85.7	0.0194	10	54.3	0.159	14	146.1	0.0216	2
91.0	6.485	5	93.4	1.323	2	95.0	0.0216	6	77.1	0.179	5	155.2	0.0268	2
105.0	5.740	2	107.7	1.460	4	104.3	0.0318	6	99.8	0.209	3	164.5	0.0268	2
115.0	7.609	11	115.9	1.452	2	113.9	0.0482	4	114.9	0.194	8	177.8	0.0288	4
124.7	7.647	11	124.4	1.455	11	124.2	0.0442	8	124.6	0.207	5	185.2	0.0305	2
142.1	8.062	5	138.1	1.512	3	135.1	0.0431	5	142.1	0.252	5	193.9	0.0319	5
154.2	8.921	11	145.2	1.873	2	144.2	0.0464	5	153.6	0.240	5			63
164.5	9.796	7	156.8	2.207	3	155.7	0.0457	5	164.0	0.255	6			
186.3	8.889	6	164.9	2.030	6	165.1	0.0519	7	181.0	0.241	4			
245.5	10.853	2	173.6	2.072	8	171.8	0.0423	3	259.6	0.318	5			
259.5	11.683	6	185.1	2.138	4	184.7	0.0508	5			149			
293.2	13.878	2	195.0	2.069	4	207.5	0.0455	4						
347.8	14.721	2	204.8	2.238	3			136						
		194	215.7	1.854	1									
			226.0	2.291	5									
			236.4	2.144	5									
			240.6	2.495	1									
			255.1	2.355	4									
			263.5	2.503	3									
			274.4	2.687	3									
			294.6	2.545	3									
			314.6	2.872	5									
			323.8	2.616	3									
			332.0	2.714	2									
			358.7	2.901	1									
			373.4	2.852	1									
			397.0	2.583	1									
			454.0	3.429	1									
					121									

BODY WEIGHT	THYROID ♂	NO.	BODY WEIGHT	SUPRARENALS ♂	NO.	BODY WEIGHT	SUPRARENALS ♀	NO.	BODY WEIGHT	HYPOPHYSIS ♂	NO.	BODY WEIGHT	HYPOPHYSIS ♀	NO.
5.5	0.0018	6	5.4	0.0020	24	5.2	0.0021	20	5.4	0.0005	5	9.2	0.0009	2
7.0	0.0022	4	9.4	0.0022	32	8.7	0.0030	12	13.7	0.0012	2	16.2	0.0010	2
11.8	0.0025	10	15.9	0.0053	12	13.9	0.0049	36	15.0	0.0009	2	21.2	0.0018	3
14.1	0.0041	5	23.5	0.0085	13	22.2	0.0080	5	19.0	0.0013	5	64.3	0.0032	4
23.5	0.0071	13	36.1	0.0120	3	45.2	0.0115	11	47.6	0.0030	2	90.0	0.0057	3
34.5	0.0103	2	46.7	0.0122	8	66.1	0.0222	2	64.0	0.0035	5	104.3	0.0062	8
47.6	0.0103	2	64.0	0.0176	5	95.5	0.0243	3	83.3	0.0033	6	116.0	0.0074	9
64.0	0.0116	5	74.5	0.0255	2	120.4	0.0344	3	95.2	0.0040	3	127.4	0.0073	4
74.5	0.0126	2	86.6	0.0218	2	146.1	0.0405	2	105.0	0.0047	2	136.6	0.0096	4
86.6	0.0163	2	105.0	0.0212	2	156.3	0.0494	3	115.7	0.0053	6	147.2	0.0088	6
105.0	0.0207	2	115.2	0.0191	3	164.5	0.0403	4	123.9	0.0050	8	155.5	0.0114	7
115.2	0.0193	3	124.5	0.0266	5	177.8	0.0406	3	137.7	0.0057	3	163.7	0.0106	7
124.5	0.0251	5	136.1	0.0317	2	185.2	0.0507	4	154.5	0.0062	8	174.2	0.0112	8
136.1	0.0274	2	154.3	0.0296	5	193.9	0.0493	5	178.8	0.0065	4	184.5	0.0127	6
154.3	0.0263	5	186.4	0.0323	3			113	202.5	0.0082	2	202.5	0.0162	5

TABLE 2—Continued

BODY WEIGHT	THYROID ♂	NO.	BODY WEIGHT	SUPRA RENALS ♂	NO.	BODY WEIGHT	SUPRA RENALS ♀	NO.	BODY WEIGHT	HYPOPHYSIS ♂	NO.	BODY WEIGHT	HYPOPHYSIS ♀	NO.
186.4	0.0313	3	202.5	0.0370	2				214.6	0.0077	2	242.0	0.0153	2
202.5	0.0373	2	211.4	0.0400	2				235.3	0.0083	4			80
211.4	0.0306	2	224.4	0.0339	2				244.9	0.0104	2			
224.4	0.0374	2	240.8	0.0404	3				284.0	0.0109	2			
240.8	0.0376	3	252.6	0.0444	2				310.4	0.0104	2			
252.6	0.0331	2	269.5	0.0490	2				356.8	0.0125	3			
269.5	0.0390	3	284.2	0.0353	3						78			
284.2	0.0449	2	293.9	0.0445	4									
293.9	0.0466	4	348.6	0.0450	4									
348.6	0.0477	4			145									
		91												

In all charts the observed mean values are indicated by either black dots (male) or clear circles (female) while those calculated from the formulas are represented either by continuous (male) or discontinuous (female) lines. Since the calculated values represent the observed data very closely the formulas may be used not only for the purpose of interpolation, but as the basis for the comparison of the growth of the various organs.

In connection with the amount of fluctuation, it should be recalled that the number of individuals was not large, particularly in the case of the heavier rats, in which a greater fluctuation is to be expected, so that with a larger number of cases, the fluctuations would probably be much diminished.

To those using the formulas which are given below, the following practical suggestions are offered:

1. When its value differs markedly from the normal, the body weight should be corrected to the observed body length, as the body length is least modified in animals exhibiting emaciation or abnormal fat deposition. The proper correction can be made according to the following formula (Donaldson '09).

$$\text{Body length} = 143 \log (\text{Bd. wt.} + 15) - 134$$

Or expressing the body weight in terms of body length, we have

$$\text{Body weight} = 10^{\frac{\text{Body length} + 134}{143}} - 15$$

2. Under some conditions the growth of rats is considerably accelerated in both body weight and body length for given ages. Such rats show generally a marked disproportion in the weight relation between body and organs; that is, the viscera are, as it were, overgrown by the remainder of the body. In such cases the body weight normal for the viscera may be estimated from the weight of any one of the organs, such as the weight of the heart. Thus one determines the body weight from the observed heart weight, and then compares the weights of the other organs as observed with those computed for the body weight thus obtained.

3. In any experiment where the controls are compared with experimented animals, modifications in the experimented group can be determined in the following manner: First determine the percentage deviation of the controls from the calculated values as given by the formulas and then the corresponding percentage deviation of the experimented animals. The algebraical difference of the two values thus obtained will be the difference between the experimented and controls. This method is important when the body weights of the two groups of rats (experimented and controls) differ widely since the weight relations between body and organs are not constant but vary with the change in body weight.

We now turn to a brief description of the formulas[1] for the weights of the viscera and the graphs which represent them.

[1] In order to bring together all the formulas which so far have been worked out in connection with the albino rat the following additional formulas are inserted here. This addition will, I believe, be very convenient for reference.

Weight of brain = 0.569 log (Bd. wt. − 8.7) + 0.554 [Bd. wt. > 10]

Weight of spinal cord = 0.585 log (Bd. wt. + 21) − 0.795

Tail length, male = 0.853 Bd. wt. + 38.8 log Bd. wt. − 90.5

Tail length, female = 0.874 Bd. wt. + 43.2 log Bd. wt. − 98.1

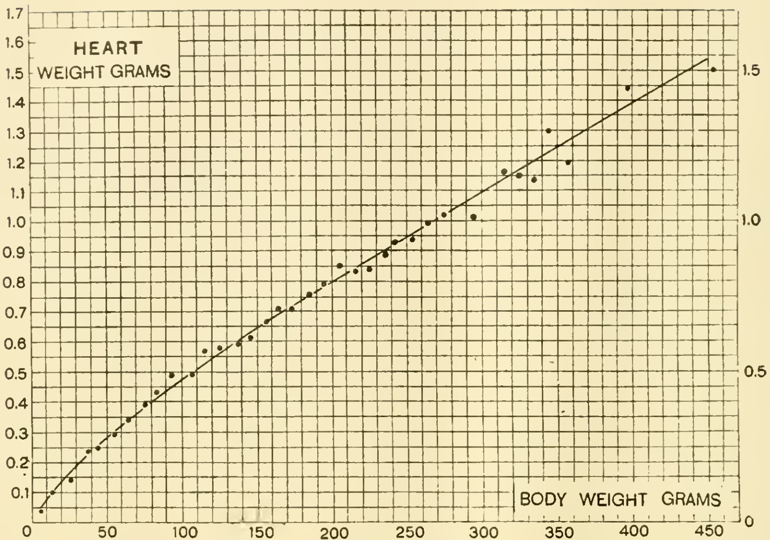

Chart 1 Showing the heart weight of the male albino rat according to body weight. The observed weights are represented by 134 male rats.
● Observed weight. ——Calculated weight.

1. Heart.[2] The growth of the heart in weight is represented by the following formula: Weight of heart =

$$0.0026\ (\text{body weight} + 14) + 0.249 \log\ (\text{Bd. wt.} + 14) - 0.336$$

The constants of the formula were determined from 134 male rats and the relation between observed and calculated values is shown in chart 1. The greater fluctuation which occurs in the rats of larger size has already been noted and explained. As has been mentioned earlier, variations in body growth alter more or less the relation between heart weight and body weight. The heart weight is usually high when the rat is small for a given age

[2] The weight relation between heart and body has been examined by numerous investigators at various times. A list of important papers, as well as a table which illustrates this relation found in the various mammals is given by Joseph ('08) including his own extensive observations on dogs of various sizes.

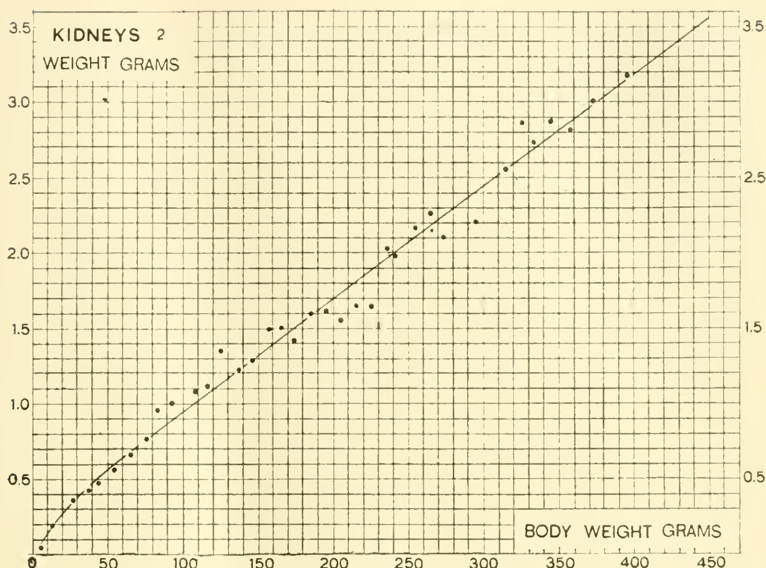

Chart 2 Showing the weight of kidneys of the male albino rat according to body weight. The observed weights are represented by 136 male rats.
● Observed weight. —— Calculated weight.

and it is usually low when the rat is large. A change in the relative weight of the heart is usually accompanied by a corresponding change for all the other organs.

2. Kidneys. The growth of the kidneys in weight is represented by the following formula: Weight of kidneys =

$$0.00718\ (\text{Bd. wt.} - 3) + 0.132 \log\ (\text{Bd. wt.} - 3) - 0.009$$

The constants of the formula were determined from 136 male rats and the relation between observed and calculated values is shown in chart 2. We notice in this case a much greater fluctuation than in the case of the heart, but at the moment no adequate explanation for this can be given. Possibly it is due to the neglected factor of age.

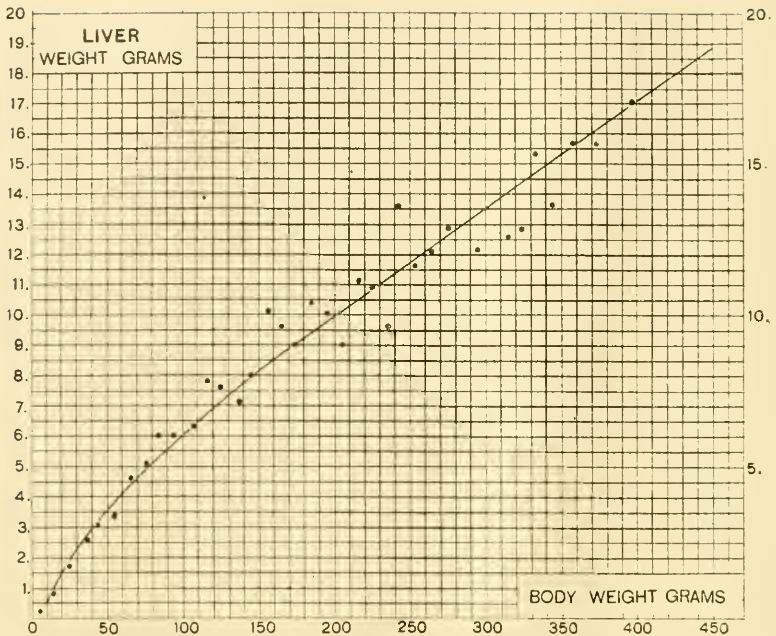

Chart 3 Showing the weight of liver of the male albino rat according to body weight. The observed weights are represented by 136 male rats.
● Observed weight. —— Calculated weight.

3. Liver. The growth of the liver in weight is represented by the following formula: Weight of liver =

$$0.0303\,(\text{Bd. wt.} + 5) + 3.340 \log\,(\text{Bd. wt.} + 5) - 3.896$$
$$[\text{Bd. wt.} > 10]$$

The constants of the formula were determined from 136 male rats and the relation between the observed and calculated values is shown in chart 3. We notice here a much greater fluctuation of the observed values than in the two previous cases. Since the liver is the seat of food storage, as well as performing several other important and complex functions, one might naturally expect a high variability as the consequence of its varied physiological activities. It is possible also that the weight of the liver may

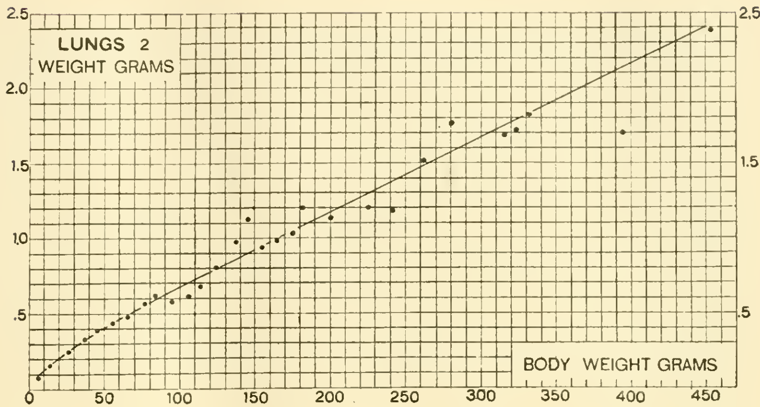

Chart 4 Showing the weight of lungs of the male albino rat according to body weight. The observed weights are represented by 90 male rats.
● Observed weight. —— Calculated weight.

alter according to the nature of the diet as well as according to other conditions not yet studied. It is interesting to note that a heavy liver is usually associated with a heavy spleen.

4. Lungs. The growth of the lungs in weight is represented by the following formula: Weight of lungs =

$$0.00471\ (\text{Bd. wt.} + 2) + 0.122\ \log.\ (\text{Bd. wt.} + 2) - 0.056.$$

The constants of the formula were determined from 90 male rats and the relation between the observed and calculated values is shown in chart 4. All evidently pathological cases were eliminated from the records used. Nevertheless on account of difficulty in determining the infection at an early period, some diseased cases may have been retained. This pulmonary trouble, commonly called 'pneumonia,' seldom appears in rats less than 100 grams in body weight, but after this period almost 90 per cent of the ordinary rat population is affected. This is true not only for the albino rat kept in captivity but for the Norway rat when freshly trapped. Although an elimination of the pathological records was difficult on account of the great frequency of

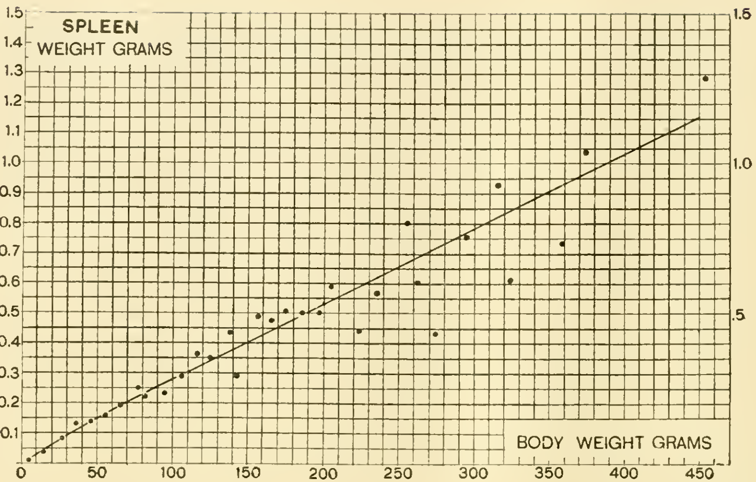

Chart 5 Showing the weight of spleen of the male albino rat according to body weight. The observed weights are represented by 87 male rats.
● Observed weight. —— Calculated weight.

infection, nevertheless when this was made as far as possible, the resulting data were quite uniform as will be seen from the chart.

5. *Spleen.* The growth of the spleen in weight is represented by the following formula: Weight of spleen =

$$0.00245 \text{ Bd. wt.} + 0.0301 \log \text{ Bd. wt.} - 0.025$$

The constants of the formula were determined from 87 male rats and the relation between observed and calculated values is shown in chart 5. The weight of the spleen is highly variable owing to a great frequency of cases of 'enlarged' spleen. In treating the records I have excluded all such plainly altered spleens. Such enlarged spleens are usually darker in color, soft to the touch, and exhibit all over the surface dark or grayish patches. A slight acquaintance with this organ will remove any difficulty in distinguishing the 'enlarged' from the normal spleen.

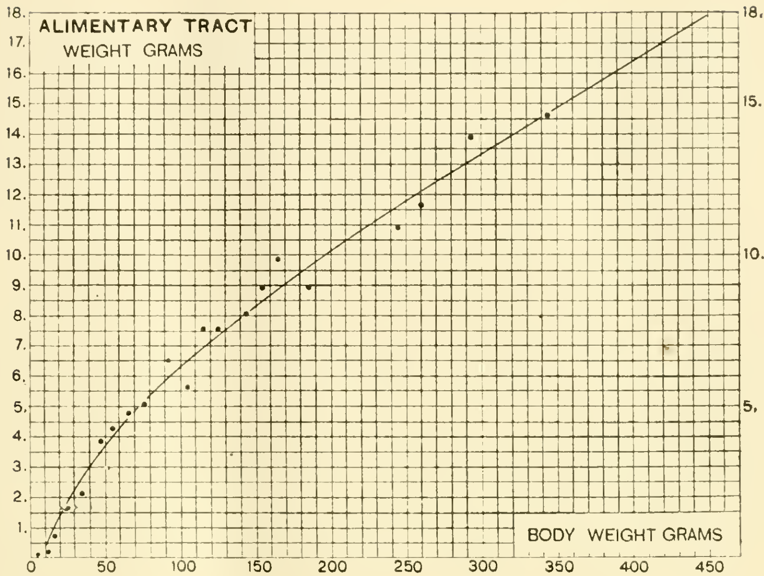

Chart 6 Showing the weight of alimentary tract of the male albino rat according to body weight. The observed weights are represented by 112 (Jackson) rats, below 50 grams in body weight, and 82 (Wistar) rats, above 50 grams in body weight.

● Observed weight. —— Calculated weight.

The cause of this enlargement, as found in the rat, has not been studied.

6. Alimentary tract. The growth of the alimentary tract in weight is represented by the following formula: Weight of alimentary tract =

$$0.0245 \text{ Bd. wt.} + 4.720 \log (\text{Bd. wt.} + 7) - 5.753$$

The constants of the formula were determined from 194 male rats and the relation between observed and calculated values is shown in chart 6. Despite the fact of some difficulty in removing this organ, the weight of the alimentary tract is highly uniform in the rats up to 150 grams in body weight, but beyond this period it becomes decidedly variable owing to the greater frequency of intestinal disturbance. This intestinal disturbance seems to

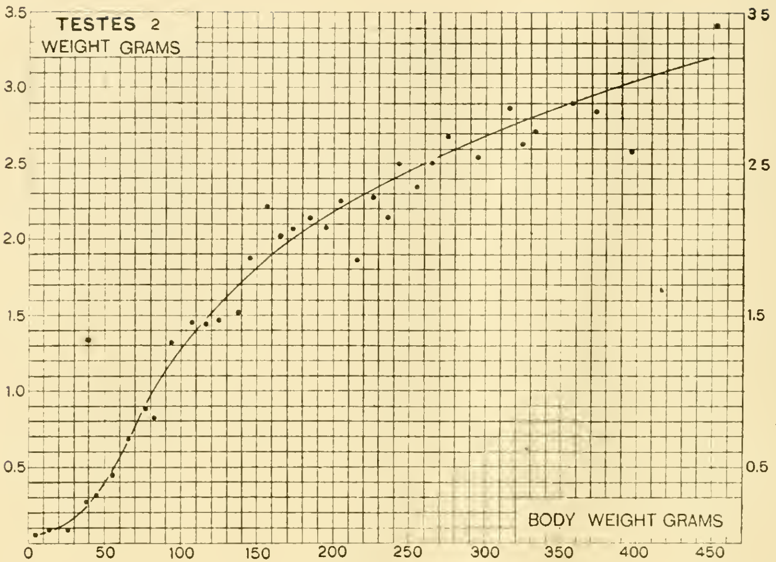

Chart 7 Showing the weight of testes of the male albino rat according to body weight. The observed weights are represented by 121 male rats.
● Observed weight. —— Calculated weight.

be associated mainly with the infected lungs. It is quite safe to conclude that the alimentary tract is more or less altered in cases where pulmonary infection is well advanced. This means that nearly 90 per cent of adult rats are unfitted for this determination. The alteration in question causes a loss of weight. The present formula was determined from the rats in apparently good health and thus practically free from this infection. Consequently the upper end of the graph will naturally be higher than when based on the values obtained from a random sampling of the general population without regard to the condition of the alimentary tract.

7. *Testes.* Owing to dissimilar rates of growth during the earlier days of life, the graph illustrating the growth of the testes has three distinct phases. The first phase however occupies such a brief interval (5 to 10 grams in body weight) that it was thought, from a practical standpoint, not worth while to work

out a special formula for it. Consequently the following formulas represent only the two remaining phases. The weight of the testes on body weight between 5 and 10 grams may be calculated with a sufficient degree of accuracy from the values given by a straight line which joins the observed weight at 5 grams with the calculated weight at 10 grams in body weight.

$$\text{Testes} = 0.043 - 0.000966 \text{ Bd. wt.} + 0.000163 \text{ Bd. wt.}^2$$
$$[10 < \text{Bd. wt.} < 80]$$
$$\text{Testes} = 2.910 \log \text{Bd. wt.} - 4.520 \quad [\text{Bd. wt.} > 80]$$

The constants of the formula were determined from 121 male rats, and the relation between observed and calculated values is shown in chart 7.

Although I have not considered such factors as the season and age, yet from the functions of these organs, it seems probable that data controlled for both those factors would be less variable than that here presented.

Chart 8 Showing the weight of ovaries of the female albino rat according to body weight. The observed weights are represented by 136 (Jackson) rats.
○ Observed weight. ---- Calculated weight.

8. Ovaries. The growth of ovaries in weight is even more complicated than that of the testes. We notice in the ovaries

three distinct phases of growth. The first and last phases are represented by the logarithmic curves, while the second is represented by a parabolic curve. Weight of ovaries:

$$\text{(Phase 1)} = 0.010 \log (\text{Bd. wt.} + 3) - 0.0082 \qquad [\text{Bd. wt.} < 50]$$

$$\text{(Phase 2)} = 0.0425 - 0.00121 \text{ Bd. wt.} + 0.0000108 \text{ Bd. wt.}^2 \qquad [50 < \text{Bd. wt.} < 80]$$

$$\text{(Phase 3)} = 0.007 \log (\text{Bd. wt.} - 105) + 0.0352 \qquad [\text{Bd. wt.} > 110]$$

The constants of the formulas were determined from 136 female rats and the relation between observed and calculated values is shown in chart 8. The data used here were obtained by Dr. Jackson from the rats kept at the University of Missouri. *These rats were all unmated* excepting a few of the oldest (at age of one year). It is therefore possible that the ovaries belonging to the mated females may show some deviation from the values given by the present formulas.

9. Suprarenal glands. In this gland a sexual difference is clearly shown. Consequently the growth of the suprarenals in weight is treated separately according to sex and is represented by the following formulas respectively.

Weight of suprarenals:

$$\text{Male} = 0.0000855 (\text{Bd. wt.} + 3) + 0.0113 \log (\text{Bd. wt.} + 3) - 0.0093$$

$$\text{Female} = 0.00023 \text{ Bd. wt.} + 0.00388 \log \text{Bd. wt.} - 0.0020 \qquad [\text{Bd. wt.} > 30]$$

The constants of the formulas were determined from 145 males and 113 females respectively. The relation between observed and calculated values is shown in chart 9. As is shown in the chart, the sex difference becomes clearly marked in the rats of about 30 grams in body weight. This difference becomes greater as the rats increase in weight. The calculated value of the female glands is represented by the discontinuous line, and the observed values, by circles. The male values are given by the continuous line record and the black dots. The physiological significance of the greater weight of the suprarenals of the female has still to be investigated.

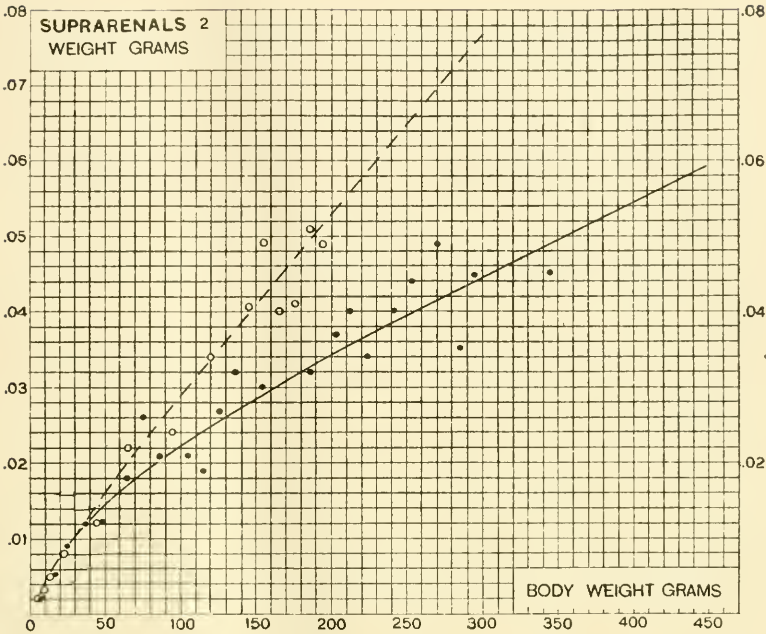

Chart 9 Showing the weight of suprarenals of the albino rat according to body weight. The observed weights are represented by 92 (Jackson) male rats, below 50 grams in body weight, and 53 (Wistar) male rats, above 50 grams in body weight; and 84 (Jackson) female rats, below 50 grams in body weight, and 29 (Wistar) female rats, above 50 grams in body weight.

● Observed weight, male. ○ Observed weight, female.
—— Calculated weight, male. - - - - Calculated weight, female.

10. Hypophysis. Like the suprarenal glands, the hypophysis also shows a distinct sex difference in weight. This appears in rats weighing more than 50 grams. Consequently the growth of this gland in weight is represented after 50 grams in body weight by the two formulas, one for each sex. Weight of hypophysis:

$$\text{Male} = 0.0000257\,(\text{Bd. wt.} + 3) + 0.00140 \log.\,(\text{Bd. wt.} + 3) - 0.00097$$
$$\text{Female} = 0.00205 + 0.000081\ \text{Bd. wt.} - 0.00196 \log \text{Bd. wt.}$$
$$[\text{Bd. wt.} > 50]$$

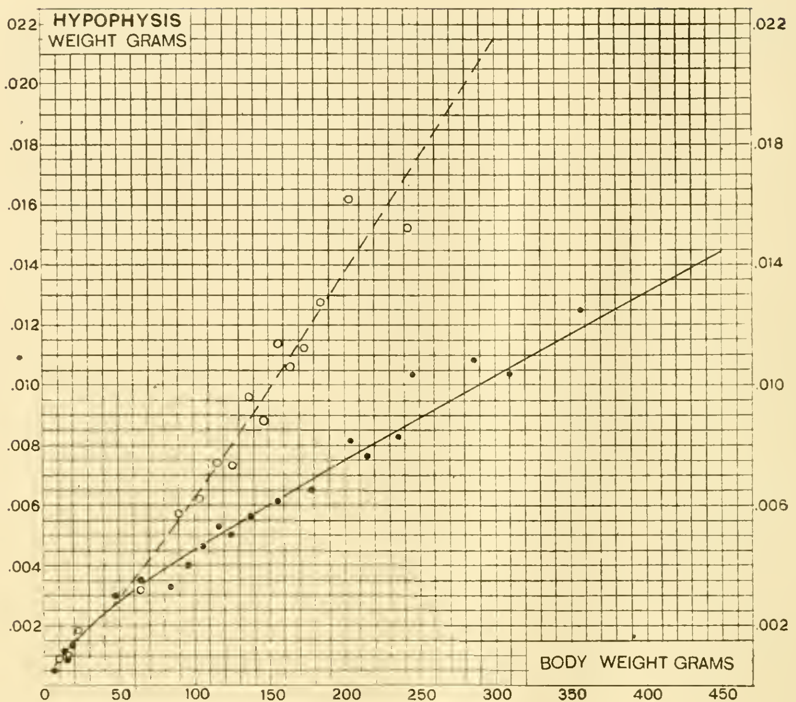

Chart 10 Showing the weight of hypophysis of the albino rat according to body weight. The observed weights are represented by 78 male and 80 female rats.

● Observed weight, male. ○ Observed weight, female.
—— Calculated weight, male. - - - - Calculated weight, female.

The constants of the formulas were determined from 78 males and 80 females. The relation between observed and calculated values is shown in chart 10.

The sex difference is clearly noticeable in the rats at and after 50 grams in body weight, and it becomes greater as the rats increase in weight. Before 50 grams in body weight the sex difference is not evident, consequently the weight of the female hypophysis prior to 50 grams should be calculated from the male formula. This sex difference in the weight of the hypophysis has not been shown previously in any mammal.

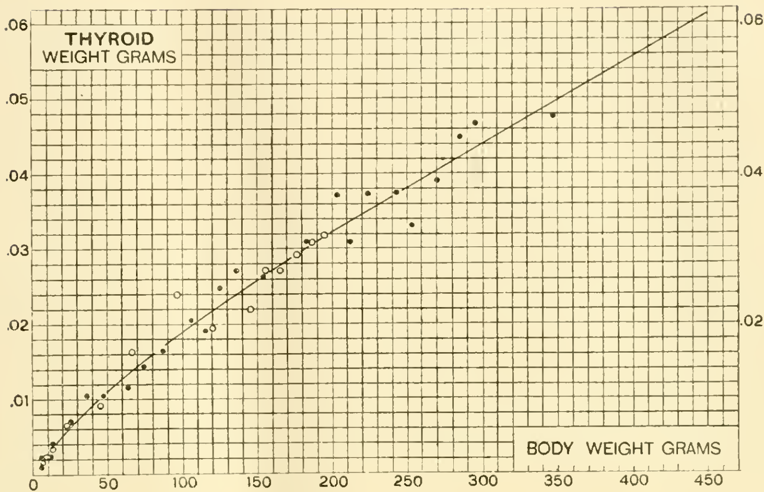

Chart 11 Showing the weight of thyroid gland of the albino rat according to body weight. The observed weights are represented by 42 (Jackson) female rats, below 50 grams in body weight, and 49 (Wistar) male rats, above 50 grams in body weight; and 36 (Jackson) female rats, below 50 grams in body weight, and 27 (Wistar) female rats, above 50 grams in body weight.

● Observed weight, male. —— Calculated weight for both sexes.
○ Observed weight, female.

11. Thyroid gland. Unlike the two ductless glands already mentioned, the thyroid gland does not exhibit any weight difference between the two sexes. The growth of the thyroid gland in weight is represented by the following formula: Weight of thyroid =

$$0.0000973\ (\text{Bd. wt.} + 27) + 0.0139 \log\ (\text{Bd. wt.} + 27) - 0.0226.$$

The constants of the formula were determined from 91 males and 63 females. The relation between calculated and observed values is shown in chart 11. We notice in the chart that the two sexes do not differ fom each other, as the observed mean values for the two sexes are not segregated and those for both sexes cluster round the theoretical line.

The thyroid gland is the most variable among all the ductless glands treated in the present paper. The variation is due to cases

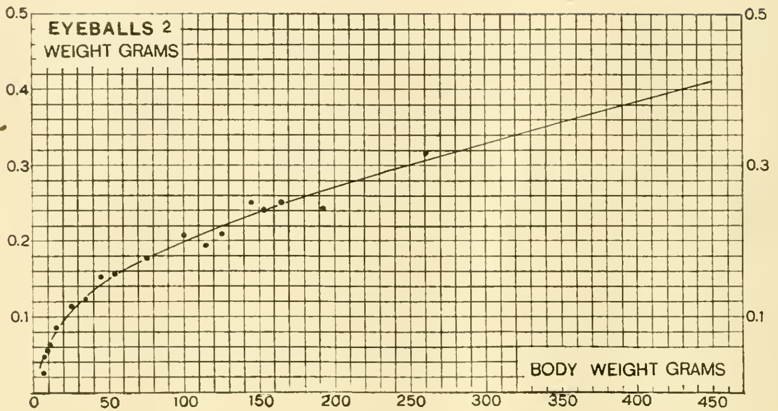

Chart 12 Showing the weight of eyeballs of the male albino rat according to body weight. The observed weights are represented by 149 male rats (Jackson)
● Observed weight. —— Calculated weight.

of enlargement. In some instances this enlargement amounts to almost ten times the average value. From the data here used, all hypertrophied cases were excluded. The enlargement of the gland can be easily recognized not only by the size, but also by the color. The color of the normal gland is pink while that of the abnormal gland is milky owing perhaps to an excessive accumulation of the colloidal material. A slight acquaintance removes any difficulty in distinguishing the abnormal from the normal gland.

12. Eyeballs. The growth of the eyeballs in weight is represented by the following formula: Weight of eyeballs =

$$0.000428 \text{ Bd. wt.} + 0.098 \log \text{Bd. wt.} - 0.041$$

The constants of the formula were determined from 149 male rats. The relation between observed and calculated values is shown in chart 12. The entire data used were furnished by Dr. Jackson and obtained by him from the rats belonging to the colony kept at the University of Missouri.

For the weight of the *Brain* and of the *Spinal Cord*, formulas have already been published (Donaldson '08) (Hatai '09).

As already stated, in the case of the *Thymus* no treatment of the data has yet been attempted.

Types of graphs

A comparison of the graphs reveals several interesting points touching the form of growth characteristic for each organ. A little study of the graphs makes it evident that we can class them under three general types.

Type 1. The graph represented by the growth of eyeballs. This is characterized by very rapid growth in weight at an early period and after this period the rate of growth is much reduced. This type is characteristic for the growth of the brain and the spinal cord in weight (Donaldson '08).

Type 2. The graphs representing the growth of all the other viscera except the sex glands. These graphs are characterized by a relatively rapid rise at an earlier period followed by an almost straight line which makes an angle of varying degree with the base line; an angle always much greater than that of Type 1.

Type 3. The graphs representing the growth of the sex glands. These graphs are characterized by fluctuating rates of growth between birth and sexual maturity. For example, in the case of the ovaries, the first phase (from birth to 50 grams) is represented by a logarithmic curve. This is followed by a phase of rapid growth represented by a parabolic curve (50 to 110 grams). This second phase is followed in turn by a phase of slower growth represented again by a logarithmic curve. Similarly, the growth of the testes has three phases, though the time relations of the corresponding phases are not the same as in the case of the ovaries.

The difference between Type 1 and Type 2 is very clear when the graphs are plotted to an equal scale on one sheet. When this is done the graph for Type 1 appears almost parallel to the base line soon after the very rapid early rise is completed, while the graph of Type 2 continues to rise at a more rapid rate. When

however, animals lower in the zoological scale are taken, Type 1 tends to resemble Type 2.

This can be seen from the graphs for the growth of the brain weight in the fish (Kellicott '08) and in the frog (Donaldson '03). Type 3, however, seems to be quite characteristically differentiated from the rest even in the fish, as shown by the graph for the gonads. The relative growth of the organs represented by these three types of graphs is represented in table 3.

The data giving the percentage growth of the brain and spinal cord (Donaldson '08) are included for comparison. In the case of each organ in table 3 the percentages were calculated by taking the final weight of the organ, at a body weight of 450 grams for the male, as 100 and then expressing the preceding weights as percentages. In the case of the female, the final organ weight (100) was taken from the rats of 300 grams in body weight. This is about the average maximum body weight the female can attain.

Examination of table 3 reveals many interesting points. First of all the three types of growth are clearly shown; that is the eyeballs and the nervous system are characterized by a precocious growth in an earlier stage. Fifty per cent of the final weight is attained by the brain in rats when still below 20 grams in body weight—in the case of the spinal cord, below 100 grams and in the case of the eyeballs, below 130 grams. In the viscera and ductless glands on the other hand, 50 per cent is attained at a body weight of about 200 grams or more. In the case of the sex glands, half of the final weight is attained at about the same stage as the eyeballs 130 grams. Nevertheless the characteristic double or triple phases of growth serve to distinguish clearly the type of the gonads from that of the eyeballs or nervous system.

It is highly interesting to notice the similarity of growth rate of all the abdominal and thoracic viscera and ductless glands, especially after the 50 per cent increment has been attained.

This similarity of the growth rate of the organs just named throughout this first third of the span of life suggests a close quantitative interrelation between these various organs and their

TABLE 3

Showing the percentage growth of various organs. The percentages were calculated by taking the final weight of the organ given in the table as 100 and then expressing the preceding weights as percentages.

BODY WEIGHT	HEART	KIDNEYS	LIVER	LUNGS	SPLEEN	ALIMENTARY TRACT	THYROID	SUPRA RENALS ♂	HYPOPHYSIS ♂	TESTES	BRAIN	SP. CORD	EYEBALLS	SUPRA RENALS ♀	HYPOPHYSIS ♀	OVARIES
5	2.7	1.3	1.4	3.3	0.7	0.7	2.3	2.7	3.5	1.3	11.2	4.3	7.3	2.1	2.3	1.6
10	4.6	4.3	2.6	5.5	2.6	1.7	4.6	7.4	6.3	1.4	30.1	10.1	14.8	5.2	4.2	5.7
15	6.8	6.2	5.6	7.3	4.1	5.3	6.6	10.8	9.0	2.0	49.0	15.0	19.7	7.9	6.1	8.4
20	8.7	7.7	8.2	8.8	5.4	8.4	8.5	13.7	10.4	2.8	56.0	19.4	23.1	10.1	7.0	10.5
30	12.2	10.6	12.4	11.6	8.0	13.4	12.1	18.1	13.9	5.0	63.7	26.5	28.4	13.9	9.3	13.7
40	15.4	13.1	15.9	14.2	10.5	17.5	15.3	21.8	16.7	8.3	68.1	32.4	32.3	17.6	11.2	15.8
50	18.2	15.5	19.1	16.6	12.9	21.1	18.2	24.8	19.4	12.5	71.6	37.4	35.7	21.1	13.0	17.6
70	23.6	20.1	24.7	21.3	17.5	27.3	23.8	30.4	24.3	24.2	76.4	45.6	41.3	27.9	19.1	20.9
100	30.8	26.8	32.2	23.7	24.2	35.2	31.2	37.5	31.2	40.6	81.2	55.0	48.1	37.7	28.8	57.5
130	37.5	33.3	39.0	34.4	30.8	42.2	38.1	44.1	37.5	50.9	84.6	62.4	54.0	47.3	39.1	87.8
170	46.0	41.9	47.4	42.8	40.0	50.6	46.7	52.1	45.8	61.5	88.0	70.1	60.8	60.0	53.5	93.4
200	52.2	48.2	53.5	49.1	46.1	56.5	52.8	57.8	52.1	67.9	90.0	75.0	65.6	69.3	63.7	95.6
230	58.1	54.5	59.4	55.3	52.7	62.2	58.9	63.2	57.6	73.4	91.8	79.2	70.2	78.7	74.9	97.5
270	66.0	62.8	67.1	63.5	61.3	69.5	66.8	70.1	65.9	79.7	93.8	84.0	76.1	91.1	80.0	98.9
300	71.7	69.1	72.7	69.6	67.8	74.8	72.5	75.4	71.5	83.9	95.1	85.9	80.2	100.0	100.0	100.0
350	81.2	79.5	82.0	79.8	78.5	83.4	81.8	84.0	81.2	90.0	97.0	92.0	87.0			
400	90.7	89.8	91.1	89.9	89.3	91.8	91.0	92.3	90.2	95.2	98.6	96.2	93.6			
450	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0			

relative independence of both the gonads and the nervous system. A direct comparison between the two sexes with respect to the relative growth rate, as here given, cannot be made because the final body weight taken in the case of the female is different from that taken for the male. Nevertheless a study of both table 3 and the charts shows that both the ductless glands and the ovary grow in the same manner as the corresponding organs in the male.

GENERAL CONSIDERATIONS

The usual view that the viscera are highly variable, in man for example, seems to have arisen largely (1) from the use of individuals past the prime of life, that is, more than thirty years of age; (2) from confusing normal and pathological material, and (3) from treating the data without proper regard for the controlling factors of race, age, body weight, stature and sex. Indeed from a standpoint of curve fitting the viscera are probably no more variable than other parts of the body.

This optimistic view is based on the data presented in this paper. It will be noticed that the total number of rats used is certainly not large, when the range of body weight is considered. Nevertheless, the resulting data show a considerable uniformity and the observed values do not usually deviate to any great extent from those calculated.

It should be possible, however, to get even a better agreement between the observed and computed values for the albino rat were it possible to control certain important modifying conditions. What these conditions are may be briefly stated.

Factors modifying the relative weights of the organs

1. Food, care and physical condition of rats. It is generally assumed that the rat can eat anything and can grow and multiply under almost any conditions. It is true that one can raise rats with an ordinary bread and milk diet, or even with some artificial diet, for one or two generations if the original strain was thoroughly sound.

Our experience with rats for many years, however, shows that they can not be kept in normal health and reared for any length of time without great care and a highly complex diet. For a time rats may grow and multiply under almost any condition, but if one takes the trouble to record the fertility, death rate, growth rate, and so forth, the importance of the proper food and surroundings will at once be recognized. The relations between the body and organs in weight are clearly modified according to whether the growth of the individual was retarded, accelerated, or normal. Although I have not arranged the data to demonstrate such modifications, the records for such a demonstration are at hand. Recent work by Watson ('10) shows more or less histological alteration of various organs following the different kinds of food given to the rats.

2. Different strains. Another cause of fluctuations in the records may be due to the use of different strains of rats. How many strains are there, and how these strains originated or further how stable such strains will be under varying conditions we do not yet know.

Nevertheless, it has come to our notice that certain strains, represented by rats coming from a new source, tend to show constantly some degree of deviation always in the same sense. For example, we had a strain called "the Ridley Park strain." The rats belonging to this strain had for a given body weight a relatively shorter tail and body and possessed a markedly heavier brain. We had again a strain called "the summer strain"—animals which had been born and reared under unfavorable summer conditions. The rats belonging to this strain had a small body weight for their age and an unusually small nervous system. Such strains may occur under various climatic, nutritional or other environmental conditions. It is important therefore to consider the conditions of the particular colony of rats which may be used for any series of observations. If the fluctuations found are not merely statistical, but deviate constantly in one direction, this fact must be given its proper weight when comparing these animals with those represented in the tables here presented.

We found by comparing our data with those obtained by Dr. Jackson from his rats that our rats had a constantly, though slightly, smaller heart and kidneys, but a heavier liver. Just what the cause of these differences may have been was not determined.

3. Influence of age and season. A third cause of disturbance may be due to the age and season. As has been already stated, I have not taken either age or season into account, but have purposely taken the rats at random in order that my data may meet the usual conditions. However, there can be little question that age and season influence the weight of some of the organs at least. For instance, the weight of the heart is slightly larger in older than in younger rats of the same body weight. The weight of the sexual glands also may vary slightly according to the season. Recent work by Seidell and Fenger ('13) demonstrates a seasonal variation of the iodin content of the thyroid gland.

4. Sex differences. The sex differences are so marked in the case of some of the organs that different formulas were needed to represent the two sexes. However, in the majority of instances, we failed to distinguish any difference according to sex, so far as weight relations are concerned, and consequently only one formula for each organ, namely, that based on the male data, was worked out. It may however so happen that certain organs react differently according to sex under similar conditions. For instance, in the albino rat removal of the sex glands from the male produces an enlargement of the hypophysis, while in the spayed female, the hypophysis is not affected (Hatai).

Doubtless a more precise analysis of the environmental conditions would enable us to extend the list of factors modifying the growth of the organs, but those which have just been enumerated are evidently worth consideration and the control of them will doubtless go far to reduce the variability of the data.

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THE NUCLEUS CARDIACUS NERVI VAGI AND THE THREE DISTINCT TYPES OF NERVE CELLS WHICH INNERVATE THE THREE DIFFERENT TYPES OF MUSCLE

EDWARD F. MALONE

The Anatomical Laboratory of the University of Cincinnati

THREE FIGURES

When one has carefully and critically studied in series of Nissl preparations the brains of various mammals, there is revealed the presence of constant cell groups whose cells invariably possess certain definite characteristics as to size, form and structure. A separation of two groups of cells based merely upon differences in their histological characters is justified in the present state of our knowledge, only when such differences are constant and striking. When these conditions are fulfilled we may conclude that such constant and striking differences in cell character correspond to a difference in cell activity, just as in other portions of the body. I have pointed out elsewhere that very real differences in cell character have been neglected by experimental workers, and that their results have been rendered thereby of less value. Since the dorsal motor (sympathetic) nucleus of the vagus is known to contain centers for the control of both heart muscle and smooth muscle, one would suppose that any real difference in the cell character of various portions of this nucleus would at once claim the attention of the experimental worker and that he would attempt to inform us as to the relation of these different types of cells to the various functions of the vagus nerve. But such is not the case; we are informed casually that some cells are large and others small, and thereafter the cells are considered as if they were all of the same type.

The discovery of two different types of cells in the sympathetic vagus nucleus was not accidental; I was led to look for this difference on the following grounds. In the first place, I had recently shown that all cells concerned in transmitting efferent impulses to striated muscle possess a fundamental similarity of structure, whether the axone of the cell be in direct relation to the muscle or whether the cell act on the muscle through the mediation of one or more efferent neurones. This observation naturally strengthened my belief in the significance of the relation of cell character to cell function. In the second place, I had observed the striking difference between the cells supplying striated and those supplying smooth muscle. Since in a recent paper Molhant had shown that all fibers of the vagus supplying heart and smooth muscle arise from the sympathetic vagus nucleus, I concluded that in cells having such diverse functions there must exist a fundamental difference in histological character. As was anticipated, two different types of cells were found; the evidence in favor of ascribing to the cells of one type the innervation of heart muscle, and on the other hand, to cells of the other type the innervation of smooth muscle, will be considered later.

The material available consisted of two complete series. The first was a series of the brain of a lemur, while the second was of the brain of macacus rhesus. Both brains were fixed in 95 per cent alcohol, and after the usual treatment with absolute and chloroform, were imbedded in paraffin. Serial sections were stained in a 1 per cent aqueous solution of toluidin blue (Grübler), differentiated in 95 per cent alcohol, dehydrated in absolute, cleared in xylol, and mounted in Canada balsam. Series of brains of other forms will have to be prepared and studied before I feel justified in committing myself upon many points, and the present article has therefore been limited, especially as to the exact location and distribution of the different types of cells.

The efferent fibers of the vagus nerve arise from two distinct columns of cells. From the nucleus ambiguous arise the fibers which supply striated muscle, while from the so-called dorsal motor nucleus arise fibers which innervate heart muscle and

smooth muscle. This fundamental difference as to function, which has been proved beyond doubt by the recent investigation of Molhant, had not been clearly recognized; this obscurity was probably favored by the fact that the nucleus ambiguous, together with the motor nuclei of the eleventh, seventh and fifth cranial nerves have often been regarded as visceral, regardless of the fact that their cells cannot be distinguished either histologically or functionally from the cells of the other motor nerves supplying striated muscle. Thus this classification giving undue emphasis to a condition which in mammals no longer exists, has contributed to the general lack of appreciation that the dorsal motor nucleus of the vagus is composed of cells which differ radically both histologically and functionally from those of the nuclei supplying striated muscle, regardless of whether the striated muscle be of somatic or of visceral origin. The name "dorsal motor nucleus" does not indicate the true function of this cell group, and I shall use the name "sympathetic or visceral nucleus of the vagus."

The location and extent of the sympathetic nucleus of the vagus is well known and will not be considered in this paper, except to call attention to the fact that it extends as a long column of cells dorso-lateral to the hypoglossus nucleus from the lowest portion of the medulla to almost the level of the oral pole of the inferior olive. An excellent description of the location of this nucleus is given in Jacobsohn's monograph. The oral portion of the nucleus is composed of small cells of the type shown in figure 2; this is true both in the case of the lemur and the monkey. As one follows the nucleus caudally a second type of cell begins to appear (fig. 1). The portion of the nucleus in which both types of cells occur is at the level of the oral portion of the hypoglossus nucleus, and here the sympathetic nucleus attains its greatest diameter. The cells of each type are partly separated from each other, although no sharp line of separation is evident. In the lemur the large cells (fig. 1) form a fairly compact group dorsal from the small cells, whereas in the monkey their relative position is reversed. Proceeding further in a caudal direction, the small cells become rapidly less numerous

and finally disappear. After the disappearance of the small cells the sympathetic nucleus, consisting now entirely of the large cells (fig. 1) proceeds caudally as a well developed and definite group. In the most caudal portion of the medulla the sympathetic nucleus is much reduced; only a few cells are seen in each section, and these cells become smaller and have the appearance of the smallest cell in figure 1; in this portion of the nucleus (the caudal end) are probably also cells of the type shown in figure 2, that is, similar to those in the oral portion of the nucleus, but at present I cannot be absolutely sure of this, as the surrounding cell groups have not been sufficiently studied. The smallest cell shown in figure 1, is probably a transition type between the other cells of figure 1, and those of figure 2. To sum up, the sympathetic vagus nucleus consists of three portions: (a) an oral portion whose cells are of the type in figure 2; (b) a middle portion whose cells are shown in figure 1; and (c) a caudal portion composed of cells shown in figure 2 (same as oral portion) and also of cells such as the smallest cell in figure 1 (probably a transition type).

It is not my intention to present in this paper a detailed description of the types of cells in the vagus sympathetic nucleus, but rather to point out the fact that there are very definite differences in histological character between the cells of the various groups; a study of the illustrations will make this evident. Since these differences in cell character exist, and since such differences must necessarily be an indication of corresponding differences of cell activity, we may now consider whether these different cell groups of different character may be brought into relation with definite functions. In the first place, it has been shown by Molhant, in his excellent and extensive work on the vagus nerve, that the sympathetic nucleus of the vagus gives origin to all the fibers of the vagus which supply smooth and heart muscle, and that all its cells give origin to such fibers. Further, he has shown that the oral portion supplies smooth muscle (stomach, lungs), the function of the extreme caudal portion is doubtful (possibly connected with the trachea and bronchi), while the intermediate portion supplies

heart muscle, but he has failed to connect these different functions with different types of cells. Concerning the function of the caudal portion of the nucleus, which is composed of cells of the type shown in figure 2, together with cells resembling the smallest cell of figure 1, we can draw no definite conclusion. The oral portion consists exclusively of the type of cells shown in figure 2, and we may conclude that this type of cell supplies smooth muscle; of course this does not justify us in concluding that this type of cell (fig. 2) is the only type of cell which may supply smooth muscle, or that this type may not in other regions have a different function. Overlapping the cells supplying smooth muscle (fig. 2) and extending caudally unaccompanied by other types of cells is the type of cell shown in figure 1, and this portion of the sympathetic nucleus has been shown (Molhant) to supply heart muscle.

It is evident therefore that the cells of figure 1 supply heart muscle, while those of figure 2 supply smooth muscle (stomach and lungs). In addition there is purely histological evidence to support the functional relations of these two types of cells (figs. 1 and 2), *since the cells supplying heart muscle* (fig. 1) *are a type intermediate in histological structure between those supplying smooth muscle* (fig. 2) *and those supplying striated muscle* (cells of hypoglossus nucleus, fig. 3). The relative size of the Nissl bodies in the three types of motor cells illustrated in figs. 1 to 3 is especially worthy of notice. The fact that nerve cells supplying heart muscle are of a type intermediate between those supplying striated and smooth muscle constitutes one of the strongest arguments in support of the importance of the relation of cell character to cell function, since heart muscle is histologically intermediate between the two other types of muscle.

The cell group which supplies heart muscle, composed of the characteristic cells shown in figure 1, I shall name provisionally "nucleus cardiacus nervi vagi." I do not feel justified in assigning any name to the other portions of the vagus sympathetic nucleus, but shall be content with pointing out that the cells of the oral portion which supply smooth muscle are of a definite type (fig. 2). A further division is at present not advisable

because the functional relations of the caudal group are not understood, and because the pigmented cells described by Jacobsohn in man, have not been identified and studied (of course, in lower animals pigment is wanting, although homologous non-pigmented cells may exist). Further subdivision of the sympathetic nucleus, together with a detailed description of the location and extent of the various cell types, the consideration of transition types, and of the relations of the nucleus to the cells of surrounding nuclei, must await a thorough study of numerous series of various animals (including man).

CONCLUSIONS

1. The histological character of a nerve cell is an indication of its function. Differences in connections with portions of the organism which differ merely in spatial relations do not involve a difference in the character of the nerve cells, but are associated merely with the *location* of the nerve cell; for instance, arm and leg muscles, flexors and extensors are all innervated by the same type of cell, although such differences in peripheral connections correspond to differences in the position of the corresponding nerve cells.

2. The three types of muscle are innervated by three distinct types of nerve cell, which, however, are related to one another in such a manner that the cell innervating heart muscle is of a type intermediate between the other two types of cells. Heart muscle, smooth muscle, and striated muscle are innervated by cells such as are illustrated in figures 1, 2 and 3 respectively, the cells of figure 1 constituting a type intermediate between the other two.

3. The nucleus cardiacus nervi vagi is situated in the middle portion of the sympathetic nucleus of the vagus and is composed of cells shown in figure 1.

4. The time has passed when experimental workers can afford to neglect to inform themselves of the existence of definite types of cells situated in the region under investigation, and to attempt to bring cell character into relation with cell function.

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PLATE 1

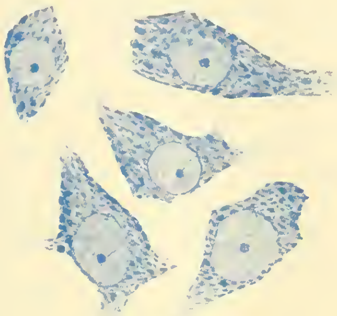
EXPLANATION OF FIGURES

1 to 3 The cells illustrated in the three figures were all drawn from the same section with the aid of the camera lucida, and for all cells the magnification is 580 diameters. I have attempted to reproduce as nearly as possible the actual appearance of the cells, combining to a certain extent different levels of focus. These three figures clearly show that the cells supplying heart muscle (fig. 1) are histologically intermediate between the cells supplying smooth muscle (fig. 2) and those supplying striated muscle (fig. 3).

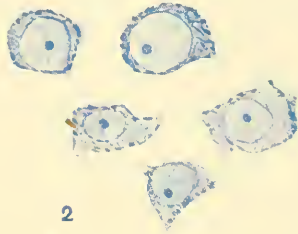
1 Cells from nucleus cardiacus nervi vagi of lemur. The smallest cell represents probably a transition type to the cell type of figure 2, and this type occurs more frequently in the caudal portion of the vagus sympathetic nucleus where it is found together with the cells of the type shown in figure 2. 580 diameters.

2 Cells of vagus sympathetic nucleus of lemur which innervate smooth muscle. In the oral portion of the sympathetic vagus nucleus these cells occur alone; more caudally they occur together with the cells of the nucleus cardiacus (fig. 1) in the most oral portion of this nucleus. In the caudal portion of the sympathetic nucleus such cells probably reappear and are accompanied by the small cell type shown in figure 1 (smallest cell). 580 diameters.

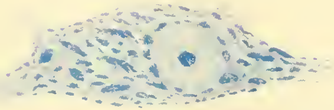
3 Cells from hypoglossus nucleus of lemur, innervating striated muscle. 580 diameters.

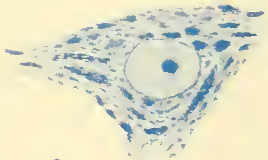


1



2





3

HISTOGENESIS AND MORPHOGENESIS OF THE THORACIC DUCT IN THE CHICK; DEVELOPMENT OF BLOOD CELLS AND THEIR PASSAGE TO THE BLOOD STREAM VIA THE THORACIC DUCT

ADAM M. MILLER

The Anatomical Laboratory of Columbia University

TWENTY-EIGHT FIGURES (SEVENTEEN PLATES)

I. INTRODUCTION

The study of the development of the jugular lymph sac in the chick, the result of which was published in this Journal,[1] (1) led to the investigation also of the developing thoracic duct and the means whereby its communication with the lymph sac is established. This investigation has been carried on with the advice and under supervision of Dr. Huntington and in the light of his recent work on reptiles (2) and the cat (3).

Within the past few years different investigators have shown that in the frog (4), (5), the chick (1), (8), the rabbit (6), the cat (9), and in man (7) each jugular lymph sac develops directly from a venous capillary network adjacent to the junction of the early precardinal with the postcardinal vein to form the duct of Cuvier. It has also been pointed out by Huntington (14) that the jugular lymph sacs, regarded as of venous origin, constitute the connecting links between the hemal vascular system and the general system of lymphatic vessels.

The origin of the systemic lymphatic vessels is a problem on which investigators are sharply divided. A summary of the different views, including the bibliography, can be found on pages 10–13 of Huntington's monograph in the Memoirs of The Wistar Institute (3).

[1] References, by number, will be found on page 162.

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SEPTEMBER, 1913

As regards the particular case of the thoracic duct, Sala (10) in 1900 published the results of his work on the chick in which he holds that it develops by canalization of solid mesenchymal cords. In 1902 Sabin (11), after working with the injection method, published her account of the development of the lymphatic system in pig embryos. In this she reinforces and extends the view of Langer (12) and Ranvier (13), that lymphatics arise from veins by a process of sprouting and centrifugal growth, maintaining that the system as a whole is developed by blind ducts that 'bud off' from the veins of the cervical and inguinal regions, widen out to form sacs from which lymphatics grow to the skin, stating also that "at the same time a growth of ducts occurs along the dorsal line following the aorta to make a thoracic duct from which lymphatics grow to the various organs." The two views expressed by Sala and Sabin are thus diametrically opposed, the one being that the lymphatics arise in the mesenchyme independently of the veins, the other that the lymphatics are outgrowths from the veins.

In 1905 Lewis (6) expressed the view that in the rabbit the lymphatic system is derived directly from the embryonal veins, multiple detached portions of these becoming confluent to form the permanent systemic lymphatics, stating that the thoracic duct "arises from a plexus of lymphatics surrounding the aorta" (p. 109).

In 1907 Huntington and McClure (25), studying the development of lymphatic vessels in their relation to the veins in embryos of the cat, found that "the lymphatics begin as extra-intimal spaces along the course of the primitive embryonal veins. They subsequently become confluent and form continuous vascular channels" (p. 42).

Huntington, in 1908 (14), while retaining this view of the genesis of the systemic lymphatic vessels, as distinguished from the jugular lymph sacs, defined the latter as the connecting links between the hemal vascular system and the general system of the lymphatic vessels, which "arise, not by transformation of veins, but by the formation of spaces lying outside the intimal lining of the veins, which spaces, becoming confluent, form the general lymphatic channels of the body" (p. 25).

In the same year (1908) McClure (21) abandoned the view previously held jointly by him and Huntington as regards the development of the thoracic and right lymphatic ducts in cat embryos, and states:

> The anlages of the thoracic and right lymphatic ducts consist of a series of independent outgrowths which first appear along the common jugular and innominate and then along the azygos veins exactly in the line subsequently followed by these ducts; these outgrowths are subsequently split off from the veins, by a process of fenestration, in the form of a series of isolated, more or less spindle-shaped spaces which later become confluent with one another and with a process of the jugular lymph sac to form a continuous system disconnected from the veins, ... (p. 542).

In 1909 Sabin (7) reiterates her original view, based on the study of injected pig embryos, stating that the "presumption seems to lie on the side that the thoracic duct develops in the same manner as all other ducts," namely, "from endothelial sprouts from the sacs" (p. 58).

McClure in 1910 (23), after further studies of critical stages in lymphatic development, retracted his former view and stated:

> The venous line . . . . along which the cat's thoracic duct develops is topographically replaced by the lymphatic channel, not directly, as assumed by me (in 1908), but secondarily by extra-intimal lymphatic space development, ... the 'extra-intimal theory,' as originally outlined by Huntington and myself (in 1906), establishes a fundamental principle of development for the main systemic lymph channels in mammals (p. 105).

Sabin, in a later article ('11) (22), states that "the thoracic duct develops in part as a down growth of the jugular sac and in part, especially its dilated portion or cisterna chyli, as a direct transformation of the branches of the azygos veins" (p. 424). This expression of opinion seems to be a correction of her earlier statements and a partial adoption of Lewis's view. In place of her former concept of an uninterrupted centrifugal lymphatic growth from the sacs, she now appears to hold that in addition a portion of the thoracic duct develops as the result of direct transformation of azygos venous tributaries into lymphatics.

Huntington again in 1911 confirmed and elaborated his former view by extensive observations on reptiles (2) and the cat, stating specifically on page 13 of the first number of the Memoirs of The Wistar Institute (3) that "the entire extensive system of lymphatic vessels proper of the adult animal, including the thoracic and right lymphatic ducts and their tributaries, is formed by the confluence of the extravenous intercellular mesodermal spaces," and that "these spaces are lined by a lymphatic vascular endothelium which is *not* derived from the hemal vascular endothelium, but develops independently of the same," and giving his summary and conclusions in remarkably clear terms on pages 153–171 of the same publication. He also points out that the systemic lymphatic development in the mammalian embryo is "by no means *confined* to the immediate environment of degenerating embryonic veins. The same field, which shows the above described histogenetic processes in the development of extra-intimal lymphatic spaces surrounding and replacing a decadent venule, will at the same time contain *numerous equivalent lymphatic mesenchymal clefts and spaces* which continue to develop independently of any association with retrograding veins" (p. 49).

Sabin in 1912 (24) still maintains that "the thoracic duct (in the pig) arises in part as a downgrowth from the left jugular sac and in part from a plexus of lymphatics which buds off from the veins of the Wolffian body" (p. 336).

Recently Kampmeier (15), after studying serial sections of both uninjected pig embryos and one of Sabin's injected specimens, concluded that

> the actual genesis of the thoracic duct is initiated by the appearance of blind mesenchymal lymphatic spaces either around or not immediately in contact with the venous derivatives, or veno-lymphatics, which become detached from their venous trunks and break up into degenerating segments . . . . During their inception and growth the walls of the discontinuous thoracic duct anlagen are composed of mesenchymal cells . . . . Injected specimens of the early lymphatic stages certify the reality of blind uninjectible anlagen beyond the farthest points to which the injecta have penetrated, demonstrating that discontinuities in a developing lymphatic channel are not 'appearances' found only by the study of uninjected embryos (pp. 463–464).

Stromsten (16) in his account of the development of the thoracic duct in turtles arrived at a like conclusion, stating that "the development of the peri-aortic lymphatic plexus in the loggerhead turtle is immediately preceded by the formation of isolated, independent spaces. They (the spaces) cannot be injected . . . ." (p. 354). "The intercellular spaces thus formed enlarge and fuse together to form lymphatic lacunae. At a later stage the lacunae acquire an endothelial lining and become the isolated anlagen of the thoracic duct" (p. 356).

As regards the thoracic duct in the chick, it will be my object in this article (1) to demonstrate its origin by confluence of intercellular spaces in the mesenchyme, independent of the veins, and to reconsider the significance of the mesenchymal cords described by Sala in their relation to the developing lymphatics; (2) to discuss the establishment of the morphological drainage line of the thoracic duct and the means by which this duct and the jugular lymph sacs communicate; and (3) to show that the organization of the avian thoracic duct corresponds in type with that established in reptiles and mammals.

II. MATERIAL

Embryos of the domestic fowl (Gallus gallus) have been used chiefly on account of the certainty in procuring the critical stages. Some embryos of the English sparrow (Passer domesticus) have also been used. Of this material I have examined thirty-two individual embryos in serial sections, comprising twenty-seven chicks and five sparrows. Four of the chicks were injected with India ink through the umbilical vein (table 1).

Unfortunately the sparrow embryos, of which series nos. 123, 124, 126, 154 and 509 were examined, were not measured and could be judged as to stages of development only by comparison with the chicks.

The embryos were fixed in vom Rath's, Bouin's, or Zenker's fluid (some in Zenker-formol), Bouin's fluid giving the best results with the least shrinkage. The sections were cut in paraffin and stained on the slide by one or the other of the following methods. After fixation in vom Rath's mixture, the staining was

TABLE 1

CHICK EMBRYOS SERIES NO.	TIME OF INCUBATION IN HOURS	GREATEST LENGTH IN MM., AFTER FIXATION
415	96	7.0
371	108	6.75
336	120	9.0
355	120	11.5
356	120	12.0
326	130	12.5
411	132	9.0
410	132	9.25
370	132	9.5
357	132	10.75
412	145	10.75
340	145	11.0
428	145	11.0
414	145	11.25
339	145	13.5
426	160	13.5
465	165	14.0
463	166	16.0
464	166	16.5
520	171 injected	
521	192 injected	
522	192 injected	
519	206	
523	216 injected	
320	230	
524	240	
483	260	

done with a much diluted Delafield's hematoxylin, followed by a weak solution of picric acid in alcohol. After all the other fixatives the sections were overstained in Weigert's hematoxylin, decolorized in water acidulated with HCl, and counterstained in a weak solution of Orange G in distilled water. The blood cells are clearly differentiated by either method; the cytoplasm of those containing even a trace of hemoglobin shows some tinge of yellow. Developing muscle tissue and nerve fibers also are yellow. Other elements are stained by the hematoxylin, the delicate processes of the irregular mesenchymal cells showing especially well.

III. HISTOGENESIS

Sala, in his account of the development of the thoracic duct in the chick (10), states that the anlagen of this lymph channel appear as isolated mesenchymal spaces which become clothed with endothelial cells derived from the mesenchyme, and which subsequently coalesce to form continuous vessels. He thus implies a denial that the lymphatics in this region arise from veins and that the lymphatic endothelium is derived from hemal vascular endothelium. He also calls attention to accumulations of mesenchymal cells which contrast clearly with the surrounding tissue. They appear to consist in large part of elements exhibiting all the characteristics of young connective tissue cells, with roundish forms, no processes and large intensely colorable nuclei. Among these elements appear red corpuscles in larger or smaller numbers. The accumulations or clumps of cells develop first on the mesial aspect of the superior vena cava, and then extend caudad to the level of the celiac artery. Without describing the histogenesis of these masses of cells, Sala states further that within them in large part the anlagen of the thoracic duct are excavated or 'hollowed out.' The details of the 'hollowing' process are not given.

In the main I can confirm the results of Sala's observations so far as he has carried them. As for the isolated mesenchymal spaces which he describes as the anlagen of the thoracic duct my study of their histogenesis in the closely graded series of chick embryos leads to the conclusion that they do arise independently of the veins and that their endothelial lining is derived from the indifferent mesenchymal cells bordering upon them. The details of the process I shall attempt to demonstrate in the following pages. After tracing the development of the accumulations of cells in the mesenchyme and their subsequent history in relation to the developing thoracic duct, it seems to the writer that a different and greater significance can be attached to them than was given by Sala. Since the cells composing them correspond so closely in their history to the developing blood cells as described by Dantschakoff (17) in the extraembryonic area of the chick,

undoubtedly they should be considered as collections of intra-embryonic developing blood cells.

These clumps of cells, as noted by Sala, develop in the vicinity of the aortic arches, especially the sixth, and along the dorsal aortic roots and the aorta as far as the exit of the superior mesenteric artery. The early stages in the formation can be clearly seen in embryos of 100 to 110 hours.

Some of the stellate elements of the mesenchymal reticulum (syncytium) become differentiated from their neighbors. Their processes are retracted and separated from the general reticulum, the cells thus becoming rounded (figs. 1 and 2). The cytoplasm acquires a more strongly basophilic character, increases in amount and becomes more homogeneous than that of the true mesenchyme. The nuclei contain a relatively small amount of chromatin and one or two, usually two, distinct nucleoli. Mitotic figures are occasionally seen (fig. 2). While the cell contours are generally regular, there are sufficient irregularities to denote an ameboid character. These cells appear to be identical with the large mononuclear cells which Dantschakoff describes as differentiating from the blood islands in the area vasculosa of the blastoderm and later in the capillaries of the yolk sac, and which she calls lymphocytes.

The cells here under consideration increase in number not only by their own proliferation but also by continued differentiation from the mesenchymal syncytium (fig. 2). For the most part they lie in compact groups, which gradually increase in size as the cells increase in number, but some cells usually appear in the vicinity of the groups (figs. 4, 5, 7, 8 and 10, *16*) and not infrequently at some distance from them. After their differentiation and separation from the mesenchymal syncytium all the cells, both members of the groups and isolated, lie free in the spaces among the stellate elements of the mesenchymal tissue (figs. 3 and 4). No blood vessels or lymphatics are present in the immediate vicinity of the cell groups when the latter first develop and consequently the cells are extravascular (figs, 1, 2, 3 and 4). It will be shown later that after the lymphatics develop in this region the cells for the most part are included within them and thus become intravascular elements.

The application of the term 'blood islands' to the accumulations of cells has been avoided because there is no real condensation of the mesenchyme whereby the differentiating elements fuse to form a solid protoplasmic mass. Each stellate element differentiates individually (figs. 1 and 2) and condensation occurs only as a subsequent aggregation of the differentiated cells. The resulting large mononuclear cells, however, so closely correspond to the first lymphocytes that develop in the area vasculosa of the blastoderm, the subsequent histories of the cells arising in the two localities being identical, the conclusion is warranted that the differentiated mesenchymal elements in question serve as an intraembryonic source of blood cells.

While the present investigation has not been of such a nature as to determine whether the mononuclear basophilic cells which have been described give rise to granular leucocytes, they certainly give rise to red blood cells. The cytoplasm of some of the cells gradually loses its basophilic character and acquires a stronger affinity for the plasma dyes, at the same time becoming quite homogeneous. These changes are concomitant with the addition of hemoglobin. The nuclear changes comprise an increase in the amount of chromatin, its arrangement into a rather heavy reticulum and the disappearance of the nucleoli (fig. 2).

Cells thus modified and containing a moderate amount of hemoglobin, as indicated by the reaction to plasma stains, are erythrobalsts. Without further visible structural changes these acquire more hemoglobin until eventually they are indistinguishable from the red blood cells in the general circulation. They have therefore become erythrocytes. It is probable also that they are definitive rather than the larger primitive erythrocytes described by Dantschakoff. The changes described above occur both in the cells composing the groups and in the isolated cells, so that fully developed red blood cells as well as the earlier developmental stages are seen not only in the groups but also in the mesenchymal spaces more or less remote from the groups.

Among the developing erythroblasts, especially in the later stages, are many small cells which apparently go through the same processes of differentiation as the red blood cells themselves and

resemble the latter in every respect except size. These are undoubtedly microcytes, the earlier stages being microblasts.

After the middle of the fifth day the masses of developing blood cells increase rapidly in size by proliferation of the component cells and continued differentiation of the branching elements of the mesenchymal syncytium. They increase in number also by the same processes of differentiation in other localities. Up to about the beginning of the sixth day all the developing blood cells in question remain extravascular, that is, free in the intercellular mesenchymal spaces which are not lined by endothelium.

By the end of the seventh day the aggregations of cells reach the height of their development. At this time they extend in two main lines from the level of the aortic arches along the dorsal aortic roots to the confluence of the latter to form the aorta (figs. 6, 20 and 22, *16*). At this level, or a little further caudal, the two main lines unite to form a single line which extends about to the level of the superior mesenteric artery (figs. 7, 8, 10, 20 and 22, *16*). At their greatest development the larger groups together form an almost continuous mass of the cells which in places is greater in diameter than the aorta. Usually there are also numerous smaller outlying groups which belong to the same general line (figs. 7 and 8).

While the main lines above described are established in all the embryos examined, yet there is a wide range of variability in the form of the groups and their arrangement in the lines. In most of the embryos examined a large mass of cells or a collection of smaller groups had developed in the region dorsal to the aortic roots and esophagus (figs. 6 and 22, *16a*). One of the most interesting and one of the most important features of the masses of developing blood cells is the fact that the main lines in their general arrangement correspond with the lines of the thoracic duct (figs. 20, 22 and 24, *16, 17 ,17a*).

To anticipate, it may be stated here that as the multiple anlagen of the thoracic duct develop the masses of developing blood cells are in a large part included within them, and thus become strictly intravascular (see fig. 10, *16, 17*). The details of this process will

be considered in the subsequent description of the formation of the lymphatic spaces and channels. After the seventh day, as the lymphatic channels in this region develop and establish communication with the jugular lymph sacs, the intravascular masses of blood cells decrease and, by about the eleventh day, are reduced to a few groups scattered through the lymphatic plexus (cf. figs. 24, 27 and 28). A considerable number of extravascular groups persist, however, until the fourteenth or fifteenth day, or even later. In the later stages the vast majority of the cells in these groups are practically mature erythrocytes lying free in the mesenchymal spaces in the vicinity of the lymphatics (fig. 11).

Inasmuch as the reduction in the masses of blood cells in the lymphatics follows the coalescence of the lymphatic spaces to form continuous channels and the establishment of communication between the latter and the jugular lymph sacs, and since the lymph sacs open into the great veins, the blood cells in question eventually reach the general circulation by way of the thoracic duct and jugular lymph sacs. The thoracic duct at one period acts, therefore, as conveyor of the erythrocyte series of hemal cellular elements which have developed from the indifferent mesenchyme along the line of the duct.

A consideration of the histogenesis of the lymph channels constituting the anlagen of the thoracic duct leads on to controversial ground. As stated earlier in this paper, the controversy hinges upon the question whether the lymphatics, exclusive of the lymph sacs or hearts, arise as sprouts or outgrowths from pre-existing vascular channels or de novo from the intercellular mesenchymal spaces.

It is the opinion of the writer that in the chick the lymph channels which constitute the anlagen of the thoracic duct arise through enlargement and coalescence of intercellular spaces in the mesenchymal tissue, and that the endothelial lining of these channels is derived directly from indifferent mesenchymal cells[2] that chance to border upon the spaces. In the material studied there

[2] It should be understood that when 'mesenchymal cells' are spoken of, they are considered as the irregularly stellate masses of protoplasm the processes of which anastomose to form the mesenchymal syncytium, or reticulum.

is no evidence of any growing out, budding or sprouting from the endothelium of pre-existing blood vessels, the tissue in which the thoracic duct develops being non-vascular. The lymphatics in question, and their endothelial lining, arise independently of pre-existing vascular channels.

Prior to the appearance of any specialized spaces or channels in the mesenchyme in the region of the future thoracic duct, the mesenchymal syncytium consists of irregularly stellate protoplasmic elements the slender processes of which anastomose freely with like processes of neighboring elements. The cytoplasm is finely granular and the nuclei, while relatively large and vesicular, contain little chromatin and one or two distinct nucleoli. Among the protoplasmic components of this tissue are the correspondingly irregular interstices or spaces which also are continuous with one another. These are called the mesenchymal intercellular spaces. The tissue as a whole might be compared with a sponge, the anastomosing protoplasmic parts representing the parenchyma of the sponge and the intercellular spaces the pores. While some of the protoplasmic elements during this time differentiate into blood cells, as previously described, our conception of the general syncytium and its spaces is in no way invalidated.

The first changes in the mesenchyme leading toward the formation of definite channels occur during the latter half of the sixth day of incubation. These changes, instead of involving the mesenchyme generally, begin in several localities. In one of the localities, for example, the intercellular spaces increase in size and coalesce, most of the protoplasmic elements of the syncytium being pushed farther apart or broken. In this manner a considerably larger space is formed out of a number of the original smaller mesenchymal spaces (fig. 9, *17*). For the most part the smaller spaces of the surrounding tissue open freely into the larger space, although in places along the edge of the latter the protoplasmic elements lose some of their stellate character and become flattened on the side toward the larger space.

The phenomena in general indicate the accumulation of the fluid filling the mesenchymal spaces, the cells and their processes

being subjected to pressure and friction incident to the flow of the interstitial substance. Thus there arises in the mesenchyme a space larger than the original interstitial spaces but derived directly from them by their enlargement and coalescence (cf. figs. 12, 13 and 14). So far as there is any definite lining for the new space, it is formed by the partially or wholly flattened mesenchymal cells upon which the fluid in the space impinges (fig. 13).

The further changes in one of these larger spaces consists in the main of its elongation through the enlargement and addition to it of other intercellular mesenchymal spaces, a progressive flattening of the cells along its sides, and an approximation of the edges of the flattened cells to form a definite lining of endothelium. There is thus formed a distinct channel in the mesenchyme. For the most part it is lined and its lumen is separated from the surrounding interstitial mesenchymal spaces by a layer of endothelial cells which represent metamorphosed stellate elements of the mesenchyme (figs, 15 and 16). At or near its ends the channel opens freely into the adjoining mesenchymal tissue spaces which in the further course of development are added to the lumen of the channel and thus become a part of it (figs. 15, 16, 17 and 18). The endothelial lining already formed merges near the ends of the channel with the mesenchymal syncytium which in turn, as the channel elongates, gives rise to more endothelium by differentiation of certain of its elements (figs. 15, 16, 17 and 18).

As stated in a previous paragraph, the larger spaces appear in several localities in the mesenchyme. Consequently the channels resulting from their further development are for a time isolated; that is, they are not directly connected with one another or with the jugular lymph sacs or any part of the hemal vascular system. These isolated spaces and channels constitute the multiple anlagen of the thoracic duct (fig. 21).

In succeeding stages each of the channels in question increases in size, especially in length, until it meets and coalesces with its neighbors. The increase in length is due in the main to the addition of more mesenchymal tissue spaces to its ends and the concomitant transformation of more stellate mesenchymal cells into

endothelial cells. There is probably also some proliferation of the endothelial cells, although in the study of the sections mitotic figures were not seen.

The coalescence of the originally unconnected lymphatics results in a network or plexus of channels (cf. figs. 21, 22 and 24). This is constantly being augmented by the coalescence of other independently formed spaces and channels with one another and with the previouly established plexus. Most of the vessels composing the plexus lie longitudinally in the embryo along the line of the aorta and dorsal aortic roots, and out of this plexus is eventually crystallized the main drainage lines of the thoracic duct. The establishment of these lines is best considered, however, in the section on morphogenesis.

The correlation of groups of developing blood cells in this region, the formation of which has already been described, and the lymph spaces and channels constituting the anlagen of the thoracic duct now remains to be discussed. As stated earlier in this paper, the blood cells that are differentiated from the mesenchymal syncytium, whether they are arranged in groups or isolated, lie free in the tissue spaces. In case the tissue spaces enlarge and coalesce in the region where the blood cells are situated the latter are then allowed to become free also in the larger space resulting from the enlargement and coalescence. The larger space becomes lined with endothelium, in the manner previously described, to form a definite vessel or channel. The blood cells, therefore, which were originally free in the tissue spaces are included in any of the lymphatics developing in that particular locality (fig. 10, *16*, *17*).

In that manner some of the blood cells become intravascular elements during the earliest stages of lymphatic development. Occasionally an entire group of cells is included in a lymph channel; in other cases only part of a group or a few scattered cells. It is true also that a great many lymphatics develop in the mesenchyme quite apart from the blood cells (figs. 15, 16 and 17).

In the earlier stages of lymph vessel formation there are great numbers of blood cells, in various degrees of differentiation, in the mesenchymal tissue spaces in the vicinity of or more or less

remote from the lymph spaces and channels. In part at least these cells become intravascular when new lymphatics are formed out of the tissue spaces in which they lie and join the general plexus of previously formed vessels.

Thus far, therefore, the admission of the blood cells to the lymph vessels depends merely upon the topographical relationship in the development of the two sets of structures. There are, however, other factors which in all probability enter into this process. Two are of especial interest and importance in the case under consideration.

It has been pointed out by Dantschakoff that the primitive blood cells in the area vasculosa of the blastoderm and the large mononuclear cells derived from them are capable of ameboid movement. The developing blood cells in the region of the thoracic duct anlagen are demonstrably of the same type as those in the area vasculosa. Hence it is reasonable to conclude that some of the developing blood cells pass from the tissue spaces into the vessels by virtue of their ameboid character.

The other factor has hitherto, so far as the writer is aware, been considered only in the study of living tissues. It seems justifiable, however, to extend the conclusions drawn therefrom to fixed tissues. In their study of chick blastoderms *in vitro*, McWhorter and Whipple (18) have observed the to and fro movement, synchronous with the heart-beat, of blood cells not only in the isolated, endothelium-lined spaces which eventually coalesce to form blood vessels but also in the tissue spaces. Furthermore, they have observed the entrance of blood cells into the general circulation following their to and fro movements in the tissue spaces. These phenomena certify the pulsation of the fluid substance in the tissue spaces in response to the heart-beat. It is not unreasonable, therefore, to conclude that some of the blood cells in the region of the developing thoracic duct are driven or sucked into the lymph spaces or channels which, as pointed before, open freely into the mesenchymal tissue spaces.

As a corollary to the phenomena mentioned in the preceding paragraph, an additional factor in the formation of endothelium might be suggested. Granting that the blood cells lying free in

the tissue spaces move to and fro in response to the heart-beat, as has been clearly observed in the living chick blastoderm, the assumption is justifiable that their movement, with the resultant friction and pressure upon the adjacent protoplasmic elements of mesenchymal tissue, assists in the flattening of these elements and the consequent formation of endothelial cells.

The hydrodynamic factors of pressure and friction of the interstitial fluid substance upon the protoplasmic elements of the mesenchyme, which were first discussed by Thoma (19) in connection with blood vessel formation and have already been noted in this particular case of development of lymphatics, together with the additional factor of pressure and friction of oscillating blood cells would, in the opinion of the writer, afford adequate mechanical means of changing the irregular plastic elements of the mesenchymal syncytium into the endothelial cells of the vessel wall.

IV. MORPHOGENESIS

Up to this point we have been considering the histogenetic changes which occur in the mesenchymal tissue, resulting in the formation of lymph channels and their endothelial lining and of blood cells. It has been demonstrated that aggregations of the developing blood cells, identical with the mesenchymal 'cords' described by Sala, appear along the lines of the future thoracic duct. It has been shown also that the rudiments of the thoracic duct develop as isolated spaces and channels in the mesenchyme, that the endothelial lining of the channels is derived directly from the mesenchymal cells forming their borders and that the channels coalesce to form a plexus. It is our object, under the head of morphogenesis, to trace the subsequent history of the isolated channels and the plexus formed therefrom.

Chick embryo of six days and sixteen hours, 13.5 mm. (Columbia Embryological Collection, series no. 426). Reconstruction, ventral view. Figure 20. The incipient stages of thoracic duct development are shown about this time. The lymph spaces (*17*) are three in number, two on the right side and one on the left, situated in the mesenchyme ventro-lateral to the aorta (*1*) about midway between the exits of the celiac (*5*) and superior mesenteric arte-

ries. They are isolated, for absolutely not any direct connection with any other vessel can be traced even with high powers of magnification. They open freely into the surrounding intercellular spaces, as show in figures 12, 13 and 14, which are photographic reproductions of three successive sections of the embryo.

The spaces fit the previous description, given in the section on histogenesis, of accumulations of an intercellular fluid in the mesenchyme. The objection that they may be shrinkage spaces is nullified by the fact that the preservation of the tissue is practically perfect, and that in this and in other stages the more nearly perfect the preservation the more clearly defined are the spaces. Furthermore, in cases of poorer preservation where there are obvious shrinkage spaces in the mesenchyme the boundaries of such spaces are almost invariably ragged and irregular and do not anywhere exhibit a smooth endothelial lining.

The masses of developing blood cells in this particular embryo (six days and sixteen hours) are perhaps unusually extensive (fig. 20, *16*). They extend in irregular groups from about the level of the superior mesenteric artery forward along the ventral and ventro-lateral aspect of the aorta (*1*) to the level of the celiac artery (*5*), with a tendency to cluster around the last named vessel (cf. fig. 7, *16*). They then divide into two general lines, one on each side, which bend laterad and extend forward along the mesial aspect of the ducts of Cuvier (*12*) and precardial veins (*10*), ending rather abruptly in a large mass which lies at the level of the sixth aortic arches (*3*) and extends across the mesial line ventral to the dorsal aortic roots (*2*).

The three spaces representing the first anlagen of the thoracic duct (*17*) bear no particular relation to the large groups of developing blood cells (*16*), although a few isolated blood cells lie in the tissue spaces around the larger rudimentary lymph spaces.

Chick embryo of six days and twenty-one hours, 14 mm. (Columbia Collection, series no. 465). Reconstruction, dextro-ventral view. Figure 21. In this embryo there is a considerable increase in the size and number of lymph spaces and channels which constitute the early anlagen of the thoracic duct (*17*). They are situated for the most part in the mesenchymal tissue ventral and ventro-

lateral to the aorta (*1*) near the exit of the celiac artery (*5*). Other spaces and channels of a similar nature are seen along the dorsal aortic root (*2*), thus continuing the same general line of lymphatics toward the jugular lymph sac (*15*) with its thoracic duct 'approach' (*15a*). Some of these early lymphatics are of considerable length and have acquired a distinct endothelial lining. Others are simply enlarged spaces in the mesenchyme exactly like those described in the preceding stage (figs. 13 and 14). There is not yet any plexiform arrangement of the channels.

The principle underlying the formation of the longer channels is in the main the enlargement and coalescence of two or more of the original smaller spaces or channels. For instance, two isolated spaces or channels lying near each other in the mesenchyme increase in size until eventually they flow together to form a single space or channel. Or, the same process occurs in several spaces or channels in a discontinuous series until all the members of the series flow together and thus form a continuous channel.

There is also in all probability some proliferation of the endothelium lining the channels. In fact there is no valid reason for not assuming that, *after the inception* of a given vascular channel, either hemal or lymphatic, along with its increase in size there is a concomitant proliferation of its endothelial cells. The crucial point, however, is the *origin* of the channels. The evidence at hand points clearly to the origin of the lymphatics constituting the rudiments of the thoracic duct directly from the mesenchymal interstitial spaces in the manner previously described, the endothelium of the lymphatics representing mesenchymal cells which are modified in accordance with the new conditions of pressure and friction.

In this embryo (series no. 465) the rudiments of the thoracic duct (fig. 21, *17*) are isolated. There is no connection between these and the hemal vascular system; nor on the other hand is there any communication with the jugular lymph sacs (*15*). The thoracic duct 'approach' of the lymph sac, which the duct eventually joins, is well developed at this stage (*15a*) but a considerable distance intervenes between the 'approach' and the rudimentary duct.

The masses of developing blood cells in this embryo are unusually scarce. A few small groups (*16*) are situated in the mesenchymal tissue ventral to the aorta (*1*), and three larger groups (also marked *16*) are seen between the dorsal aortic root (*2*) and the jugular vein (*10*).

Chick embryo of six days and twenty-two hours, 16 mm. (*Columbia Collection, series no. 463*). *Reconstruction, ventral view. Figure 22.* In this embryo the lymphatics (*17*) are in approximately the same stage of development as in the preceding embryo. They are situated for the most part along the ventral aspect of the aorta (*1*). A few spaces are situated ventro-lateral to the dorsal aortic roots (*2*).

The masses of developing blood cells (*16*) are much more extensive than in the preceding embryos of about the same stage, thus exhibiting the variability of the structures. The groups associated with the rudimentary thoracic duct (*17*) lie ventrolateral to the aorta (*1*), with some tendency to cluster around the celiac artery (*5*). The rest of the groups extend in a continuous mass on each side along the ventro-lateral aspect of the dorsal aortic roots (*2*) and along the mesial aspect of the jugular veins (*10*) (cf. fig. 6, *16*) as far forward as the fourth aortic arch (*4*). A large aggregation of developing blood cells (*16a*) also lies between and somewhat dorsal to the aortic roots (*2*) and the arches (*3, 4*) (cf. fig. 6, *16a*), being connected with the left lateral groups by cords extending ventral and dorsal to the roots and arches. Associated with this large mass are a few lymphatics (*19*) (cf. fig. 6, *19*) which subsequently join the thoracic duct and may also communicate with the cephalic end of the jugular lymph sac.

One of the most interesting features of this particular stage is the well developed thoracic duct 'approach' of the jugular lymph sac (fig. 22, *15a*). A reconstruction to show this structure was made on a larger scale and is illustrated in figure 23. The lymph sac iself (*15*) lies dorsal to the jugular vein (*10*) and fits into the angle between the latter and the subclavian vein (*11*). The thoracic duct 'approach' (*15a*) is situated on the mesial aspect of the jugular-subclavian angle and thence extends a short distance caudad along the mesial side of the precaval vein. It lies for the

most part between a mass of developing blood cells (*16*) and the vein (*10*) and ends blindly (see also fig. 19, *16*, *15a*). A short distance farther caudad are two spaces in the mesenchyme which represent the extreme cephalic end of the rudimentary thoracic duct (fig. 23, *17*). These spaces are isolated, for with high magnification there cannot be discerned any connection between them and the thoracic duct 'approach' (*15a*) or the other rudiments of the thoracic duct lying still farther caudad.

The thoracic duct 'approach' of the jugular lymph sac in the chick is without doubt the homologue of a similar structure described by Huntington and McClure (9) in the cat. Like the lymph sac itself, it is of venous origin in the chick as in the mammal, and forms an integral part of the sac. It arises from some of the more mesially and caudally situated components of the early venous plexus in the region of the jugulo-subclavian angle. When fully formed it extends caudad and mesad for some distance along the mesial aspect of the precaval vein, and terminates blindly (figs. 19 and 23, *15a*). Subsequently when it is joined by the thoracic duct, it serves as the portal of entry of this duct into the lymph sac.

Chick embryo of seven days (*Columbia Collection, series no. 512*). *Reconstruction, ventral view in figure 24; view from left side, figure 25.* In this embryo there is considerable advance in the development of the rudiments of the thoracic duct. The spaces and channels, which in the preceding stage were unconnected, have here coalesced to form an extensive plexus (*17*) of channels extending from the level of the junction of the dorsal aortic roots (*2*) nearly to the exit of the superior mesenteric artery. The plexus lies for the most part ventral to the aorta (*1*), but a few of its components extend around on the lateral aspect of this vessel. Most of the channels in the network are much larger than the original spaces and channels, leaving but small areas of mesenchymal tissue among them; in part they have even fused to form irregular sinuses. The celiac artery (*5*) in most of its longitudinal course penetrates the plexus (*17*). The plexus as a whole is still isolated, being connected neither with any part of the hemal vascular system nor with the thoracic duct 'approach' of the jugular lymph sac (*15a*).

Along the dorso-lateral aspect of the aorta (*1*) a number of spaces and channels have also developed (fig. 25, *20*). In addition, a few smaller lymphatics have appeared along the lateral aspect of the aorta. The dorsal aorta is thus almost completely encircled by a group of spaces and channels comprising the large ventral lymphatic plexus described in the preceding paragraph and the dorsal and lateral sets of lymphatics.

These encircling lymphatics are in the aggregate homologous with the dorsal set of peri-aortic sinuses in reptiles, as described by Huntington (2). They are also homologous, in all probability, with the azygos portion of the thoracic duct in the cat, as described by Huntington (3), and in Tragulus, as described by Tilney (20). A comparison with Stromsten's (16) description and figures indicates, too, that the lymphatics associated topographically with the dorsal aorta in the chick are collectively the homologue of the peri-aortic network of lymph vessels in the loggerhead turtle, or, more specifically, with that portion of the network surrounding the dorsal aorta. In addition, the lymphatics around the dorsal aorta in the chick, as previously described, may be placed in the same phylogenetic line as the postcardinal and supracardinal divisions of the thoracic duct in the pig, as recently worked out by Kampmeier (15).

Returning to a further consideration of conditions in the chick at this stage (seven days, fig. 24), it is seen that a few small lymph spaces (*17a*), also isolated, have appeared along the ventro-lateral aspect of the dorsal aortic roots (*2*) in the interval between the plexus previously described (*17*) and the thoracic duct 'approach' of the jugular lymph sac (*15a*). These small spaces represent the beginning of the connection between the thoracic duct 'approach,' on each side, and the unpaired portion of the thoracic duct itself, here composed of the large plexus (*17*).

The conditions in the chick thus correspond so closely to those in other forms that it is possible to draw a clear homology between the lymphatics joining the thoracic duct 'approach' to the unpaired portion of the duct itself in the chick and the preazygos segment of the thoracic duct in the cat (Huntington) and Tragulus (Tilney), with the cephalic portion of the peri-aortic lymph

plexus in the loggerhead turtle (Stromsten), and with the precardinal division of the thoracic duct in the pig (Kampmeier).

In the mesenchyme between and dorsal to the aortic roots and arches in this embryo (fig. 24) a number of lymph spaces have developed. Some of these lie within the masses of developing blood cells in this region (*16a*) while others are situated at some distance from them. A few have coalesced to form a distinct channel (*19*). The subsequent history of these lymphatics will be given in the discussion of later stages.

A few isolated lymph spaces are also found in and near the root of the dorsal mesentery at the level where the celiac artery enters the mesentery (figs. 24 and 25, *21*). These belong to the category of mesenteric lymphatics, but do not yet communicate with the developing thoracic duct.

The masses of developing blood cells in this embryo are extensive (figs. 24 and 25, *16*, *16a*). Those associated with the main portion of the developing thoracic duct lie either in the meshes of the lymph plexus (*17*) or are already included within the vessels composing the plexus. A plate-like mass extends over the mesial aspect of the pleural cavity on the right side. The blood cells still, as in preceding stages, tend to cluster about the celiac artery (*5*). There is no connection in this stage between the masses just mentioned and those which lie farther forward along the mesial aspect of the great veins (*10*, *12*), and which are associated with the thoracic duct 'approach' of the jugular lymph sac (*15a*). In the region between and dorsal to the dorsal aortic roots (*2*) and aortic arches (*3*) there is seen a large mass (*16a*) with which certain lymphatics (*19*), previously referred to, are associated. Situated farther caudad are also several masses associated with the lymphatics which lie along the dorso-lateral aspect of the aorta (fig. 25, *16a*).

A feature shown in this reconstruction, and not in any other, is a portion of the splanchnic plexus of veins (figs. 24 and 25, *14*).[3]

[3] Splanchnic venous plexus is the name given by Dr. A. J. Brown, in his yet unpublished work on the development of the pulmonary veins, to the network of venous capillaries in the wall of the alimentary tube in the earlier stages of development.

This is seen near the cephalic end of the large ventral lymph plexus (*17*) but has no connection with the latter.

At this point it may be well to state that in the chicks of the Columbia Collection I have found no evidence of extra-intimal or perivenous origin of the lymphatics that make up the thoracic duct, such as described by Huntington in the cat and Kampmeier in the pig. This mode of development appears to be a strictly *mammalian* specialization. In fact Huntington (3), speaking of the extra-intimal replacement of veins by developing lymphatics, states (p. 155):

> The association of these (lymphatic) channels, in the mammalian embryo, with certain embryonal venous lines is purely a secondary, mechanical and topographical relationship, expressed by the condensed term of 'extra-intimal' development of mammalian systemic lymphatic vessels, and absolutely devoid of genetic significance. This is, without reference to other vertebrate classes, proved by the development within restricted areas in the mammalian embryo of systemic lymphatic channels through the direct confluence of intercellular mesenchymal clefts, not related topographically or in any other sense to the embryonal veins. It is true that in the mammal this independent lymphatic genesis is extremely limited, and that the majority of the lymphatic vessels develop in close association with embryonal veins, as products of the confluence of perivenous extra-intimal spaces. But this is merely, as shown by *comparison with other amniote embryos*, the expression of the peculiar relations obtaining in the mammal between the venous and lymphatic circuits of the vascular system, developed independently of each other.

Further, Huntington (2), describing the independently formed system lymphatic channels of the reptilian embryo, by confluence of intercellular mesenchymal spaces, states concerning the latter:

> They are not complicated by close topographical relations to adjacent temporary embryonic venous plexuses, as in the mammal, but develop independently by themselves in mesenchymal territory not occupied by hemal vascular elements. . . . . In both lacertilian and chelonian embryos the greater part of the enormously enlarged systemic lymphatic channels develop without any reference whatever to embryonic veins, in mesenchymal areas where the latter are extremely scanty or entirely wanting (pp. 271–273).

Stromsten (16) has reached similar conclusions in studying the development of the prevertebral peri-aortic sinuses in chelonian embryos.

These finding in reptiles, and my own results in the bird, warrant the conclusion that the sauropsids agree absolutely genetically with the mammals in the development of the main axial lymphatic lines by confluence of independently formed intercellular mesenchymal spaces, but that the latter are characterized by close topographical association of these spaces with temporary embryonic veins which they in large part replace, while the former present no such association. In them the progress of thoracic duct development is not, as is the case in the mammal, complicated by the presence of an extensive azygos venous system, and axial lymphatic development, especially in the reptile, occurs chiefly by confluence of mesenchymal spaces surrounding the main arterial trunks.

Chick embryo of eight days (Columbia Collection, series no. 513). Reconstruction, ventral view. Figure 26. The next important change in the lymphatics constituting the thoracic duct comprises the further development of certain isolated spaces situated along the ventro-lateral aspect of the dorsal aortic roots in the interval between the cephalic end of the large ventral plexus and the thoracic duct 'approach' of the jugular lymph sac. The incipient stage in the formation of these spaces was illustrated in figure 24, *17a*. In the embryo now under consideration they have enlarged and coalesced to form a continuous channel (figure 26, *17a*). This in turn has united with the ventral unpaired portion of the thoracic duct (*17*) and with the thoracic duct 'approach' of the lymph sac (*15a*). There is thus established a direct and free communication between the ventral lymph plexus (*17*), which had arisen as an independent and isolated structure, and the jugular lymph sac (*15*). Therefore, as clearly shown in figure 26, the thoracic duct 'approach' (*15a*), which was previously described as an integral part of the jugular lymph sac (see fig. 23, *15a*), serves as the portal of entry of the thoracic duct into the lymph sac (fig. 26, *15*).

The writer has in a previous article (1) shown and will here again point out in a later stage that in the chick, as in reptiles and mammals, a communication is established between the jugular lymph sac and the great veins in this region through one or more

taps. And inasmuch as the thoracic duct opens into the lymph sac, the latter serves as the portal of entry of the systemic lymphatics into the venous system, a point upon which emphasis has already been laid by Huntington (2) (3) in his work on reptiles and the cat.

In the embryo of eight days the large ventral lymph plexus of the preceding stage (cf. fig. 24, *17*) has undergone further coalescence of its component channels to form a large irregular sinus with a few fenestrae (fig. 26, *17*). The sinus lies ventral to the aorta (*1*) and extends from the junction of the dorsal aortic roots (*2*) to the level of the exit of the superior mesenteric artery (*9*). At its cephalic end it branches off into the two slender trunks, one on each side, which extend cephalad and laterad to join the 'approaches' (*15a*) of the lymph sacs (*15*). These trunks, which are the last components of the lymphatic drainage line to develop, constitute in the bird, according to the anatomical terminology, the right and left thoracic ducts.

The lymphatics (*19*) situated between and dorsal to the aortic roots (*2*) and arches (*3, 4*) are here seen to comprise a plexus and a few outlying isolated spaces and channels (cf. fig. 24 and 26, *19*). This plexus now communicates with the chain of lymphatics (*20*) lying dorso-lateral to the aorta (*1*), which in turn communicate with the large ventral plexus through channels formed by coalescence of spaces lateral to the aorta (cf. fig. 25, *20, 17*). The entire group of lymphatics in the region of the dorsal aortic roots and the dorsal aorta as far back as the superior mesenteric artery, with the exception of the mesenteric lymphatics (fig. 26, *21*), which have not yet joined the thoracic duct, drain into the jugular lymph sacs.

The masses of developing blood cells were omitted from the reconstruction of the eight day stage (fig. 26). A careful study of the serial sections showed that they were fewer and smaller than in the preceding stage. This may be due to the variability characteristic of the masses, or it may be due to actual reduction of the masses since the blood cells, as stated in in the section on histogenesis, now have access to the jugular lymph sacs through the recent connection established between these structures and

the chain of lymphatics with which the blood cells have for the most part been associated.

Chick embryo of nine days and fourteen hours (*Columbia Collection, series no. 320*). *Reconstruction, ventral view and view from left side. Figures 27 and 28.* At this stage the main features of the adult thoracic duct have been established. The large plexus (*17*) ventral to the aorta (*1*) now drains through the right and left thoracic ducts (*17a*) into the 'approaches' (*15a*) of the jugular lymph sacs (*15*) and then through the sacs into the great veins (*10, 12*).

The ventral plexus (*17*), the isolated anlagen of which were the first lympathics to develop in the thoracic duct line, is relatively smaller and the component channels less dilated than in the preceding stages. This is probably due to the outflow of the contents into the lymph sacs and veins through the more recently formed channels which have been called the right and left thoracic ducts (*17a*). The mesenteric lymphatics (*21*) have increased in size and for the most part coalesced to form sinus-like channels. Between these and the thoracic duct chain no connection can be detected at this stage, although little tissue intervenes. Farther cephalad another isolated group of lymphatics (*22*) is associated with the esophagus.

The right and left thoracic ducts (*17a*) are longer than in the preceding stage (cf. fig. 26, *17a*). The left is considerably greater in diameter than the right. Each opens into the corresponding 'approach' (*15a*) of the jugular lymph sac. The 'approach' on each side is still patent as a branch of the main portion of the sac.

There is now free communication between the lymph sacs (*15*) and the great veins through recently formed taps, of which two are present on the left side of the embryo and one on the right. Of the two on the left side, one is situated on the dorsomesial side of the superior vena cava (*12*) just below the level of the jugulo-subclavian junction (*10, 11*); the other is situated farther forward on the mesial side of the jugular vein (*10*). The tap on the right side of the embryo is located on the dorso-mesial side of the superior vena cava at the level of the jugulo-subclavian junction.

In a previous article on the development of the jugular lymph sac in birds (1) the writer stated, in the description of tap formation, that "it is not improbable that a study of later stages will reveal a homologue of the common jugular tap in the mammal" (loc. cit., p. 486). There is little doubt that the tap on the mesial side of the left jugular vein, referred to in the preceding paragraph, fulfils the requirement. Moreover, the other tap on each side near the jugular-subclavian junction in the chick is in all probability homologous with the jugulo-subclavian tap in the mammal (26), although it is slightly different in position.

The previously described lymphatics situated dorsal to the aortic roots and arches and dorso-lateral to the aorta here constitute a long chain of continuous channels reaching from the level of the jugular lymph sac to the level of the celiac artery (figs. 27 and 28, *19*, *20*; cf. figs. 24, 25 and 26, *19*, *20*). At the extreme cephalic end one of the channels of this series opens into the right lymph sac. The probable significance of this opening will be considered in the subsequent discussion of the masses of developing blood cells. The plexus (*19*) in the region of the aortic arches is considerably reduced as compared with preceding stages. The portion of the dorsal plexus (*20*) associated with the dorsal aorta communicates with the large ventral plexus (*17*) through small channels which curve around the lateral aspect of the aorta. It is seen, therefore, that the entire group of lymphatics associated with the aorta and dorsal aortic roots, with the exception of a few still isolated spaces and channels, can now discharge the contents of the channels into the great veins.

The most interesting and, in fact, from the standpoint of the hemophoric[4] function of the thoracic duct, the most important feature of this stage is the great reduction in size and number of the masses of developing blood cells. It has been shown in the foregoing pages that the differentiating blood cells associated with the developing lymphatics are admitted or gain access to the lymph spaces and channels and that when the communication is established between the thoracic duct and the jugular lymph sacs the blood cells can thus reach the sacs. Now since the taps

[4] Hemophoric—blood bearing or carrying—is a term suggested by Dr. Schulte as a coördinate with hemopoietic—blood producing.

between the lymph sacs and great veins have been formed, the blood cells are admitted to the hemal vascular system.

In this connection there are two aspects of the question which are especially worthy of consideration. In the first place there are few blood cells within any of the channels forming a part of the thoracic duct system, while in earlier stages, before the thoracic duct had open communication with the lymph sacs and great veins, many of the lymph channels were filled with the hemal cellular elements. In view of this, and the fact that there are no other means of egress for the blood cells formerly contained in the lymphatics, it must be concluded that the blood cells reach the hemal vascular system *via* the thoracic duct, the jugular lymph sacs and the recently formed taps between the sacs and the great veins.

The other feature is the great reduction in the size and number of the extravascular masses of developing blood cells. The diminished masses are shown in yellow in figures 27 and 28, the conditions in which should be compared with those in figures 22 and 24. A few fairly extensive groups are found in the mesenchymal tissue among the channels composing the large ventral plexus (*17*). These are for the most part quite closely associated with the lymphatics. A few small groups still are found in the region of the thoracic duct 'approach' (fig. 28, *15a*). The extensive masses associated in earlier stages with the lymph plexus dorsal to the aortic arches have almost wholly disappeared. This plexus itself is considerably reduced, and it is not unreasonable to assume that the connection between the cephalic end of the plexus and the jugular lymph sac, previously alluded to, is in some way associated with the discharge of the numerous blood cells differentiated in this region into the lymph sac.

In view, therefore, of the intimate relationship between the developing thoracic duct and blood cells in the same general region, and of the sudden and marked reduction in number of these blood cells, both intravascular and extravascular, following the establishment of communication between the duct and the jugular lymph sacs and great veins, the importance of the thoracic duct as a carrier of hemal cellular elements for a period of embryonic life in the bird can scarcely be doubted.

V. SUMMARY

Prior to the appearance of lymphatics in the region of the future thoracic duct, namely, along the aorta and dorsal aortic roots, the mesenchyme comprises a syncytium of irregular strands with correspondingly irregular interstitial spaces. The tissue is non-vascular.

The initial change in conditions is manifested in the appearance of distinct lacunae in the mesenchymal tissue along the ventro-lateral aspect of the aorta at the level of the celiac artery. The lacunae are bounded by unmodified protoplasmic elements of the mesenchymal syncytium, and open freely into the adjacent intercellular—interstitial—spaces. Obviously the lacunae represent enlarged intercellular spaces, and the inference is justifiable that they are filled with the intercellular fluid.

In a slightly advanced stage of development in general there is a greater number of lacunae in the same region in the embryo and also an increase in size and a difference in the appearance of some of the lacunae. The increase in size certainly depends in part upon actual dilatation and in part upon addition of more of the adjacent mesenchymal intercellular spaces, for every possible gradation can be seen between the smallest and the largest. The difference in appearance is observed to be due to the presence of flat cells which form a distinct boundary or wall, although not usually complete. Morphologically these cells are equivalent to endothelial cells. Inasmuch as they shade by invisible gradations into the unmodified mesenchymal cells bordering upon the rest of the lacuna, we conclude that they are derived directly from the indifferent mesenchymal cells. The differentiation, we may also infer, is due in part to pressure and friction incident to the flow of the tissue fluid. Another factor in the differentiation may also be pressure and friction incident to the to and fro motion of blood cells in the tissue spaces and lacunae in response to the heart-beat, a phenomenon observed in living blastoderms.

Studies of later stages show these isolated lacunae to be the rudiments of the thoracic duct, and the conclusions that they are direct derivatives of the mesenchymal intercellular spaces and

that the flat cells forming their endothelial walls are differentiated in situ from the mesenchymal cells are based upon prolonged and thorough studies, with high magnification, of serial sections of chick embryos in practically perfect states of preservation. The writer is therefore forced to ally himself unequivocally with the advocates of the view that the thoracic duct originates independently of the veins and lymph sacs.

Studies of subsequent stages also show that the numerous isolated lacunae, or rudiments of the thoracic duct, enlarge still further, principally in a longitudinal direction, and coalesce with one another to form a plexus of lymph channels which lies ventral to the aorta. Other similar isolated lymphatics develcp along the dorso-lateral aspect of the aorta and in the region dorsal to the aortic roots and arches, and then coalesce to form plexuses. Eventually all the plexuses intercommunicate.

In the meantime a connection is established between the large ventral plexus and a branch of each jugular lymph sac known as the thoracic duct 'approach.' All the components of the thoracic duct system thus drain into the jugular lymph sacs. Communications, or taps, are established between the lymph sacs and the great veins, and the thoracic duct then drains into the hemal vascular system, the lymph sacs serving as portals of entry.

In the region of the developing thoracic duct, namely, along the aorta and dorsal aortic roots, and also in the region dorsal to the aortic arches, a great number of blood cells arise. The genesis of these cells is indicated by certain changes in some of the irregular elements of the mesenchymal syncytium, comprising a marked increase in the basophilia of the cytoplasm, a rounding of the cell body and a separation from the general mesenchymal reticulum. The resulting cells thus lie free in the interstitial spaces, and structurally are similar to the large mononuclear cells (lymphocytes of Dantschakoff) in the area vasculosa of the blastoderm. They increase in number both by mitosis and by constant differentiation from the mesenchymal syncytium.

Many, at least, of these basophilic cells are transformed into erythrocytes through the addition of hemoglobin to the cytoplasm and certain nuclear modifications comprising the

disappearance of the nucleoli and the rearrangement of the chromatin into a heavy reticulum.

These developing blood cells, at first scattered in the mesenchymal intercellular spaces, become aggregated, following the increase in their number, into extensive masses which lie along the line of the thoracic duct and also in the region dorsal to the aortic arches.

When the lymphatics comprising the rudiments of the thoracic duct develop, some of the developing blood cells, even some of the smaller groups, are seen to be contained within them, while others are still free in the mesenchymal intercellular spaces, that is, extravascular. Subsequently more and more of the cells are observed to be intravascular. It may be concluded that the developing blood cells become intravascular by simple inclusion as the lymph channels develop, or by virtue of their ameboid character, or as a result of their motion to and fro in the tissue fluid in response to the heart-beat.

The blood cells that are admitted to the lymph channels constituting the thoracic duct system during its development rapidly diminish in number after communication is established between the thoracic duct and the jugular lymph sacs and between the latter and the great veins. It can be inferred then that the blood cells which develop along the line of the thoracic duct reach the blood stream *via* this duct and the lymph sacs. Considering the vast number of hemal cellular elements, especially erythrocytes, arising in this region and the probability that they reach the general circulation *via* the thoracic duct, this duct assumes an additional phase of importance in the chick in that it performs a hemophoric, or blood carrying, function.

In conclusion, I wish to thank Dr. Huntington and Dr. Schulte for their valuable criticism and suggestions, Dr. McWhorter for his painstaking work in making the photomicrographs, and Mr. Petersen for his careful execution of the color plates.

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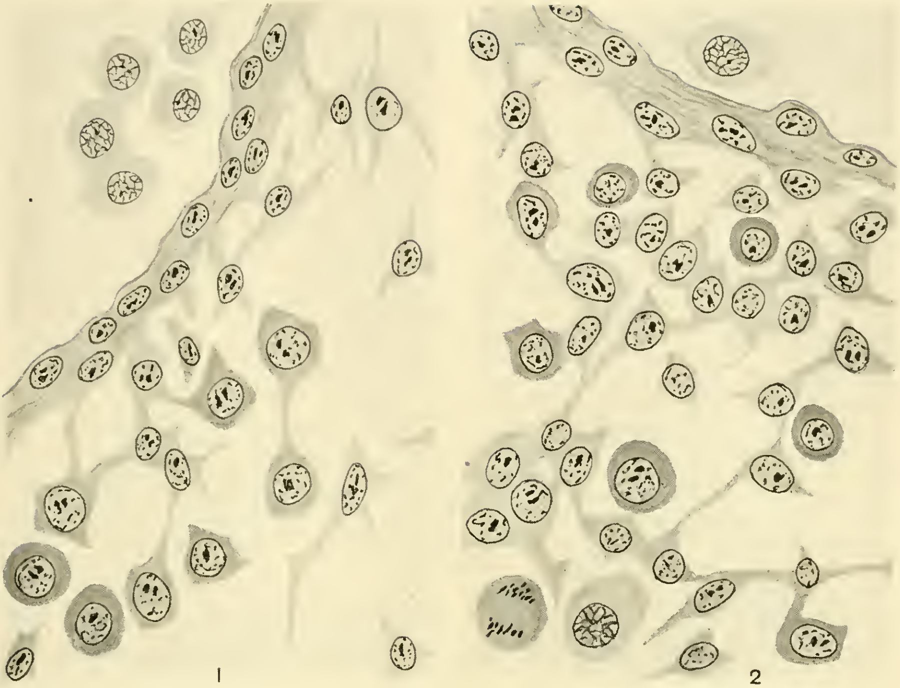
PLATE 1

EXPLANATION OF FIGURES

1 From a section of a chick embryo of 108 hours, 6.75 mm. (series no. 371; slide II, section 80). Outlines drawn under Edinger projection apparatus, × 1500; reduced to ×1000. In the upper left corner is a portion of the aorta with a few erythrocytes. Outside of the aortic wall is an area of mesenchyme in which some elements show stages of differentiation leading toward large basophilic cells, two of the latter lying free in the tissue spaces at the lower left corner of the figure. These are apparently identical with the developing blood cells (lymphocytes) in the area vasculosa of the blastoderm as described by Dantschakoff.

2 From the same embryo as figure 1 (slide II, section 97). Outlines drawn under Edinger projection apparatus, × 1500; reduced to × 1000. A small part of the aorta is shown in the upper right corner. Some of the protoplasmic elements of the mesenchymal syncytium are shown in various stages of differentiation leading toward the free basophilic cells (lymphocytes of Dantschakoff). In the lower left corner is seen one of these cells in the anaphase of mitosis. At the right of this is a practically mature erythrocyte lying free in a mesenchymal intercellular space.

3 From a section of a chick embryo of 132 hours, 9 mm. (series no. 411; slide IV, section 16). Outlines drawn under Edinger projection apparatus, × 1500; reduced to × 1000. This figure, taken just ventral to the aorta, shows a number of the rounded basophilic cells lying free in the mesenchymal intercellular spaces.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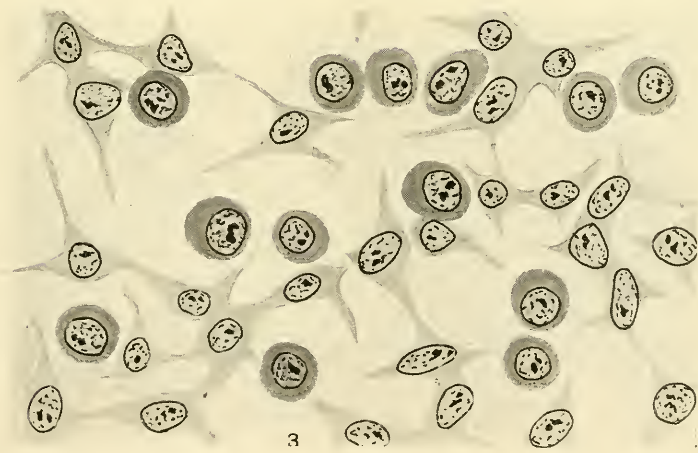
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PLATE 2

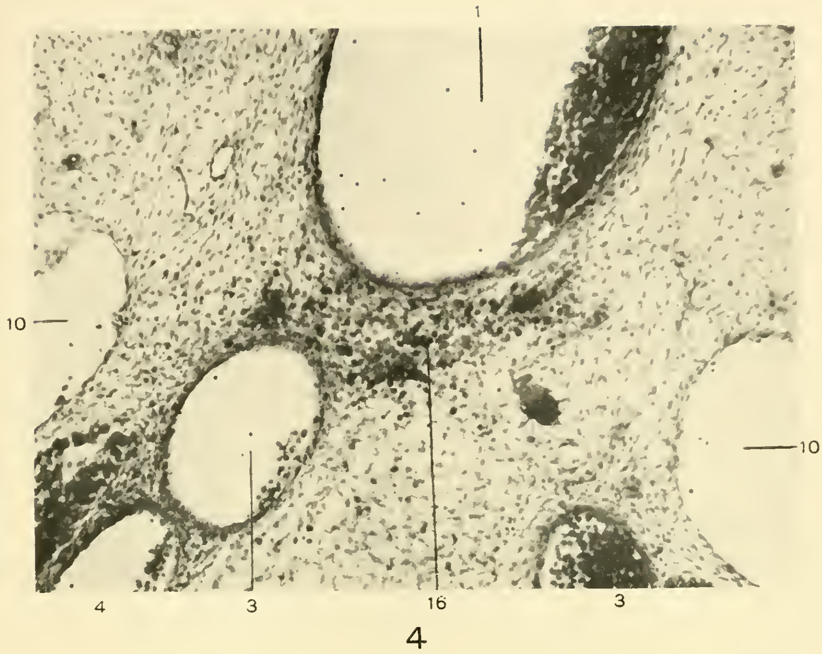
EXPLANATION OF FIGURES

4 From a section of a chick embryo of 5 days, 12 mm. (series no. 336; slide IV, section 7). Photomicrograph. × 166.

1, aorta
3, aortic arch VI
4, aortic arch IV
10, precardinal vein
16, group of developing blood cells

5 From a section of a chick embryo of 6 days, 13.5 mm. (series no. 339; slide VIII, section 35). Photomicrograph. × 166.

2, dorsal aortic root
3, aortic arch VI
16, groups of developing blood cells
27, vagus nerve



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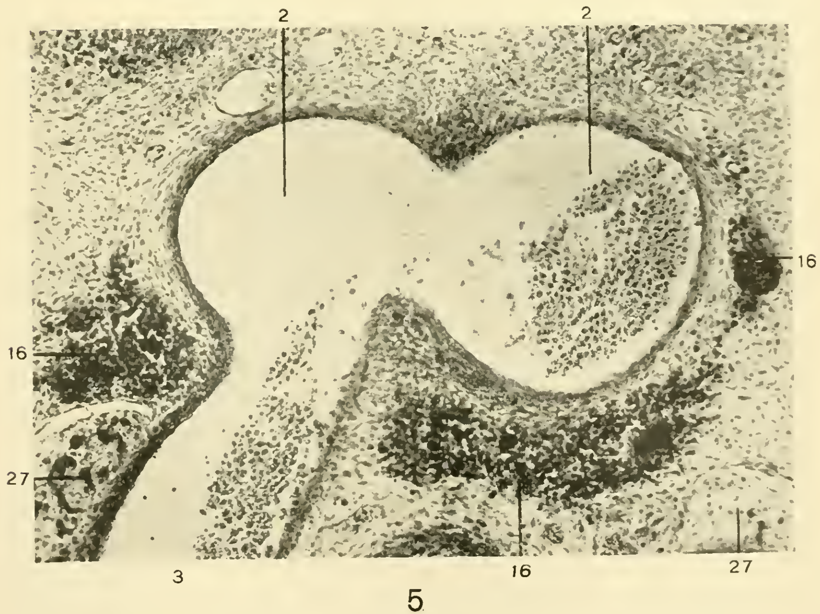

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PLATE 3

EXPLANATION OF FIGURES

6 From a section of a chick embryo of 7 days (series no. 512; slide III, section 15). Photomicrograph, × 80.

2, dorsal aortic root
3a, pulmonary artery
10, precardinal vein
16, groups of developing blood cells along precardinal vein
16a, groups of developing blood cells dorsal to esophagus
19, lymphatics dorsal to aortic arches and esophagus
27, vagus nerve
esophagus in center of figure
bronchi at lower border of figure

7 From a section of a chick embryo of 6 days and 16 hours, 13.5 mm (series no. 426; slide XI, section 21). Photomicrograph, × 133.

1, aorta
5, celiac artery
8, dorsal somatic artery
16, groups of developing blood cells
23, celom (pleural cavity)
25, mesonephros
26, lung anlage

6

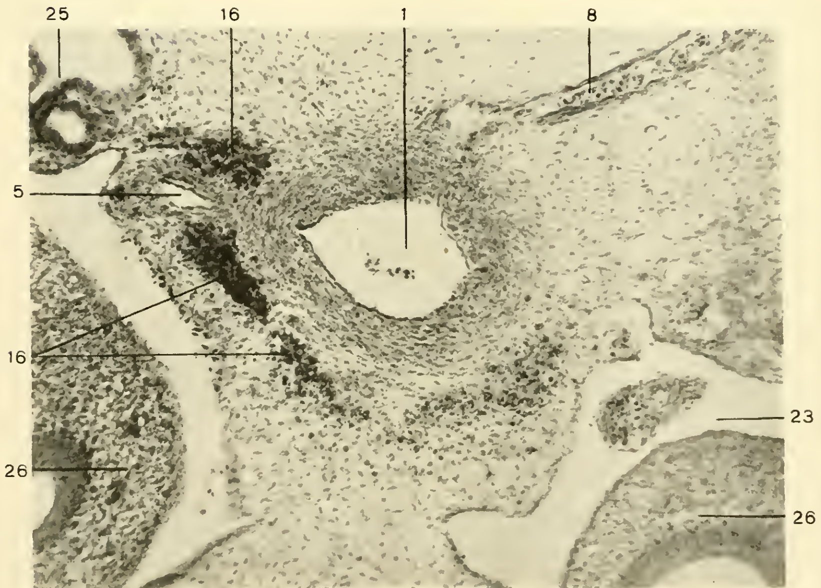

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PLATE 4

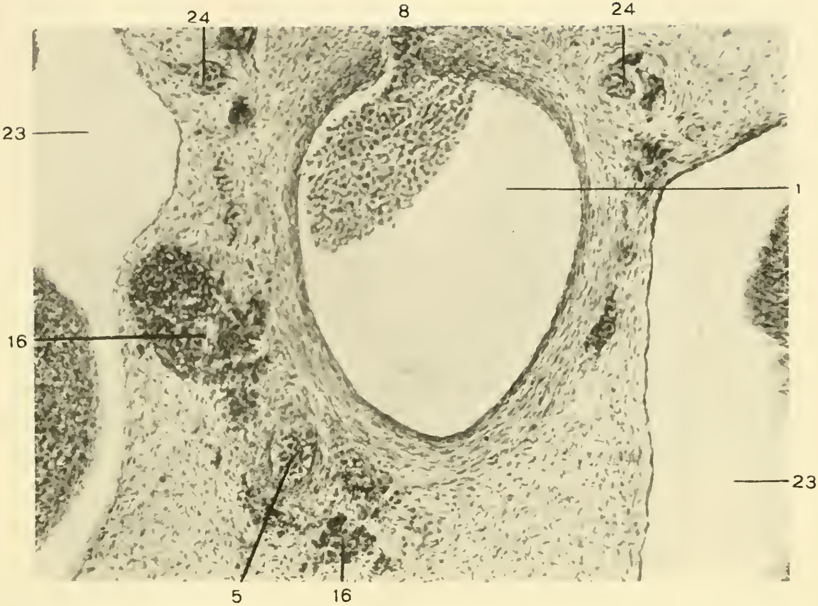
EXPLANATION OF FIGURES

8 From a section of a chick embryo of 6 days and 22 hours, 16.5 mm. (series no. 464; slide XXII, section 22). Photomicrograph, × 133.

1, aorta
5, celiac artery
8, dorsal somatic artery
16, groups of developing blood cells
23, celom (pleural cavity)
24, sympathetic nerves

9 From a section of a chick embryo of 6 days and 16 hours, 13.5 mm. (series no. 426; slide XVIII, section 3). Photomicrograph, × 233.

1, aorta
16, developing blood cells
17, lymph spaces, rudiments of thoracic duct
23, celom (abdominal cavity)



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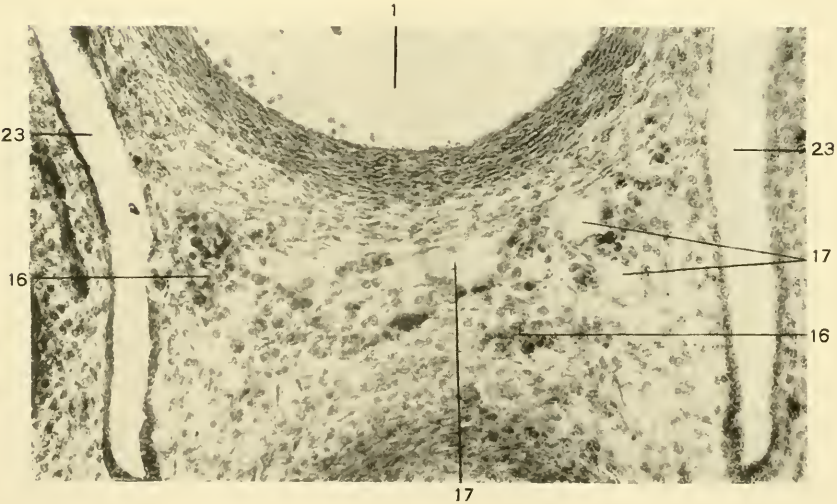

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PLATE 5

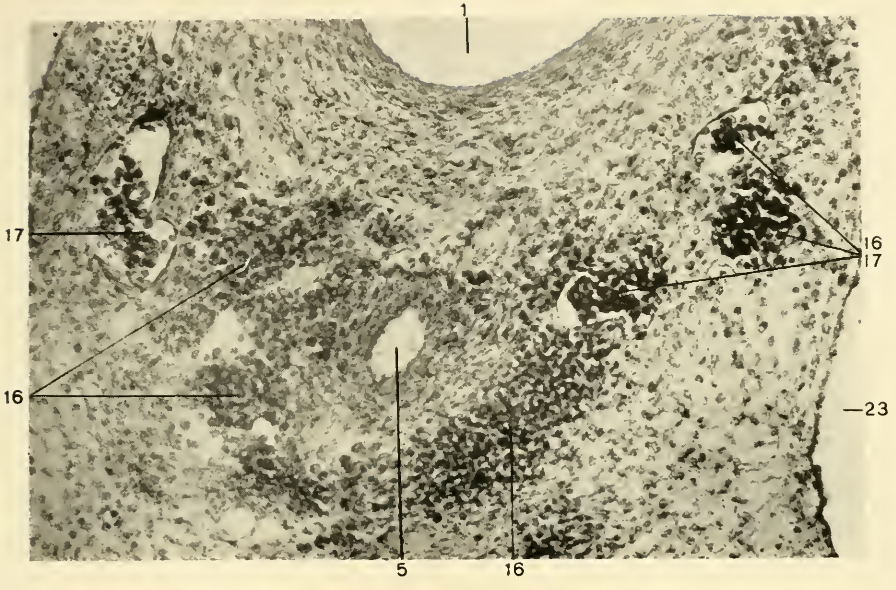
EXPLANATION OF FIGURES

10 From a section of a chick embryo of 7 days (series no. 512; slide VII, section 1). Photomicrograph, × 233.

1, aorta
5, celiac artery
16, groups of developing blood cells
17, lymphatics, rudiments of thoracic duct
23, celom (abdominal cavity)

11 From a section of a chick embryo of 14 days (series no. 518; slide XIII, section 9). Photomicrograph, × 133.

1, aorta
16, blood cells, mostly mature erythrocytes, in tissue spaces
17, lymphatics (part of thoracic duct system)



10

17
16
1
17

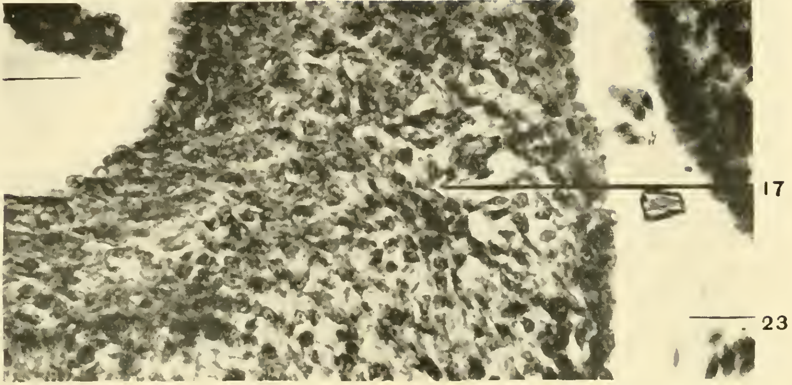
11

PLATE 6

EXPLANATION OF FIGURES

12, 13, 14 From three successive sections of a chick embryo of 6 days and 16 hours, 13.5 mm. (series no. 426; slide XII, sections 9, 10 and 11). Photomicrographs, × 350.

In figure 13 the arrow (*17*) points to a lymph space in the mesenchyme. At its right side the lymph space is seen clearly to open into the adjacent mesenchymal intercellular spaces. The preceding section in the series (fig. 12) shows no space (the arrow points to the same locality as in fig. 13). In figure 14 the arrow points to a light area in the mesenchyme which represents the opening of the space of figure 13 into the adjacent mesenchymal intercellular spaces; this can be seen much more clearly with the microscope by changing the focus. The lymph space shown in figure 13 is the same one represented in figure 20, *17* at the right of the aorta. Aorta, *1*; celom (abdominal cavity), *23*.



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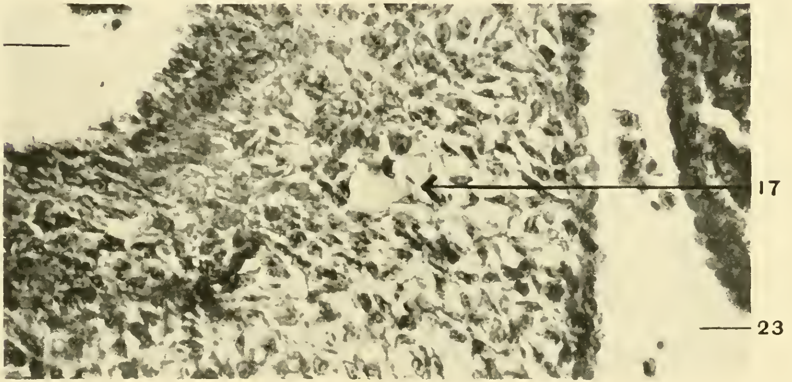

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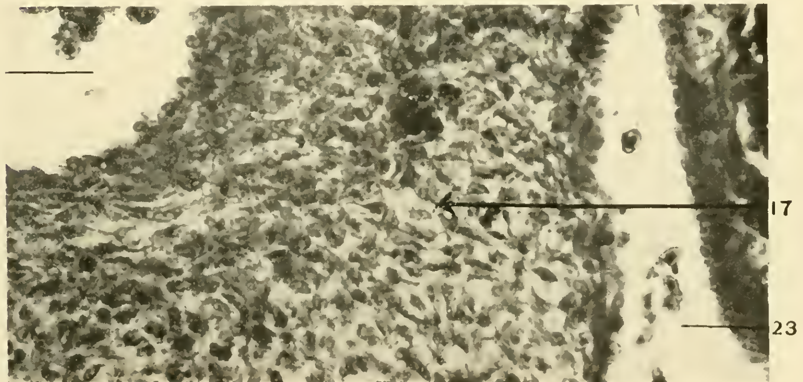

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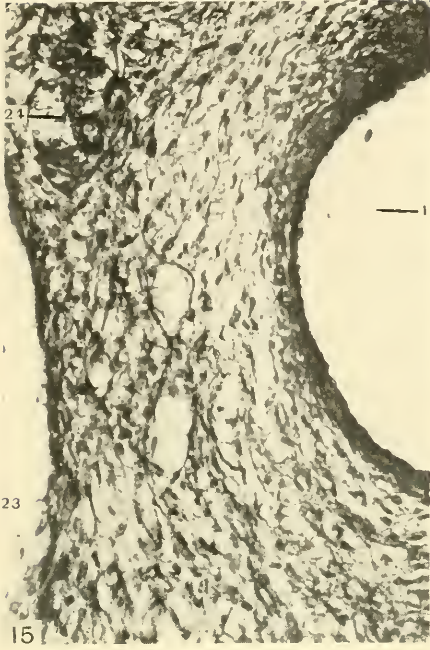
PLATE 7

EXPLANATION OF FIGURES

15, 16, 17 and 18 From four successive sections of a chick embryo of 7 days (series no. 512; slide VII, sections 21 and 22; slide VIII, sections 1 and 2). Photomicrographs, × 250.

In figure 15 four lymph spaces, in part lined with endothelium, are seen at the left of the aorta (*1*). The one nearest the aorta, while in part lined with endothelium, opens freely into the adjacent mesenchymal intercellular spaces. The largest space also opens below in a similar manner. In figure 16, from the succeeding section in this series, only one of the spaces appears, opening above into tissue spaces. The others have simply become continuous with tissue spaces and therefore do not appear as distinct lacunae; this can be clearly demonstrated with the microscope by changing the focus on the rather thick sections (20 micra). In figure 17 the arrow points to the termination of the distinct lacuna of figure 16; the free communication with the tissue spaces is quite obvious. In figure 18 there are no lacunae, all those of the preceding sections having opened into the mesenchymal intercellular spaces. Aorta, *1*; part of sympathetic nervous system, *24*.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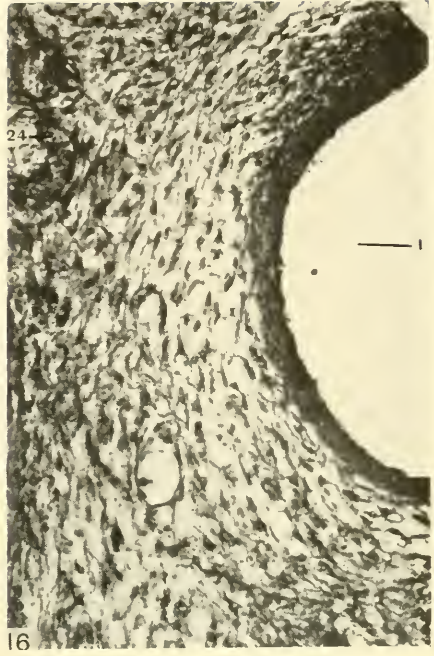
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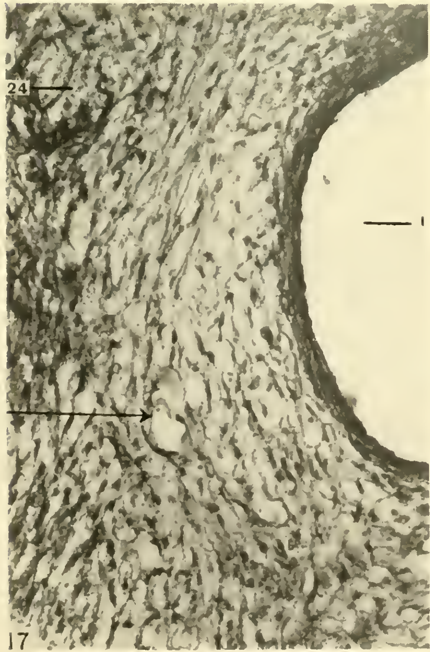
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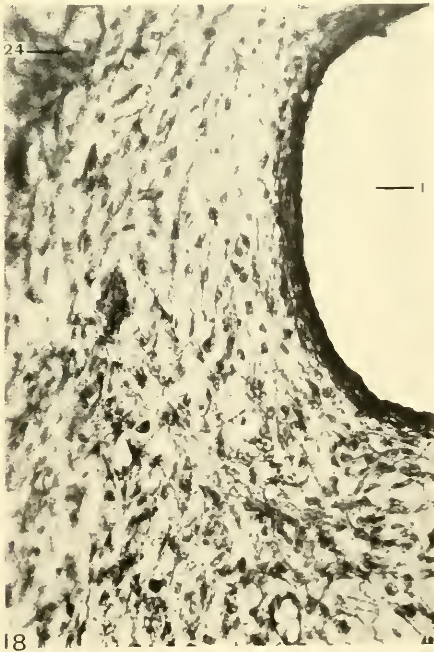
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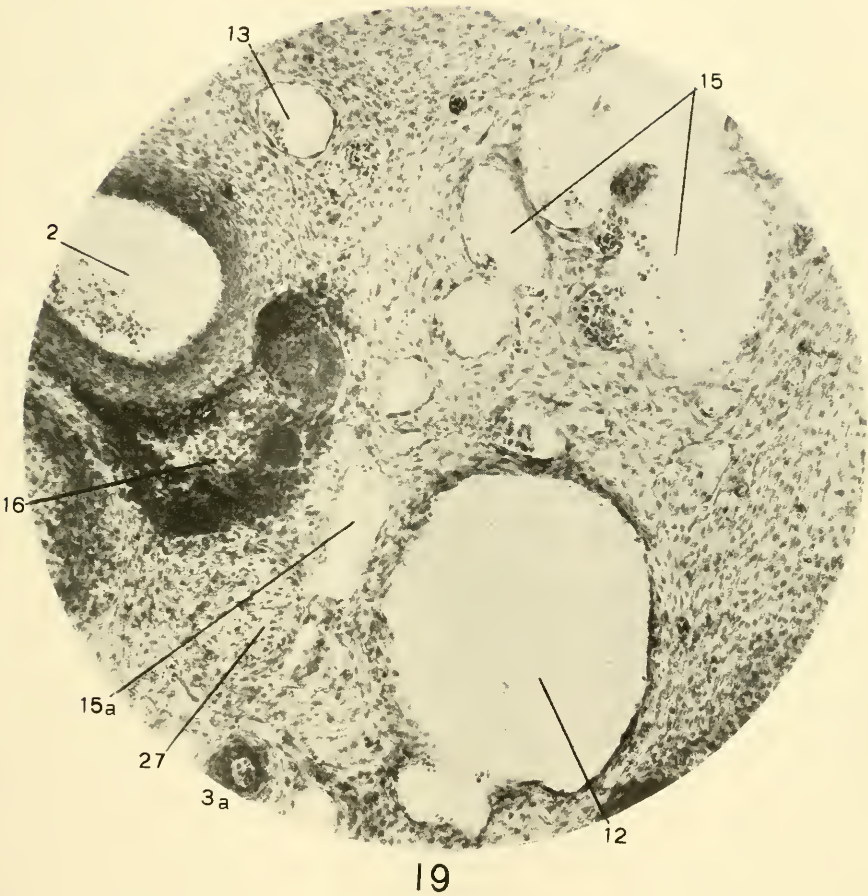
18

PLATE 8

EXPLANATION OF FIGURE

19 From a section of a chick embryo of 6 days and 22 hours, 16 mm. (series no. 463; slide XVI, section 9). Photomicrograph, × 160.

2, dorsal aortic root
3a, pulmonary artery
12, precaval vein
13, vertebral vein
15, jugular lymph sac
15a, thoracic duct 'approach'
27, vagus nerve



19

PLATE 9

EXPLANATION OF FIGURE

20 Drawn from a reconstruction of a chick embryo of 6 days and 16 hours, 13.5 mm. (series no. 426). Ventral view.

1, aorta
2, dorsal aortic roots
3, aortic arch VI
3a, pulmonary artery
4, aortic arch IV
5, celiac artery
10, precardinal vein
11, subclavian vein
12, duct of Cuvier
13, vertebral vein
15, jugular lymph sac
16, groups of developing blood cells
17, first rudiments of thoracic duct

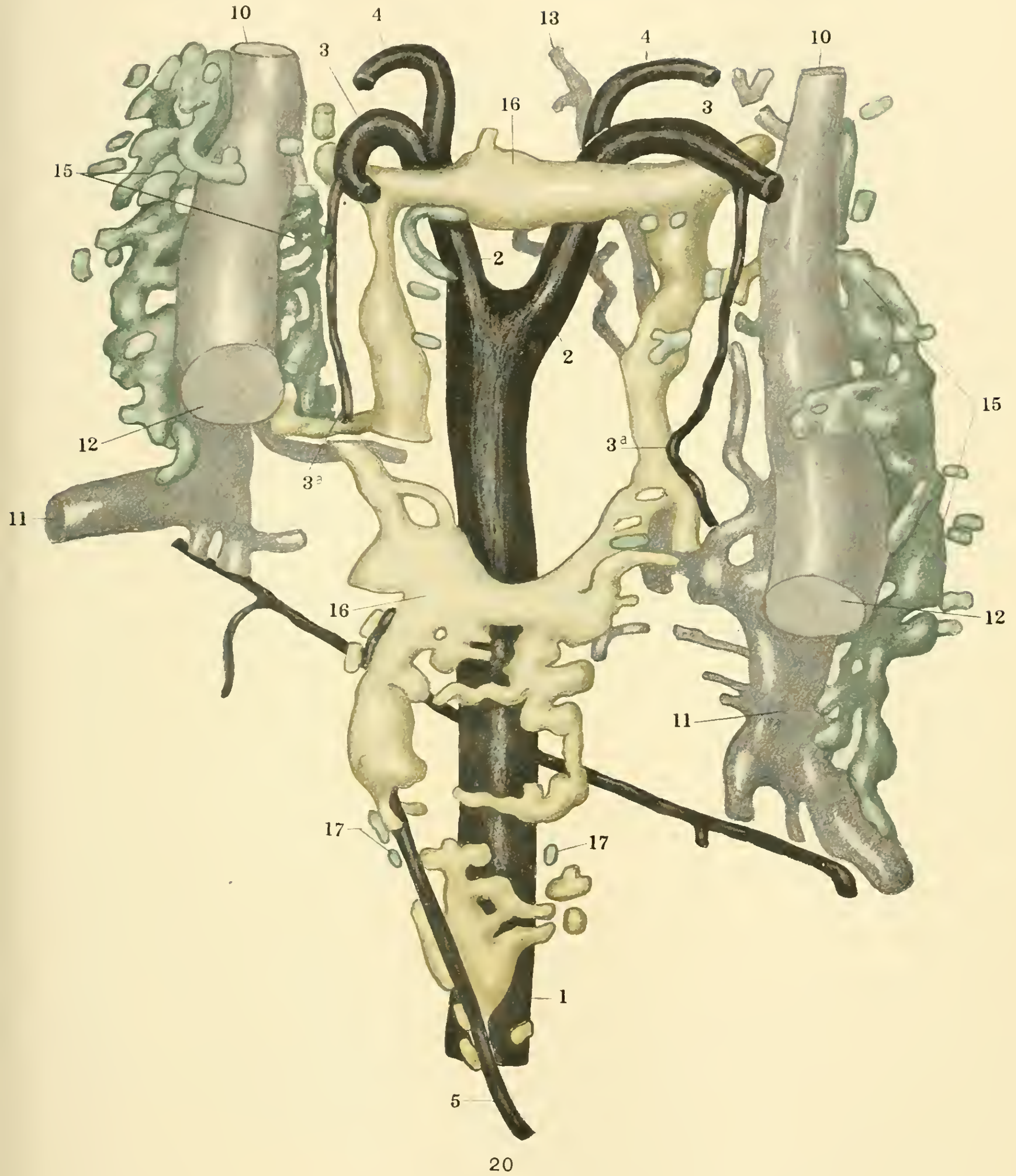


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PLATE 10

EXPLANATION OF FIGURE

21 Drawn from a reconstruction of a chick embryo of 6 days and 21 hours, 14 mm. (series no. 465). Ventro-mesial view.

1, aorta
2, dorsal aortic roots
3, aortic arch VI
3a, pulmonary artery
5, celiac artery
6, notocord
10, precardinal vein
11, subclavian vein
12, duct of Cuvier
13, vertebral vein
15, jugular lymph sac
15a, thoracic duct 'approach' of jugular lymph sac
16, groups of developing blood cells
17, rudiments of thoracic duct

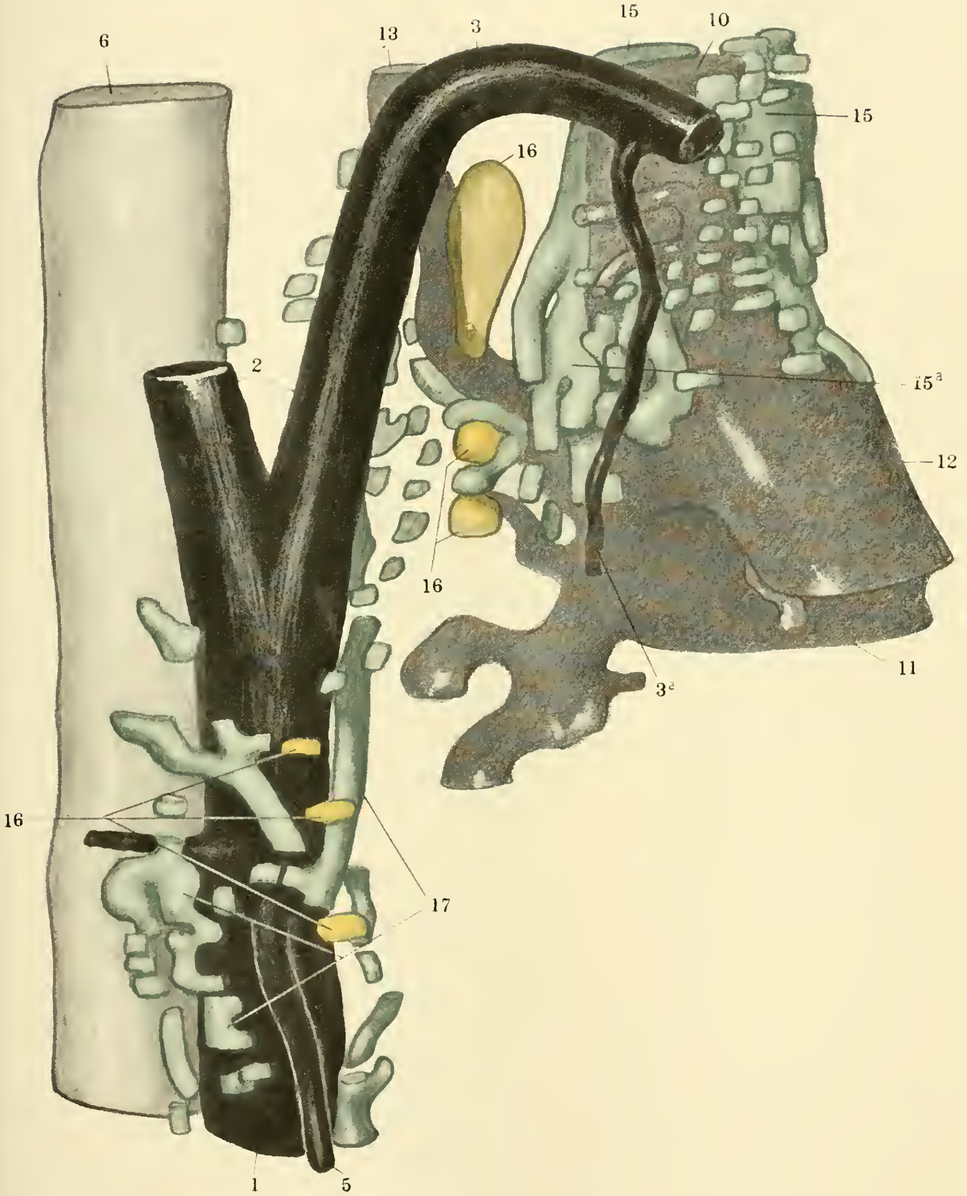

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PLATE 11

EXPLANATION OF FIGURE

22 Drawn from a reconstruction of a chick embryo of 6 days and 22 hours, 16 mm. (series no. 463). Ventral view.

1, aorta
2, dorsal aortic root
3, aortic arch VI
3a, pulmonary artery
4, aortic arch IV
5, celiac artery
10, precardinal vein
11, subclavian vein
12, duct of Cuvier
13, vertebral vein
15, jugular lymph sac
15a, thoracic duct 'approach' of jugular lymph sac
16, groups of developing blood cells
16a, groups of blood cells dorsal to aortic roots and arches
17, rudiments of thoracic duct
18, lymphatics along aortic arches
19, lymphatics dorsal to aortic roots and arches
21, mesenteric lymphatics

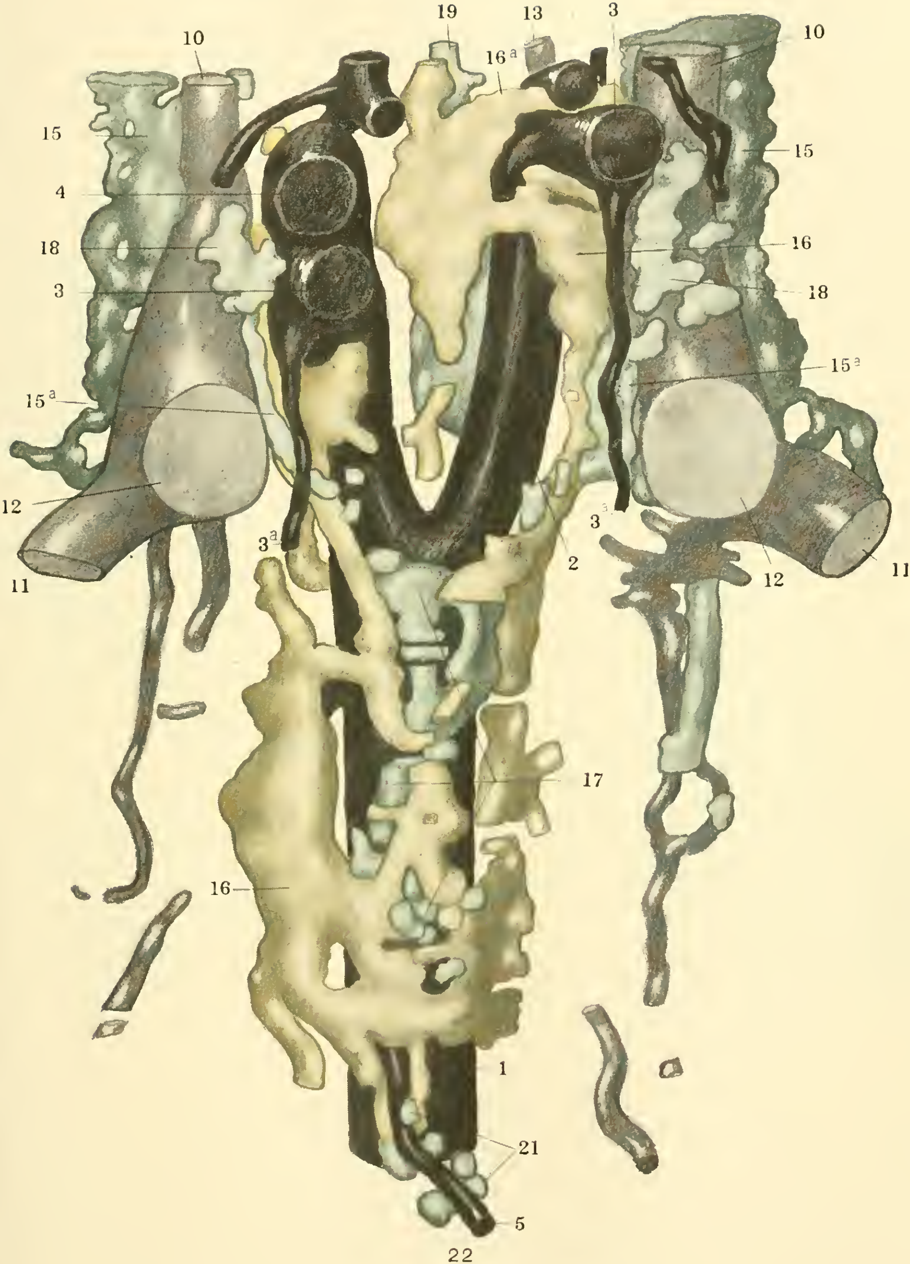

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PLATE 12

EXPLANATION OF FIGURE

23 Drawn from a reconstruction of a chick embryo of 6 days and 22 hours, 16 mm. (series 463). Mesial view.

10, precardinal vein
11, subclavian vein
12, duct of Cuvier
15, jugular lymph sac
15a, thoracic duct 'approach' of jugular lymph sac
16, groups of developing blood cells
17, rudiments of thoracic duct, extreme cephalic end

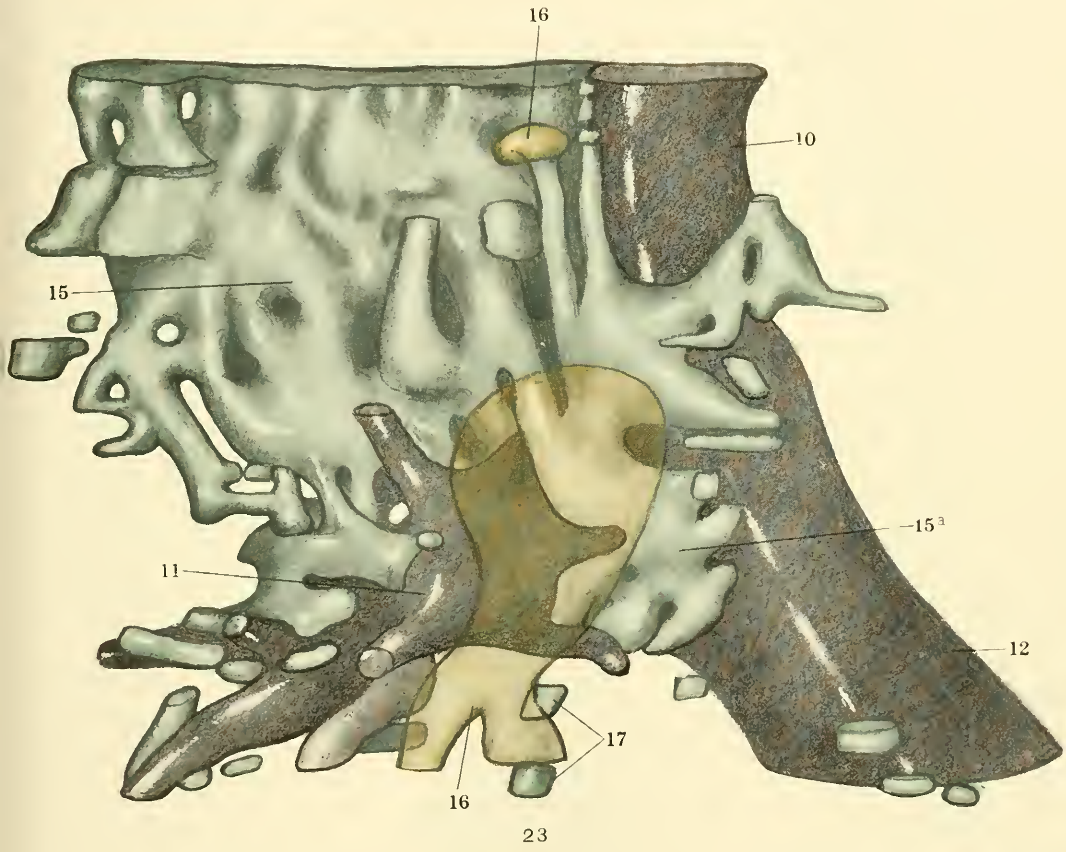

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PLATE 13

EXPLANATION OF FIGURE

24 Drawn from a reconstruction of a chick embryo of 7 days (series no. 512). Ventral view.

1, aorta
2, dorsal aortic roots
3, aortic arch VI
3a, pulmonary artery
5, celiac artery
7, carotid artery
10, precardinal vein
11, subclavian vein
12, duct of Cuvier
14, part of splanchnic plexus of veins
15, jugular lymph sac
15a, thoracic duct 'approach' of jugular lymph sac
16, groups of developing blood cells
16a, blood cells dorsal to aortic roots and arches
17, thoracic duct, ventral plexus (homologue of azygos segment)
17a, thoracic duct (homologue of preazygos segment)
18, lymphatics along aortic arches
19, lymphatics dorsal to aortic roots and arches
21, mesenteric lymphatics

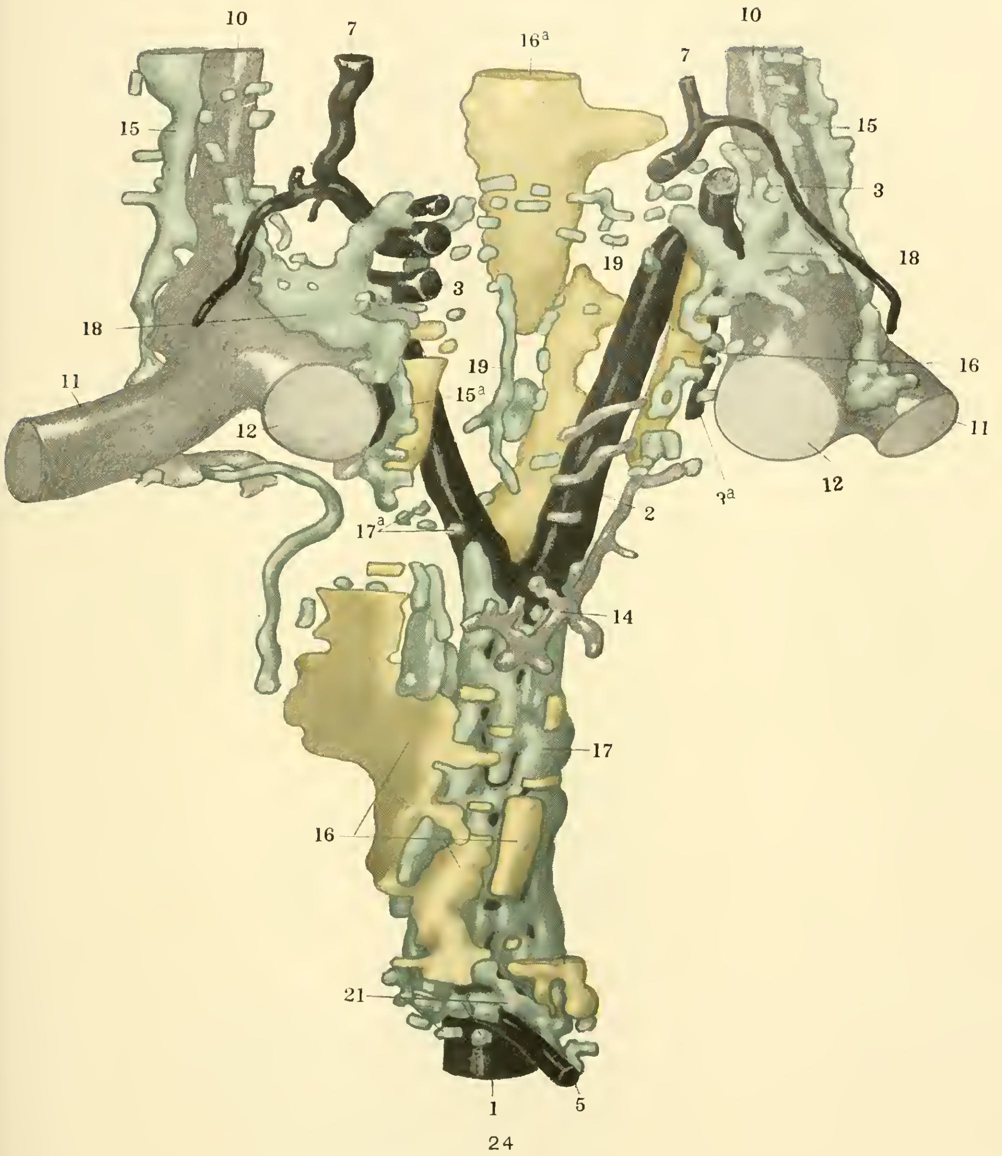

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PLATE 14

EXPLANATION OF FIGURE

25 From same reconstruction as in fig. 24. View from left side.

1, aorta
3, aortic arch VI
5, celiac artery
7, carotid artery
8, dorsal somatic arteries
10, precardinal vein
11, subclavian vein
12, duct of Cuvier
13, vertebral vein
14, part of splanchnic plexus of veins
15, jugular lymph sac
16, groups of developing blood cells
16a, blood cells dorsal to aortic roots
17, thoracic duct, ventral plexus
18, lymphatics along aortic arches
20, lymphatics dorso-lateral to aorta
21, mesenteric lymphatics

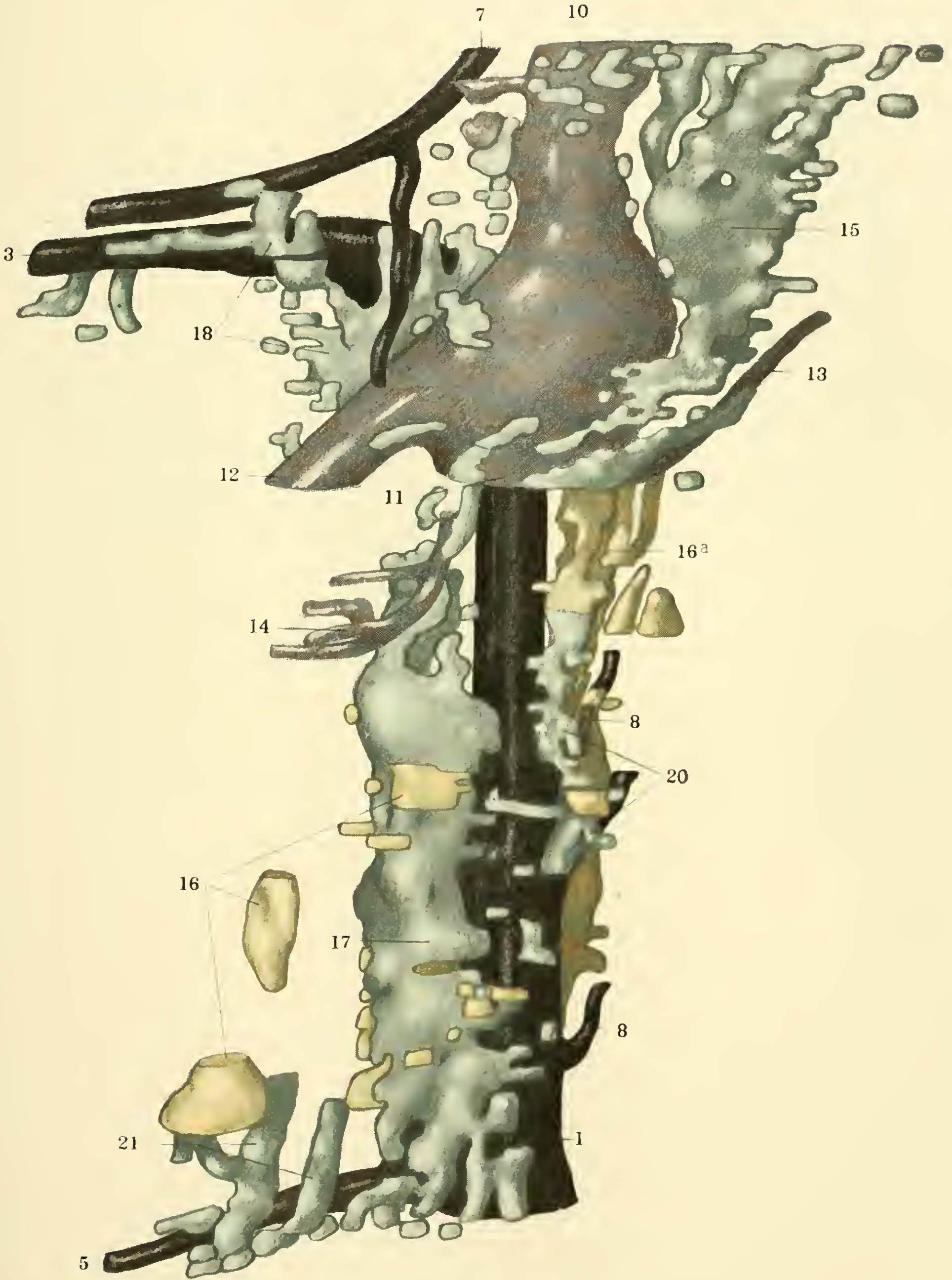

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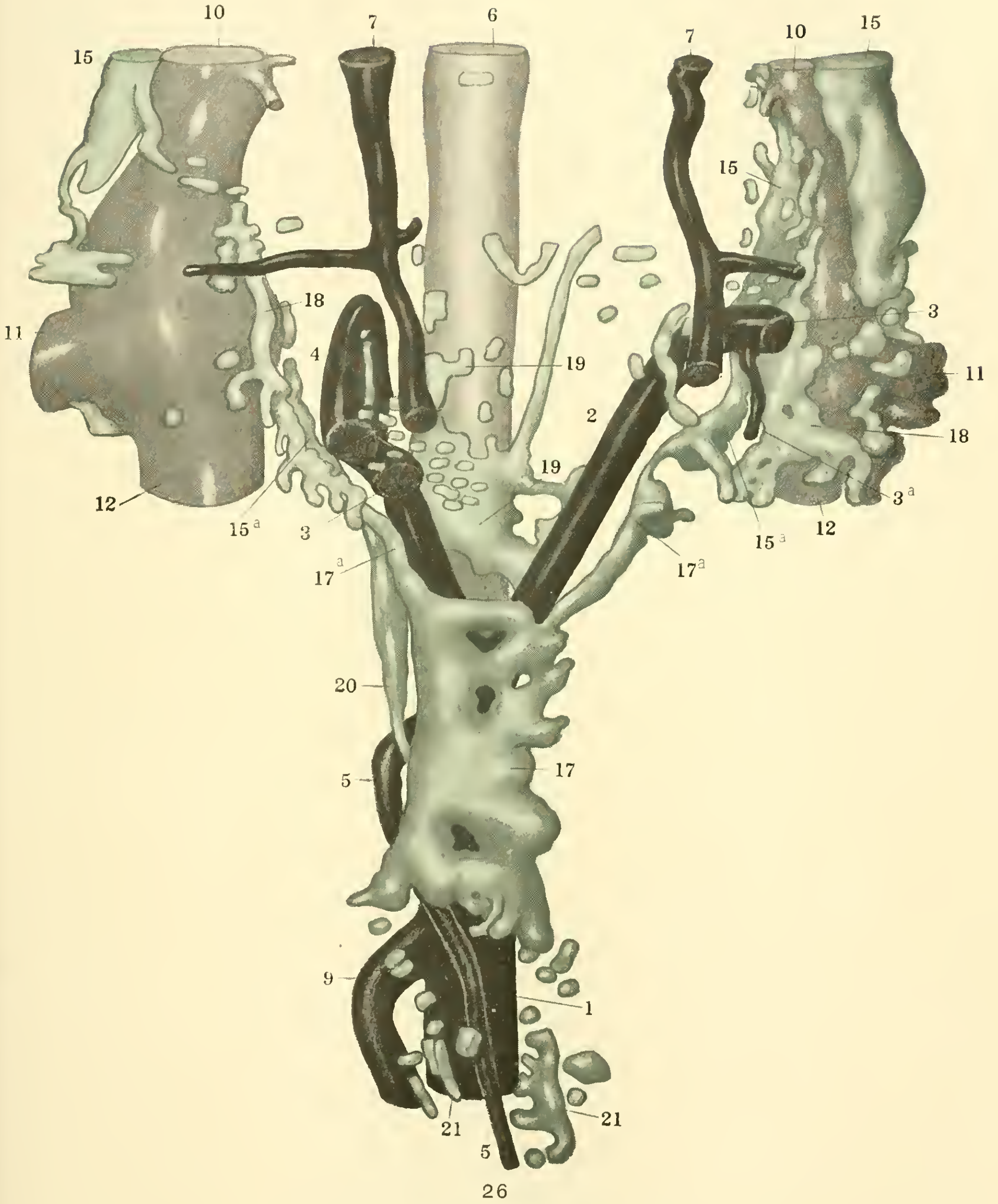
PLATE 15

EXPLANATION OF FIGURE

26 Drawn from a reconstruction of a chick embryo of 8 days (series no. 513). Ventral view.

1, aorta
2, dorsal aortic roots
3, aortic arch VI
3a, pulmonary artery
4, aortic arch IV
5, celiac artery
6, notocord
7, carotid artery
9, superior mesenteric artery
10, precardinal (jugular) vein
11, subclavian vein
12, duct of Cuvier
15, jugular lymph sac
15a, thoracic duct 'approach' of jugular lymph sac
17, thoracic duct, ventral plexus (homologue of azygos segment
17a, thoracic duct (homologue of preazygos segment)
18, lymphatics along aortic arches and duct of Cuvier
19, lymphatics dorsal to aortic roots and arches
20, lymphatics dorso-lateral to aorta
21, mesenteric lymphatics



26

PLATE 16

EXPLANATION OF FIGURE

27 Drawn from a reconstruction of a chick embryo of 9 days and 14 hours (series no. 320). Ventral view.

1, aorta
2, dorsal aortic roots
3, aortic arch VI
3a, pulmonary artery
4, aortic arch IV
5, celiac artery
7, carotid artery
9, superior mesenteric artery
10, p ecardinal (jugular) vein
11, subclavian vein
12, duct of Cuvier
15, jugular lymph sac
15a, thoracic duct 'approach' of jugular lymph sac
16, groups of developing blood cells
17, thoracic duct, ventral plexus (homologue of azygos segment.
17a, thoracic duct (homologue of pre-azygos segment.
18, lymphatics along aortic arches
19, lymphatics dorsal to aortic roots and arches
21, mesenteric lymphatics
22, lymphatics associated with esophagus

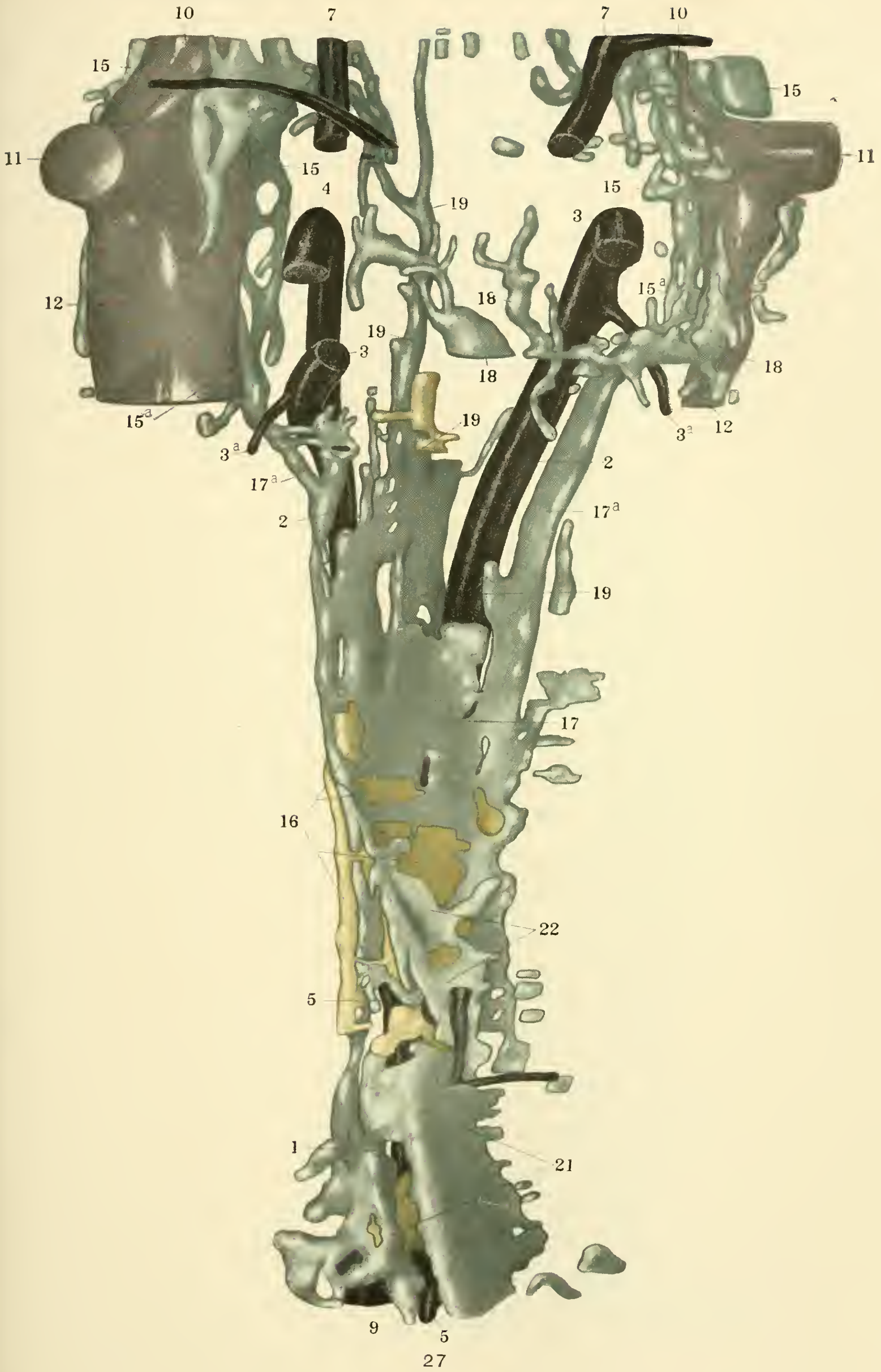


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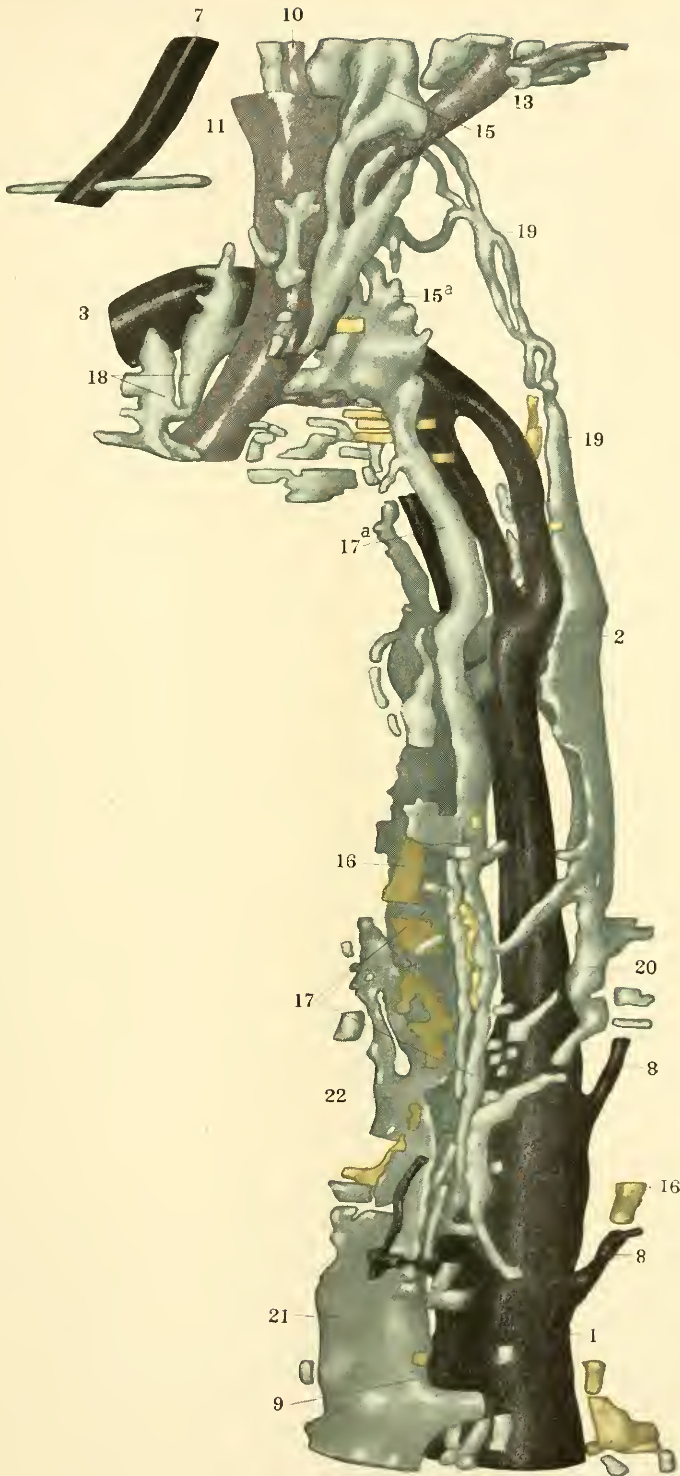
PLATE 17

EXPLANATION OF FIGURE

28 From same reconstruction as figure 27. View from left side.

1, aorta
2, dorsal aortic roots
3, aortic arch VI
7, carotid artery
8, dorsal somatic arteries
9, superior mesenteric artery
10, precardinal (jugular) vein
11, subclavian vein
12, duct of Cuvier
13, vertebral vein
15, jugular lymph sac
15a, thoracic duct 'approach' of jugular lymph sac
16, groups of developing blood cells
17, thoracic duct
18, lympathics along aortic arches
19, lymphatics dorsal to aortic roots and arches
20, lymphatics dorso-lateral to aorta
21, mesenteric lymphatics
22, lymphatics associated with esophagus



28

ON THE PRENATAL AND NEONATAL LUNG

WILLIAM H. F. ADDISON AND HAROLD W. HOW
The Anatomical Laboratory of the University of Pennsylvania

EIGHT FIGURES

INTRODUCTION

Of the various changes occurring in the lung at birth, we have studied especially the increase in size of the spaces within the respiratory lobules, and the change in the character of the contents of these spaces. Whenever this portion of the lung's history is considered, the statement is made that, with the first pulmonary inspirations after birth, the spaces become distended, but we have found no data expressing the amount of this distention. As to the question of the existence of fluid contents in the spaces of the prenatal lung, it is seldom mentioned in current literature, although it would seem to be of importance, especially in connection with the beginning of breathing.

In making comparisons, we have used material from experimentally controlled animals, in order to obtain specimens of prenatal and neonatal lungs, which would be comparable in all respects. This was done by using animals of the same litter, of which some had breathed, and others had not breathed.

LITERATURE

Measurements made on the minute parts of the lung have been recorded by a comparatively small number of investigators. Friedrich Merkel, in Bardeleben's Handbuch ('02), reproduces from Rossignol ('46) a table of sizes of lung alveoli at different ages. The first item in the list gives the mean size of alveoli of newborn children which have not breathed, or have breathed

only a few hours. It is exactly between these two classes of individuals that we have sought to study the differences. Kölliker made numerous lung measurements but apparently none directly contrasting the two conditions seen in the prenatal and postnatal lung. Miller's ('95) table of sizes of the various air-passages in dogs is from material of one stage only. Concerning the phenomenon of expansion from a more general point of view, there are many observations. For instance, the fact that all parts of the neonatal lung do not expand equally has been recorded a number of times. Dohrn ('91) says that at least two days are required to unfold the lungs completely. Jalan de la Croix ('83), found in the lung of a child which lived seven days groups of alveoli which persisted airless. In dealing with the lung of children which have survived only a few days after birth, there is the possibility, however, of the organ being premature or pathological, and either of these conditions would affect its expansion. The question of the contents of the spaces in the fetal lung has for the most part been referred to only incidentally, by observers interested in premature respiratory movements. The experiments recorded by Preyer ('85) in his "Specielle physiologie des embryo," are the most definite we have found, and these will be referred to later.

A complicating factor in studying the appearance of the normal fetal lung is the possible occurrence of intrauterine respiratory movements. These have been described in the study of living human fetuses by a whole series of clinical observers (for instance, Ferroni '99) and have even been recorded by means of tracings. The current opinion as expressed by Howell ('11) is that the mammalian fetus under normal conditions makes no respiratory movements while in utero. Ballantyne ('02) in his "Antenatal pathology and hygiene" accepts the results of the clinicians, but says it is doubtful if such movements are strong enough to draw liquor amnii into the lungs. It would also seem from our experiments that if these movements are really similar to respiratory movements, they are not to be compared in point of intensity with the postnatal efforts, and do not affect the structure of the lung to any extent.

METHOD OF PRESERVATION OF MATERIAL

In order to preserve, as nearly as possible, the normal relations of the lung to surrounding structures, and to prevent its collapse, all animals were injected with 5 or 10 per cent formalin, either through one of the umbilical vessels, or through the aorta, without opening the thoracic cavity. After the lungs had hardened in situ, portions were removed, embedded in paraffin or celloidin and sectioned.

CONTENTS OF THE SPACES WITHIN THE FETAL LUNG

When a living fetus, near full-term, is exposed within the uterus, with the amniotic sac still unruptured, it can readily be stimulated to make respiratory movements. The angles of the mouth begin to twitch, and are drawn slightly upwards, the abdomen enlarges, evidently due to the descent of the diaphragm, and almost simultaneously the nostrils dilate, and the mouth slightly opens in a yawning manner. The result is the drawing into the respiratory tract of the amniotic fluid. We found that the mere manipulation necessary to expose the fetus is sufficient to bring about these movements, if the animal is very near the end of gestation. A surer method is to clamp the umbilical cord, but neither method will act if the animal is not sufficiently advanced in its development. Several have previously recorded the above or similar observations. Winslow is quoted by Preyer as having written in 1787, "Liquorem amnii respirare videntur." Leclard ('15) clamped the neck of a still living fetus, and on opening the trachea found there a fluid analogous to amniotic fluid. When a colored fluid had been injected previously into the amniotic fluid that in the bronchi was likewise colored. Preyer ('85, p. 148) repeated and verified Leclard's experiment with a guinea-pig near the end of gestation. He found that the fuchsin which he injected into the amniotic sac not only colored the lips, tongue, palate and all the pharynx, but also the lungs and the inside of the stomach. Geyl ('80) added to the experiment in the following manner. With all aseptic precautions, he injected aniline blue into the amniotic sacs of seven fetuses of a

rabbit, which was nearly three weeks pregnant. Three days later the seven young were born, three dead and four alive. The three former had their lungs colored blue, as had also one of the latter. While there is no doubt that liquid is present within the lungs after these inspirations of amniotic fluid, none of the observers have directed their attention especially towards seeing the contents before such inspirations had taken place. One can easily deduce that liquid is present all the time during the development of the fetal lung, but in order to obtain, if possible, direct evidence several simple experiments were performed. The first series was with large sheep fetuses, obtained from an abattoir, with membranes and uterus intact. In two cases the following procedure was followed. The uterus was opened, and the fetus, 35 cm. in length, exposed within the unruptured amniotic sac. By means of a needle carefully passed through the amnion, a strong ligature was drawn through the tissues of the neck of the fetus, behind the trachea, and out of the amnion again at the point of entrance. With the head of the fetus covered by the amniotic fluid, the ligature was tied tightly and the trachea constricted. Our special aims were to prevent liquid escaping from the amniotic sac and air from entering it. The trachea and lungs were carefully dissected out, without injuring the visceral pleura, and placed in a large jar of water, from which all air-bubbles had been previously removed. After they had been allowed to sink to the bottom of the jar they were agitated in order to remove adherent air. Different parts of the lung were then cut with scissors and crushed. A yellowish-red fluid escaped from the crushed masses of tissue and diffused through the water but no bubbles were seen to escape.

In a third sheep fetus the trachea was ligated at its upper and lower ends and then dissected out. The closed segment of trachea was carefully cleaned and dried, before being opened over a dry glass plate. It was found to contain a faintly yellowish slightly viscid fluid, which was pressed out on the plate. When this fluid was tested with acetic acid there was a reaction showing the presence of mucin. The bronchi contained a similar fluid, and when the lungs were compressed this fluid was forced out.

In order to secure histological material for a study of the normal appearance of these lungs, another fetus of approximately the same size was injected through the umbilical vein with 10 per cent formalin. Later the thorax was opened and after the relations of the lung were observed, pieces of the lung were taken for histological sections. The appearance as evidenced by characters to be described later, was that of a lung which had not made premature respiratory movements.

The second series of experiments were repetitions of the foregoing, performed on a litter of living dog fetuses lying within the uterine horns of the mother. The parent animal was anesthetized and the young carefully exposed one at a time without unnecessary manipulation. In order to prevent the entrance of amniotic fluid, due to premature respiratory movements, the trachea of each fetus used was clamped with an artery forceps as soon as it was exposed and before the amnion was ruptured. As a result of this stimulation the animal at once began to make violent efforts to breathe, but if the trachea was well clamped these were ineffectual and the animal soon died of asphyxiation. The lungs were removed as described for the sheep fetuses, and experiments carried out in a parallel way. The results were similar to those of the first series, so it may be concluded that normally in the fetal trachea, bronchi and lungs there exists a liquid which resembles the amniotic fluid in appearance. The question arises as to what becomes of this liquid when breathing begins. From observations on fetuses removed from the uterus, the first act in breathing is always inspiration. As the lung enlarges, due to the contraction of the diaphragm and other respiratory muscles part of the liquid in the upper parts of the tract is drawn downwards and part remains distributed along the walls of the air-passages. When the fetus inspires amniotic fluid before its removal from the sac, it is always much handicapped. It does not breathe so strongly, and its efforts become weaker, and farther apart until they cease altogether. If in these circumstances, as we tried with young dogs, the animal be held with head downwards, and the thorax compressed at short intervals, the difficulty in breathing is often relieved, and the animal im-

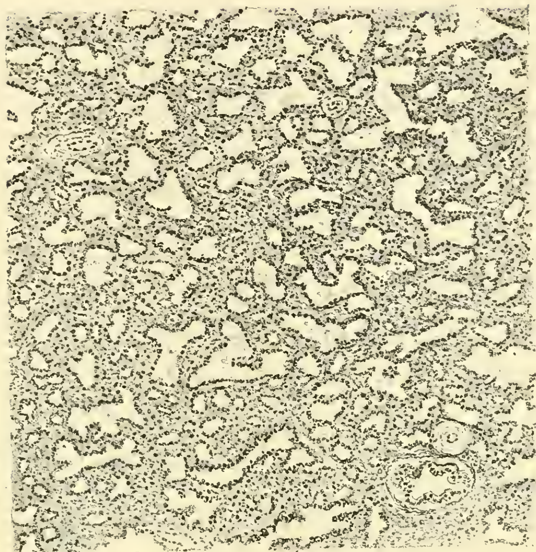
proves and lives. As is known from both clinical medicine and experimental studies, the lung has a marked capacity for the absorption of liquids, and the quantity of fluid present in normal birth is readily disposed of.

MATERIAL FOR BREATHING AND NON-BREATHING LUNG

In order to secure examples of the breathing and non-breathing lung which would be strictly comparable, we used pregnant dogs near the end of the gestation period. By the manner described in experiment on the contents of the prenatal lung, some of the fetuses were removed and not allowed to breathe. Others were removed from the membranes as quickly as possible, before any attempt at breathing had been made. After the umbilical cord was tied and severed, they were laid in a warm place. They soon showed signs of activity, crying and crawling about. These were etherized at the end of an hour, and injected with formalin. Fetal material was also obtained by etherizing the parent animal after several fetuses had been taken out and allowing the remainder of the young to die before removing them. For comparison with the new-born, pups two days old were used, and these were injected with formalin through the abdominal aorta.

STUDY OF PRENATAL LUNG

The appearance of sections from the fetal lung obtained in the above described manner is shown in figures 1 and 2. The texture of the organ is quite gland-like, with the mesenchyme framework present in large amount. The spaces are variable in size and shape on account of the structures being cut in different planes of section, but the distribution of the spaces is fairly uniform. In order to study the relative area occupied by lung tissue and the intervening open spaces, drawings were made with the Edinger drawing apparatus, on cross-ruled millimeter paper. Various magnifications were used, varying from 60 to 220. The results of a series of such drawings showed that the percentage of area occupied by lung tissue was 70 to 80 per cent and by the intervening spaces 20 to 30 per cent. In view of this condition it does not seem correct to call the fetal lung 'solid,' as do some



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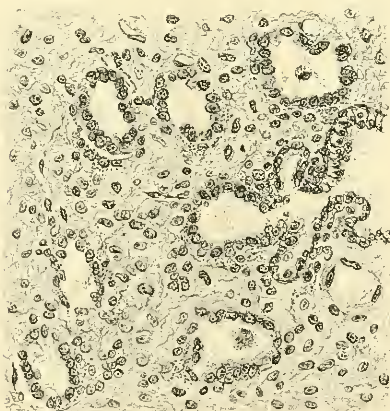


2

Fig. 1 Fetal lung of dog, at end of gestation. × 62.

Fig. 2 Fetal lung of dog, at end of gestation, with the granular reticular substance in the spaces, and two of the free mononuclear cells. × 280.

of the present-day text-books of physiology and pathology. On the other hand, it is very easy to increase the size of the spaces artificially. In J. M. Flint's paper on the development of the lungs ('06), he has an illustration of the lung of a fetal pig 27 cm. long, which is a stage shortly before birth. For the purpose of preservation the lung was injected intra-tracheally with the fixing fluid and this fact is responsible for the appearance of the lung in section. The appearance resembles very closely that of a lung which has breathed, as one can see by comparing it with his next figure, no. 29, that of a two-day-old pig. This is because the spaces were distended by the injection of the fixing fluid and indeed, as we found with sheep fetuses, relatively little force is required to distend the fetal lungs when introducing fluid through the trachea. In consequence of this distention the lining cells of the respiratory lobules are artificially stretched and flattened and no longer show the normal condition.

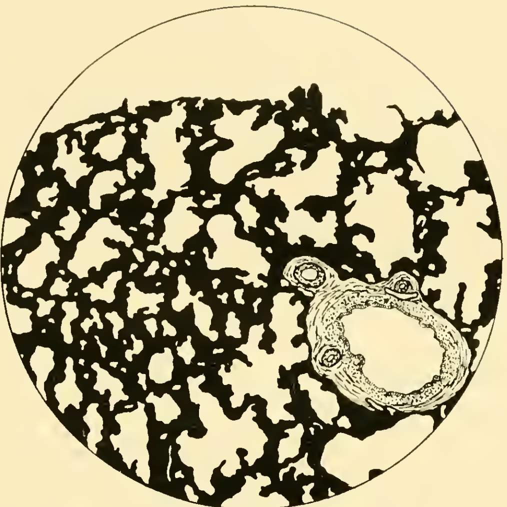
When the spaces are examined they are seen not to be entirely empty, but to contain here and there light pink-staining irregular masses of a finely granular substance. This is apparently a precipitate derived from the liquid existing within the spaces, and was clearly seen at the period just before birth in all well-preserved fetal lungs not only of dog, but also of cat, rat and man. The origin of the mucin constituent of the fluid is at least partly from the goblet cells of the trachea, for in sections of the latter we found them numerous and characteristically stained. Mention may also be made of conspicuous large rounded cells lying free within the spaces. They are distributed rather evenly but not in large numbers, and usually occur singly. They measure 11 to 14 μ in diameter, and have a single nucleus which is eccentrically placed. The nucleus may be round, oval or indented, and varies from 6 to 7 μ in its greatest dimension. If flattened in shape, the width is 3 to 4 μ. These cells in the dog have a distinctly granular cytoplasm, which stains well with eosin. They probably belong to the same variety of cells as those present in the air-spaces of the normal breathing lung which take part in removing carbon particles and other foreign matter from the alveoli. In the fetal lung they may have the similar function of

removing cellular débris, from the liquid-filled spaces. They also resemble the 'heart-failure cells' of the lung which are seen in certain pathological conditions. Several views are held as to the nature of these phagocytic cells in the breathing lung, but the study of these very similar appearing cells in the fetal lung, leads one to agree with Kölliker ('02 vol. 3, p. 310), that they are a form of 'Wanderzellen,' and not desquamated epithelium.

NEONATAL LUNG ONE HOUR OLD

When the respiratory muscles first begin to act at the beginning of neonatal life, the thoracic cavity is enlarged. In consequence of the relation between thorax and pleural sacs the lung is likewise enlarged and inspiration is the result. With the increase in size of the lungs to occupy a larger volume, the framework of the organ is stretched and put on a tension, while the spaces become larger. This is easily apparent when one compares figures 4 and 3, showing the lung of a dog which had breathed one hour, and a fetal lung respectively. By examining sections across the entire lung and sections from different regions, it can be seen that expansion does not take place equally in all parts of the lung, nor in any particular area do all the alveoli and air-sacs increase alike. As a result it was not easy to arrive at a quantitative estimation of the area of lung-tissue and of the air-passages, as seen in the sections. In regions, which had not been much inflated, and did not appear much more open in character than fetal lung, the framework still occupied 60 per cent of the total area. But in regions where the respiratory channels were more dilated, it occupied only 40 per cent or in restricted areas even less. Thus there is a variation in the ratio, but in general it may be said that the tissue occupies 40 to 60 per cent of the area of the cross-section. The fact that the lung does not expand at once after birth was seen also in newborn albino rats. These were obtained from The Wistar Institute of Anatomy with the aid of Dr. Stotsenburg. These young were taken immediately after birth, before they were dry and before they had begun to feed. Examination of their lung (fig. 6) shows that some areas have been inflated very little, in contrast with other parts, which are quite expanded.

3



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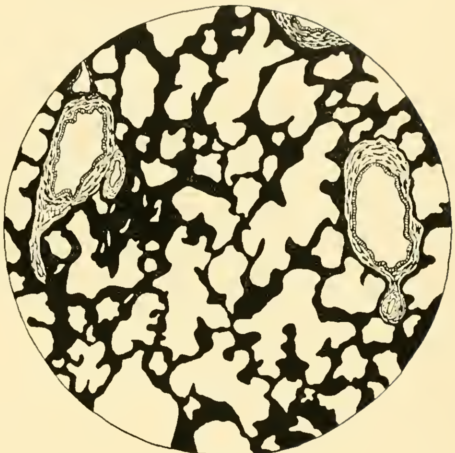

5

Fig. 3 Fetal lung of dog at end of gestation, showing texture of lung before breathing. × 40.
Fig. 4 Lung of one-hour-old dog of same litter, showing expansion of lung after breathing one hour. × 40.
Fig. 5 Lung of two-day-old dog, showing expansion of lung after breathing two days. × 40.

A careful examination shows that the granular reticular substance may still be found usually lying in contact with the walls of the air-spaces. Its presence here indicates how the fetal liquid

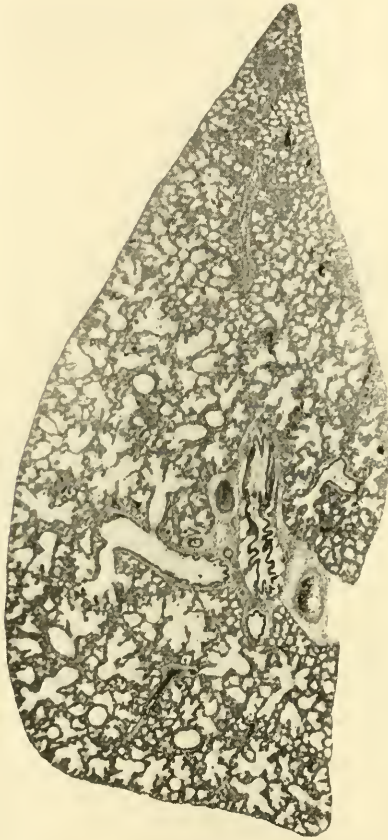

Fig. 6 Lung of albino rat, one-half hour after birth, showing partial expansion. × 25.

is disposed of when breathing commences. When the lung expands and the fluid in trachea and bronchi is drawn downward, part of the fluid remains adherent to the inside of the walls. In many sections it appears that more of the granular precipitate

is seen in the peripheral portions of the lobes, immediately under the pleura, as if more of the liquid had found its way to the ends of the lobules.

The large mononuclear cells which were seen scattered about in the fetal lung are now difficult to find, although they may still be seen by searching. In comparing the prenatal and neonatal lungs of kittens and other litters of dog, this same condition was noted.

LUNG OF TWO-DAY-OLD DOG

The structure of the lung at this stage resembles, in general, the mature lung. Reference to figure 5 shows the texture to be very open in character. By means of outline drawings on cross-ruled millimeter paper the framework is found to be only 20 to 30 per cent of the entire area, the open spaces constituting the remainder 70 to 80 per cent. As the lung enlarges with the further growth of the animal, the spaces still further increase. As it is generally expressed, the growth of the lungs does not keep pace with the growth of the thorax, as a whole, and consequently the framework of the lungs becomes more and more stretched, and the spaces are thereby also enlarged.

In the study of the lungs of these prenatal and postnatal animals other points are seen. The behavior of the lungs when the thorax is opened is different in animals which have breathed, and those which have not breathed. In the former the lung retracts quickly into the dorsal parts of the thoracic cavity, while in the latter little change takes place. The best method for comparing the positions of the lungs within the chest cavity, in these two conditions, is to inject the blood vessels with formalin without opening the thorax. It is true that the formalin causes a swelling of the tissues of the organs, but the relations are well-preserved. When one opens the thorax under these conditions, the fetal lung is found not to extend so far forward and not to cover as much of the heart as the postnatal lung, but in general the relations are very similar. The differences are the result of the increase in size of the lungs due to inspiration, while the size of the other organs remains the same.

The fetal lung is opaque, and dense-looking, being often compared in consistency with the thymus. Its color is dull grayish-red. The breathing lung has a translucent pinkish color due to the greatly increased flow of blood, and to the presence of air. It floats upon water in contrast to the non-breathing lung, and this is utilized as a medico-legal test. When the breathing lung is moved between thumb and finger, the characteristic crepitations are elicited.

In sections the postnatal lung shows congested blood-vessels, in striking contrast with the fetal lung, which shows but little blood. It would seem from examples of lung, which we obtained from still-born animals, that the increased flow of blood begins immediately, when the thoracic cavity is enlarged under the action of the muscles. These examples were from kittens, which were born at full-term. Two of them were found with the fetal membranes intact, and had never breathed air, while one of the litter had breathed freely. In the lungs of the non-breathing animals, the spaces were distended slightly more than in the normal fetal lung and the blood-vessels were much congested. The animals had evidently tried to begin to breathe air, but had only succeeded in inspiring the liquor amnii. It would appear that, as soon as the negative pressure commences, more of the blood stream is deflected into the pulmonary arteries, less going through the ductus arteriosus, and this occurs irrespective of what is being drawn into the lung spaces.

APPEARANCE OF LUNG OF PREMATURELY BORN ANIMAL

An important factor in the expansion of the lung is the stage of development of the fetus at birth, that is, whether it is prematurely born or not. In a kitten born one week before the remainder of the litter, we had an opportunity of seeing this condition. This kitten had the appearance of a healthy normal animal, although somewhat undersized. It crawled about and cried vigorously when disturbed but lived only about twelve hours. Sections from the lung of this animal showed evidence of the struggle to start and maintain respiration. The general appearance is given in the outline drawing, figure 7. The bronchi are

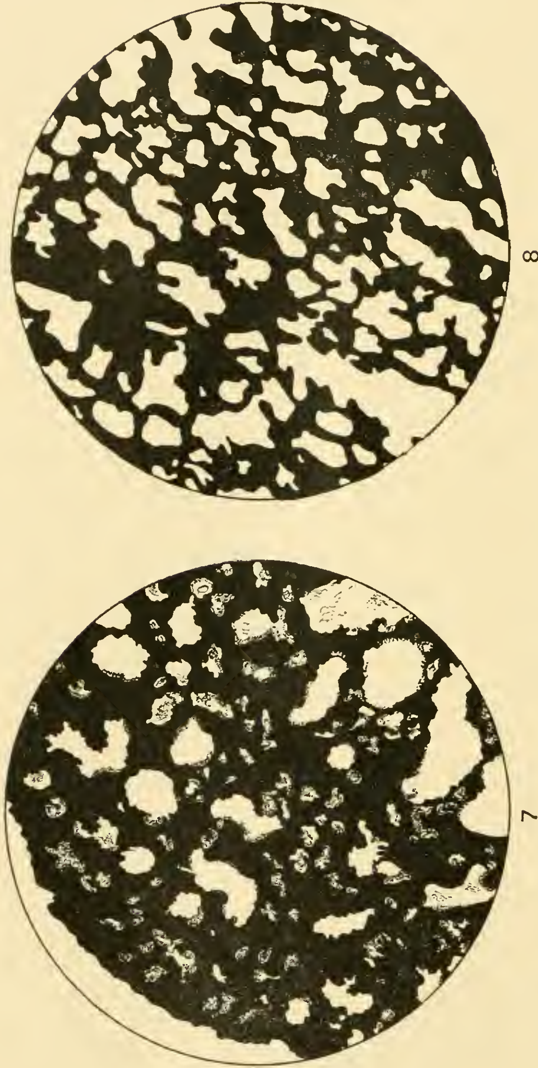

Fig. 7 Lung of kitten, born one week prematurely, which died twelve hours after birth, showing several enlarged spaces but many unexpanded or compressed. × 40.

Fig. 8 Lung of kitten of same litter, born at normal time, which was allowed to live twelve hours. × 40.

all much distended, but only a few of the respiratory spaces have been affected. Those which have been distended, however, are often over-expanded, as may be seen by comparison with figure 8, from a kitten of the same litter, born at normal time, and which had breathed twelve hours. The remainder of the lung is denser than the ordinary fetal lung, and has apparently been compressed by the over-distention of a few of the spaces. Evidently respiration was begun before the lungs were sufficiently developed to assume their normal function. A point of interest seen in examining the sections is that the alveoli which are situated directly on the bronchioles are distinctly distended. No doubt it was these alveoli which played a large part in aerating the blood of the animal during the period that it survived, and indeed it must be these which function first at the beginning of normal respiration.

We here wish to express our thanks to Professor Piersol for helpful advice and criticism and to Mr. E. F. Faber for assistance with certain of the drawings.

SUMMARY

1. During prenatal life the future respiratory passages are filled with a liquid. With the first inspirations in air, the thoracic cavity is enlarged by the action of the respiratory muscles, the lung is thereby enlarged, and the liquid in the trachea and bronchi is drawn down into the lung, and is distributed along the walls of the alveolar and other spaces.

2. In microscopic sections of lung taken from fetal animals, there is seen a finely granular substance (a precipitate from the fluid present) widely scattered through the spaces; and large mononuclear cells, probably phagocytic in function are found uniformly distributed in small numbers.

3. In sections of lung of neonatal animals the finely granular substance is still found, usually close to the walls of the air-spaces and fewer mononuclear cells are seen.

4. Before breathing, the lining epithelial cells of the alveoli are irregularly cuboidal with rounded nuclei; but after breathing, with the increased area of the walls of the alveoli, the nuclei are

spaced farther apart, the cytoplasm is drawn out and the cells are very thin and flat. After breathing has begun, the mesenchyme appears denser, with its nuclei more compacted, and the blood-vessels are distended and more conspicuous.

5. Measurements on cross-ruled millimeter paper show that the lung tissue in sections of fetal lung of dog constitute 70 to 80 per cent of the entire area; in sections of lung of dog which has breathed one hour, 40 to 60 per cent; and in sections of lung of dog which has breathed two days, 20 to 30 per cent.

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THE DEVELOPMENT OF THE CEREBRAL VENTRICLES IN THE PIG

CHESTER H. HEUSER

Harvard Medical School, Boston, Massachusetts

TWENTY-SIX FIGURES

In this study of the cerebral ventricles of pig embryos three methods have been employed—wax reconstruction, dissection, and the making of minute casts. Wax reconstructions of the ventricles have the advantage of large size. The volume of the various cavities of the brain can best be estimated by immersing portions of such reconstructions in water, and observing the displacement. From these observations the actual size of the ventricles can be readily calculated. But dissections and casts are more accurate for showing details, as, for example, the neuromeral grooves; and most of the drawings have been made from such preparations.

Brains of pig embryos measuring from 12 to 45 mm. were dissected under the binocular microscope, with finely ground instruments. Some of the dissections were designed to give a median sagittal view of the right half of the entire brain. The embryo, fixed preferably in Zenker's fluid, was held in a mass of cotton wet with alcohol, between the thumb and index finger of the left hand; and under a small stream of alcohol, a longitudinal cut was made with a sharp safety razor blade. Very perfect cuts can thus be prepared, such as will pass between the hemispheres and thence backwards, sectioning the infundibulum, corpus mamillare and other divisions of the brain almost exactly in the median plane. Mesenchyma which covers the hemisphere, olfactory lobe, or other portions which it is desired to expose, can be easily removed with delicate needles and forceps.

Casts of the ventricles, in embryos measuring from 12 to 22 mm., were made after the following method. Longitudinal sections of the entire embryo, made slightly to the right side of the median plane, were prepared as above described. Then, under the binocular microscope, obstructing portions of the brain walls were dissected off, and the ventricles were cleaned of all precipitation with a small brush and syringe. Next the embryo was taken from the alcohol and pinned down to a piece of cork and with narrow pointed strips of filter paper all the alcohol was removed from the cavities of the brain. In a large measure the success of the cast depends upon the completeness of the removal of the alcohol. The cast was made by filling the cavities with black wax with the aid of a fine electrically heated needle. A small piece of wax was carried with a cold needle to the proper place and then melted with the electric needle. This process was repeated until the ventricles had been filled. Then if desired, white wax was melted over the upper surface of the dissection for holding the parts more closely together. Moreover, the white background thus provided contrasts sharply with the black cast. Lastly the preparation was turned upside down and the brain wall was carefully dissected away from the wax. For making a permanent preparation of the cast, it is best to mount it in a dry cell on a microscopic slide. Casts thus made show a little more than one-half the ventricles. They can be studied with the binocular microscope, and drawings can be made quite as satisfactorily as from larger models. After embryos exceed 20 mm. in length the interventricular foramen is so small that it is difficult to get the lateral ventricles completely filled, and after 24 mm. the lateral recesses can hardly be cast.

For the electric needle a piece of no. 18 copper wire is coated, except for about 15 mm. at one end, with a thin layer of some insulating paste which will harden and be resistant to heat. The commercial 'caementium' is very satisfactory. The bare end is ground down to a fine point which is the working part of the instrument. Behind this, covering a length of about 20 mm., a piece of no. 32 German silver wire, about 20 cm. long, is wrapped around the insulated copper wire, the coils being well insulated from each other and covered with caementium. Attached to the ends of the German silver wire are small copper wires which run

to a lamp board. The lamp board, with the sockets connected in parallel, serves as a rheostat, and any desired temperature can be obtained by turning on the proper lights. One 50 c.p. globe allows about the desired amount of current to flow. A larger electrically heated smoother for wax models and an electrically heated knife for cutting wax plates, which were used in making the models, will be described at a future date.

Those who study the nervous system from the standpoint of comparative physiology regard the "functional system of neurones" as the real unit of the nervous system. Thus Herrick ('08), in discussing the morphological subdivisions of the brain, refers to the influence of metamerism as primitive, dominating the subdivision of the nervous system of lower vertebrates. He further states that transverse divisions, such as the diencephalon and mesencephalon, are not 'natural regions' because the primary metamerism has ceased to be an important factor. But the anatomical subdivisions exist no less than the physiological, and although their significance may not be profound, they form a convenient basis for description. In the following account the primary divisions of the brain will be considered in this order—fore-brain, mid-brain, hind-brain. Reference will be made especially to the casts and models of the ventricles, but the wall of the brain must also be studied as the changing structure which modifies the cavities within.

FORE-BRAIN

A model of the cerebral ventricles of a 5.1-mm. embryo, the youngest one studied, shows that the three divisions of the brain are distinctly marked off from each other, and that the fore-brain is already subdivided into telencephalon and diencephalon. Johnston ('09) states that the boundary between the telencephalon and the diencephalon is determined in mammals, as well as in lower vertebrates, by the velum transversum above and the primitive optic groove or postoptic recess below. This subdivision is distinct in the young pig studied as seen in figure 4. In the model very slight lateral swellings from the telencephalon indicate the cavities of the rudimentary cerebral hemispheres. The diencephalon shows a division into two parts or neuromeres, the first

or parencephalon, and the second or synencephalon of Kupffer ('03). The relatively large cavities of the optic vesicles extend out from the antero-ventral part of the diencephalon. Almost one-fourth of the length of each vesicle, representing the optic stalk, extends nearly straight out laterally, and the remainder bends sharply toward the mid-brain.

In an embryo of 12 mm. the lateral ventricles have expanded considerably and they now slope outward forward and slightly upward from the interventricular foramen. The foramen is almost circular in outline and has an area of about 0.428 sq. mm. In the cast (fig. 5) the rounded mass of wax filling the lateral ventricle shows a slight concavity below, which has been produced by the developing corpus striatum. This body arises as a thickening of the ventro-lateral wall of the hemisphere, as seen in the dissection, figure 6. This figure, it may be noted, bears a very striking resemblance to the reconstruction of the brain of Anguis fragilis (40–50 somites), published by Kupffer ('03, p. 220, fig. 224). In both, the parencephalon and synencephalon are sharply marked off from each other. Two swellings, which correspond with these subdivisions, are seen in the cast (fig. 5). The parencephalon is larger than the synencephalon, and its cavity produces a more extensive swelling, which however, is quite low.

The mamillary recess and the infundibulum below it, neither of which had appeared in the 5.1-mm. embryo, are now very distinct. The velum transversum, a portion of which is shown in figure 7, is well developed, producing a deep inward bend of the anterior wall of the fore-brain above the hemisphere.

The brain of a 17-mm. pig embryo, as seen in median section (fig. 8), shows a prominent corpus striatum, above which is the interventricular foramen. The foramen is now crescentric, since it has been invaded from below by the corpus striatum, but notwithstanding the growth of the entire embryo, its area has become actually reduced. It measures about 0.315 sq. mm. As seen in the dissection, the lower portion of the corpus striatum is bounded behind by a deep groove which is continuous with the optic recess. This groove appears as a ridge upon the cast (fig. 9), and in front of it the position of the corpus striatum is indicated by an excava-

tion. Above this hollow is seen the inferior horn of the lateral ventricle. The outline of the ventricle is no longer round, as in the 12-mm. embryo. It is prolonged anteriorly or downward to form the first indication of the olfactory lobe, and posteriorly or above, it is somewhat flattened. This upper portion terminates in the inferior or descending horn. Fraser ('94) has referred to this horn in an abnormal human adult brain as in "reality ascending" and Thompson ('08) similarly states that "from the developmental point of view, this descending horn is in reality an ascending horn." In pig embryos, however, as seen in figures 5 and 9, its primary direction is apparently ventral to the cerebral axis so that the inferior horn may be said to descend, even in early stages. The cavity of the lateral ventricle is seen laid open in figure 10. It differs from that of the 12-mm. pig embryo (fig. 6) since it has been invaded by a prominent chorioid fold. This fold is a lateral extension of the velum transversum, and it consists of vascular mesenchyma covered by the thin wall of the brain. At this stage its ventricular surface is perfectly smooth, but in a 22-mm. embryo the chorioid fold has developed a vascular fringe which appears as a plaited frill. This is shown in figure 13.

The walls of the diencephalon in the 17-mm. embryo have thickened considerably so that the external depression between the parencephalon and the synencephalon has become almost imperceptible. Their cavities however, can be distinctly seen when the dissection of the brain is studied (figs. 8 and 9). The mamillary recess now projects out some distance from the third ventricle. Midway between this recess and the infundibulum the floor of the brain has thickened to form the tuber cinereum. The optic thalami, represented by the thickenings of the lateral walls of the diencephalon, have developed to such an extent that they have already partially fused with the corpus striatum. The place of fusion appears as an interruption of the groove between the interventricular foramen and the optic recess.

The median section of a brain of a 22-mm. embryo (fig. 11), as compared with that of the 17-mm. specimen, shows several new features. Along the cut edge dorsally, the pineal body has appeared as a slight elevation. Within the third ventricle,

the thalamus has enlarged, and the corpus striatum appears as if pushed forward before it. Between the corpus striatum and the upper part of the thalamus, the interventricular foramen is seen as a slit, which arches up over the corpus striatum, being widest immediately under the velum transversum. The area of the aperture has become further reduced. Between the corpus striatum and hypothalamus, the optic recess appears as in the 17-mm. specimen. Somewhat further back, and parallel with it, is the recessus infundibuli, and between the two grooves made by these recesses, is the pars optica hypothalami. This is convex toward the ventricle. Between the hypothalamus below and the thalamus above is the sulcus hypothalamicus (B.N.A.) or sulcus limitans (His '93)—a groove which continues from the mid-brain downward and forward becoming somewhat indistinct above the pars optica hypothalami. Reichert ('61) described this groove in the brain of a pig embryo (also in cat and human embryos) as extending from the foramen of Monro to the entrance of the aqueduct of Sylvius, and he named it the sulcus of Monro. In fig. 12 an extension may be traced from the sulcus to the lower end of the interventricular foramen; another extension proceeds toward the recessus postopticus. But the main continuation is probably that which ends in the optic recess. This accords with the opinion of His ('93, p. 177), and also of Johnston ('09, p. 517). The sulcus limitans is indicated in the embryo of 17 mm. and in younger specimens, but it is more distinct at 22 mm.

The median section of the 22-mm. embryo shows the uncut median surface of the right hemisphere, ending below in a rounded olfactory lobe. Extending from the lower border of this lobe toward the notch below the velum transversum, is a groove, which, with the lamina terminalis, bounds a triangular portion of the hemisphere. Smith ('03) named this triangular thickening the 'paraterminal body'. Herrick ('10) proposes to divide this body into a ventral component, the 'corpus precommissurale,' and a dorsal component, the 'primordium hippocampi,' but these subdivisions do not appear externally in the 22-mm. embryo.

In the dissection of a 45-mm. embryo (fig. 14) most of the paraterminal body together with the adjacent medial wall of the right

hemisphere have been cut off, thus displaying the structures within the ventricle. The corpus striatum is seen to be divided by a vertical groove into two portions. The groove is deep below but fades out above. Thompson ('09) has described a similar groove in a cat embryo 20 mm. in length, and he refers to the larger lateral and the smaller median subdivisions which are set apart by the groove as 'roots' of the corpus striatum. His ('89) described the corpus striatum as being composed of three limbs. In the 45-mm. pig there are also three limbs, but only two are seen in figure 14. The small median limb, which is not shown, fuses with the lamina terminalis just below the interventricular foramen. The groove is shown at x in figure 18, which is a frontal section through the head of a 45-mm. pig. The plane of this section is indicated in figure 14. The groove is best seen in the right side of the section, where the median subdivision may be observed to pass down toward the paraterminal body. In a section further back (fig. 19) the groove is quite shallow. Above the corpus striatum (figs. 14 and 19) the chorioid plexus is seen projecting into the lateral ventricle. The fold, surmounted by a frill, which was seen in the 22-mm. embryo, has given place to a very thin layer with many reduplications and subdivisions. Villi are nowhere present, but in thin sections the smaller processes extending out from the main folds may simulate them. Under the binocular microscope it is clearly seen that this plexus consists only of folds with secondary subdivisions. The plexus is attached along a fissure measuring 2.1 mm. in length, which extends backward from the interventricular foramen.

The caudal portion of the plexus—about one-fourth of the entire length—is very much attenuated. It forms a short free projection extending 0.18 mm. beyond the end of the fissure, and the free portion shows no secondary folds (fig. 21). The anterior portion of the plexus forms a larger free projection which extends 0.72 mm. past the front end of the fissure.

In the 22. mm. embryo (fig. 13) a slight invagination of the medial wall of the hemisphere, above the chorioid plexus and parallel with it, extends from the interventricular foramen backward into the inferior horn. This represents the hippocampus,

which is better developed in the 45-mm. embryo. It is seen in figures 19, 20, and 21 (marked +).

Considered as a whole, the lateral ventricle has expanded greatly and may readily be divided into three parts—the anterior horn, which is in front of the corpus striatum; the body, which is above it; and the inferior horn, descending behind it. A cast of the left lateral ventricle is shown in side view in figure 16, and in ventral view in figure 17. The latter shows the large excavation made by the corpus striatum. Toward the olfactory lobe this concavity is bisected by a ridge, which lies between the large roots of the corpus striatum, as described in connection with figure 14. Below the concavity, in figure 17, there is a ridge which is fissured, posterior to the interventricular foramen, to receive the chorioid plexus. This ridge separates the hollow for the corpus striatum above, from that for the hippocampus below.

The lateral ventricle communicates with the third ventricle by an interventricular foramen which is smaller than in the preceding stages. The third ventricle has become reduced to a slit-like space, owing to the great thickening of the walls of the diencephalon. A portion of it has become practically obliterated. This is where the thalami have grown against each other (figure 21). They have not yet fused, however, to form the massa intermedia; that is the ependymal layer over each thalamus is still uninterrupted. Along the dorsal margin of the cleft, which represents the ventricle, there is a thin lateral expansion on either side (fig. 15). The expansion begins at the interventricular foramen, where it is broadest, and it diminishes backward, ending a short distance in front of the pineal recess. Thus the expansion is wedge-shaped when seen from above. The brain-wall which overlies this expansion is the tela chorioidea, which has a corrugated surface in relation with the vascular mesenchyma. Below and behind the thalamus is a deep groove—the sulcus limitans—which, as already described, sends prolongations to the interventricular foramen, recesses postopticus and recessus opticus. The sulcus is seen in section in figure 20, with the thalamus above and the pars optica hypothalami below.

The oldest embryo studied measured 260 mm. Its brain was dissected out, embedded in celloidin, and cut into sections 0.2 mm. thick. From these sections a wax model of the ventricles was constructed, as shown in figures 25 and 26. The three subdivisions of the lateral ventricle have become highly developed. The anterior horn, which in the 45-mm. embryo ended in a short and slightly pedunculated olfactory bulb, now extends through the olfactory stalk and terminates in the expanded ventricle of the olfactory bulb. The body of the lateral ventricle is corrugated above, where it is in relation with bundles of fibers in the corpus callosum. The inferior horn not only descends, but extends forward as a slender prolongation, which ends blindly in the temporal lobe of the brain. As seen from below, the cast of the lateral ventricle shows the concavities for the corpus striatum and the hippocampus respectively, separated by a ridge into which the chorioid plexus is invaginated. The ridges bounding the corpus striatum correspond with those seen in the 45-mm. embryo.

In the 260-mm. specimen, the interventricular foramen has expanded considerably, and its area was found to be 6.92 sq. mm. Extending backward from the foramen, between the region occupied by the thalamus below and the tela chorioidea above, there is a tubular expansion of the third ventricle, somewhat flattened dorso-ventrally. This corresponds with the much smaller expansion, T-shaped in section, which was described in the 45-mm. embryo. In the 260-mm. embryo there is a long caudal extension of the dorsal part of the third ventricle which curves slightly upward and forms the suprapineal recess. It measures about 5.6 mm. in length and extends backward with quite a uniform diameter of 1.4 mm. This conspicuous feature is not represented in the 45-mm. specimen. The cast of the cavity (fig. 25) is deeply corrugated on all sides by longitudinal folds. These accomodate branches of the medial cerebral vein. A large branch of the vein on either side produces a deep groove along the dorsal expansion of the third ventricle. Between the suprapineal recess above and the pineal recess below there is a well marked lateral compression of the ventricle, produced by the hab-

enular ganglion. The conspicuous ridge at the lower margin of this habenular concavity continues caudally into the pineal recess. Between the pineal recess and the groove for the posterior commissure there are two slight projections of the third ventricle (infrapineal recesses). The very extensive fusion of the thalami, forming the massa intermedia, has produced a hole in the model of the third ventricle. It is oval in outline, measuring about 4.6 mm. by 3 mm.

MID-BRAIN

It is well known that in young embryos the cavity of the mid-brain is very large, forming the middle cerebral vesicle. In the adult it is generally described as "reduced to a narrow slit—the aqueduct of Sylvius." Cunningham states that the mid-brain "is tunnelled by a narrow passage, the aqueduct of Sylvius, which extends between the fourth and third ventricles," and Bensley ('10) writes: "The mesencephalon is noteworthy in a mammal as lacking a ventricle. Its cavity is a narrow canal, the aqueduct of Sylvius . . . ." But Retzius ('00) described a spindle-shaped expansion in the middle portion of the cavity of the adult human mid-brain, and named it 'ventriculus mesencephali.' It will be shown that a distinct cavity exists in the mid-brain of the adult pig, comparable with that found by Retzius in the human brain.

In the 5.1-mm. pig (fig. 4) a slight constriction separates the caudal part of the diencephalon from the cavity of the mid-brain. Another slight constriction marks the position of the isthmus between the mid-brain and the hind-brain. The angle formed by a line passing through the axis of the diencephalon with another through the isthmus and cavity of the medulla, is very acute (30°) in this stage. In the adult it is very wide.

The cavity of the mid-brain continues to expand and becomes sharply marked off from that of the hind-brain at the isthmus, as shown in the 12-mm. embryo (fig. 5). Between the mid-brain and fore-brain, however, the cavity shows only a slight constriction, which is limited to the lateral surface. As seen in the cast

neither the mid-dorsal nor the mid-ventral line is indented at this point.

Figure 9, of a 17-mm. embryo, shows that the dorsal surface of the mid-brain has become flatter than before and its caudal end projects further toward the hind-brain. This projection, on either side, marks the position of the future inferior colliculus. The ventricle of the mid-brain is somewhat quadrilateral in cross section, and in the cast it shows a prominent longitudinal ridge corresponding with the line of maximum width. The groove in the wall of the brain corresponding with the ridge may be referred to as the lateral sulcus (sulcus lateralis internus). It must not be confounded with the sulcus limitans which, in the cast, is a much less conspicuous ridge, more ventrally placed. The sulcus limitans is more evident in the dissection, figure 8. It separates a small thick-walled ventral zone from an extensive thin-walled dorsal zone. The ventral zones, on either side of the mid-ventral sulcus, form low rounded eminences projecting into the cavity of the ventricle which constitutes the tegmental fold of His. The lateral sulcus passes longitudinally through the dorsal zones, terminating on either side, in the projection which forms the inferior colliculus.

In a slightly older embryo (22 mm.) the dorsal wall of the mid-brain has become relatively thinner, and its ventricle has expanded greatly. At this stage it forms a very conspicuous portion of the whole ventricular system. Its volume is estimated at 4.1 cu. mm. Separate recesses for the inferior colliculi can now be recognized. In earlier stages the posterior overhanging portion of the mid-brain ended abruptly in a straight transverse line. In the 45-mm. embryo this border shows a deep median cleft, and each arm of the bifurcation thus formed represents the cavity of an inferior colliculus. In the 22-mm. specimen the cavities projecting backward from the dorso-lateral corners of the mid-brain ventricle have just begun to develop.

In the 45-mm. embryo the third ventricle is connected with the cavity of the mid-brain by a more elongated constricted portion than in previous stages. At the anterior end of the mid-brain ventricle there is a median dorsal extension 1.2 mm. caudad

to the pineal recess. This outgrowth (figs. 14 and 15) which lies just behind the posterior commissure is the incisura postcommissuralis. It is not present in the 20-mm. pig but is very distinct in the 45-mm. embryo. The ependymal and mantle layers but not the ectoglial layer of the brain-wall make a distinct outward bend above it. Caudad to the incisure, the dorsal surface of the mid-brain cavity slopes gradually upward to a height of 0.6 mm. and then extends nearly straight backward over the body of the cavity. The inferior collicular recesses are very much more distinct than in younger pigs.

The length of the mid-brain, including its collicular recesses is 5.0 mm. and the width, in the widest part is 2.6 mm. The volume of the cavity is very much greater than in preceding stages and is now about 6.9 cu. mm.

A cast of the cavities of the mid-brain in an embryo measuring 110 mm. is of an irregular prismatic form as shown in figure 24. It presents a median dorsal ridge which extends from the incisura postcommissuralis posteriorly, ending in a median depression between the eminences representing respectively the cavities of the right and left inferior colliculi. The outer margin of the cavity of each inferior colliculus is continued forward as a prominent ridge, corresponding with the sulcus lateralis. This ridge flattens out rather sharply and completely a short distance behind the posterior commissure. At the base of the cavity of the inferior colliculus, a prominent ridge ascends from the isthmus, but it can be followed only a short distance behind the posterior commissure into the territory of the mid-brain, where it terminates. This ridge represents the sulcus limitans of the isthmus. Ventrally there is another ridge which may be regarded as the sulcus limitans of the mid-brain, although it is not continuous with the structure just described as the sulcus limitans of the isthmus. It arises beneath the latter in or near the median ventral sulcus. It then passes laterally, diverging from its fellow on the opposite side. The point of widest separation is soon reached, after which the two sulci gradually converge, coming together in the mid-ventral line beneath the incisura postcommissuralis. The surfaces between the ridges on the cast are

all concave, and in the central part of the mid-brain there are five surfaces—two dorso-lateral, bounded by the median dorsal ridge and the lateral ridges; two ventro-lateral, extending downward to the sulci limitantes; and a ventral surface between these sulci.

The form of the mid-brain ventricle in the embryo measuring 110 mm. has been described at length, since subsequently it undergoes only slight modifications. This was determined by modelling the ventricles in the 260-mm. pig (figs. 25 and 26) and by making dissections of the adult. The ventricle of the mid-brain continues to increase in size but it does not keep pace with the growth of the adjoining cavities. The grooves remain as described, and the ventral surfaces, between the sulci limitantes, are always subdivided posteriorly by a forward extension of the median ventral sulcus.

HIND-BRAIN

In the 5.1-mm. embryo the cavity of the hind-brain or third cerebral vesicle is elongated and quite straight. Behind the ventricle of the mid-brain (fig. 4) a well marked constriction indicates the future isthmus. Caudad to this, the cavity, or fourth ventricle, widens somewhat, and then slopes gradually through the medulla to the spinal cord. The low ridge which follows the line of greatest width corresponds at this stage with the line of attachment of the thin roof-plate, and not with the sulcus limitans. Along the ventral surface of the cast there is a sharp median ridge which represents the sulcus medianus of the rhomboid fossa.

Seven neuromeral grooves can be identified. The first produces a low but broad ridge on the cast anterior to the widest part of the rhombencephalon. Dorsally it flattens out before reaching the line of maximum width and ventrally it does not extend quite to the median sulcus. The second neuromeral groove is situated opposite the widest portion of the fourth ventricle. The next four are about equally spaced. They are very prominent in the ventral zone and some of them appear to reach upward a short distance into the dorsal zone. The last one is

represented on the cast by a prominent mound, but the elevation does not extend as far dorsally or ventrally as do those immediately preceding it. Caudally it blends with the sulcus limitans. Bradley ('04 and '05) described seven neuromeres in a nineteen-day embryo.[1]

The pontine flexure soon becomes definitely established as shown in figure 5, which is a cast from a 12-mm. embryo. In this embryo the median ventral edge of the cast is very sharp around the flexure and backward toward the spinal cord. At the pontine flexure there are six neuromeral grooves. The relation of the grooves to the median sulcus is clearly shown in the dissection, figure 7, The former seventh groove has been taken up by the sulcus limitans so that it is no longer recognizable. Above the grooves the ventricle has become quite wide. In dorsal view the body of the ventricle slopes out laterally quite rapidly behind the isthmus, attaining its maximum width in the region of the future lateral recesses, which become distinct in embryos of about 15 mm.

In the 17-mm. embryo the isthmus leading from the mid-brain to the body of the fourth ventricle is diamond-shaped in cross section; the ventral zones are here more extensive than the dorsal, which is the reverse of the condition in the mid-brain. The ventral median sulcus is narrow and deep, so that in the cast there is a sharp edge on the ventral surface of the fourth ventricle extending around the pontine flexure, and onward caudally to the spinal cord. There are now five neuromeres in the hind-brain. The body of the fourth ventricle has expanded a great deal since the last stage described, and the lateral recesses are now well indicated. Slight concavities on either side of the body of the ventricle between the isthmus and the lateral recesses, in the dorsal zones, are caused by the thickening of the brain in this region to form the lateral portions of the cerebellum. Behind the lateral recesses the thin roof of the hind-brain has become deeply invaginated to form the chorioid plexus. About 100 villi are developed on the fold.

[1] According to Keibel's Normentafel, pig embryos of nineteen days measure from 4.5 to 8 mm.

In a 22-mm. embryo (figs. 11 and 12) the fundamental arrangement of parts has not been altered. The cerebellar thickenings have enlarged considerably, producing correspondingly greater depressions in the dorsal zones along the body of the fourth ventricle. The sulcus limitans, for the same reason, has been made very much more evident. The lateral recesses extend further to each side and are comparatively much narrower than in the younger stage considered. The chorioidal lamina extends out to the ends of the lateral recesses, and the number of villi springing from it has increased to about 150. Caudad to the chorioid plexus the dorsal part of the cavity is slightly distended or puffed up dorsally, thus forming the 'caudal protrusion' (Blake '00). In advanced embryos of dogs, cats, pigs, sheep and also the chick, according to Blake, there is a marked caudal protrusion. He wrote, "This protrusion is completely closed and resembles the finger of a glove." It can hardly be described in the pig as 'finger-like' since, as shown in figure 11, it forms a rounded dome.

Taken as a whole, the fourth ventricle in the 45-mm. pig (figs. 14 and 15) has the same parts as seen in the 22-mm. embryo. The cavity of the isthmus has increased in dorso-ventral diameter from 0.75 to 0.85 mm. As seen by comparing figures 11 and 14, it has become relatively low, but laterally it has become further expanded. Its thin lateral edge, as seen in the cast (fig. 15), extends backward and becomes continuous with the body of the ventricle a short distance in front of the lateral recess. Where this thin edge fuses with the body there are three small wrinkles—the lateral remnants of the neuromeral grooves. The two halves of the cerebellum have become very much thicker and meet at an angle of about 130°. The medial part of the cerebellum is also thicker than before, so that all together the broad median fissure of younger stages has been appreciably reduced. The lateral recess, while absolutely larger than before, appears much slimmer. The floor and roof of the recess have almost come together, the cavity being largely filled with the chorioid plexus. In figure 23, in the left recess, is seen a long stretch of the chorioid fold. Sections a short distance caudad

to this one show the fold to be continuous from one recess to the other. The marked villous character of the chorioid plexus is illustrated well in this section (fig. 23). It shows also the rhomboid fold projecting into the cavity of the hind-brain below the chorioidal lamina. The rhomboid fold appears in the model (fig. 15) as a sharp rim, extending along the upper part of the body of the ventricle behind the lateral recess. It extends to the caudal protrusion of the fourth ventricle, which has expanded to a very great size; its volume is more than one-half that of the remainder of the ventricle. This protrusion, from above and from behind, appears almost spherical. The posterior boundary slopes downward making almost a right angle with the narrow cavity of the caudal part of the medulla. When the body of the fourth ventricle is viewed from below, the ventral median sulcus and the two sulci limitantes are seen converging towards the cervical flexure. The ventral median groove is nearly straight and produces with the slim cavity of the cord an angle of about 125°.

In the 260-mm. embryo (figs. 25 and 26) the cavity of the isthmus is very broad and flat. On the ventral surface a deep ridge—the median ventral sulcus—is continuous with the similar ridge of the mid-brain ventricle. On account of the extensive growth of the cerebellum the dorsal surface of the body of the ventricle has become deeply concave. Only a very slight caudal protrusion is now present. Bradley ('05) described the first appearance of openings in the lateral recesses—foramina of Luschka—in embryos of about 100 mm. Because of these openings and on account of the growth of the chorioid plexus the cavities of the lateral recesses, as seen in the model, have been reduced to slim irregular bodies.

MEASUREMENTS OF THE BRAIN

In order to show the change in the volume of the cavities during development, measurements of wax models were made by the immersion method. The results of these measurements, reduced to cubic millimeters, are given in table 1.

TABLE 1

Volume of the cerebral ventricles in cubic millimeters

EMBRYO	FORE-BRAIN	THIRD VENTRICLE	LATERAL VENTRICLE	MID-BRAIN	HIND-BRAIN	TOTAL CAVITY OF BRAIN
mm.						
5.1	0.0948			0.022	0.206	0.3227
12	1.716	1.374	0.1712	0.357	3.38	5.453
20	7.11	2.148	2.481	2.96	3.407	13.477
24	9.893	2.357	3.768	5.181	3.57	18.644
45	30.49	4.19	13.15	6.904	5.84	43.234
260	711.58	94.6	308.4	10.07	35.5	757.15

From table 1 it is seen that each part of the entire ventricular system increases in size continually during development. The increase is relatively greater at first, and as would be expected, it becomes gradually less toward the adult. For a time—until embryos measure between 12 and 20 mm.—the fourth ventricle is the largest part of the ventricular system. After the lateral ventricles have expanded appreciably the cavity of the fore-brain is the largest part.

The cross-sectional area of the interventricular foramen was determined from wax models by measuring the area of the foramen cut through the narrowest portion.

The cross-sectional area of the interventricular foramen becomes progressively greater up to about the 12-mm. stage after which it becomes both relatively and absolutely smaller for some time. In later stages it again expands considerably; in the adult it is very much larger than in younger stages.

TABLE 2

Area of the foramen interventriculare

EMBRYO	FORAMEN INTERVENTRICULARE
Length in mm.	*Area in square millimeters*
12	0.42
20	0.26
45	0.18
260	6.92

The mid-brain angle in early stages is very acute. For measuring the angle in the different embryos during development it

is difficult to establish lines whose loci will be absolutely comparable throughout the series. The apex of the angle has been located in the middle point of the mid-brain ventricle; one line has been drawn from it to the optic recess, and the other to the middle point in the cavity of the central canal opposite the cervical bend.

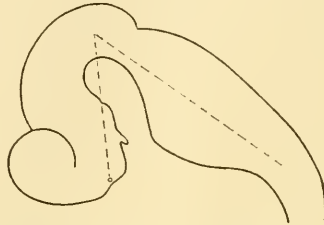

Fig. 1 Outline from figure 5, showing the position of the mid-brain angle

TABLE 3

Increase in the mid-brain angle

EMBRYO	MID-BRAIN ANGLE	EMBRYO	MID-BRAIN ANGLE
mm.	*degrees*	*mm.*	*degrees*
5.1	30.0	45	84.0
12	48.5	260	146.0
17	59.5	Adult	152.0
22	68.0		

The angle increases from 30° in the 5.1-mm. embryo to 152° in the adult. The curve of growth of this angle rises up relatively rapidly at first and slowly in later stages to the adult.

CONCLUSIONS

The well-known series of models of the developing human brain made by His and the papers which they illustrate make it possible to compare the conditions which have been described in the pig with those in man. Comparisons with the embryonic brain of other mammals cannot be made, since it appears that no one has attempted to extend the work of His here referred to, or even to repeat it critically. The first impression derived

from comparing His's models of the interior of the human embryonic brain with those of the pig is that they are strikingly similar, especially in the earlier stages; but more careful study shows very considerable differences. The 6.9-mm. human embryo (His, '89, Taf. 1, Fig. 2) is somewhat more advanced than the pig embryo of 5.1 mm., as indicated by the greater development of the hemisphere, and of the pontine flexure. In His's model the cavities of the parencephalon and synencephalon are not indicated and the neuromeres of the hind-brain are not shown. It may be questioned however, whether these structures are actually absent from the human brain at this stage. On the other hand, the model of the human brain shows a prominent tegmental fold passing from the mid-brain toward the fore-brain, but no such fold occurred in the pig embryo. In a model of a pig embryo of 5 mm. Johnston ('09) has shown the neuromeres of the fore-brain and hind-brain but there is no tegmental fold. This fold is the portion of the tegmentum which projects dorsally into the cavity of the mid-brain; the fold on either side extends from the median ventral sulcus to the sulcus limitans. Altogether Johnston's model of the brain of the 5-mm. pig agrees very closely with the first model described in the present paper, and it is evident that we have more detailed and accurate knowledge of the shape of the brain in the pig embryo than in the human embryo of corresponding stage.

His's model of the human embryo of 10.2 mm. corresponds quite closely with the pig embryo of 12 mm. and his 13.6-mm. specimen is comparable with the pig of 17 mm.; the human embryo of the third month, which completes His's series of models, is the stage intermediate between those dissected in pigs of 22 and 45 mm. In all of His's models, except the oldest, the tegmental fold is very prominent; and in the embryo of three months it is perfectly distinct. The sulcus limitans is accordingly well defined in the mid-brain, but it does not form a distinct continuous longitudinal groove from the spinal cord to the optic recess in any of His's models. In fact, His described the sulcus as becoming leveled off in the territory of the isthmus

by the growth of the walls—being interrupted at the junction of the isthmus with the mid-brain.

In the casts of the ventricles of the pig, in early stages, there is a broad line of maximum width corresponding with the sulcus limitans.

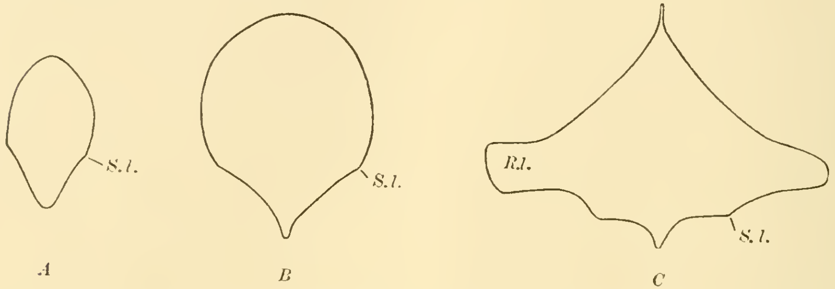

Fig. 2 Sections through the hind-brain of pig embryos, (*A*, 3.9 mm.; *B*, 10 mm.; *C*, 23.6 mm.), showing the change in position of the sulcus limitans.

This groove which is lateral in the cord becomes a ventral groove in the medulla as described by His, and at the junction of the isthmus with the mid-brain it comes to an end. On account of the expansion of the roof-plate in the hind-brain the sulcus limitans soon falls below the line of maximum width. Thus in the diagram (fig. 2, *B*) the sulci are ventrally placed, and the width of the fourth ventricle between them is less than it is slightly further dorsally. In an older embryo (fig. 2, *C*) the sulci are close together and on the floor of the ventricle. In the mid-brain there is early a line of greatest width, which becomes shifted so as to form the outer border of the tegmental fold, as shown in the diagram, figure 3.

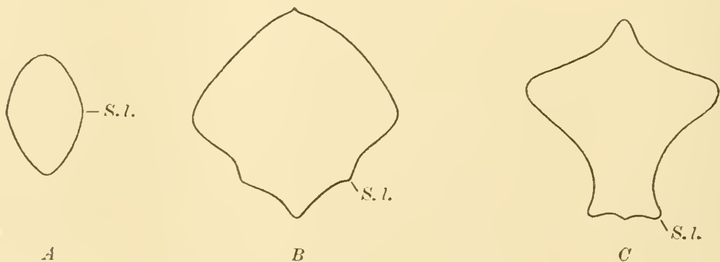

Fig. 3 Sections through the mid-brain of pig embryos, (*A*, 3.9 mm.; *B*, 23.6 mm.; *C*, 110 mm.); showing the change in position of the sulcus limitans.

This shifting in the mid-brain is brought about by the expansion in the dorsal zone to form the cavities in the colliculi. The line of maximum width begins behind the constriction between the mid-brain and fore-brain in the superior collicular cavity and terminates at the caudal end of the mid-brain in the inferior collicular recess. Anteriorly the hypothalamic sulcus is seen to connect with the tegmental sulcus. This connection is most distinct in the 22.mm. embryo.

His first called attention to the dorsal and ventral zones of the brain and cord. A demarcation of the sensory from the motor portion of the central nervous system is obviously of fundamental importance and Johnston ('09) has described the sulcus limitans as the most important landmark in the brain. Diagrams have appeared which show an uninterrupted line, extending from the optic recess backward through the brain and spinal cord. However, there is no real picture which shows a sulcus continuous throughout the brain. It is distinct in each division of the brain, but at the cephalic end of the isthmus it is interrupted.

The hemisphere of course is very much larger in the human embryos than in approximately corresponding stages of the pig. The thalamus and habenular region are also further advanced in the human stages. The tegmental folds never form conspicuous projections into the ventricle of the mid-brain as they do in early human embryos. The olfactory lobe becomes progressively more conspicuous in the pig series—the reverse of the condition in human embryos, of later stages especially. In the latter, the olfactory lobe is covered over by the rapidly expanding pallium. The olfactory lobe in the pig always extends some distance in front of the end of the hemisphere. The roof of the fourth ventricle is not present in any of the His models, hence there is no indication of a caudal protrusion of the fourth ventricle. This protrusion is first well indicated in pig embryos about 22 mm. long. It expands very rapidly until the cerebellum grows downward into this region. There being no foramen of Majendie in the pig, the posterior medullary velum stretches nearly straight across the caudal part of the fourth ventricle so that the caudal protrusion is just recognizable in the adult.

The form of the ventricles in the adult pig has apparently never been studied by means of casts or models. Figures 25 and 26, representing the cerebral ventricles in an embryo of 260 mm., are however very similar to those which would be obtained from an adult and they may be compared with Dexler's figure of the cavity of the brain in the horse, and with any one of several figures of the ventricles in the human brain. The first of these were published by Welcker ('78) who filled the ventricles with wax, injecting through the infundibulum, and published figures and a brief description of the model thus obtained. Testut ('97) figured and described a plaster cast of the ventricles. Barratt ('02) constructed a wooden model from measurements obtained from thick sections (12.5 mm.). From the method employed accuracy of detail could scarcely be expected. By far the most delicate and satisfactory figures are those of Retzius ('00) who made casts by the use of Wood's metal. Harvey ('10) has recently used Wood's metal for the same purpose with similar results.

In the human brain immediately above the pineal body there is a backward extension of the cavity of the third ventricle which forms the supra-pineal recess. This term was introduced by Reichert ('61, Bd. 2, S. 69) who described the structure and showed its relations in median sections of the brain. In his figures the recess measures nearly 5 mm. in length. In Welcker's plaster cast it appears to be somewhat larger and in the cast by Retzius it measures 10 mm. But Testut and Harvey show only very slight protrusions. The recess may be variable in the human brain. In Dexler's model of the ventricles in the horse it is a very conspicuous object, nearly 25 mm. in length. In the adult pig it measures 5.5 mm. In proportion to the size of the third ventricle it is very much longer than in man but much shorter than in the horse. The significance of this recess has not been determined.

A very conspicuous feature of the casts of the third ventricle is the aperture made by the massa intermedia. In the several models of the human brain this aperture varies considerably in size, but in no case is it as large as in the pig or the horse. In

the pig it appears to be somewhat smaller than in the horse, but in both these animals the opening is very large. The area of fusion in the 260–mm. pig measures about 8.4 sq. mm., and in the adult pig about 61.6 sq. mm.

The lateral ventricles in pig, horse and man differ very greatly. In man there is a posterior horn which is absent in the horse and pig. But the latter both show an extension of the ventricle into the olfactory lobe, whereas in man, the anterior horn ends bluntly. In the horse the pedicle of the cavity of the olfactory stalk is very long and markedly concave dorsally, it ends in an elongated irregular ventricle. The pedicle in the pig is nearly straight and much shorter. It terminates in a flattened expansion which in the anterior end is compressed dorso-ventrally.

In connection with the lateral ventricle it may be noted that its chorioid plexus in the pig is not provided with villi. Meek ('07) in his discussion of the general morphology of the plexus writes: "Villi are scarce in the chicken, duck and pigeon, but more abundant in the hog, while they reach a considerable development in the horse, ox, and especially among porpoises, crocodiles, and some of the selachians (Pettit '02–'03)." Findlay ('99) writes similarly of this plexus: "The surface of the chorioid plexus is beset with a large number of highly vascular villous projections. These are of all sizes, and the largest may branch and subdivide many times before the ultimate villi are formed." The study of the brain of the pig has shown that the chorioid plexus of the lateral ventricle first develops as an extension of the velum transversum into the lateral ventricle. The free border rapidly becomes much longer than the attached part so that it is early thrown into folds. These primary folds increase in number and give rise to secondary folds, but as shown by means of the binocular microscope, villi are not developed in this plexus.

In pig embryos of 35 to 40 mm. and in all older stages, there is a median dorsal recess behind the posterior commissure. This postcommissural recess is shown by Retzius who labels it the 'incisura postcommissuralis.' It is shown also in Harvey's lateral view. None of the other authors have an indication of it in their figures. In the adult pig the recess is very prominent.

Finally, it should be noted that the aqueduct of Sylvius remains as a distinct ventricle of the mid-brain in the adult pig. One of Retzius's figures alone gives a good lateral view of the mid-brain of man. This he described as 'ventriculus mesencephali.' The cavity of the mid-brain in the horse stands out also as a distinct ventricle. In the pig the mid-brain has throughout development and in the adult a well defined ventricle which constantly increases in size as long as the brain grows.

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ABBREVIATIONS

B.olf., bulbus olfactorius
C.pr., caudal protrusion
Cbl., cerebellum
Ch.op., chiasma opticum
Coll. i., colliculus inferior
Coll.s., colliculus superior
C.inf., cornu inferius
C.par., corpus paraterminale
C.pin., corpus pineale
C.str., corpus striatum
For., foramen interventriculare
F.plx., fissure for plexus chorioideus
Hab., habenula
Hip., hippocampus
Inc.pc., incisura postcommissuralis
I., infundibulum
Is., isthmus
L.ch., lamina chorioidea epithelialis
Mes., mesencephalon
$N_1 - N_7$, neuromeres
N.op., nervus opticus
N.tr., nervus trigeminus
Par., parencephalon
Plx., plexus chorioideus
R.i., recessus infundibuli
R.l., recessus lateralis
R.m., recessus mamillare
R.op., recessus opticus
R.pin., recessus pinealis
R.post., recessus postopticus
R.sup., recessus suprapinealis
R.f., rhomboid fold
S.l., sulcus limitans
Syn., synencephalon
T.ch., tela chorioidea
Th., thalamus
V.b.olf., ventriculus bulbi olfactorii
V.l., ventriculus lateralis
Vel.tr., velum transversum
V.op., vesicula optica

PLATE 1

EXPLANATION OF FIGURES

4 Wax model of the cerebral ventricles of a 5.1-mm. pig. H.E.C. series 1904. × 20 diam.

5 Cast of the cerebral ventricles of a 12-mm. pig. × 15 diam.

6 Dissection of the brain of a 12-mm. pig. × 15 diam.

7 Dissection of the brain of a 12-mm. pig. × 15 diam. The plane of the cut edge of this dissection is indicated by arrows in figure 5.

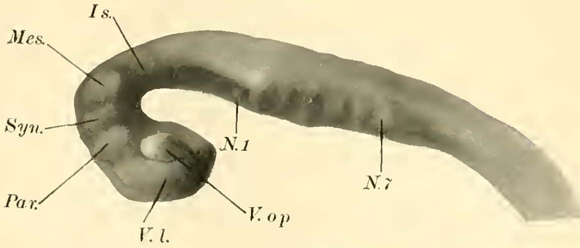

Fig. 4

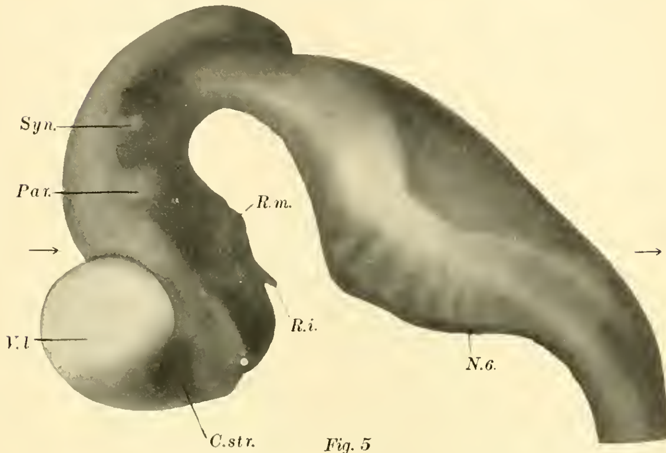

Fig. 5

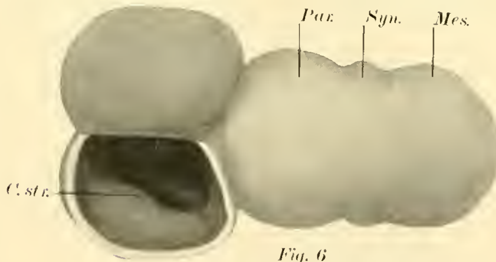

Fig. 6

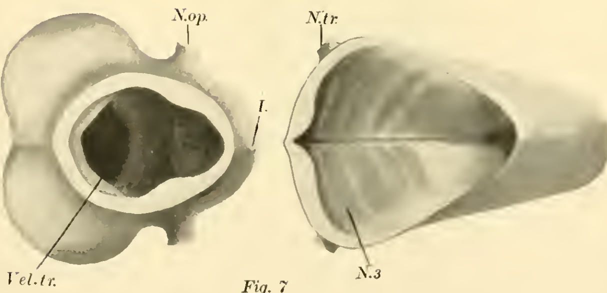

Fig. 7

PLATE 2

EXPLANATION OF FIGURES

8 Dissection of the brain of a 17–mm. pig. × 10 diam.
9 Cast of the cerebral ventricles of a 17-mm. pig × 10 diam.
10 Dissection of the brain of a 17-mm. pig. × 10 diam.

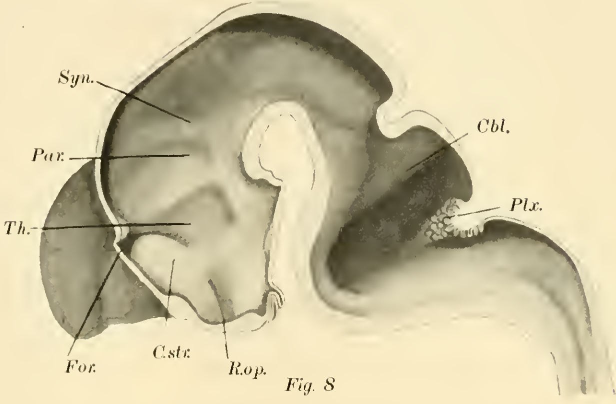

Fig. 8

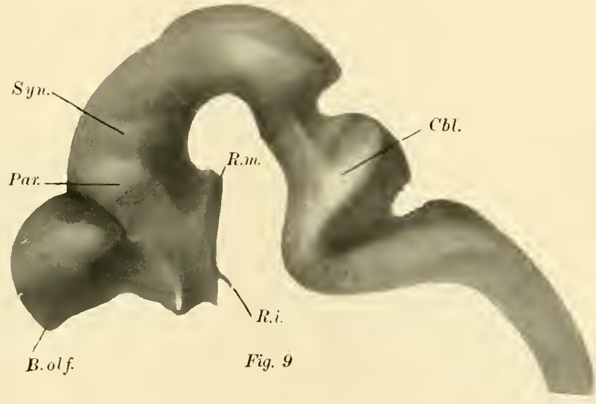

Fig. 9

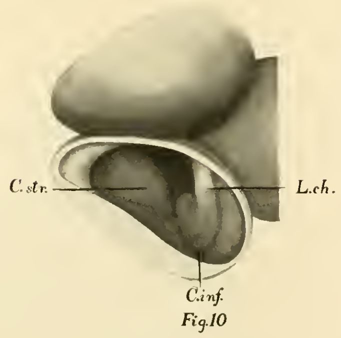

Fig. 10

PLATE 3

EXPLANATION OF FIGURES

11 Dissection of the brain of a 22-mm. pig. × 10 diam.
12 Cast of the cerebral ventricles of a 22-mm. pig. × 10 diam.
13 Dissection of the brain of a 22-mm. pig. × 10 diam.

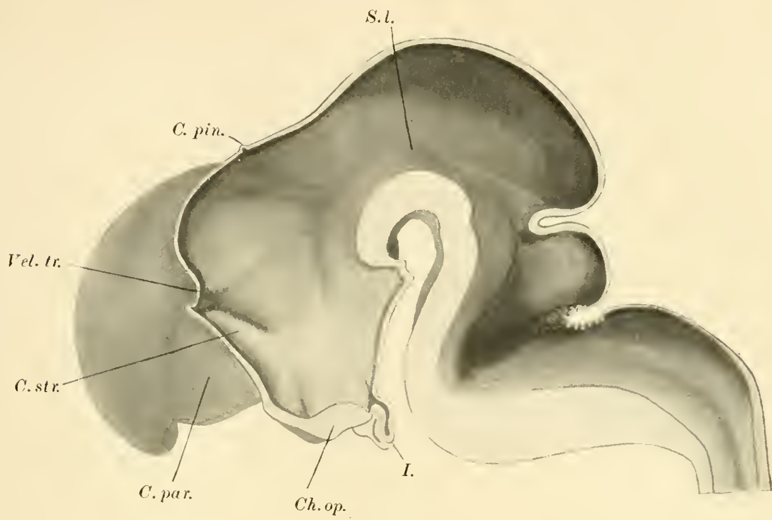

Fig. 11

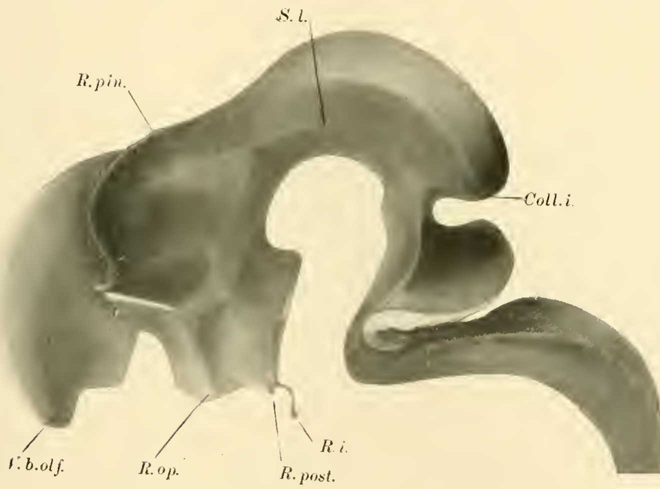

Fig. 12

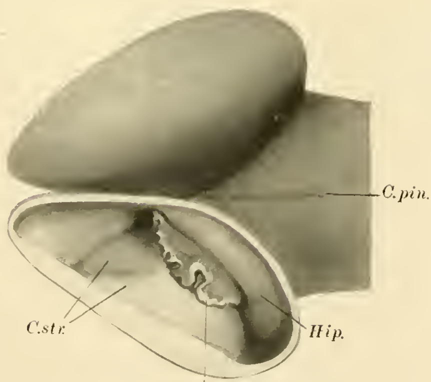

Fig. 13

PLATE 4

EXPLANATION OF FIGURES

14 Dissection of the brain of a 45-mm. pig. × 6.5 diam.
15 Wax model of the cerebral ventricles of a 45-mm. pig. × 6.5 diam.
16 Left lateral ventricle of the model shown in Fig. 15.
17 Ventral view of the same ventricle.

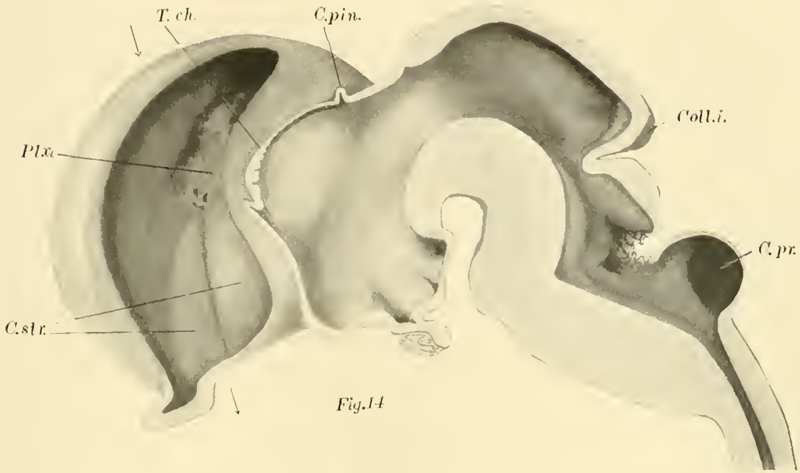

Fig.14

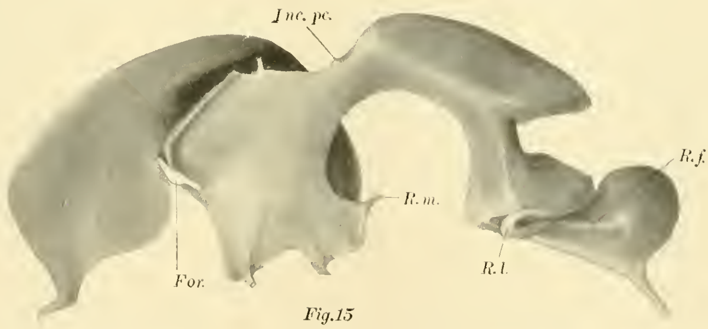

Fig.15

Fig.16

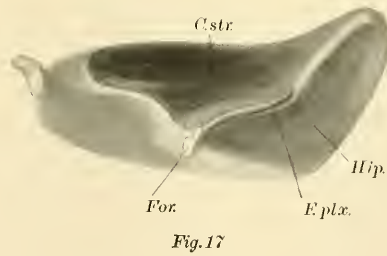

Fig.17

PLATE 5

EXPLANATION OF FIGURES

18 to 23 Microphotographs of frontal sections through the head of a 45-mm. pig. H.E.C. series 1826. × 5 diam. The position of section 563 is indicated by arrows in figure 14.

18 Section 563.
19 Section 629.
20 Section 687.
21 Section 755.
22 Section 1067.
23 Section 1115.

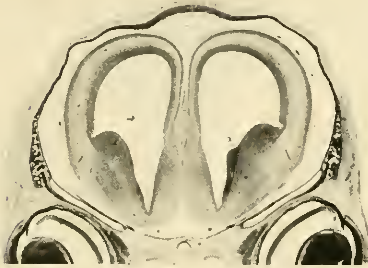
Fig. 18

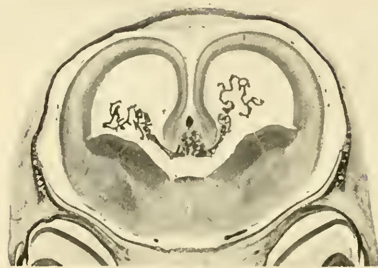
Fig. 19

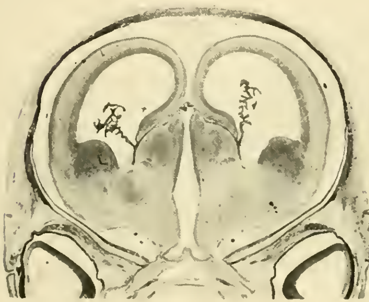
Fig. 20

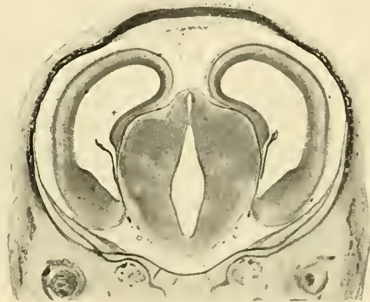
Fig. 21

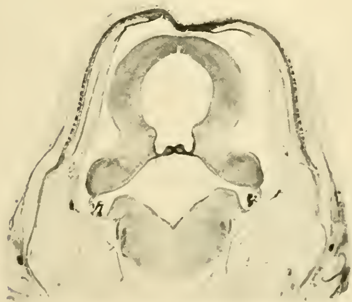
Fig. 22

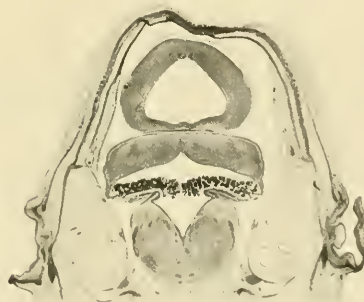
Fig. 23

PLATE 6

EXPLANATION OF FIGURES

24 Wax model of the mid-brain ventricle of a 110-mm. pig, viewed slightly from below and behind. × 15 diam.

25 Wax model of the cerebral ventricles of a 260-mm. pig. × 3 diam.

26 Ventral view of the same model.

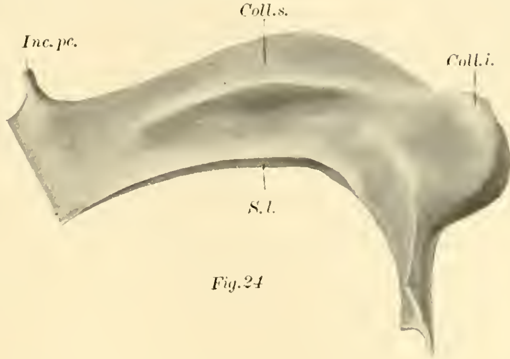

Fig. 24

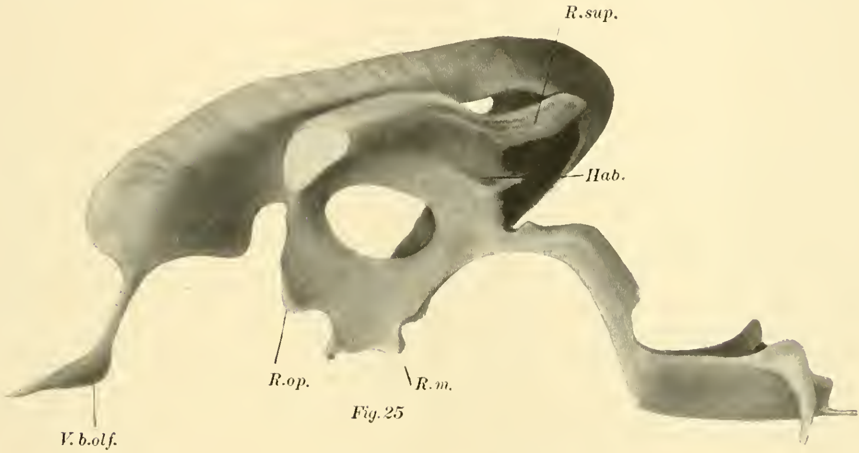

Fig. 25

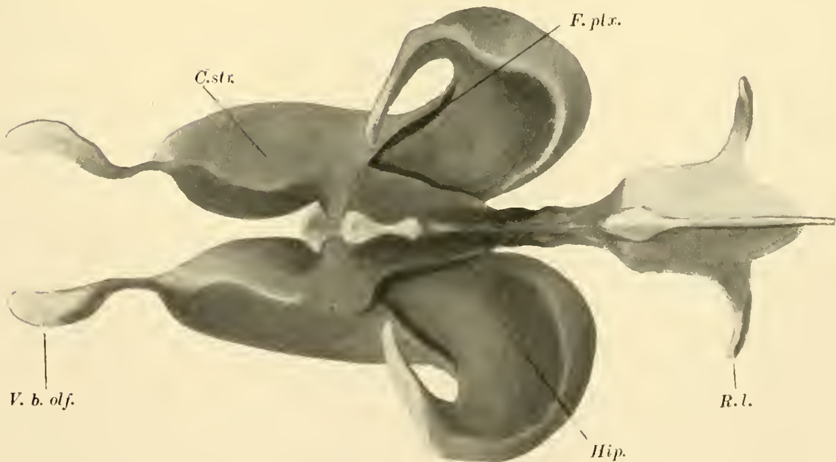

Fig. 26

AN EXPERIMENTAL STUDY OF THE POSITION OF THE OPTIC ANLAGE IN AMBLYSTOMA PUNCTATUM, WITH A DISCUSSION OF CERTAIN EYE DEFECTS

CHARLES R. STOCKARD

Department of Anatomy, Cornell University Medical College, New York City

NINE FIGURES

INTRODUCTION

Numerous investigations have recently been directed towards an analysis of the processes concerned in the development of the vertebrate eye. Both mechanical and chemical methods of experiment have been employed and at the present time most of the results seem open to explanation. Various theories and speculations, however, have been advanced which will probably form a source of contention until the experimental results are better or more uniformly interpreted.

By way of introduction to the experiments recorded in the present paper and a discussion of their significance, it may be well to outline briefly the status of the main problems concerned.

The writer has previously recorded the results of experiments bearing upon an analysis of the manner of origin and development of the optic cup and the optic lens. It was demonstrated that in the species studied the crystalline lens can arise entirely independent of any influence from other eye parts. It seems also equally clear from the many cases observed that the optic vesicle or cup can at some stage during its development induce the formation of a lens from the ectoderm with which it comes in contact,

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even though this ectoderm is not exactly that from which the normal lens would have arisen. Lenses were induced to arise only from head ectoderm and independent lenses invariably arose in the head region, it was thus concluded that the head ectoderm principally possessed the independent lens forming power.

A consideration of numerous eye abnormalities which occurred in these experiments seemed to throw light on the earlier conditions of origin of the optic anlage from the medullary tissues. A number of the abnormal eyes appeared to lend themselves to a common interpretation and for this reason I advanced in an entirely hypothetical manner the view that these conditions might be considered as arrests in eye development.

Spemann in two more recent contributions to the development of the eye has confirmed all of my observations that were touched upon by his experiments. In an equally general manner he has disagreed with most of the deductions which were drawn from my experimental results. An attempt to satisfy these disagreements will be made in the body of the present paper.

It is now concluded from a study of abnormal eyes and the experiments below that the eye anlage in the medullary plate is primarily median and single and normally separates or spreads into two almost equal growth regions which develop in lateral directions reaching further and further out until finally the optic vesicles come in contact with the ectoderm at the sides of the head. Provided this view is correct cyclopia is then an arrest in eye formation.

Spemann, on the contrary, holds that the eye anlagen originally arise lateral in position along the borders of the medullary plate. The cyclopean defect according to him is due to a failure of central medullary tissue to develop so that the lateral eye anlagen slump towards the median plane, *fuse* and form a single cyclopean eye. Spemann, however, presents no experimental evidence to show that the eye anlagen do occupy lateral positions since all of his operations included the median medullary tissue as well as the lateral.

The only evidence to draw upon is the interesting experiments of Lewis, in which it was found that cyclopia sometimes resulted in Fundulus embryos when the anterior end of the embryonic shield was injured by pricking. Lewis also interpreted these defects as due to a fusion of the optic anlagen, and had suggested, as Spemann now does, that the chemicals used in my experiments suppressed the development of median tissue in the medullary plate and thus caused the eye anlagen to come together, fuse and produce cyclopia. There are a great many strong objections to this hypothesis of Lewis and Spemann which have been enumerated in my previous papers and to which I shall take occasion to refer briefly in the following discussion.

An objection of primary importance to the idea of cyclopia as a result of the coming together of lateral anlagen through a failure of intermediate tissue to form is the fact that cyclopean eyes are rarely in size and extent equal to the sum of the two normal eyes combined. A cyclopean eye is, as a rule, little if any larger than one normal lateral eye and in fact is often much reduced or actually minute in size as compared with a normal eye. This fact indicates most decidedly that eye material, as such, has been injured or arrested in its development and differentiation. One is then scarcely warranted in assuming that the defect is solely due to a failure in formation of material between the eyes.

Spemann has found, although he locates the anlagen in the wrong place, that not only is the eye anlage definitely localized in the open medullary plate but actually the tapetum nigrum is distinct from the other retinal layers. How then could absence of material between the *lateral* eye anlagen cause less eye material to arise?

The differentiation of these prelocalized anlagen require definite amounts of energy. Any treatment that weakens the developing embryo at certain periods in a definite way renders the eye anlagen incapable of differentiation so that they do not arise from the brain.

The entire problem is readily open to experimental test. The contention may resolve itself into the question: Where are the

optic anlagen originally located in the medullary plate or tube? The present communication will present the results of experiments aimed towards an answer to this question. Certain interpretations put forward by Spemann regarding the origin and development of the primary components of the eye will also be considered.

MATERIAL AND METHOD

The material used in all the experiments has been the developing embryos of the salamander, Amblystoma punctatum. Amblystoma eggs are surrounded by masses of jelly-like material from which they may readily be separated. The eggs live perfectly without the jelly mass, provided they are well covered with fresh water.

These eggs are of sufficient size to render it possible to cut out with fair accuracy definite regions or groups of cells from the medullary plate or groove.

The method of experiment has been entirely by mechanical operation. This method is particularly useful for the problem in hand since definite areas may be removed and the results studied. The operations were made under a binocular microscope. Fine steel needles and the smallest scissors were used as instruments.

The embryos were kept from three to five days after the operation in water which had been previously boiled. They were killed in a mixture of one part formalin to three parts saturated corrosive sublimate, left for three hours, rinsed in water and put into 70 per cent alcohol with iodine. Others were fixed for three hours in Bouin's fluid, formalin and picroacetic. The sectional embryos were stained with Delafield's hematoxylin and eosin.

CONSIDERATION OF THE EXPERIMENTS

1. Sticking the future brain portion of the medullary plate with needles

The anterior portion of the medullary plate was stuck with steel needles in such a manner as to disturb the cells over an area extending from the anterior border of the medullary fold back to the constricted portion of the plate and laterally from fold to fold. The needle was inserted below the outer layer of cells and raised so as to push the cells apart; this was done a number of times with each specimen. The needles were also swung to the right and left in the medullary tissue until the cells were considered to be disturbed to quite an extent. The object in such an experiment was to determine how severe an injury to the cells was necessary in order to prevent the development of the optic vesicles.

Twenty-three embryos were treated in this manner, and all were killed four days after the operation. Under a high power binocular microscope most of them distinctly showed that the optic cups were well pushed out laterally and in contact with the ectoderm at the sides of the head. Both eyes were clearly seen in seventeen of the individuals, while six seemed to have eyes yet not so well developed. These six doubtful specimens were sectioned and studied microscopically.

Both eyes were present and apparently normal in structure in five of the six embryos. The sixth individual showed eyes which were slightly irregular in form and poorly developed, yet both eyes were distinctly present.

The experiment would indicate that a disturbance, of the type employed, of the cells constituting the eye anlage in the medullary plate was not sufficient to prevent the normal development of the eyes in these embryos.

Another point of interest might have been attacked by such an experiment provided the embryos had been allowed to develop sufficiently long after the operation. That is, whether or not the cells destined to form the tapetum nigrum layer might be

intermixed with those cells destined to form other retinal layers, and so produce an eye with the pigment cells scattered irregularly through the retina. Spemann's recent experiments on cutting out and reversing certain areas of the open medullary plate indicate that the tapetum cells are fully localized and separate from the other retinal cells in certain amphibian embryos. The embryos in my experiments had not differentiated the retinal layers sufficiently far to determine with certainty whether there was a persistent disarrangement of the cells, yet the general appearance of the eyes seemed perfectly normal.

2. The median region of the anterior part of the medullary plate cut out, reversed and transplanted in the medullary plate

This experiment is similar to those performed by Spemann on several amphibian embryos. Spemann found that pieces of the medullary plate when cut out and turned around continued to develop with their original orientation undisturbed, thus indicating the early prelocalization of certain future parts of the brain and eyes. When the operation chanced to cut the eye anlage so that part of the future eye material was anterior to the cut and remained in position, while part was contained in the cut-out piece which was then turned around and transplanted, carrying the future eye cells to a more posterior position, two eye regions developed on each side. One arose from the anterior undisturbed cells and the other from the transplanted posterior cells.

The reversed pieces in the present experiment were not long enough to carry the eye back to a distant posterior position, and the cut extended so far foreward that the eye anlage was not divided transversely as in Spemann's operations. The operations were done chiefly to test whether the eye anlage in Amblystoma was well localized and would develop after such reversal of tissues.

Eight embryos were studied after having had antero-median pieces of the medullary plate cut out, reversed and transplanted. Seven of the eight developed both eyes, many of which showed

indications of their misplacement. One individual showed one abnormal eye while the other was probably indicated by a doubtful eye structure situated within the archenteron. The general structure of the eyes seemed normal and only slight indication of their reversed origin was shown by a study of these early stages.

Thus if the eye anlage was contained within these cut-out pieces the reversal and transplantation of the pieces did not show any very detrimental effects on the future development of the eyes, though in one of the eight cases one eye was abnormal and the other was greatly misplaced and indistinctly indicated.

The experiments of sticking and disturbing the cells in the anterior end of the medullary plate without actually removing the cells does not prevent the subsequent development of the optic vesicles in an apparently normal manner. Cutting out rectangular pieces of the medullary plate which contain the eye anlage, reversing and replanting them merely cause the eyes to develop in misplaced positions. These two experiments demonstrate the fact that unless the future eye material is well removed by the operation the optic vesicles may later form; this is important in estimating the value of the results in the experiments to follow.

3. Lateral regions cut from the anterior part of the medullary plate

Four embryos were operated upon, as shown in figure 1. The indicated region of the right lateral portion of the flat medullary plate was cut entirely away with fine scissors. The area removed did not extend quite to the median line except that in one case it may have or probably did remove tissue beyond the median line. The embryos were killed three days later, cut into sections and studied microscopically.

One of the four lacked both eyes or had eyes so small and poorly formed that they could not be recognized, and the brain showed on one side indications of the operation. Both eyes had thus been removed by an operation which extended at most

slightly beyond the median line and certainly did not include the left lateral medullary tissue.

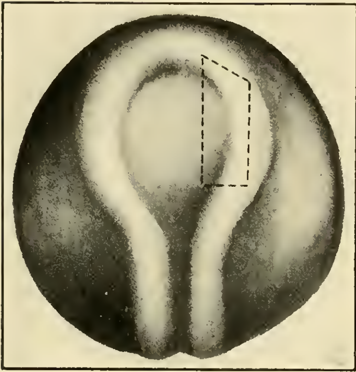
The other three embryos had both eyes present, though one eye in one specimen and both eyes in another were abnormal or defective. In these cases the cut did not extend close enough to the median line to remove all of the eye substance of that side; yet if the eye anlagen at this time had been lateral in position, one would have been completely removed.

A similar experiment was performed on seven somewhat earlier embryos. In these cases where the medullary plate was younger and wider in extent the removed area was confined to a lateral position and did not extend close to the median line, yet it extended laterally into the medullary fold (fig. 2).

Three days later the embryos were killed. On studying the sections of the head region *six of the seven embryos were found to possess two perfect eyes of normal proportions.* The seventh individual had only one well defined eye while the other eye was absent.

The removal of this lateral area of tissue which has been considered the position of the early eye anlage by certain investigators gives no effect on the development of the future eye unless the cut be made to extend very close to the median line. *In eleven operations of this type nine individuals did not suffer the loss of either eye,* while one specimen lacked both eyes and another one had only one eye. The case in which both eyes were absent might have been due to the fact that the cut extended a little way beyond the median line and removed cells destined to form the material of both eyes. Experiments recorded below would indicate that this was the case. A second possibility is that the operation so weakened the individual—which may have been below normal in vigor, though apparently perfect specimens were selected for all the operations—that it lacked the power to differentiate the eyes from the medullary tissue.

Six of the nine specimens which possessed both eyes showed no effects of the removed material in either the size or form of their eyes. The other three embryos had one or both eyes some-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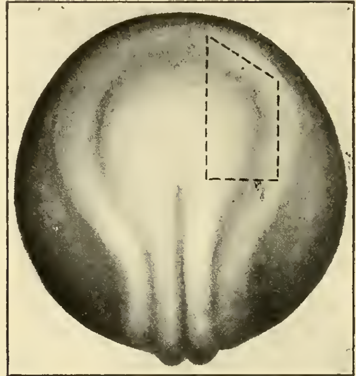
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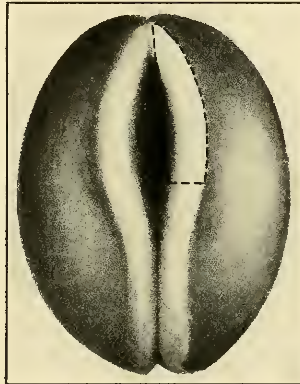
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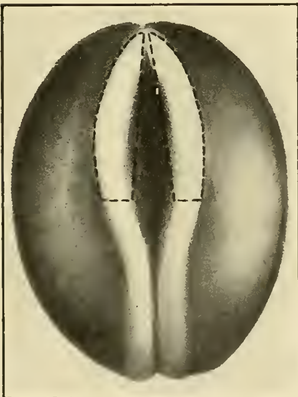
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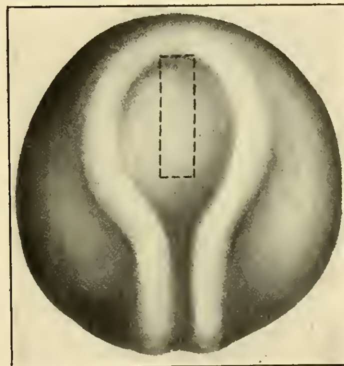
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what smaller than usual. The cases with only one eye defective are perhaps clearly understood on the ground that the cut encroached upon the material destined to take part in the development of this eye. In the cases with both eyes defective it scarcely seems possible that the opposite eye could have been injured by these cuts without entirely removing the eye on the side where the cut was so extensive. The only plausible possibility is that the material for both eyes was medially located and the two eye anlagen closely connected. Any injury to this region might later be shown by both eyes if the injury were at all extensive, and if less extensive and confined entirely to one side of the median line only one eye might show the effect.

I realize that this explanation might be interpreted in part as opposed to Spemann's experiments in which he found the future eye materials so consistently definite in the medullary plate. When the eye anlage was cut transversely and the posterior portion reversed and planted in a distant position it was found that if the forward part of one eye was small the part which had been cut from this and placed posteriorly was large. This fact does not indicate entirely that the eye stuffs are separate; only that when the eye anlage, even if it be median and single, is cut across obliquely and the posterior part removed and transplanted it continues development in a normal consistent manner, the small eye part coming off on the side where less material exists and vice versa.

4. The anterior lateral part of the neural fold and a portion of the medullary wall cut from one side

These experiments were performed on embryos older than those used above in order to test whether the eye anlagen shortly before the coming together of the neural folds might be situated laterally along the border of the folds. In other words, do the eye anlagen primarily arise in the median line or region of the medullary plate and later occupy more lateral positions?

The operation consisted in cutting away one side of the anterior part of the medullary fold and also including in the removed

part some of the material constituting the lateral wall of the medullary groove (fig. 3).

Four embryos were operated upon in this manner. Three days after the operation they were killed and prepared for study. A careful examination showed that three of the four specimens possessed both eyes, while one had no eye, or a questionable formation, on the operated side although a perfect eye was present on the unoperated side. Thus in three-fourths of the few cases used, neither of the future eyes had been injured by the operation.

Six other embryos were operated upon in a similar fashion. These were killed two days after the operation. Studying the six embryos in cross section it was found that three possessed both eyes in perfect condition, one individual had two eyes, yet one eye was small and defective on the operated side, and two of the specimens had no eye on the operated side. Thus four of the six specimens had two eyes each, and two of the six had only one eye each.

Combining the results of both experiments it is shown that, *of the ten operated embryos seven possessed both eyes and three lacked an eye on the operated side.* One of the seven which had both eyes present showed a defective eye on the operated side.

A comparison of these experiments with those recorded in the preceding section in which the operation was performed on earlier embryos would indicate that the eye anlage has possibly widened out so as to extend into more lateral regions in these later stages. The weight of evidence, however, would indicate that the position is not directly lateral along the edge or border of the medullary plate, as Spemann has assumed.

It must be remembered that in the folding process which converts the medullary groove into a tube the original borders or edges of the medullary plate come to meet in a middorsal line. After the medullary tube is thus formed the optic vesicles push out from a lateral or really ventro-lateral region and certainly do not in any sense come from the original borders of the medullary plate which are now dorso-median in position.

5. Both anterior lateral parts of the neural fold cut away just before the folds close together

The operation consisted in clipping away with scissors the lateral wall of the neural groove from both sides; that is, the raised folded parts and the dorsal portion of the lateral neural wall indicated in figure 4. The actual crest of the neural folds is of course not future lateral material as in the closure of the furrow to form the neural tube the crest becomes dorsomedian as just mentioned.

Four embryos were successfully operated upon in the given manner. Three days after the operation they were killed and prepared for study. All of the embryos were found to possess both eyes in well developed conditions.

Five other embryos in a similar stage of development were operated upon so as to remove the lateral neural folds as indicated in figure 4.

Four days after the operation the embryos were killed. Studying these embryos in section showed that four of the five possessed both eyes in a well formed condition giving no indication of the injury while the fifth individual possessed only one eye. This last specimen was evidently cut too near the median line on one side so that the future eye material of that side was destroyed.

Thus in nine specimens with both sides operated upon, eighteen operations, only a single eye was missing. The other seventeen operations did not cause any noticeable effect in either the development or nature of the resulting eye.

These experiments clearly demonstrate that the eye anlage is not located along the lateral edge or border of the medullary plate or groove, as Spemann holds. The importance of such facts in connection with opposing explanations of certain eye abnormalities will be fully considered in a following section of this paper.

The next experiments to be presented were performed in order further to substantiate the fact that the eye parts do occupy a

median position in the medullary plate or groove. I have been led to think from a study of a number of eye abnormalities that the eye anlage is more or less median in its original position.

6. The removal of anterior median strips of cells from the wide medullary plate

In these operations a narrow median strip was cut from the anterior region of the wide medullary plate. The strip in all cases was narrow, being in fact only about one-third or one-fourth the entire width of that portion of the medullary plate lying between the medullary ridges, as shown in figure 5.

Four embryos with wide medullary plates were operated upon in the above manner. Four days later they were preserved for study.

Three of these four specimens were eyeless, showing that the operation had removed the entire eye anlage from the *middle* of the medullary plate. The remaining one of the four embryos had one poorly formed eye while the other was only slightly indicated, so that in this case almost all of the ability to develop eyes had been lost as a result of the operation.

Five other embryos were subjected to a similar operation to that indicated in figure 5 except that the median strip removed was still narrower than in the preceding experiment. These embryos were of different stages but all showed the medullary folds far apart so that the medullary groove was wide open. Three days after the operation the embryos were prepared and studied.

One of the specimens was eyeless, one had two poorly developed eyes, two had one well formed eye while the other eye was questionable or absent, one had both eyes present. Thus the removal of a *very* narrow median strip gave less decided effects than the removal of somewhat wider strips in the experiment above. Yet four out of the five embryos showed eye defects, two having both eyes affected and two having one normal eye and one eye questionable or absent.

Combining the results of the two experiments it is found that the removal of narrow median strips from wide medullary plates exerts the following influence on the future development of the eyes: *Of nine embryos thus operated upon four failed entirely to develop either eye. Two showed two defective eyes. Two individuals developed one perfect and one defective or questionable eye. Only one of the nine embryos showed two apparently normal eyes.*

Since six of the nine embryos had the development of both eyes either entirely suppressed or decidedly affected, and two of the remaining three had one eye affected, it seems most certain that cells destined to take part in eye formation are located in the median region of the medullary plate and are removed by the operation employed. One must conclude that the median optic anlage occupied at least one-fourth or one-third of the width of the medullary plate in the anterior region.

A general statement of the results of certain of the experiments described above may be expressed as follows: Thirty embryos studied after various operations in which lateral portions of the medullary plate were removed at slightly different developmental stages (sections 3, 4, 5) showed in twenty-four individuals, or 80 per cent of the cases, subsequent development of both eyes, while only six specimens or 20 per cent of the cases, showed absence of the eye. In one case the presence of the eye is questionable, in five cases one eye and in one case both eyes were absent. The absence of eyes in the latter cases is possibly due to the cut having been made in a more median position than was intended.

Nine embryos studied after having been operated upon so as to remove a narrow median strip of cells from the anterior portion of the medullary plate (section 6) showed in four cases, or about 45 per cent of the specimens, entire absence of eyes. In four other individuals the eyes were highly defective, one specimen having one poorly formed eye while the other was questionably present. In only one of the nine embryos did both eyes approach the normal condition, from this specimen an extremely narrow piece had been cut away. The optic anlage in this case might have been sufficiently wide at the time of the

operation to allow its median portion to be removed and yet enough material remain on either side of the cut to give origin to the two eyes. According to the views of several investigators the removal of this median material should have caused cyclopia, yet it did not. I shall presently attempt to show that cyclopia is not due to the coming together of lateral material in the median line but to a failure of median material to spread laterally.

When the results of these two classes of experiments are contrasted one must conclude that: *The eye anlage in the medullary plate occupies an antero-median position as shown by the various abnormalities incurred when this region is cut away. The failure to injure the development of the eyes in the great majority of cases when the lateral portions of the medullary plate are removed by operation indicates further that the eye anlagen do not occupy lateral positions during this stage of development.*

A DISCUSSION OF PREVIOUS IDEAS REGARDING THE GENESIS OF CERTAIN EYE ABNORMALITIES IN THE LIGHT OF THE ABOVE RESULTS

There has lately been considerable discussion regarding the way in which the cyclopean defect occurs. The experiments described above may serve to concentrate the case on a definite developmental period and, to my mind, settle the question as far as the medullary plate stage is concerned. Whether it is possible to carry any question of vertebrate eye abnormalities, as such, further back in development than this stage seems doubtful, since here it is that the localization of the eye anlage is first known to exist. It is certain, of course, that this anlage does come from cells which are present before the medullary plate has formed. Whether these cells are localized and are entirely future eye anlagen cells, and not indifferent ectoderm cells which might have the power of forming any portion of the brain or central nervous system, one is at present unable to state.

As the case stands, it seems possible to explain the entire genesis of the cyclopean defect from the earliest time at which the optic anlage is definitely known to be localized. This as-

sumption is not made solely from the material presented in the present paper, but from the facts furnished by these experiments together with the observations made upon the large number of cyclopean eyes and brains which the writer has studied during the past several years.

Various authors have at different times thought that cyclopia was due to a fusion of the eyes after they had arisen from the brain. The earlier in development the fusion occurred the more intimately associated the two eye components became. This view has been proven incorrect by actual observation on cyclopean monsters where it is found that the cyclopean condition of the eye, whether large and hour-glass shaped or of small size resembling a normal eye, is present from the earliest appearance of the optic vesicle from the brain. In other words, the several degrees of the cyclopean eye come off from the brain in their final conditions.

The idea of the fusion of the eye parts was deep rooted, however, and now exists in the recent views of Spemann in a refined form. Spemann believes, as several others had previously suggested, that cyclopia is due to an absence of non-ophthalmic tissue in the median region of the medullary plate or groove. This lack of median tissue allows the eye anlagen which he holds to be lateral in position, near the borders of the medullary plate, to come together and fuse in the median plane and later give rise to a cyclopean eye. Cyclopia, according to this idea, occurs in a more or less passive manner, and is, after all, actually a fusion of the eye anlagen of the two sides during development.

I am certain that this fusion explanation which has now been forced entirely back into the medullary plate, is as false as its bolder predecessor which assumed the fusion to take place outside of the brain tissues after the optic vesicles or cups had arisen. Spemann did not advocate this late-fusion view, but claimed from his beautiful experiments on Triton that the cyclopean eye arose out of the medullary tissues in its final condition. He now, however, assumes the rôle of a most ardent supporter of the fusion of the optic anlagen within the medullary plate.

The present writer's opinion of the cause of cyclopia, which was advanced in 1909, was to the effect that this deformity is the result of a weakened development. Spemann has termed this view the 'laming hypothesis,' since my assumption was that the various chemical substances employed in producing cyclopean defects in fish embryos have a tendency to lower the developmental energies and so reduce the power necessary to accomplish the processes involved in the outpushing of the optic vesicles from the brain.

Considering the probable manner in which the cyclopean defect occurs, Adami ('08, p. 241) has theoretically concluded that it is due to developmental arrest or lack of vigor. While I am unable to agree with the details in Adami's argument of a primary growth point at the anterior tip from which is budded off successively the paired parts of the two sides, the anterior ones necessarily arising last after the other parts had been left in more posterior positions, the final conclusion that a weakening of particular developmental processes results in cyclopia is confirmed by all my experiments.

The different types or degrees of the cyclopean defect depend upon the stage in development at which the arrests occur as well as upon the strength or severity of the treatment employed. I shall now attempt to defend this position with the evidence at hand, and in so doing shall as decidedly prove the mistake in considering the defects as the result of any failure to arise of median medullary tissue (other than future eye tissue) and the subsequent fusion of the *lateral* optic anlagen. There is no median tissue between the eye anlagen. The median tissue is the eye anlage itself and will subsequently go to form some portion of the eye, either optic cup or optic stalk, depending largely upon its position and the extent of normal development attained.

The writer had ('07, '09, '10) recorded a number of eye conditions which are considered to be different degrees of cyclopia using the term in a general sense. At any rate, these several conditions differ only in degree and grade perfectly into a continuous series. There is no qualitative difference between them. Spemann has objected to including among these defects certain

modified types which are at least closely related to the series in manner of origin, though they may not actually intergrade. (We are now considering only the cyclopean series, not the monster monophthalmica asymmetrica which will be dealt with later.)

One finds on referring to my paper of 1909 (p. 293) that the cyclopean series, A to G, passes from the normal individual through different degrees of association of the two eyes to a median cyclopean eye only as large as one normal lateral eye, then to a cyclopean eye of smaller dimensions until it is extremely minute and may finally be deeply buried beneath the brain as a small pigmented vesicle, as is shown in figure 52, page 321. Only one step further and the eyes fail to arise entirely so that eyeless individuals exist which with a slightly greater power of differentiation or more developmental energy might have given cyclopean monsters. The last assumption is warranted since these eyeless specimens actually resemble the cyclopean monsters in other structures; for instance, the mouth is a narrow proboscis similar to that in the cyclopean monster instead of the usual laterally spread mouth of the normal embryo.

Why should every step and gradation in this series exist if several, or any of the conditions are of a different quality or type? It seems certain that one examining the large number of cyclopean fish on which my study was based would be forced to admit the correctness of the statement that these individuals exhibit different degrees of one and the same defect.

The question then follows, if cyclopia were due, as Lewis, Spemann and others assume, to a failure to develop of median medullary tissue so allowing the eye anlagen to come together in the median plane and fuse, why is not every cyclopean eye equal in mass to the two normal eyes fused? Spemann does not suggest in any place that eye material also fails to arise. He shows in his recent experiments that the future eye is fully laid down in the medullary plate. Not only is the eye present in the medullary plate but the cells destined to form different layers are distinct. Spemann found that certain cells cut out of the medullary plate and planted in more posterior positions formed only the tapetum nigrum layer. If the eye is thus so

definitely predetermined in the medullary plate and Spemann believes that cyclopia is due to failure of median medullary cells other than future eye cells then cyclopean eyes ought always to be large or double in size.

The fact is that these predetermined eye cells in the medullary plate in most cases of cyclopia are incapable of perfect differentiation on account of insufficient energy, so they remain in the brain, or only part of them is capable of differentiation and thus small defective cyclopean eyes result.

The actual 'Lähmungs' or suppression is of the eye material itself. *Cyclopia is an eye defect,* and an injury of the eye forming material is the cause. The brain may also be defective as an accompaning abnormality, although in some cases the brain, with the exception of the optic tracts and parts, may be structurally and functionally perfect, as is indicated by the normal life and behavior of many of the cyclopean Fundulus embryos as well as by the existence of the huge cyclopean ray, Myliobates noctula, reported by Paolucci in 1874.

The chemical substances employed by the author in producing the cyclopean defect and a number of others with which McClendon has obtained similar results, all tend to suppress or arrest the development of the eye material in the brain. This future eye material is assumed to occupy a median position. When the arrest is complete, and necessarily taking place at early stages, no eye parts arise from the brain nor are any differentiated within the brain substance itself. Thus a completely eyeless individual is produced.

Spemann states that the eye is capable of differentiation even though it be contained within the brain substance as he has found in amphibia and as Mencl recorded in a teleost. It must be realized that these are *exceptional cases.* A certain amount of energy is necessary for differentiation of the eye to take place even within the brain and when only this amount of energy is present the eye may differentiate within the brain, but when the required energy for any reason is not available the eyes are incapable of any differentiation. Many eyeless individuals have been observed in my experiments which have no indication what-

ever of eye parts within the brain. Could any one ask, whether this be due to the failure of non-ophthalmic parts of the medullary plate to arise or, on the other hand, to a failure of the future eye forming cells to arise, or to differentiate after they have arisen?

It is not meant to convey the idea that all eyeless brains are related to the cyclopean series, as this is not the case. The future eye forming cells may in some cases have been absent from the start. In other specimens the future optic vesicles might have been in positions to arise normally and laterally and for some reason were incapable of outpushing or of differentiation.

Certain eyeless brains, however, such as those in individuals having the proboscis-shaped mouth, do belong to the series and must be caused in the same way as are the various cyclopean conditions. They have merely responded to a more exaggerated degree.

The most extreme cases of cyclopia with actual eye structures are those in which a small pigmented vesicle arises from a ventromedian part of the brain, as is shown in my figure 52, 1909. This pigment layer of the retina seems to be its most persistent portion, as it may appear when all other recognizable retinal parts fail to arise. There is a possibility that the small tapetum nigrum groups of cells which in some cases formed from Spemann's transplanted portions of eye anlagen may not be due to the fact that only the anlagen of such cells were transplanted, but that all other retinal cells except these were incapable of differentiation when so small a piece of future eye tissue was isolated by the operation.

The next degree of cyclopia is exhibited by an individual having a median eye that is much smaller than one normal lateral eye. We then have a median cyclopean eye of about the same size as one normal lateral eye. The latter case has been termed the 'perfect' cyclopean condition.

All these abnormalities are best explained as follows: The future eye forming cells occupy a median position in the medullary plate and the cells destined to form the two eyes are arranged in one group. This median group of future eye cells

normally widens or spreads laterally while two centers of active growth become established which gradually assume more lateral positions until they push out as the two optic vesicles. In the degrees of cyclopia mentioned in the preceding paragraphs the median eye anlage does not widen or spread laterally but is arrested in its primary condition; thus the two growth centers are not sufficiently separated and only a single center exists, and even more than this, the arrest is to such an extent that the entire or normal amount of optic material does not differentiate. Hence, one finds a median cyclopean eye consisting of an amount of eye material far below that normally present.

Other individuals are found in which greater masses of eye material have succeeded in differentiating, and development has been vigorous enough to allow the early eye anlagen to spread to a greater or less degree and establish the two eye forming centers. Such specimens finally present a cyclopean eye showing distinctly its double composition, the two retinae are more or less distinct and the eye large consisting of a greater amount of material than a normal lateral eye, yet less bulky as a rule than the sum of the two normal eyes of the species.

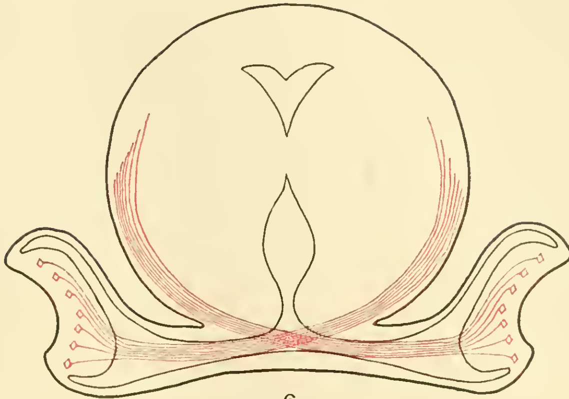
The hour-glass eye or incomplete cyclopia is commonly observed in the experiments. This case is due to a later or less complete arrest in development than those mentioned above. Both eyes have differentiated out of the medullary tissue but the embryo was not vigorous enough to permit their normal separation and outpushing. Thus the eyes come off from their original ventro-median position and remain in close contact or actual union.

Finally one observes individuals in which the eyes are separate and distinct yet unusually close together. Such embryos are able to differentiate their eye material and this material is capable of pushing out from the brain but a slight weakness or arrest has occurred on account of which the optic stalks are short and the optic cups are unable to take a normal position so that they remain unusually near together and look in an abnormal direction.

In the ordinary individual the future eye forming material is first located in a median position in the medullary plate. This material becomes more extensive or widens laterally, and two growth centers are established, the material between the centers finally becomes the median ventral layer of cells of the optic stalks. Later the incipient optic vesicles begin to evaginate or push in a ventro-lateral direction and finally turn dorsally and laterally to reach their usual places at the sides of the head. The optic stalks, however, still lead back to the ventro-median position and there in the fish the optic fibers, following the optic stalks as paths, cross and in higher vertebrates form the optic chiasma always in the ventro-median plane below the brain floor and from here the optic tracts proceed to their centers in the brain.

The median position of the optic chiasma outside and below the brain is an important structural fact in the present consideration. Figure 6 is a diagram of a transverse section through an early brain with the optic cups in their usual position. The optic stalks connect with the brain and the median ventral cell layer is actually part of the stalks. As development proceeds the optic fibers arising from the cells in the retina follow along the optic stalks to reach the brain. The investigations on the development of the optic nerve have shown that the optic stalks become solid and form the supporting paths or neuroglial scaffolding along which the optic nerve fibers grow. The fibers from one retina meet those from the other in the median plane *below* the brain and in the fish the fibers from the two retinae cross directly while in higher forms partial crossing takes place and the optic chiasma is formed outside the brain. Figure 7 is a sketch representing a cross section of the actual condition of the early optic nerves in a fish embryo. This position of the optic cross is only possible if the median tissue be optic stalk tissue.

Suppose, on the other hand, that the eyes primarily originate from lateral medullary tissues and between the two eyes other brain tissue is present. The optic stalks are then attached to the lateral regions of the brain from which the optic vesicles



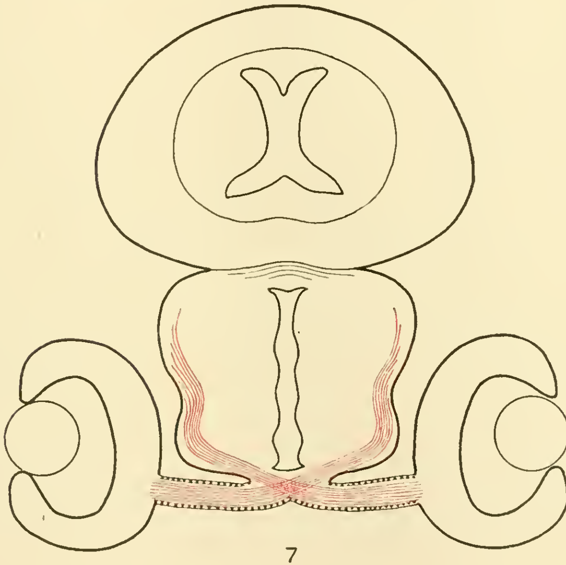


Fig. 6 A diagrammatic cross section through the brain and optic cups of an early embryo, with all of the median ventral medullary tissue represented as being part of the optic stalks. The future fibers of the optic nerves, shown in red, follow the optic stalks to the median plane where they cross and afterward enter the brain to pursue their course as the optic tracts. The optic cross is entirely beneath and outside the brain.

Fig. 7 A sketch representing the actual outlines of a cross section through the brain and eyes of a late fish embryo. The optic nerves cross below and outside the brain and are surrounded by cells derived from the original optic stalks.

pushed out. Figures 8 illustrates diagrammatically a cross section of this condition. In the course of development the fibers of the optic nerve following the stalk reach the lateral position and must enter the brain and continue within its tissue in order to meet the nerve of the opposite side and form the cross or chiasma. Brain tissue would lie beneath the optic chiasma and the chiasma would necessarily be within the brain. This condition is never found in any normal vertebrate.

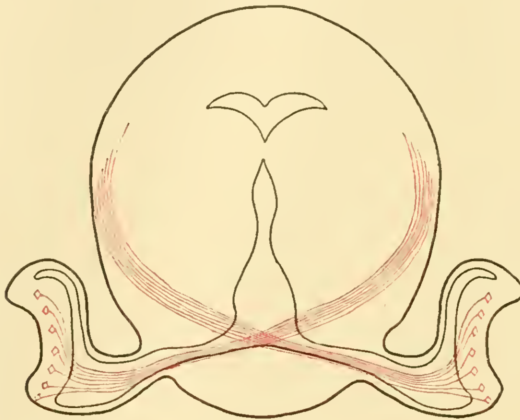


Fig. 8 A diagrammatic cross section through the brain and optic cups in an imaginary case in which the optic connections are lateral with median brain tissue originally lying between the eye anlagen. The future optic fibers growing along the optic stalks as paths reach the lateral points on the brain from which the stalks arose and then enter the brain tissue before having formed the cross. Continuing to grow, the optic nerves meet and cross in the median plane. The cross is within the brain itself and lies above the median mass of tissue which has always existed between the eyes. No vertebrate brain exhibits such a condition.

One might claim that the optic fibers on reaching the brain ran ventrally and formed the cross beneath, but no such change in direction is seen at any stage of their development. The structural relationships seem to depend upon a median origin and connection of the optic stalks.

The fact that a cyclopean eye may have no well formed optic stalk and is entirely median in position not necessitating the

absence of any other brain part is in entire accord with the above facts.

It is believed, therefore, that the various degrees of the typical cyclopean condition from eyes unusually close together, to median double or hour-glass eyes, to the large oval eye, to a median round eye of usual size, to the median eye smaller than one normal eye, and finally to complete failure of eye material to arise from the brain are probably all due to developmental arrest. The arrest in development is the result of some influence which has reduced the developmental vigor below the normal so that the energy is not available to carry out the usual processes of differentiation and growth.

The author has figures and described other cases of eye defects which do not fall exactly into the series of cyclopia as considered above; yet these cases are modified conditions which are closely related to the cyclopean series both in point of origin and in their final condition. The curtain-like eyes which face the median plane and often have a single lens between them might be considered as delayed cases of cyclopia. The eye anlage widened, the eyes became separated and continued their differentiation, yet they were unable to turn out and assume their normal lateral positions so that they faced in a ventro-median direction with their anterior walls closely approximated as is shown in figure 38 ('09) and figures 1, 4, 5, 6, 13, 14 ('10 a). Such eyes often excite a single stimulus upon a more or less ventral ectodermal region which responds by forming a single lens lying between the two eyes.

The writer has illustrated by a diagram (fig. 15, A and B '10 a,) the difference between these eyes with their choroid surfaces against the lateral ectoderm of the head and their concave retinal surfaces facing medially, and the eyes of a normal individual ('10 a p. 380). As was then stated, the experiments did not give a definite clue to indicate the position of the optic anlagen in the early brain, thus my explanation, or 'laming hypothesis,' referred mainly to the pushing out and lateral development of the optic vesicle and cup. All these cases are decidedly

of a type which would suggest lack of developmental energy necessary to attain the normal.

Another group of eye anomalies were extremely common under the same experimental conditions which caused the cyclopean series. These individuals possessed one normal eye in the usual lateral position while the eye of the opposite side in the numerous specimens showed various degrees of imperfection from a condition slightly below the normal in size to complete absence of the eye. Such anomalies were termed 'monophthalmica asymmetrica' in contrast to the symmetrical one-eyed monsters with a median cyclopean eye.

The genesis of the asymmetrical defects is not entirely clear, yet they also are probably due to developmental arrest or suppression of the one eye. The growth centers representing the two future eyes of an individual are rarely equally vigorous and it is frequently noticed that one eye arises slightly before its mate and develops at a little faster rate. It might be that at some critical point in development one of the future eye centers is affected after the growth centers had begun to localize in more or less lateral positions.

In treating the eggs with alcohol a number of embryos occurred in which both eyes were small and defective even though they arose from the brain and attained more or less lateral positions. One might assume this to occur as a result of an arrest in development which affected both eye forming centers after the centers became separate or distinct from one another. Part of the eye forming material is suppressed or its differentiation is prevented so that each eye is decidedly under size and defective. In some of the cases of cyclopia mentioned above the eye was also very small and defective, in these cases the two growth centers which would give rise to the future lateral eyes did not become sufficiently separated so that only a single median eye arose and the reduced vigor permitted this to form only as a small and poorly differentiated structure.

In some instances where an embryo possessed only one member of the normal eye pair this eye was unable to attain its usual

lateral position so that it faced the median plane, and if the lips or periphery of the optic cup failed to reach the ectoderm the eye was without a lens.

In none of the specimens, although many were old with the central nervous tissue highly differentiated, was I able to detect any material within the brain which might be considered as representing the missing eye.

Eye conditions such as these are doubtless due to the action of some inhibitory influence which prevents the complete origin and differentiation of eye material, or when it does arise allows it to develop only in a weakened or defective manner. Since monophthalmica asymmetrica occurs so persistently in the same experiments with cyclopean individuals as Lewis, McClendon and I have all found, it is not improbable that the cause is the same in the two cases.

The above considerations have been entirely from the standpoint that the ophthalmic defects under discussion originate during the medullary plate stage. The cause of cyclopia or the tendency to produce such a defect might of course have its origin much earlier in development. It might, in fact, go back to the germ cells themselves or finally it might possibly occur as an hereditary variation. All experimental cyclopia, however, furnishes evidence directly contrary to the latter possibilities. This point I have considered in a previous paper and have clearly demonstrated as have several other investigators that the condition is not due to a germinal variation but may be induced as the result of external stimuli applied during the early development of the eggs.

The cyclopean abnormality may be caused in Fundulus embryos by subjecting the eggs to various chemical stimuli after they have developed normally for as long as fifteen hours. A fifteen-hour Fundulus embryo has the germ ring beginning to form and descend over the yolk sphere, the embryonic shield is scarcely indicated but appears very soon afterwards. Embryos of later stages subjected to the same treatment develop normally, or do not show cyclopia, while stages younger than fifteen hours and

as early as the first cleavage are much more readily affected in such a manner as to cause the cyclopean defect. The optic vesicles appear at about thirty hours after fertilization, but the stimulus must be applied at a time sufficiently long before this process occurs, since a number of important steps in eye formation are doubtless taking place before the visible signs of optic vesicles are present.

The fact that cyclopia may be produced after the beginning of the germ ring and embryonic shield in the teleost embryo indicates directly that the defect may occur in what would be the medullary plate stage of amphibian embryos. Thus explanations of the cause of cyclopia must consider it as occurring in the medullary plate stage.

Spemann ('12 b, pp. 38–39) has taken exception to my statement regarding the non-occurrence of cyclopia when eggs are treated later than fifteen hours after fertilization (although the optic vesicles do not arise until about the thirtieth hour) "*since insufficient time exists for the substances to act on the eye anlagen.*" The paragraph following this statement in my paper (p. 388, '10a) is: "The solutions are effective up to a stage in development preceding the formation of the germ ring and embryonic shield, and *the action of the Mg on the eye anlagen probably takes place while the embryonic shield and outline of the embryo are forming.*"[1]

This statement seems to me perfectly direct and clear: Yet Spemann intimates in one sentence that I mean to infer that fifteen hours are necessary for the substances used to penetrate the egg membrane! He then states that I showed in 1907 that KCl would penetrate within a few minutes and stop the embryonic heart beat. The Mg and other solutions used may pass through the membrane equally as rapidly, this I have not fully tested. The fact that it does has no bearing on the *fact* that stimuli do not have sufficient time to *act upon the optic anlagen to induce cyclopia* when applied to the eggs at later periods in normal development than fifteen hours after fertilization since they must act on the anlagen in the embryonic shield, and to act later is

[1] The last clause was not originally in italics.

too late. The reason for the nonoccurrence of cyclopia when eggs were treated at periods later than fifteen hours after fertilization or fifteen hours before the appearance of the optic vesicles is that the optic anlagen and all other embryonic parts are constantly changing during development and have passed beyond the critical stage. It is not only a question of how quickly one may stimulate but in what condition the anlagen are at the time of stimulation. It is safe to say that cyclopia can *not* be produced after the optic anlagen have proceeded to some definite stage in their normal development. Even if Mg should penetrate all the membranes within a few seconds its action could never induce cyclopia in an embryo with the two optic vesicles visibly formed. There was no question of the time necessary for penetration, but an important question of the embryonic condition to be *acted* upon, the critical condition of the optic anlagen.

In the above connection it may be mentioned that such substances as alcohol and ether, when administered to an early embryo may cause it to develop into a decided monster. A late embryo or foetus may respond to the same treatment by producing an individual exhibiting no gross morphological defects yet showing decidedly abnormal nervous reactions, while a similar treatment might exert little or no effect upon a fully formed individual. A stimulus could not cause the mature individual to change into a structural monster. The developmental period of administration is of as high importance in determining the result as is the nature of the stimulus used, unless of course the stimulus be entirely destructive.

Again Spemann misinterprets a statement regarding the action of Mg. It was remarked that the cyclopean embryos developing in the Mg solutions were, except for the eye defects, more perfect than those arising from treatments with alcohol, ether, and so forth. The Mg cyclops often had apparently normal brains, could swim in normal fashion, took food and reacted to stimuli much as normal embryos did, while those embryos treated with alcohol and ether had various defects of the brain and cord. In this connection it was cited as of interest that Mayer had re-

cently found in studying nerve muscle preparations that Mg salts seemed to prevent activity by affecting the muscle directly without apparently affecting the nerve. There is, of course, no direct connection between these facts and cyclopia; the mention was made merely in a general way, and most decidedly did not intend to convey the notion that muscle contractibility and the outpushing of the optic vesicles were phenomena of similar nature. They are similar only in that both are dynamic processes and require energy for their accomplishment.

There is no necessity for further discussing the *fact* that a number of eggs when subjected to the same solution do not all respond in a like manner (Spemann '12 b, p. 37). This is a typical case of differences in individual resistance and vigor which is observed among any one hundred individuals of any living species. It is equally true that the two sides of a so-called bilateral individual are rarely, if ever, identical.

Spemann is no doubt correct in stating that the relationship between cause and effect in my chemical experiments on cyclopia is not clear. Yet it seems to me that it is not entirely dark, the entire relationship between cause and effect in biological experiments is rarely if ever clear step for step.

I should like, however, to point out that the chemical experiments did one thing in proving that cyclopia could be caused from normal embryos through the action of the environment. This fact did away with all theories of germinal origin of the defect, one of which was strongly presented by Wilder about the same time. The experiments also make clear the stage in development at which cylopia may occur, and they further supply the richest amount of material yet available for the study of this defect. Finally, they prove to my mind that cyclopia is a developmental arrest and may be due to any cause which lowers developmental vigor at certain critical stages in the formation of the eye anlagen. These important points in the study of this defect have certainly *not* been made clear by the mechanical experiments though I do not deny that they might possibly have been.

To quote again from Spemann:

> Gegen diese Defekthypothese erhebt Stockard (1909b, p. 172) die Fragen: warum sollte bei den Magnesiumembryonen gerade das Gewebe zwischen den Augen ausfallen, und keine anderen Gewebe?; warum sind die Riechgruben bei Cyclopie manchmal verschmolzen und manchmal getrennt?; . . . . ist bei den asymmetrisch-einäugigen Missbildungen der einseitige Augendefekt etwa auf die Abwesenheit der einen ersten Augenanlage zurückzuführen?—Darauf möchte ich mit der Gegenfrage antworten: sind denn all diese Tatsachen verständlicher bei Zugrundelegung der Stockard 'schen Lähmungshypothese?

I should answer now, as it was inferred above, that they most decidedly are, and an attempt to show this fact in some detail has been made in the preceding pages.

The title of the recent paper by Spemann "Zur Entwicklung des Wirbeltierauges" might better have been "The development of the vertebrate lens," as little or almost no attention is given to other parts of the eye. The problem of the lens formation is very fully considered.

"Cyclopia of the lens" is discussed from the same standpoint as cyclopia of the optic cups. In the case of the lens median ectodermal cells may be missing so that the two normally lateral lenses fuse in the median line. It seems to me that this is the one straw too much for the 'Defekthypothese.' The imagination which pictures the falling out of just the exact amount of median ectodermal tissue which would allow the primary lens forming cells of the ectoderm to fuse towards the center and keep proper pace with the movements and final position of the various cyclopean eyes which my Fundulus material presents must be most vividly active. An even more plausible possibility out of this imaginary dilemma is to consider the lens anlage as a single median group of cells that divides into two parts which come later to lie in lateral positions. This view would at least have the advantage that in cyclopia of the eye "cyclopia of the lens" would maintain the lens forming cells in a fairly median region, and in normal development the lens cells would have a more or less definite path to follow and place to reach. The pineal eye in many forms possesses a fairly definite lens which must have arisen medially and the present lens may have been somewhat more anterior yet also median. These suggestions are of the

most speculative nature and are intended merely as such. In my experiments many of the embryos which possess supernumerary lenses show, as Spemann has called attention to, that the accessory lens may actually lie more anterior than the eye. Nevertheless, others, show free lenses in lateral positions.

The presence of a lens in the cyclopean eye is explained by the fact, well established for several species, that an optic vesicle or cup possesses the power to stimulate lens formation from any region of the head ectoderm with which it comes in contact. There would be no necessity of imagining a condition of "cyclopia of the lens" even though a median lens should be observed in an anophthalmous monster. Normal lateral lenses have been observed in anophthalmic monsters (see figs. 1 and 5, and fig. 3, plate II, 1910 b).

The embryo from which my figure 4 ('10 b), was taken is of new interest in connection with a hypothetical case called for by Spemann (p. 81 '12 a). He states as a possibility that

> der cyclopische Defekt nur die Epidermis betrifft, so dass Riechgruben und primäre Linsenbildungszellen median zusammenrücken, während die Augenbecher, wie normal seitlich gelegen, sich ihre Linsen aus der dortigen Epidermis bilden. Durch solche Fälle würden in der Tat an einem und demselben Kopf beide Fähigkeiten demonstriert, die der Linse zur Selbstdifferenzierung und die des Auges zur Linsenerzeugung. Bis jetzt liegen aber derartige Fälle nicht vor.

The case however, was, recorded at the time and seems to fill the requirements set forth. The independent origin and differentiation of the lens is demonstrated in a median position slightly more anterior than the eyes, and the more or less lateral eyes have derived lenses from the ectoderm with which they came in contact as is shown by figs. 9 and 10, 1910 b, yet this ectoderm is part of the usual region from which an optic cup has the power to derive a lens. The power to form lenses, as Speman and I have claimed, is possessed by the ectoderm of the head.

According to Spemann's assumption, the embryo (fig. 4) presents true "cyclopia of the lens." The condition, however, may better be interpreted as an illustration of the high lens-forming

capacity possessed by the ectoderm at the anterior tip of the head.

Lens-forming power seems to diminish from the anterior tip of the head backward until trunk ectoderm no longer possesses the capacity to form a lens, as Spemann found in transplantation experiments. For this reason independent lenses arise, as a rule, far anterior to and often in front of the more or less lateral eyes (my figs. 3, 4, 7, 9, 10, 11, 12, 21, etc. '10 b). In fewer cases independent lenses are found in more posterior lateral regions approximately in the normal lateral eye position (figs. 1 and 2

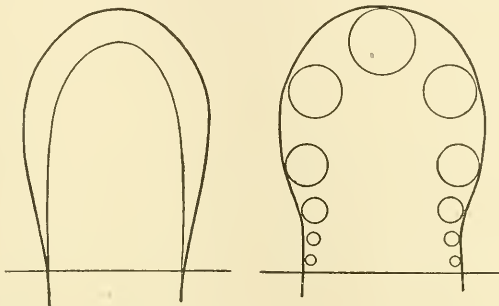


Fig. 9 Two diagrams indicating the primary lens-forming power of various portions of the head ectoderm. The lens-forming tendency is considered to be greatest at the anterior end and gradually decreases towards the trunk ectoderm until the ability to form a lens is lost where the trunk begins. The rows of circles indicate the magnitudes of lens-forming tendency in different regions and do not signify the size of the lenses. Posterior lenses may be as large as anterior ones, yet they occur less frequently as independent structures. Free lenses usually occur near the anterior tip.

and 3, plates I and II '10 b). Figure 9 may serve to illustrate diagrammatically the extent and gradation of the lens-forming power possessed by the head ectoderm. This rather diffuse localization of lens-forming cells in the general head ectoderm as demonstrated by numerous experiments seems sufficient to account for all phenomena of lens formation in cyclopia, as well as the supernumerary lenses which the writer has reported.

The median position in cyclopia of normally bilateral organs involves one other part. The nose or nasal pits in the cyclopean

fish are sometimes median and single, or occasionally bilateral and more or less normal in position. I might also add that the nasal pits are often absent. Again the 'defect hypothesis' must expect median cells to fail and so allow the two nasal pits to fuse medially. In this case the evidence is still stronger for the primary median origin of the ectodermal anlage. Dohrn's studies on Ammococtes have shown the median position and relationship of the nasal and hypophyseal invaginations. The question of monorhiny in the cyclostomes is not fully determined but evidence is certainly available to indicate a median nose anlage. These, however, are phylogenetic considerations which would only serve to prolong the present discussion.

The experimental results presented by Spemann relative to questions of lens formation agree almost entirely with the conclusions which were presented in my paper of 1910 b. He disagrees, however, with many of my interpretations, yet I believe the disagreement is not as complete as it often seems.

I had suggested that the power of the ectoderm to form a lens without the presence of an optic cup was less vigorous or efficient than when the optic cup combined its stimulus with the tendency to lens formation possessed by the ectoderm. For this reason when the ectoderm was injured by many of the mechanical operations which have been employed in the study of lens formation the injured ectoderm was unable to form independent lenses although normally it would have had such power. Attention was called to the different results of Lewis and Miss King on Rana palustris.

Spemann ('12 a, p. 49) rejects this idea although he produces evidence in his paper to prove its very probable correctness. Compare the results he obtained in the origin of independent lenses after cutting out the optic anlagen from medullary plates with glass needles, in which case the ectoderm in the primary lens-forming region was uninjured, with the results following a burning out of the eye anlagen with hot needles, in which case neighboring tissues were necessarily injured. After the latter operation only *one well formed lens occurred in five cases.* A

further comparison between the glass needle operations on early stages with open medullary plates and later stages where the ectoderm was raised and the optic vesicles cut out from beneath it show a difference in the response of the ectoderm in producing free lenses in favor of the less injured or less disturbed ectoderm.

A few of the early lenses figured by Spemann are at least questionable. For example, figure 44 ('12 a) shows on the operated side an irregular thickening within a single cell layer. Such a thickening might readily have resulted from the disturbance to which the portion of ectoderm had been subjected. This specimen is cited as evidence that the accessory or supernumerary lenses in my experiments may have arisen as buds from a common origin. The possibility of early lenses constricting or budding into two or more is freely admitted. Figures 14, 17, 18 and 20 ('10 b) show constricted or double lenses. If the constriction had been carried further two lenses might have resulted. Yet the relative positions occupied by several other of the lenses figured are difficult to account for on the above basis.

Finally, there is no question of the fact that in numbers of the fish monsters which I have figured and described small and ill-formed eye vesicles or fragments are associated with large well-formed lenses. The fact of the constant association of such optic structures with lenses whenever the optic parts chance to lie near the ectoderm makes it practically certain that the defective eyes have stimulated the lenses to arise in these positions. The size of the lens in Fundulus is not regulated by the size of the optic cup. This is further proven by the small lenses in large eyes and by large protruding lenses in rather well-formed but small eyes. The error in logic which Spemann ('12 a, p. 81) claims to exist on page 405 of my 1910 b paper in discussing these eye fragments I am unable to detect and several other embryologists have been unsuccessful in pointing it out.

SUMMARY

1. *Experiments in which certain regions are removed by mechanical operations from the medullary plate of Amblystoma punctatum seem to show that the earliest optic anlage is median in position.*

Thirty embryos from which lateral portions of the medullary plate and the anterior lateral part of the medullary fold were removed at slightly different stages gave in twenty-four cases, or in 80 per cent of the individuals, subsequent development of both eyes. In five individuals one eye was absent and in one specimen both eyes failed to arise. The absence of eyes in the latter cases was probably due to the cut having been made in a more median position than was intended.

Nine individuals were operated upon so as to remove narrow strips of cells from the anterior median portion of the medullary plate. Four of these cases, or about 45 per cent of the specimens, failed entirely to develop eyes. According to Spemann and others they should have given some degree of cyclopia. Four other individuals possessed highly defective eyes, one embryo having one eye poorly formed while the other was questionably present. Only one of the nine specimens so operated upon was capable of developing both eyes to an extent approaching the normal.

2. When the cells in the anterior portion of the open medullary plate are disturbed by being stuck and scraped in various ways with steel needles they do not loose their power of giving rise to optic vesicles and cups which are normal in appearance during the early stages, later stages were not studied.

3. If the optic anlage be cut out of the medullary plate and reversed in position and then transplanted in the medullary plate it still retains the power to give rise to optic vesicles and cups which are abnormal in position to an extent depending upon the distance the anlagen were shifted by the operation.

The facts furnished by these experiments are considered in connection with recent views regarding the genesis of certain ophthalmic defects.

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THE BREEDING HABITS, MATURATION OF EGGS AND OVULATION OF THE ALBINO RAT

W. B. KIRKHAM AND H. S. BURR

Sheffield Biological Laboratory, Yale University

EIGHTEEN FIGURES

INTRODUCTION

The present work was started by the senior author under the supervision of Professor W. R. Coe in the spring 1907, and in 1908 Professor Coe published in Science a brief statement of what had been found. In the summer of 1911 the junior member, Mr. Burr, took up the work. In the interim the literature of the subject had been enriched by three papers, and since then two additional ones have appeared. Lantz in 1910 contributed to a United States government report, on the economic importance of the rat, a short paper on the natural history of the animal. The author describes the different species of rats, their distribution, and general habits, but pays little attention to the details of their reproduction.

Sobotta and Burckhard ('10) made a careful study of the maturation and fertilization of the egg of the albino rat, and they describe and figure the ovarian egg in the stages of the first polar spindle, and first polar body with the second polar spindle, and the tube egg in the stages of the second polar spindle, fertilization, second polar body, and the pronuclei. Ovulation is stated to occur independent of pairing within thirty-six hours after the birth of a litter, and the eggs fertilized nine to twelve hours after copulation. Sobotta and Burckhard found the mature rat egg, in the ovary, to measure in preserved material 0.06 to 0.065 mm. in diameter; practically the same as the

mouse egg. These investigators never saw a definite first polar body associated with an egg in the tube.

Newton Miller's paper ('11) on reproduction in the brown rat is based solely upon observations of the living animals. He found that both sexes become sexually mature "at least by the end of the fourth month," that the litters contain from six to nineteen young apiece, and that these animals breed the year round.

Mark and Long ('12) devote most of their contribution to an extended description of the elaborate warm chamber they have devised for the study of living mammalian eggs. When it comes to the results obtained with their apparatus they have, at present, little to say. Living eggs of rats and mice obtained in a manner similar to that described by one of us (Kirkham '07) were placed on the stage of the microscope in the warm chamber and spermatozoa added, the mouse eggs underwent no change, but the rat eggs within five minutes to two hours began the formation of the second polar cell. Cleavage has never been observed, and after twelve hours the eggs begin to degenerate.

The latest contribution to the literature on the subject of rat breeding is by Helen Dean King ('13) who records for the albino rat somewhat the same phenomena previously observed by Daniels ('10) in mice. The normal period of gestation for the albino rat, according to Miss King, is twenty-one to twenty-three days. If six or more young are being carried while a previous litter of five or less are still suckling the period of gestation may be prolonged, while if more than six young are suckling the period is always prolonged, regardless of the number being carried. Unlike the mouse, the albino rat appears not to exhibit any exact relation between the number of young either suckling or borne and the extent of prolongation of the gestation period. This paper also contains evidence that the eggs of a given oestrus cycle in the albino rat may be discharged from the ovaries in two sets, with an interval of two to three days, and also that in very rare instances this interval may be extended to two weeks. Miss King would like to interpret the latter cases as instances of a distinct oestrus cycle occurring during pregnancy.

The present paper has as its object the filling in, as far as possible, of such stages as have not previously been described, and the presentation of evidence regarding the time relations in the development of individual eggs. The authors' thanks are due to Prof. W. R. Coe for the use of the notes and drawings of the eggs of the brown rat, and to Dr. T. B. Osborn of the Connecticut Agricultural Experiment Station for the designs of the cages used and for the animals with which the work was started.

BREEDING HABITS

About 150 albino rats were under observation at different times during the investigation. One large cage was used for all rats not at the time under special care. For individuals two types of cages were employed, one, a cylindrical cage of wire netting of sufficient size to accommodate two rats at a time, and the other a much larger, rectangular cage of galvanized iron, with wire netting only on the front and bottom. This second type of cage was designed primarily as a breeding cage and was large enough to house a mother rat and a litter of the largest size until the latter were sexually mature.

The food of the animals consisted of oats, corn, wheat, sunflower seeds, and dog-biscuit, together with bits of lettuce, string beans, bread, and various kinds of cooked meat and fish.

All cages were kept as clean as possible, but except when absolutely necessary litters less than two weeks old were never disturbed. At the times when we were inspecting them the rats were encouraged to come out of their cages and run about the room, and to this familiarity with us as well as to the additional exercise thus secured we attribute much of our success in rearing large litters without their being maimed or eaten by the parents.

Usually females were isolated in breeding cages as soon as they were seen to be pregnant, but in the few instances when males were left with such females until several days after the birth of the litter no mortality occurred. This fact leads us to

agree with Miller ('11) and King ('13) that mother rats, unless they are in an unhealthy condition, or have been frightened in some way, rarely if ever kill or maim their young.

Albino rats give birth to young in all seasons of the year, but it is only from April to October that ovulation as a rule occurs within 48 hours after parturition; during the remaining months they are apt to skip oestrus cycles, ovulation not occurring until some three weeks after parturition.

The senior author showed in 1910 that the albino rat ovulates regardless of whether pairing has previously taken place, and when males are continuously present copulation may occur before the ripest eggs in the ovaries have formed the first polar spindles. On several different occasions we have seen the actual pairing. It differs markedly from the condition described by Sobotta ('95) for the mouse, since the male albino rat is not prostrated by the sexual act, but walks slowly away. When a previously isolated female who is in heat is placed in a cage with several males they will all pair with her in rapid succession.

The period of gestation in the albino rat is twenty-two days when the female is not nursing a previous litter, in which event the period may be lengthened as found by King ('13). The litters varied in number from four to twelve and the birth usually took place in the late afternoon or the early evening, although probably it may occur at any hour of the day, since we have observed it at noon. The process of parturition is briefly as follows: The female in order to aid in the expulsion of the foetus flattens herself against the bottom of the cage while a series of wave-like muscular movements pass posteriorly along the body starting just behind the shoulder. As soon as the young rat is free from her body, the female rises up on her haunches, seizes in her forepaws the button-like placenta, which is still attached to the offspring by the umbilical cord, and devours first it and then the cord, cutting off the latter as close to the body of the young animal as she can get with her teeth. The female then again flattens herself out against the bottom of the cage preparatory to the appearance of the next young rat. The process is repeated until all have been brought forth. Then, and not

before, does the mother assemble the young, cleaning them up with her tongue, after which they lie close together under her to keep warm. From this time on until the young are able to crawl around by themselves the mother never leaves the nest until she has carefully covered her litter. On returning she always looks around for any that may have rolled or crawled out in her absence, and such offenders are quickly seized in her jaws and hauled back into the nest.

The albino rat becomes sexually mature, at least in some cases, as early as fifty-five days after birth, since in one instance a litter was born to rats that were only seventy-seven days old.

MATURATION AND OVULATION

The paper by Sobotta and Burckhard ('10) on the maturation and fertilization of the albino rat is by far the most complete account of the subject that has so far been published. However this report left a number of things to be cleared up.

Working with material from 81 rats we have attempted to investigate and make clear the following points: (1) the early development of the egg previous to the formation of the first polar spindle, (2) the formation of the first polar body, (3) the condition of the egg at ovulation, (4) the process of fertilization and second polar body formation.

At first the rats were watched and killed at short intervals up to forty-eight hours after pairing. This gave no data that could be depended upon for determining the stage which either the ovarian or the tube eggs had reached. However, by relating the time of killing the female to the time of parturition it was found that the approximate development of the egg could be predicted without much difficulty. We say approximate because even though the exact hour of parturition be known it is impossible to say that at a given interval of time the eggs are in a given stage of development.

The parturition of a female caged with a male having been observed, she was killed twenty-four hours later. This female yielded unfertilized tube eggs, indicating that ovulation had re-

cently occurred. Cases such as this show that ovulation usually occurs about twenty-four hours after parturition. The individual variation is so great that any complicated apparatus for determining the exact date of parturition is valueless. We have obtained the best results by killing the females at half hour intervals, beginning in the later afternoon and continuing through the early evening. By doing this practically all the stages of maturation can be obtained.

In a number of instances the senior author dissected out the Fallopian tubes, and after placing them in warm salt solution, by slitting the tubes he was enabled to obtain two eggs fertilized but unsegmented, three eggs in the two cell stage and three eggs so obscured by follicle cells as to prevent any exact information as to their condition. The technique of this operation is so simple, requiring only a binocular microscope, two needles, some warm physiological salt solution, and a female rat that has given birth to a litter at least twenty-four hours before and not more than five days previously, that we recommend the rat as highly as the mouse for obtaining live mammalian eggs for class demonstration.

In all other instances the ovaries (and also the tubes, wherever ovulation was thought to have occurred) were fixed in either Zenker's fluid or in a strong solution of Flemming, imbedded in paraffin, cut serially into sections 0.010 mm. thick and stained in Delafield's haematoxylin. Such sections as were found on subsequent examination to be worthy of detailed study were later decolorized with acid alcohol and restained with Heidenhain's iron-haematoxylin.

A study of the ovaries of the above rats showed that there is a progressive development of the egg until it is ready to leave the ovary at ovulation. The developing eggs of any adult ovary can be readily divided into six groups. The first of these (fig. 1) includes all those eggs that are in the resting condition. These vary considerably in size, as do also their follicles. The earlier stages show a small egg with a follicle consisting of from one to three layers of radially arranged follicle cells with scattered cells lying between the layers, the later stages lie in larger follicles

with many more layers of cells. The egg nucleus presents a constant appearance, a clearly defined nuclear membrane, scattered chromatin and a deeply staining nucleolus.

The second group includes those eggs which differ from those of Group I only in their size and in the fact that they lie in much larger follicles, the latter consisting of a large number of cells closely packed but showing no radial arrangement except in the layer immediately surrounding the egg. Such an egg is shown in figure 7.

The third group includes a much smaller number of eggs which lie in follicles similar to the preceding, except that the cells lying

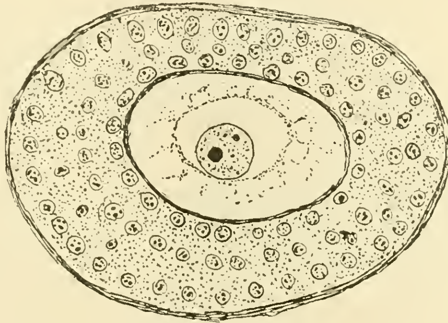

Fig. 1 Normal resting follicle. × 630

in or near the center of the follicle show a marked tendency to separate, leaving a clear space. This condition may, however, be found in follicles belonging to eggs of Group II, for the factors governing the growth of the follicle are not, according to our observation, constant, since growth may set in when the egg has reached the stage of development included in either Groups II, III or IV (figs. 2, 3 and 4). The nuclei of the eggs of this third group show a marked change. The nuclear membrane is still distinct, but the chromatin is less scattered and the nucleolus has become partially vacuolated, since it shows much less affinity for the stain. Figure 8 shows an egg of this group.

The fourth group shows further modifications. It is at this point that the maximum growth in the size of the follicle takes

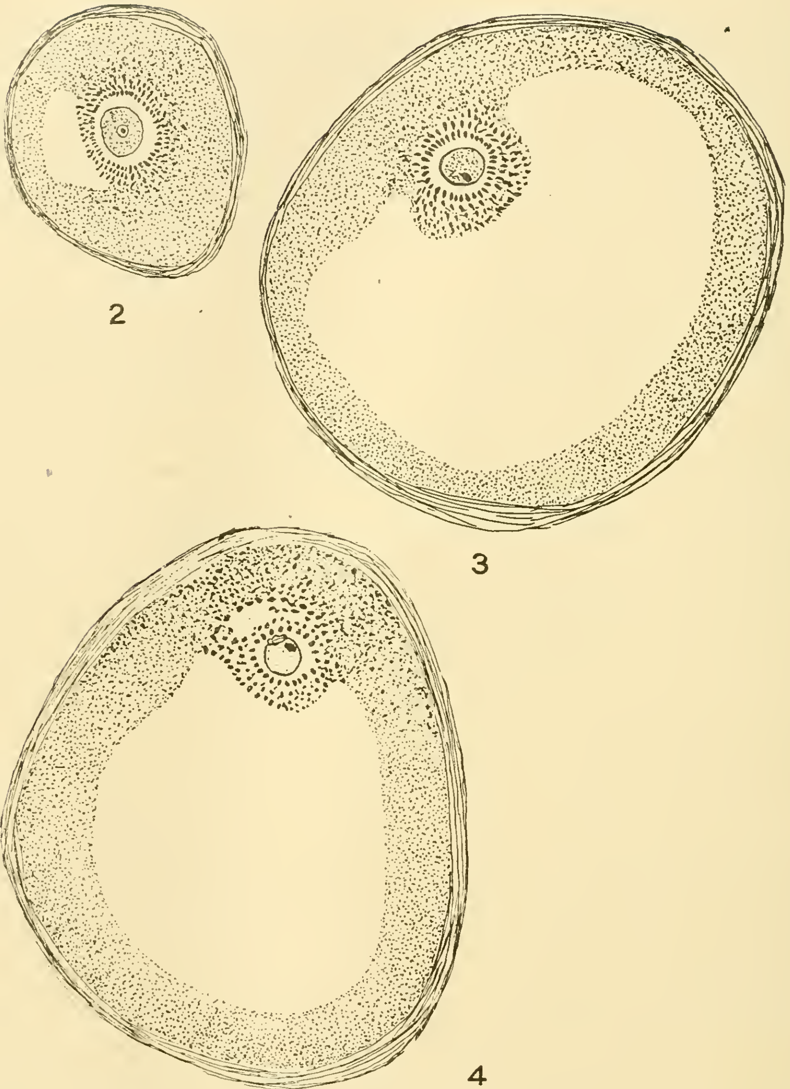

Fig. 2 Earliest observed maturation phenomena—increase in size of follicle. × 90.

Fig. 3 Follicle of egg with first polar spindle shown in figure 10 of Plate II. × 90.

Fig. 4 Follicle of egg with first polar body and second polar spindle shown in figure 11 of Plate III. × 90.

place. While growth may have started in either of the two preceding groups, the greatest growth occurs with the egg in this stage of development. Eggs of this group have been observed with follicles similar to those of the two preceding groups, and also with follicles of nearly the maximum size. The nuclei of these eggs show a diminution in the amount of chromatin present and a complete vacuolization of the nucleoli, the latter showing no affinity whatever for the stain. Such an egg is shown in figure 9.

The fifth group consists of the eggs with first polar spindles. The follicles here are typical, showing a slight tendency to be thinner in the region where the follicle is nearest to the surface of the ovary. The nucleus of the egg has disappeared, and in its place lies the first polar spindle (fig. 10).

The sixth group shows no change in the size of the follicle. The first polar body has been extruded and a second polar spindle formed (fig. 11).

In all the above divisions, with the exception of the sixth group, wherever a distinct zona radiata can be seen, very fine protoplasmic bridges can readily be distinguished crossing from the follicle cells to the egg. The presence of these very distinct filamentous processes of the follicle cells seems to have been entirely overlooked by previous investigators.

One striking thing is to be noted with regard to the above divisions—never were all six found together in one ovary at a given time. As was to be expected, Group I, since it included all the resting eggs, was present in all ovaries. Group II, on the other hand, was seen to drop out on the appearance of Group IV and to reappear on the disappearance of the latter. Group V also appeared on the disappearance of Group IV. When Group V dropped out, Group VI appeared. Group III was found in all ovaries.

From the fact that perfectly normal eggs of Groups II and III were found in the ovary just at, and also just subsequent to ovulation, it was evident that more than one oestrus cycle was necessary for the development of the egg from the resting stage to the stage of the first polar body and second polar spindle,

at which stage the egg leaves the ovary, for, if the above changes occurred in one oestrus cycle, all normal eggs in the above condition would go out of the ovary at ovulation, leaving only Group I eggs in the ovary. This condition was not seen. Hence we were forced to find some other explanation of the facts.

Figure 5 shows in the form of a table the facts described above. The vertical readings show the groups of eggs. The horizontal readings show the periods into which the oestrus cycle is divided. Period *a* is the division of the oestrus cycle extending from ovulation to the twenty-first subsequent day and covers a period of time in which there is little change in the personnel of the ovary. Period *b* covers the succeeding six hours; period *c*, the next six, and period *d*, the last six hours remaining before ovulation. The above figures are only approximate, as the individual variation is too great to permit of any exact data.

By studying the figure it will be seen that Group IV disappears at period *c*. At the same time the ovary contains Groups I, II, III and V, IV and VI being absent. During the interval between periods *c* and *d* Groups I, II and III remain unchanged, but Group V disappears and Group VI appears.

After ovulation we find in period *a*, Groups I, II and III only. But in period *b*, II disappears and IV appears.

From the above data we were led to believe that the development of an egg follows the arrows in the diagram. That is, that Group II comes from I in period *c*, remains unchanged through *d* and *a*, becomes transformed into III during period *b*, remains unchanged through *c*, *d* and *a*, grows to IV in *b*, to V in *c* and to VI in *d*, and so out at ovulation.

The above explanation of the facts rests on the assumption that the normal rate of development is approximately the same for all eggs. This assumption we think is warranted, for if II developed into IV during period *b* instead of remaining unchanged until the next oestrus cycle, the number of Group III eggs found should be very small, since the change would be a rapid one. On the other hand, if the development involved a longer period of time—that is, if Group III became a second resting stage—one would expect to find a comparatively large number

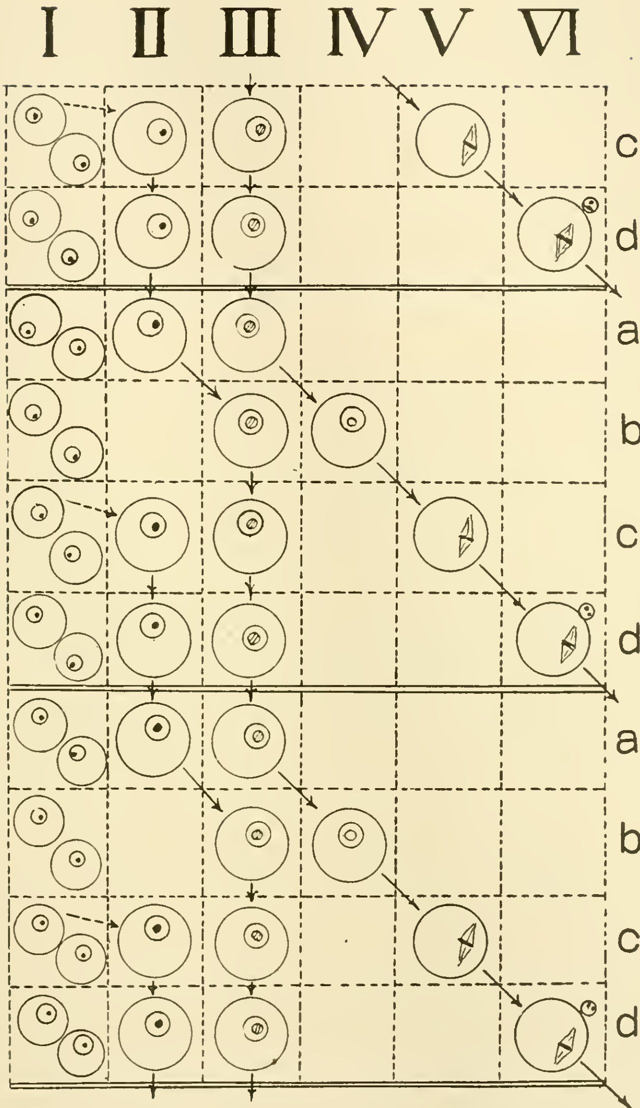

Fig. 5 Diagram showing probable development of an egg from resting stage through ovulation. Roman numerals I–VI indicate successive stages in development of eggs; *a–d* indicate periods in oestrus cycle. Arrows indicate probable course of development of individual eggs.

TABLE 1

Showing the relative number of eggs in the various stages of development at different periods of the oestrus cycle, as found in individual ovaries

	OVARY NO.	I	II	III	IV	V	VI	DEGENERATING
Period a..........	168	12	3	4	0	0	0	28
Period b..........	145	161	0	24	19	0	0	138
Period c..........	171	50	10	7	0	5	0	52
	167	34	2	3	0	5	0	35
Period d..........	152.2	22	7	14	0	0	3	120
	152	24	11	8	0	0	2	42

of such eggs in an ovary at any given time. This, however, was not the case, the number of Group III eggs found being relatively close to the number of Group I eggs.

Table I is compiled from a count of all the follicles in six ovaries, representing each of the four periods. It shows the relative number of eggs in each group present at the same time in a given ovary. The count can only be an approximation, owing to the occasional loss of a critical section and the frequent difficulty in determining with accuracy whether or not an egg was normal, but is sufficiently exact for this purpose.

We were unable to obtain any stages that intervene between the eggs of Group IV and those with the first polar spindle, so we cannot say whether the nuclear membrance disappears before or after the first appearance of the first polar spindle. With regard to this spindle, however, there are a number of details worthy of attention. It is short and broad, with well defined fibers which do not come to a sharp focus (fig. 10). The possibility of centrioles being present was mentioned by Coe ('08), but these are apparently lacking in polar spindles of the albino rat. The chromosomes are numerous, crowded, and never found in a definite equatorial plate. Most of the first polar spindles seen are parallel to the surface of the egg, and this appears to be the position in which the spindle waits for the stimulus that leads to the formation of the first polar body (fig. 6 a). When this stimulus comes the spindle rotates on its long axis, coming to lie more or less radially (fig. 6 b and 6 c).

The next stage we were able to obtain is shown in figure 11. This is an ovarian egg with the first polar body and the second polar spindle. As in the case of the mouse, the nuclear material is never gathered into a resting stage between the time of extrusion of the first polar body and the formation of the second polar spindle.

The first polar body is rarely seen in eggs outside of the ovary, but there is absolutely no reason to doubt that it is always formed, since it is almost invariably present beside normal ova-

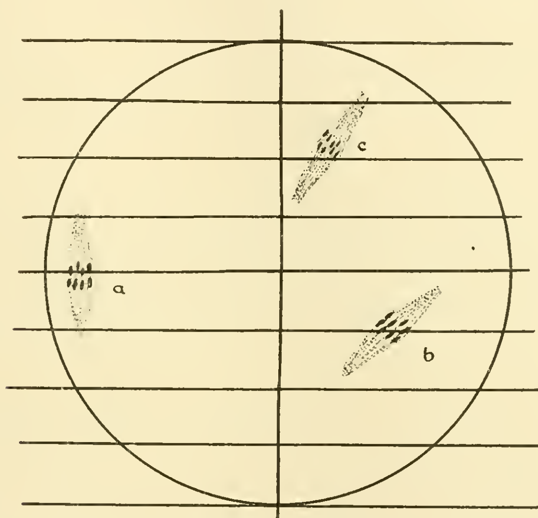

Fig. 6. Reconstructions of three spindles showing gradual rotation from the paratangential position (*a*), through (*b*), to the radial position (*c*).

rian eggs, possessing a second polar spindle. Even in the ovary, however, its protoplasm displays its characteristic tendency to undergo rapid disintegration. In such fully matured eggs as have failed to escape from the ovary and are just starting to degenerate, as well as in those about to be discharged, the second polar spindle may be sharply defined, yet a careful search fails to reveal a trace of the first polar body. The chromatin in the first polar body is always scattered, and when first formed this

polar body is, in all probability, always larger than the second, though disintegration may set in immediately upon its formation.

The second polar spindle as seen in the ovary is much longer and narrower than the first, but resembles the first polar spindle in having open ends and no centrioles. The chromosomes in the second polar spindle are almost always spherical.

TUBE EGGS

The living unsegmented egg of the albino rat measures about 0.079 mm. in diameter (the exact size varies a few thousandths of a millimeter in different specimens), and is surrounded by a zona of transparent jelly about 0.022 mm. in thickness. The two unsegmented rat eggs that were obtained sufficiently free from follicle cells to be available for detailed study, both possessed two polar bodies, measuring in one specimen 0.019 and 0.0132 mm. in diameter respectively, and in the other specimen 0.008 and 0.0065 mm. These eggs while translucent were filled with highly refracting globules scattered through the protoplasm. In one egg there was a clear area near the center, where we thought we could distinguish the two pronuclei lying side by side.

The rare occurrence of the first polar body associated with the egg in the tube is to be attributed to its rapid disintegration, which, as already stated, begins almost as soon as it is formed, and may lead to its complete disappearance before ovulation occurs. A stained and sectioned tube egg, accompanied by the first polar body, is shown in figure 12. This polar body is very small, contains only a little stainable chromatin scattered through it, and its protoplasm is much denser than that of the egg.

Until after fertilization, and if this fails to take place until it degenerates, the chromatin of the second polar spindle remains in a clearly defined equatorial plate, but in the egg in the Fallopian tubes, this spindle always appears much longer and thinner than in the ovarian eggs.

The rat spermatozoon has an exceedingly long tail (fig. 16 a), and like that of the mouse carries more or less of its tail with it

when it enters the egg, a fact mentioned by Coe, and by Sobotta and Burckhard. As soon as the sperm head begins to penetrate the cytoplasm of the egg the formation of the second polar body is started.

In the albino rat the second polar body is characterized by having the chromatin content massed, while the chromatin of the first polar body is always scattered through the cytoplasm. This distinction, however, does not hold for the Norwegian rat, of which two eggs are shown in figures 17 and 18. The chromatin left in the egg after the formation of the second polar body rounds itself up and becomes surrounded by a membrane, thus forming the female pronucleus. The sperm head on its entrance swells up and likewise assumes a rounded form with a nuclear membrane, as is shown in figure 16.

SUMMARY

1. Male albino rats rarely, if ever, are responsible for the killing or maiming of their young. Diseased condition or fright are probably the chief causes of the destruction or injury of their offspring by the females.

2. Albino rats give birth to young the year round, but only from April to October do the females regularly ovulate twenty to forty-eight hours after parturition.

3. Albino rats of both sexes are sexually mature when less than two months old.

4. Living rat eggs are easily obtainable during the four days following ovulation by dissection of the Fallopian tubes.

5. The maturing eggs in the ovary are joined to the surrounding follicle cells by very definite cell bridges.

6. The development of eggs can be traced in the ovary through two oestrus cycles preceding their discharge.

7. The first polar spindle is short and broad, and is usually formed less than twenty-four hours after parturition.

8. The first polar body is always formed in rat eggs, but its protoplasm is very unstable, and disintegrative processes often bring about its complete disappearance about the time the egg reaches the Fallopian tube.

9. The second polar spindle is long and narrow. Its appearance marks the end of maturation phenomena in the ovary, and the termination of all development of the egg unless fertilization occurs.

10. In albino rats the chromatin of the first polar body is scattered, that of the second polar body is massed.

11. The very long middle piece of the sperm tail follows the head into the cytoplasm of the egg.

June 1913

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PLATES

Figures 1 to 4 and 7 to 18 were drawn with the camera lucida. All figures, except 17 and 18 were drawn with Zeiss no. 4 oc. and $\frac{1}{12}$ oil immersion obj. giving a magnification of 1000 diameters. Figures 17 and 18 were drawn with a no. 6 oc. and $\frac{1}{12}$ oil imm. obj., giving a magnification of 1760 diameters. These figures are reduced one-third, giving a magnification in the finished plate of 1174. All other figures are reproduced at the size drawn.

PLATE 1

EXPLANATION OF FIGURES

7 Shows an ovarian egg in the resting stage. The egg (Group II) has attained approximately its greatest diameter. Nucleolus solid and deeply staining. Protoplasmic bridges well marked. A follicle cell is shown dividing at the left.

8 An ovarian egg (Group III) at a slightly later stage showing the vacuolization of the nucleolus well started.

7

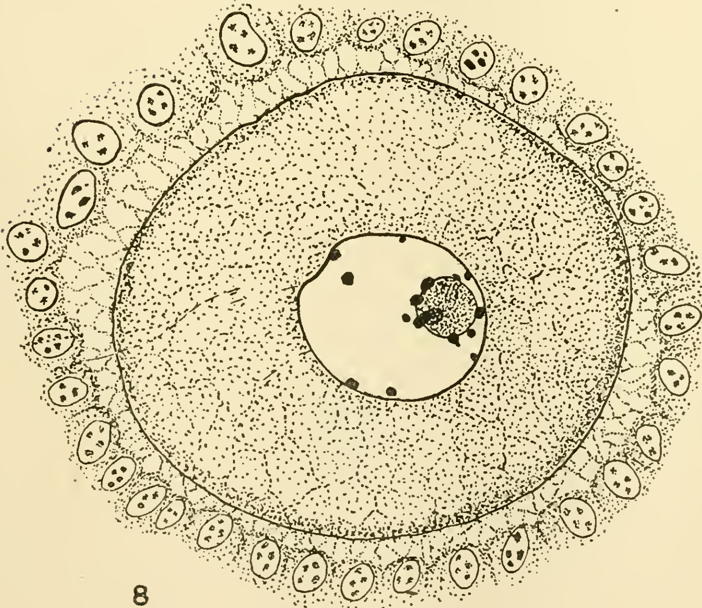

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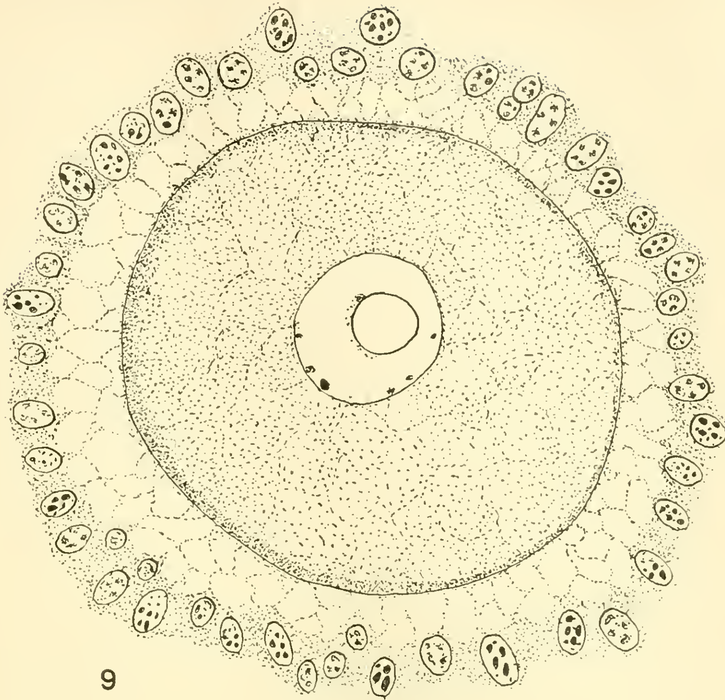
PLATE 2

EXPLANATION OF FIGURES

9 A later stage than fig. 8, showing the complete vacuolization of the nucleolus (Group IV).

10 A radial first polar spindle (Group V) showing the blunt ends.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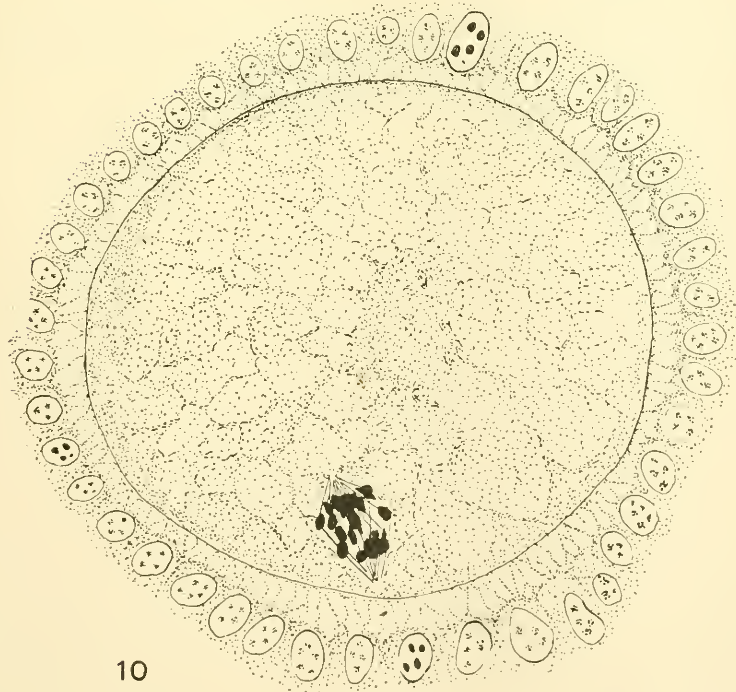
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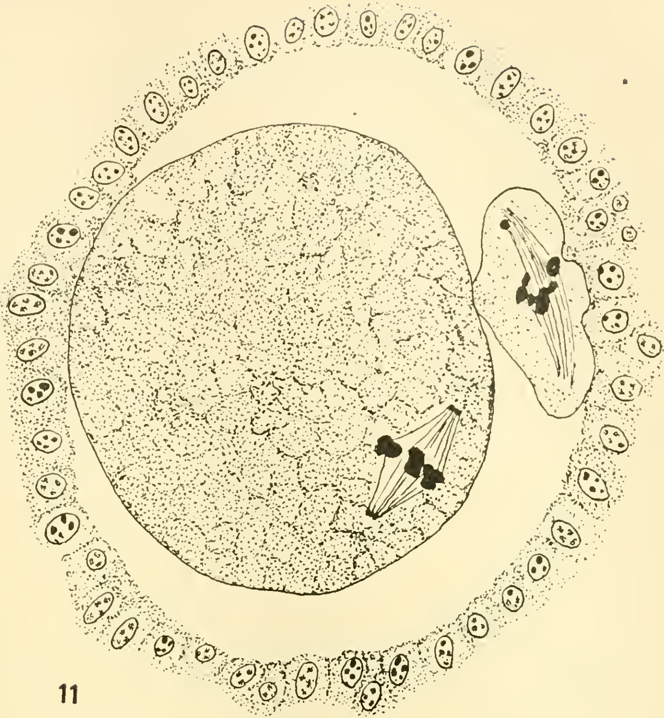
PLATE 3

EXPLANATION OF FIGURES

11 An ovarian egg (Group VI) showing the first polar body with a spindle, and the early type of short, thick second polar spindle within the egg. The protoplasmic bridges have at this stage disappeared.

12 A tube egg, showing the first polar body at the right, and the long slender type of second polar spindle at the lower left-hand margin of the egg.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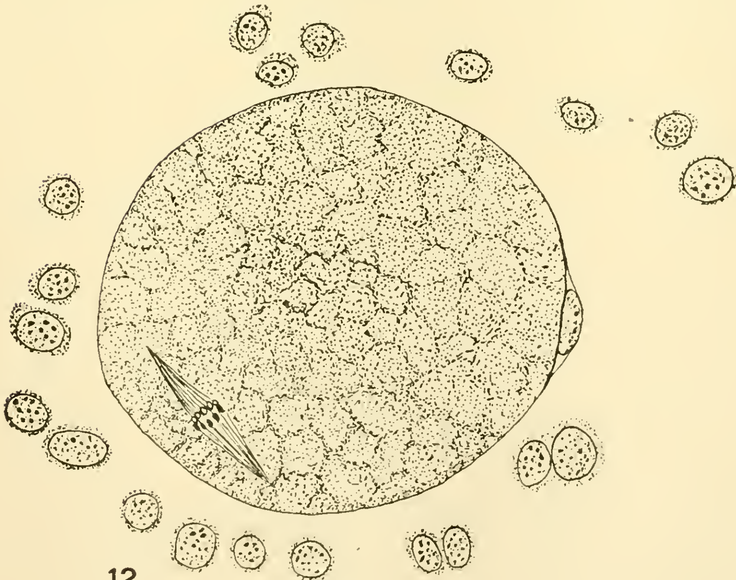
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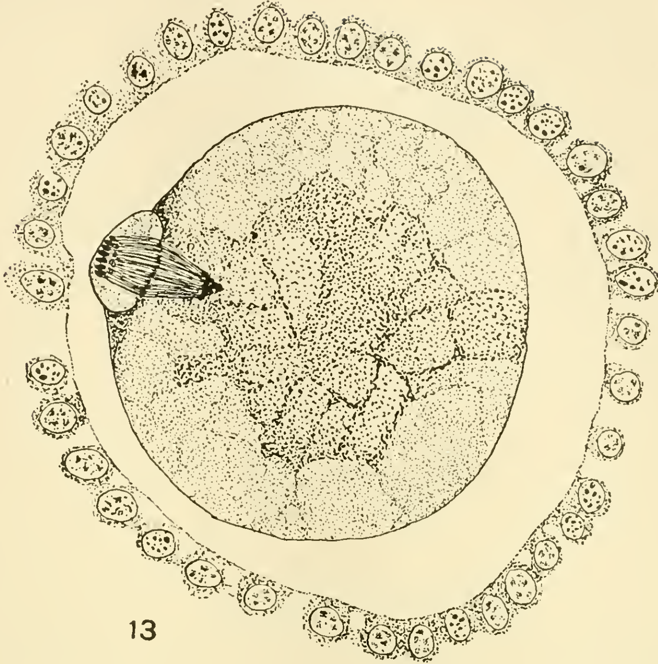
PLATE 4

EXPLANATION OF FIGURES

13 A tube egg, showing the second polar body in the process of formation. The enveloping follicle cells still retain their continuity.

14 A tube egg, showing the first polar body at the top, the second polar body in the process of formation at the lower left-hand margin of the egg, and the sperm head at the right, with a portion of the tail.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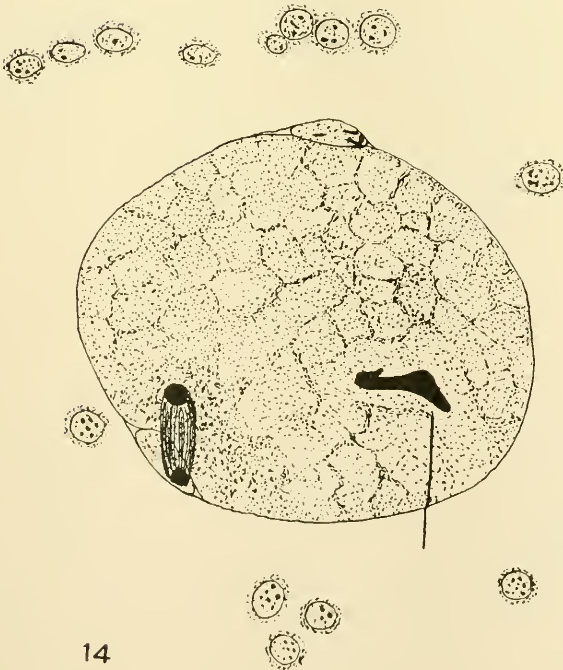
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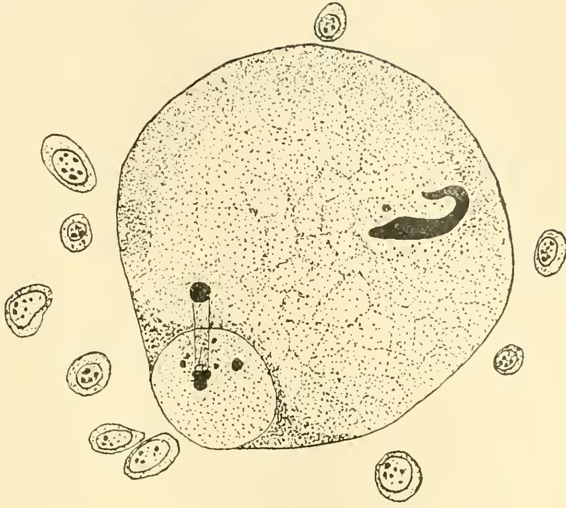
PLATE 5

EXPLANATION OF FIGURES

15 A tube egg, showing the second polar body at the left and the sperm head at the right.

16 A series of drawings showing the changes in the sperm head: *a*, a spermatozoon in the tube. The head was drawn from a stained section—the tail was added from data of a living spermatozoon, *b* to *g*, various stages in the transformation of the sperm head within the egg.



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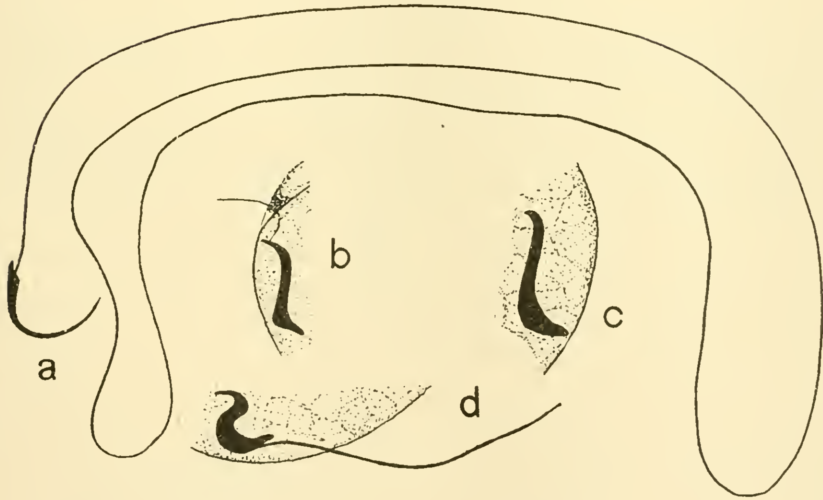

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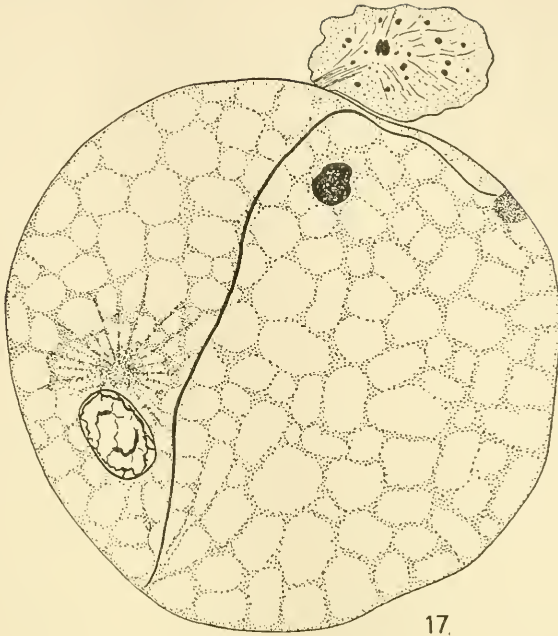
PLATE 6

EXPLANATION OF FIGURES

17 A tube egg of a brown rat, showing the second polar body at the top, below the deeply staining female pronucleus, the entrance cone to the right, the male pronucleus with the sperm aster in the lower left-hand margin and the sperm tail extending diagonally through the egg.

18 A somewhat later stage of the egg of the brown rat, showing in addition to the above-mentioned points the nucleolus vacuolated in the female pronucleus.

Figures 17 and 18 were drawn by Dr. W. R. Coe, and are here published with his kind permission.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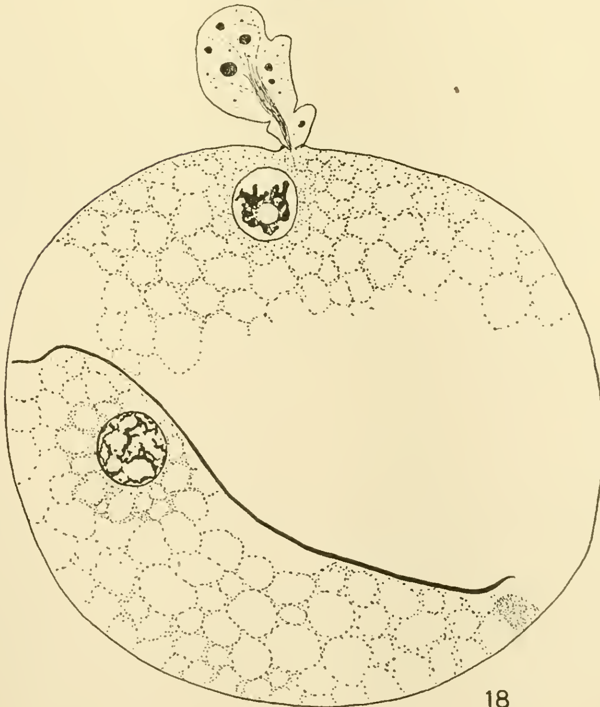
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A HUMAN EMBRYO OF THIRTEEN SOMITES

IVAN E. WALLIN

Anatomical Laboratory, Bellevue Hospital Medical College, New York City

SEVEN FIGURES

The embryo which forms the basis of this work was given to me by Dr. Rudolph Boencke in the spring of 1911. It has been placed in the collection of the Department of Anatomy at the University and Bellevue Hospital Medical College and is called embryo no. 4.

The embryo was aborted two weeks after the last menstrual period. There was no record of coitus. After fixation and with the amnion intact the embryo measured 2.3 mm. in length. It was cut into transverse sections 5 μ in thickness, and stained with iron haematoxylin. The embryo yielded 287 sections.

Wax plate reconstructions were made of the complete embryo, the heart, the foregut, also of the caudal part of the medullary tube with the hind-gut and the belly stalk vessels. A graphic reconstruction was made representing the embryo cut in the mid-sagittal plane. All the reconstructions were made at a magnification of 200.

The embryo appears to be normal in every respect and the following points of structure have been determined.

EXTERNAL FORM

In its general configuration this embryo is very similar to Pfannenstiel III described by Low ('08). The body has a regular dorso-ventral curve and has a slight twist so that the head is situated to the right of the mid-sagittal plane. The yolk sac communicates with the primitive gut by means of an extensive yolk stalk. The latter has its greatest diameter in the cephalo-

caudal direction and its lateral width is greatest at the cephalic end. Caudal and to the right of the yolk stalk the belly stalk leaves the embryo passing ventrally and curving to the right and caudad. Lateral to the yolk stalk the embryonic coelom has an extensive communication with the extra-embryonic coelom.

The heart produces a prominent bulging of the right side of the body immediately caudad to the head. The most prominent part of the bulging marks the flexure in the heart tube between the bulbus cordis and the ventricle. The neck flexure has not advanced to any prominent degree. There are two prominences on the dorsal surface of the head region, one at the cephalic end of the mid-brain and the other at the cephalic end of the hind-brain. Caudally the body curves gradually in a ventral direction. There is no distinct caudal flexure.

The medullary tube is open to the exterior at both ends. The cephalic neuropore exhibits an unusual appearance for an embryo of this age. It is very wide and gives a great breadth to the head when viewed from the ventral aspect. The lateral lips of this neuropore curve dorsally and form the ventral boundary of a deep groove which is directed cephalo-caudally. The caudal end of this groove runs into the stomodeum. This part of the nervous system which represents the forebrain has not kept apace with the development of the remainder of the tube. It apparently is a persistence of the condition which is present in an earlier stage of development. Eternod's ('95) embryo of eight somites and the embryo of seven somites described by Dandy ('10) exhibit cephalic neuropores which appear to be in about the same stage of development.

There are no indications of otic invaginations. Two pairs of entodermal pouches are in contact with the ectoderm. The points of contact are indicated on the surface by shallow depressions. In figure 2 their positions have been indicated on the surface by broken lines.

The amnion lies close on to the body of the embryo. The head fold crosses the ventral aspect of the heart at about its middle. The lateral folds follow the lateral lips of the coelom. The tail fold is situated on the dorsal aspect of the belly stalk.

NERVOUS SYSTEM

The nervous system has not proceeded very far in its differentiation. The brain flexures do not agree with the His models of this stage, but correspond more to the older embryos described by Thompson ('07) and van den Broek ('11). The most distal portion representing the forebrain is still open and is bent almost at right angles to the mid-brain. The long axis of the forebrain lies in a cephalo-caudal plane and almost parallel with the long axis of the hind-brain. The most cephalic point of the nervous system is thus represented by the junction of the forebrain and the mid-brain. Near the caudal extremity of the forebrain there is a thickening together with an evagination of the brain ectoderm. This evagination is almost in contact with the ectoderm of the stomodeum and undoubtedly represents the infundibulum. Cephalad to the infundibulum and about in the middle of the lateral expansions of the cephalic neuropore there is a slight depression of the ectoderm on each side which represents the beginning of the optic vesicles.

The mid-brain is quite extensive as is apparent from an examination of figure 3. Its floor is smooth and exhibits a thickening at the cephalic end. Caudally there is a flexure of the floor between the mid-brain and the hind-brain. The floor of the mesencephalon is thickened at its cephalic end. The trigeminal ganglion is present as a distinct mass of cells. Its position is represented in figure 3 by a broken circle. The hind-brain passes gradually into the spinal cord. A distinct neck flexure is not present.

The medullary tube has its greatest diameter at the cephalic extremity. It diminishes gradually in size caudally. At the caudal neuropore it exhibits a slight enlargement.

DIGESTIVE SYSTEM

The stomodeum is a broad and deep invagination of the ectoderm between the heart bulging and the head. It touches the entoderm of the pharynx and forms with it the beginning of an oral plate. There is no indication of an hypophysis. The ectoderm lining the stomodeum is thickened especially in the roof.

The cephalic extremity of the pharynx projects beyond the oral plate and nearly reaches the floor of the forebrain, a small amount of mesoderm intervening.

The median thyreoid anlage is a very prominent evagination of the entoderm of the floor of the pharynx. It projects between the layers of splanchnic mesoderm at the arterial end of the heart immediately caudad to the endothelial aorta and the first aortic arches. The cephalic wall of the evagination is considerably thicker than the caudal. Cephalad to the thyreoid anlage the first branchial pouches are evaginated from the lateral wall of the pharynx and immediately caudad to the thyreoid the second pair of pouches are present. The first pair of pouches are the larger. Their long axes are directed laterally, cephalad. and slightly dorsal. Opposite the venous opening of the heart the liver anlage is present as a thickening of the gut entoderm. Lung buds have not developed in this stage.

A cross section of the foregut has a crescentic outline with the concavity directed dorsally. The tube is widest at the point where the first pair of branchial pouches is developed. The cephalic part of the foregut is flattened dorso-ventrally. Caudally the dorso-ventral diameter increases gradually to the end of the foregut where it becomes greater than the lateral diamter.

The gut entoderm extending out into the yolk stalk retains its thickness only a short distance (fig. 3).

The hind-gut is shorter than the foregut. Its dorso-ventral diameter is comparatively large while its lateral diameter is small. The allantois is evaginated from the ventral wall. The lumen of the diverticulum is very small at its proximal end, but throughout the rest of its extent it is distinct. At first the allantois lies between the allantoic arteries. At its distal end it comes to lie between the venous and arterial trunks or sinuses of the belly stalk. The end of the allantois is not recurved as found by Lewis ('12) but ends as a straight tube. The hind-gut exhibits a dorso-ventral constriction immediately cephalad to the allantoic diverticulum. Caudal to the allantois the hind-gut widens out to form the cloaca. The entoderm of the ventral wall

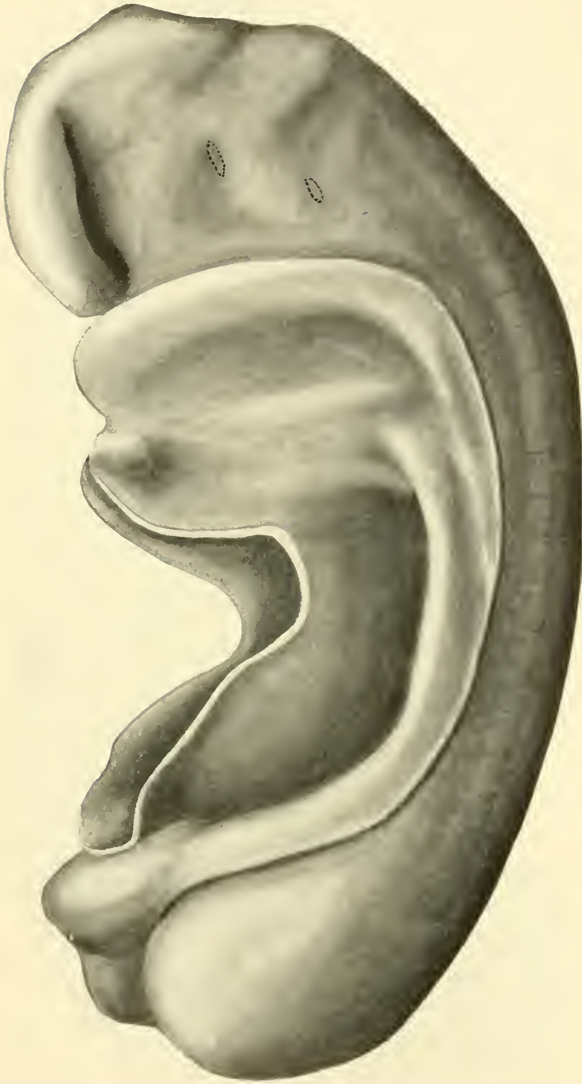


Fig. 1 Wax plate reconstruction of complete embryo seen from left side. The broken lines indicate the points where the entodermal pouches touch the ectoderm. × 100.

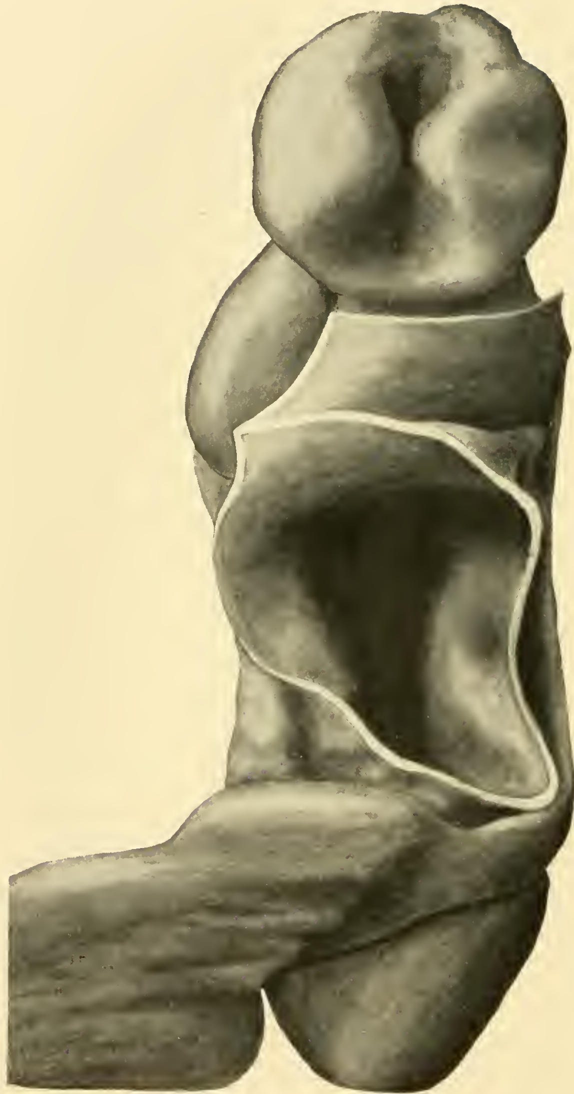


Fig. 2 Wax plate reconstruction of complete embryo seen from the ventral aspect. × 100.

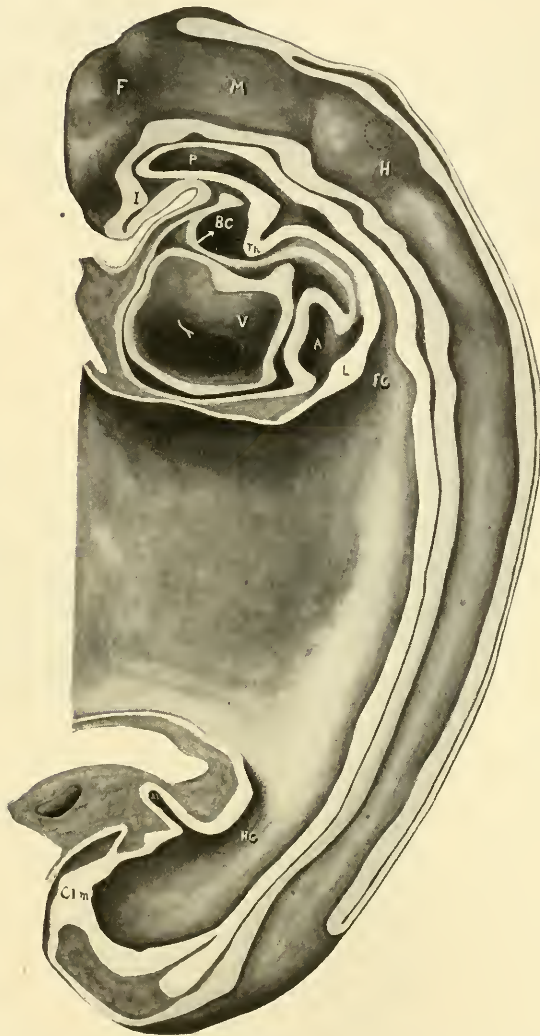
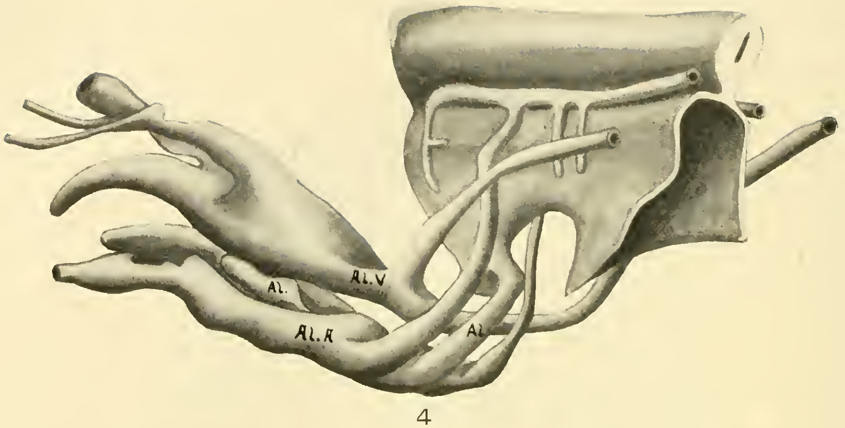


Fig. 3 Graphic reconstruction representing the embryo cut in the mid-sagittal plane. The broken circle above the letter *H* represents the position of the trigeminal ganglion. × 100.

A, Atrium
Al., Allantois
Al.A, Allantoic artery
Al.V, Allantoic vein
BC, Bulbus cordis
Cl.M, Cloacal membrane
F, Forebrain
FG, Foregut
H, Hind-brain
HG, Hind-gut
I, Infundibulum
L, Liver anlage
M, Mid-brain
P, Pharynx
Th, Thyreoid
V, Ventricle



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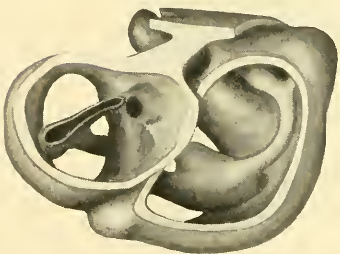

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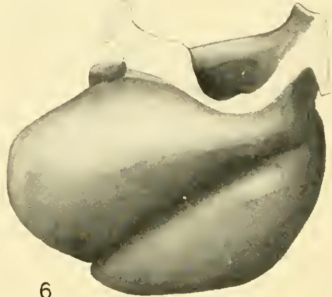

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Fig. 4 Wax plate reconstruction of caudal end of the medullary tube and hind-gut with the belly stalk vessels viewed from the side. × 100.

Fig. 5 Wax plate reconstruction of a section of the heart with the endothelial tube in position viewed from the cephalic aspect. × 100.

Fig. 6 Wax plate reconstruction of the heart viewed from the cephalic aspect. × 100.

Fig. 7 Wax plate reconstruction of the heart viewed from the caudal aspect. × 100.

of the cloaca is fused with the body ectoderm and forms a thick cloacal membrane. At the most caudal part of the cloaca there is a thickening of the entoderm together with a slight evagination which is suggestive of a post anal gut.

NOTOCHORD

The notochord is about in the same stage of development as the one described in a 2.5 mm. embryo by Kollmann ('90). The notochord is intimately connected with the gut entoderm throughout its length with the exception of the caudal end. The caudal end, or tail bud, is cut off from the entoderm and lies imbedded in the mesoderm between the neural ectoderm and the gut tube. There is no distinct notochordal canal as described by Mall ('91), Eternod ('99) and Grosser ('13). In places the cells of the notochord are vacuolated and apparently in a stage of developing a canal. The relationship of the notochord to the gut entoderm is a very intimate one. In the region of the mid-gut the notochord is composed of but a single layer of cells which appear to be a modified part of the gut entoderm. Where the notochord is composed of more than a single layer of cells the basal layer is directly continuous with the single layer of cells forming the gut entoderm. It is impossible to give any other interpretation than that the notochord is developed from the gut entoderm. In places the cells of the notochord are arranged in two lateral masses giving the appearance of bilateral symmetry. This condition is undoubtedly accounted for by the arched nature of the original notochordal plate. In the subsequent proliferation of cells they would grow laterally and unless there were an especially active growth of cells in the central part a gap would naturally intervene between the two lateral groups of cells. At the cephalic end the notochord has more the appearance of a rod and is almost pinched off from the entoderm. On account of the plane of the sections it is not possible to determine with certainty the cephalic limit of the notochord.

MESODERMAL STRUCTURE

There are thirteen pairs of mesodermal somites. These are hardly discernible on the surface. The first pair is situated at a level of about midway between the neck flexure and the hind-brain flexure. The last pair is opposite the point where the allantois leaves the hind-gut. A myocoele may be observed in most of the somites. The cells of the somite are arranged in a radial manner.

The pleuro-peritoneal coelom communicates with the extra-embryonic coelom on the two sides of the yolk stalk. In its cephalic portion it communicates with the pericardial coelom. The lateral lips bounding the open part of the pleuro-peritoneal coelom have a thickened edge produced by the allantoic veins which run cephalad in this position.

The septum transversum is present as a single layer composed of the cephalic wall of the yolk stalk fused with the caudal part of the pericardium.

The excretory system is represented by pronephric tubules. The morphological details of these, as far as I have studied them, agree with the description given by Felix ('12).

VASCULAR SYSTEM

The heart tube is composed of three parts, bulbus cordis, ventricle and atrium. The atrium is situated in the mid-line of the body immediately cephalad to the septum transversum. Its greatest diameter is transverse. From the left extremity of the atrium the atrial canal runs to the left, ventral and cephalad to the ventricle. The ventricle pursues a course from the left to right, ventrally and somewhat caudad. At the right extremity of the ventricle the heart tube makes a sharp bend so that its continuation, the bulbus cordis, comes to lie parallel with the ventricle. The bulbus cordis has a fairly constant size up to its cephalic end where it diminishes slightly. It ends in the mid-line of the body.

The ventral wall of the cephalic end of the bulbus cordis is continuous with the pericardium as is also the case with the

caudal wall of the venous end of the heart (figs. 3, 6, 7). The dorsal wall of the bulbus cordis has a distinct mesentery connecting it with the dorsal pericardium. Near the point where the atrial canal joins the ventricle the ventricle has a mesentery which joins the pericardium at the place where the mesentery of the bulbus cordis joins it. Caudal to the junction of these two mesenteries there is a small space dorsal to the atrium which is free from mesentery and represents the future transverse sinus of the pericardium.

From the dorsal wall of the bulbus cordis a tube-like diverticulum is present (fig. 5). I have been unable to find any references in literature to anything similar to this. The tube runs in the mesentery of the bulbus cordis and at its distal end it comes into close proximity to the ventricle. It is probable that this tube represents a vestige of the space between the two laminae in the closing up of the heart tube and the formation of the mesocardium. Two other tubular spaces of a similar appearance may be seen in the mesentery. They have no communication with the cavity of the myo-epicardium. I observed a similar diverticulum from the bulbus cordis in a 4.06 mm. embryo belonging to the collection of the Department of Anatomy of Syracuse University. It may be noted that this bulbus cordis diverticulum does not contain any endothelium. The endothelial fibrillae, however, appear to extend into it.

The endothelium in no place approximates the walls of the myo-epicardium. The caliber of the endothelial tube varies in the different chambers of the heart, being quite constant in the bulbus cordis, enlarged in the ventricle, and greatly reduced in the atrial canal. In the atrium it widens out into the right and left lateral expansions of the atrium. At its cephalic end the endocardium is continued by the ventral aorta which immediately divides to form the first pair of aortic arches. At the venous end of the heart the most distal part of the endocardium represents the sinus venosus. There is no constriction between the sinus venosus and the atrial part of the endocardium. The endothelial fibrillae which have been observed by various authors

and to which Mall ('12) ascribes the source of the intima may be seen in connection with the endocardium in its entire length.

The blood vessels are collapsed in places so that it is not possible to trace them in their entire extent. The communication between the first pair of aortic arches and the dorsal aortae could not be seen. The dorsal aortae are distinct throughout their course lying dorsal to the gut tube. There is no indication of a second pair of aortic arches. The first pair come off at a point cephalad to the first mesodermal somites. Vitelline vessels containing blood are easily discernible in the wall of the yolk sac and yolk stalk. Vitelline veins run dorsally in the cephalic part of the yolk stalk to gain the caudo-ventral aspect of the sinus venosus opposite the fourth pair of somites. The allantoic veins (fig. 4) begin in the belly stalk as a single trunk or sinus. As the sinus approaches the body of the embryo it bifurcates to form the two allantoic veins which diverge and run laterally and cephalad to gain the lateral lips of the coelom. In this position they run in a cephalad direction to the septum transversum where they enter the caudo-dorsal part of the sinus venosus. The allantoic arteries leave the dorsal aortae at a point opposite the place where the allantois is evaginated from the hind-gut and caudal to the last pair of somites. The arteries run ventrally on either side of the allantois in the belly stalk. At a point more distal than the bifurcation of the allantoic venous trunk the allantoic arteries anastomose to form a single trunk. I have been unable to find any trace of the anterior and posterior cardinal veins. At the cephalo-dorsal aspect of the sinus venosus on the left side there is a short bud-like diverticulum which may represent the future ductus Cuvieri.

I wish to take this opportunity to thank Dr. Boencke for this valuable embryo and Profs. H. D. Senior and F. W. Thyng for assistance and advice in connection with this piece of work.

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THE NERVE SUPPLY TO THE PITUITARY BODY

WALTER E. DANDY

Hunterian Laboratory of Experimental Medicine
The Johns Hopkins University

THREE FIGURES

It is but natural that neglect of an organ itself should yield a proportional lack of interest in its more detailed structure and even more so, in its less important adjuncts—the blood and nerve supply. Such has been true of the pituitary body.

The recent tremendous stimulus produced by Paulesco's (1) sudden transformation of the hypophysis from a structure of vestigial curiosity to a vitally essential organ, has borne its fruit in the rapid accumulation of co-working histological, (2) experimental (3) (4) (5) (6) and clinical (7) (8) observations. Though still very meager our information is now sufficient to have established a hypophyseal clinical entity, amenable in many cases to medical and surgical treatment.

Forming as it does a link in the chain of internal secreting glands, the hypophysis, essentially of hormone action, must be regulated as other glands in this system, by an autonomic nervous mechanism.

Recent studies from the Hunterian Laboratory (5) by Goetsch, Cushing and Jacobson gave evidences of hypophyseal influence over carbohydrate metabolism. It has been shown that sugar tolerance is dependent upon the functional activity of the posterior lobe of the pituitary body. It was later shown by Dandy and Fitz Simmons (observations unpublished) that a piqûre of the hypophyseal region in rabbits produced a heavy glycosuria, therefore giving results similar to a piqûre of the so-called Bernard's sugar center in the floor of the fourth ventricle. These results have been amplified by Weed, Cushing and Jacobson (6).

The combination of glandular or hormone activity and the results of mechanical stimuli (presumably of nervous origin) has suggested the possibility of a neuro-hypophyseal sugar center.

The rational interpretation of this and other physiological data has been handicapped by the uncertainty and meager evidence of the regulatory autonomic nervous mechanism. Accordingly at the suggestion of Dr. Cushing under whose direction the experimental hypophyseal investigations have been conducted, the determination of the source and distribution of the nerve supply was undertaken.

Lying as does the hypophysis in such close proximity to the carotid arteries with their abundant superimposed plexus of sympathetic nerve fibers, it is but natural to assume that this is the source of the hypophyseal nerve supply. Indeed evidence of this is found in the infrequent passing reference to a nerve filament which could be traced from this plexus to the hypophysis.

EARLY REFERENCE TO THE NERVE SUPPLY

Probably the earliest reference to a hypophyseal nerve supply is the casual mention by Bourgery ('45) that he observed sympathetic nerve fibers passing to the pituitary body. Further substantiation is subsequently given in similar casual mention by Fontona, Cloquet, Bock, Ribbes, (9) and possibly others.

In his Anatomie des Menschen ('79) Henle (9) devotes a paragraph to the hypophyseal nerve supply and supplements this description by a drawing of the carotid sympathetic system, which includes a cluster of two or three twigs running from each plexus to the pituitary body. This is the most extensive description of the hypophyseal nerve supply extant. He casts doubt upon the previous discovery of nerve fibers to this gland and concludes that on account of the inherent difficulties they have mistaken fibrous filaments of connective tissue for nerve filaments, saying, "Ohne Zweifel beruhen diese und manche ältere Angaben auf Verwechselung fibröser Bälkchen mit Nervenfasern, doch zeigte mir das Mikroskop in dem Netzförmigen zwischen Carotis und Hypophyse ausgespannten Gewebe feine Nerven-

faserbündelchen dieselben, von denen Luschka sagt, dass sie zwei bis drei jederseits, in den vorderen Lappen der Hypophyse sich einsenken." It is based upon this paragraph and drawing by Henle that an occasional brief mention of hypophyseal nerve supply is found in the more detailed and comprehensive anatomies, the majority, however, passing over the matter in silence.

The internal distribution of the hypophyseal nerves was studied by Berkley ('94) (10) in a series of Golgi stained sections. He observed numerous varicose nerve filaments in the interior of the gland, the lobus anterior and pars intermedia in particular, but some also in the posterior lobe. The external connections of the nerves were not studied. On account of his inability to observe nerve cells in the gland, he presumed they were of extraneous origin and thought they probably come from the sympathetic system.

MATERIAL AND METHODS

The purpose of this paper is to consider only the relatively grosser aspects, i.e., the origin, course and distribution of the hypophyseal nerve supply. The histological distribution and relation of the ultimate filaments to the gland cells have not been considered. It is analogous in character to a recent publication (11) dealing with the blood supply of this organ.

The difficulties of deductions and the impossibility of an accurate conception of the nerve supply based upon gross human dissection have been shown (Henle) (9) by the supposedly erroneous observations of early investigators in mistaking connective tissue trabeculae for the very delicate nerve filaments, which are almost beyond the range of naked vision. These observations are based upon the canine and feline gland, the animals used in the experimental investigations in the Hunterian Laboratory. The anatomical environment of the pituitary body in these forms is such that the difficulties of a tightly enclosed, deeply imbedded and adherent gland encountered in man and the ape are obviated. The hypophysis dangles from the brain and is readily removed with the brain after liberation of its single point of dural

attachment posteriorly, so that the entering nerves may be studied in their true relations, without tearing or distortion.

We have used almost exclusively the specific methylene blue intra vitam method of staining the nerves. For the details of this technique we are greatly indebted to the excellent contribution by J. Gordon Wilson (12). Three essentials are necessary for the successful use of this stain: the exsanguination of the tissues must be thorough in order to get a sharply defined picture of the nerves, since the combination of the methylene blue with blood presents a diffuse, indistinct picture with poorly stained nerves; the nerves must be superficial or covered only by a thin layer of tissue; the air must come in contact with the nerves, otherwise no differentiation takes place.

During the final stages of bleeding the anaesthetized animal from the femoral arteries, a $\frac{1}{20}$ per cent isotonic solution of methylene blue "nach Ehrlich" at body temperature was injected into both carotid arteries and continued until the injecting fluid emanated perfectly clear from the femorals. A tourniquet was then applied around the neck below the point of injection under a pressure sufficiently low to insure filling of the cephalic vessels without danger of diffusion or rupture.

On account of the capricious character of this stain, litters of very young puppies or kittens were injected at the same sitting, so that the defects of some might be supplemented by better staining of others. The total nerve supply then is a summation of results, a reconstruction as it were.

After a few minutes to allow penetration of the stain, the skull was opened and a block of tissue, including the hypophysis with its vessels and nerves in their normal relations, was removed from the base of the brain. The hypophysis was gently retracted so as to allow full exposure of one side to the air. The nerves then assume their differential blue. These specimens were immediately studied under the binocular microscope. The study of fixed specimens with post mortem staining was far less satisfactory, because of the collapse of blood vessels, with which the nerves are intimately associated, the more stiffened picture, and the deficient maintenance of the blue in the nerve fibers.

NERVES TO THE ANTERIOR LOBE

The key to the nerve supply of the pituitary body is the arterial supply to this organ. In a recent publication from this laboratory, it was shown (11) that the anterior lobe received an extensive blood supply from a large number of minute vessels, most of which, even when injected, were beyond the range of naked vision. These vessels radiate from the Willisian circle to the hypophyseal stalk like spokes to the hub of a wheel. The majority of these branches are from the anterior and posterior communicating arteries. The network of sympathetic nerves comprising the carotid plexus is continuous along the three main branches which result from its trifurcation. The distribution, however, is very uneven. A few fibers continue along the anterior and middle cerebral arteries for a short distance but the great majority are found on the two communicating arteries which supply the hypophysis; the posterior communicating artery is particulary well supplied. From these extensions of the carotid plexus numerous filaments are given off and pass along the blood vessels to the stalk of the hypophysis, from which they delve into the substance of the anterior lobe and are lost to view. Some arterial branches have as many as three or even four small filaments, the majority, however, only one or two. The course of the fibers is fairly direct and very few branches are given off. These filaments frequently entwine the vessels but no minute plexuses or anastomoses are visible after leaving the plexus on the main trunks. No nerves have been observed on the external surface of the anterior lobe. All nerves going to the hypophysis are in contact with the sheaths of minute blood vessels. On reaching the stalk it is of course impossible to trace this relation further. Their distribution in the gland has not been observed.

NERVES OF THE PARS INTERMEDIA

Only by dissection of the hypophysis can the nerve supply of the pars intermedia be traced. By gently separating and retracting the posterior lobe from the clasping mitten-like anterior lobe, it is often possible to trace a single nerve fiber with its branches passing down the stalk and spreading out over the pars intermedia which envelops the posterior lobe (fig. 3).

NERVES OF THE POSTERIOR LOBE

It has been shown that the posterior lobe is supplied by a median artery which is formed by the confluence of two branches, one from each carotid artery immediately after its entrance into the cavernous sinus. In the canine this vessel enters the posterior lobe at the only point of dural attachment. Vital nerve staining is somewhat more difficult in this region on account of the relatively thicker dural covering which excludes the action of the air and necessitates a delicate dissection of this vessel. For a long time we were unable to find any trace of a nerve entering the posterior lobe. Several branches were always visible at the origin of the vessels from the carotid but the fibers were lost in the dura before the posterior lobe was reached.

However, it was finally possible to demonstrate nerve fibers actually entering the posterior lobe along the artery. Certainly the disparity between the nerve supply to the posterior and anterior lobes is most striking—in the anterior lobe almost superabundant, in the posterior lobe very few. This contrast may in some measure be due to the difficulties mentioned above; we are however disinclined to lay much emphasis on them.

A most striking color contrast is demonstrated upon removing the hypophysis after vital staining. The anterior lobe is a yellowish white, the posterior a deep indigo blue, possibly due to the (autogenic?) nervous character of the posterior lobe. The blue is of a homogeneous character, no nerve fibers being differentiable under the higher magnifications of the binocular microscope. The intensity of the blue is even much more marked than that of the adjacent, deeply staining oculomotor nerve.

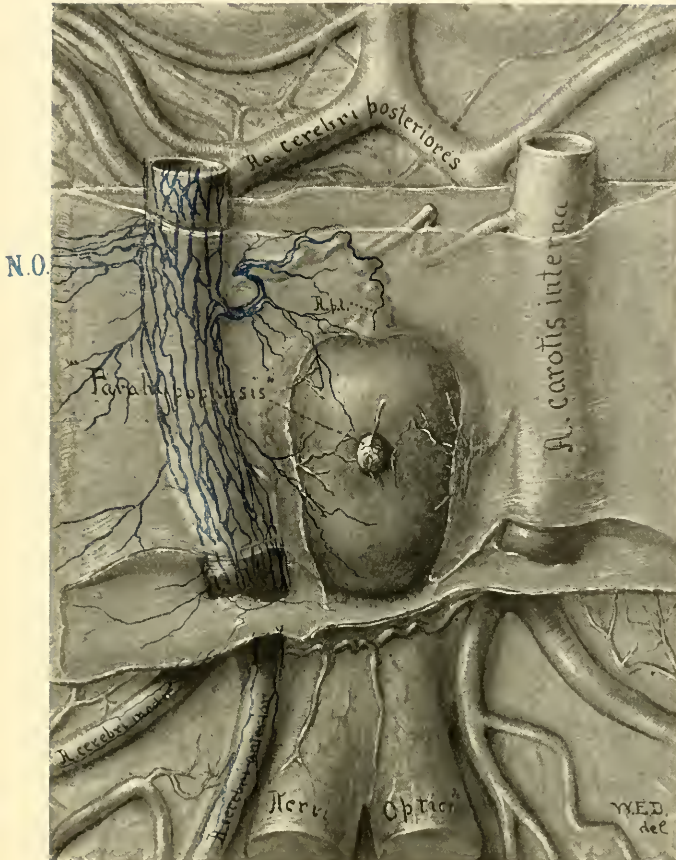

Fig. 1 Semi-diagrammatic representation of one side of the cavernous sympathetic system of a canine, showing the nerves passing to the posterior lobe along its artery. Other branches to the dura and a cluster (No) to the N. oculomotorius. The hypophyseal region is viewed from below with dura intact.

NERVES OF THE PARAHYPOPHYSIS

This little 'nubbin' resting in a small depression in the floor of the sella, usually enclosed in dura, is present in over 80 per cent of canines, and is evidently a remnant of the embryonic Rathke's pouch. In some adults it may be traced to the pars

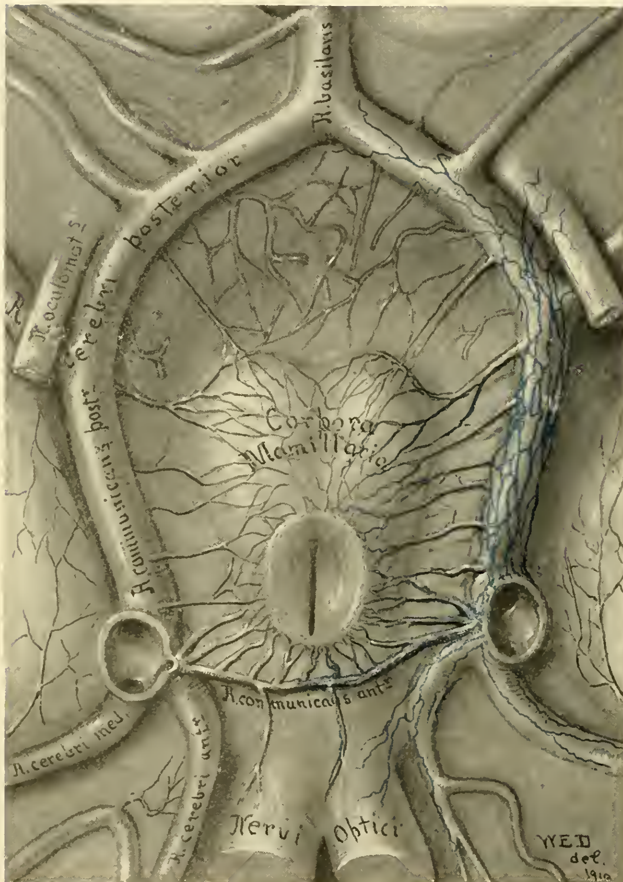

Fig. 2 Semi-diagrammatic reconstruction of sympathetic nerves passing along the arterioles to the stalk of the hypophysis to supply the anterior lobe and pars intermedia. Note relative dwindling of nerves away from the hypophyseal region. The view is from below with dura, hypophysis and carotid artery removed.

intermedia; it varies greatly in size and histological character. It has an individual blood supply, a small artery given off by each posterior lobe artery. Frequently it has been possible to trace a nerve some distance along this vessel toward this "body" but never have we been able to observe a definite nerve connection.

Fig. 3 Drawing to show the nerve passing from the plexus surrounding the posterior communicating artery, down the stalk of the hypophysis to the anterior lobe and the pars intermedia which covers the posterior lobe. The anterior lobe has been dissected from the posterior lobe and gently retracted to permit this view.

OTHER BRANCHES OF THE CAROTID PLEXUS

During observations on the hypophyseal nerve supply naturally the distribution of the sympathetic filaments were noted in the immediate vicinity. The dura of the sella region is exceptionally well supplied with filaments from the carotid plexus. Several branches run from the carotid plexus direct to the oculomotor nerve. A couple of twigs were also observed entering the

optic nerve; these branches were from the nerves in the adventitia of the anterior cerebral artery. There is thus afforded a direct nervous autonomic path between the optic and oculomotor nerves and between these and the sympathetic trunk.

SUMMARY

The nerve supply to the pituitary body is from the carotid plexus of the sympathetic system. Numerous branches radiate to the stalk along the hypophyseal vessels and are immediately lost to view in the substance of the anterior lobe.

The posterior lobe nerve supply is very scant, in marked contrast to the extensive innervation of the anterior lobe.

The pars intermedia receives its nerves from the stalk.

There is connection between the carotid sympathetic system and the oculomotor and optic nerves.

The absolute differentiation between secretory and vasomotor nerves is of course a matter of much dispute and is impossible. The impression, however, from the character and course of the nerve fibers their greatly increased number in the region of the hypophysis, and their disappearance at a distance from the hypophysis, the differences between the supply of the anterior and posterior lobes, the connections established with the other cranial nerves, leads us to regard them as secretory, in contradistinction to vasomotor, the existence of which in the cranial chamber has not been observed.

It is a pleasure to express my gratitude to Dr. Harvey Cushing for his suggestions during the progress of this problem.

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THE MORPHOGENESIS OF THE MAMMALIAN OVARY: FELIS DOMESTICA

B. F. KINGSBURY

Department of Histology and Embryology, Cornell University

THIRTY-TWO FIGURES

It is a striking peculiarity in the development of the reproductive system in man and mammals (not to consider other forms of the Vertebrata) that the duct system (modified in the female) is laid down double, the duct system of the male disappearing in the female or persisting as vestigial structures and vice versa, the organs of the female duct system and the mammary gland being vestigial in the male. The embryo in the course of development thus passes through an indifferent period during which the sex cannot be ascertained, followed by the period of sexual differentiation. In the double appearance of the reproductive fundaments development is protandrous—that is, the 'male' system appears earlier than the female—this rule applying also to the differential development of the ovary and the testis as well as the duct system. No fully satisfactory explanation has been given for this double development in the internal reproductive system. Three or four general explanations have been offered for this fundamental law. (1) The primitive vertebrate was hermaphroditic and that even in the higher forms the hermaphroditic tendency persists showing itself in the development of a double system, male and female, even causing the development in the male of a rudimentary mammary gland, in the female of a rudimentary prostate gland—organs which did not extend back in the line of vertebrate descent anywhere near the hypothetical hermaphroditic ancestral form. The occurrence of hermaphroditism among the ascidians and lower vertebrates (that is, Myxine) and the sporadic appearance of a true or false, complete or incomplete, hermaphroditism

among the higher forms, up to and including man, is regarded as supporting this contention. On this interpretation the indifferent period would be a potentially bisexual or hermaphroditic period in development. This interpretation is not necessarily opposed to the view that the sex-determining factors are already present in the egg at the time of its fertilization. (2) As a modification of the above view might be mentioned the interpretation that the early mammalian embryo during the indifferent period is truly bisexual, containing the potentialities of either sex whose subsequent determination leads to the arrest, atrophy and more or less complete disappearance of the organs of the opposite sex. These two views are related and also bear on the problem of sex determination whose cytological side is subsequently mentioned and briefly discussed. (3) It has been argued that the reason for the development of the Müllerian duct in the male is due to the fact that it was the original reproductive duct and that the utilization of the excretory duct system in the male as a reproductive duct was a secondary acquirement. This interpretation, supported by Waldeyer and Lenhossek, hardly suffices. The theory would fail to explain the appearance of the rudimentary mammary gland in the male, or the prostate and rete ovarii in the female. (4) The converse of this view might be suggested, namely, that the pronephric duct system (the male duct system) represented the ancestral system in the nephridia in pre-chordate forms, serving not only the nephric system but the reproductive system as well, and conveying both the male and female reproductive cells to the exterior. The shedding of the female reproductive cells into the coelomic cavity from the surface of the gonad and the development of a Müllerian duct in association therewith would thus be a secondary adaption.[1] (5) By no means exclusive of the preceding suggestions as to the meaning of the double character of the reproductive organs, since they deal with a distinct aspect of the problem, should be mentioned

[1] Cf. Felix, W.; Theoretische Betrachtungen über das Genitalsystem der Vertebraten. pp. 821–834, Handbuch der Entwicklungsgeschichte der Wirbeltiere, Vol. 3, 1, 1906.

the attempts to analyse the mechanism of sex inheritance and development. Upon the cytological side we have the interpretations correlated with the large amount of work being done at the present time upon the determination of sex and the chromosome pattern of the germ cells. This is not the place in which to enter into a discussion of the complicated problem of the cytological basis in the determination of sex. The careful and detailed work on a number of forms, mainly invertebrate and chiefly insects has, as is of course well known, established the existence of an extra chromatin mass (heterochromosome) or chromosome complex whose presence is correlated with the development of the female sex.

Upon the basis of this fact theoretical considerations have led generally to the conclusion that the heterochromosome is the conveyor of 'femaleness', being an embodiment of 'determiners' of the female sexual characters, and that the female is homozygous as regards sex characters, the male being heterozygous. The presence of the heterochromosome in the higher vertebrates and its significance in the determination of sex are in a far from satisfactory condition at the present time. In the case of man, Guyer, Guthertz and Winiwarter have described heterochromosomes, but their descriptions do not mutually support one another and hence there is considerable uncertainty as to what the facts may be. Their observations, however, together with those of Jordan on the opossum and bat, Newman and Patterson on the armadillo, Stevens on the guinea pig, Vejowsky on the cat, indicate the presence in the male gametogenesis of an extra chromatin mass (heterochromosome). Jordan, however, states that it is absent in the spermatogenesis of mongoose, cat, squirrel, rabbit and pig. Winiwarter and Sainmont have observed in the oogenesis of the cat a body that they suggest may be a heterochromosome. The morphological basis of fact is therefore still very scant. The statement may be vouchsafed, furthermore, that the correlation of this individual chromatin mass with the development of female sexual characters or even with the determination of the female sex is unproven.

The assumption—to speak in Mendelian terms—that the female is homozygous (homogametic, Wilson; ♀ ♀) as to sex, the male being heterozygous (digametic, Wilson ♂), which appears to be the more generally accepted view, does not seem to afford a satisfactory hypothetical basis for the explanation of the double development of the reproductive duct system of higher vertebrates, the prostate and rete ovarii in the female, the mammary gland in the male. Nor does the converse furnish an interpretation much more satisfactory—that the male is homozygous as to sex (♂ ♂), the female being heterozygous (♀ ♂). If it is believed to be of advantage to express sex in these terms, in so far as the explanation of the development of the primary sexual characters of vertebrates is concerned, it would seem necessary to consider both male and female heterozygous as to sex, the male characters being or becoming dominant in the male, the female characters recessive, and vice versa, in accordance with the early suggestion of Castle.

The only generalization that can be safely drawn, however, from the cytological conditions that have been described would appear to be that in the development of the female sex a greater amount of 'formative material' is required. This is in general accordance with the suggestions of Boveri, Goldschmidt and Wilson, and is supported by the experimental work of R. Hertwig and Kuschakewitsch, Miss King, and Riddle, indicating what has been termed a 'quantitative' rather than a qualitative factor in the determination of sex. It would seem as though on the cytological side little assistance were to be gained from the work upon the 'sex' chromosomes as carriers or determiners of sexual characters. In most of the work upon the 'x,' 'sex' or heterochromosomes, the point of departure has been the correlation of chromosomes or parts of chromosomes with the appearance in development of definite morphological characters. The problem of the early development in vertebrates of the reproductive organs appears to be closely linked with hermaphroditism in the pattern, which I believe—admittedly by Wilson—presents difficulties from the standpoint of chromosomal sex determination. The problem is, I believe, broader and there is involved the choice between what

I have termed a process interpretation and an ultimate particle interpretation of structure. Furthermore there are two aspects that may well be quite distinct so that the problem would be essentially doubled—the determination of sex on the one hand, and the origin and development of the sexual organs on the other.

From whatever point of view the development of the vertebrate reproductive system may be considered, interest must center in the essential organs—the ovary and testis—and the question as to whether or not they, as well as the duct system, exhibit the sexual dimorphism is particularly pertinent. Waldeyer ('70) suggested that the potentialities for the development of both ovary and testis existed in the same individual and that the male and female gonads developed from different portions of the germinal ridge. Van Beneden ('80) from the conditions in the adult bat advanced the hypothesis that ovary and testis were homologous or at least analogous in their morphology, comparing the medullary cords (cordons pleins) to the tubuli contorti (seminiferentes) the medullary tubes (cordons tubulaires) to the tubuli recti, the reticular body (corps reticule; that is, rete ovarii) to the rete testis (Halleri). Subsequent work on the development of the ovary by Mihalkowics ('85), Janosik ('85) ('88), Coert ('90), Allen ('04), and Winiwarter ('00) particularly have seemed to justify the suggested homologization. It is the concensus of opinion of these later writers that the medullary cords are developed from the germinal epithelium, and are hence directly of mesothelial origin, they being early formed and succeeded later by the growths that furnish the functional germ cells of the ovary, the frequently described Pflüger's egg tubes or cords. It should be stated, however, that the older interpretation of the origin of the medullary cords from the Wolffian body, as held by Waldeyer, Balfour, and Braun, is still adhered to by O. Hertwig ('11) and Tourneux ('09), the latter regarding the tubuli contorti of the testis as developed from the same source, while the former derives them from the germinal epithelium (mesothelium).

The development of the rete ovarii (and the rete testis) seems unquestionably to demand further investigation to determine its mode of origin. Coert ('90), Mihalkowicz ('85), Janosik ('85),

Allen ('04), and Felix ('11) have derived it from the mesothelium, while Sainmont ('05) in his detailed study supports the older view of its origin from the Malpighian corpuscles of the mesonephros.

The most recent study of the development of the mammalian ovary, that of man, by Felix in the Keibel-Mall "Handbook of human embryology" leaves undiscussed (apparently purposely) the question of the comparability of the medullary portion of the ovary with the testis, the presentation in the second volume contrasting in this respect with the brief statement in the first volume illustrated by means of an elaborate diagram supporting the testicular homology of the ovarian medulla. Felix, in fact, distinctly rejects this homologization, inferentially at least, by the form in which the development of the gonads in man is described. Briefly stated, in the indifferent stage, the gonad consists of an inner epithelial mass, separated from the covering epithelium by the (primitive) tunica albuginea. In the male this becomes the permanent T. albuginea and the inner epithelial mass speedily resolves itself into the spermatogenic tubules. In the case of the ovary however, the inner epithelial mass becomes again intimately associated with the mesothelial covering of the organ and a new peripheral zone closely blended with and probably derived from the inner epithelial mass becomes developed, termed by Felix the 'neogenic zone.' This is destined to form the definitive cortex of the mature ovary while the central portion of the inner epithelial nucleus or mass undergoes a progressive degeneration toward the periphery. The inner epithelial nucleus therefore furnishes the material for the functional structures in either ovary or testis, differentiation of the former proceeding peripherally, and more slowly whereas in the testis the differentiation is central and early. Any homolog of the seminal tubules of the testis is lacking in the human ovary, according to Felix.

It will be seen that the exposition of the development of the human ovary given by Felix is not in itself contrary to the acceptance of the ovarian medulla-testis homology,—previously mentioned—since the portion of the epithelial nucleus in the indifferent organ that forms the spermatogenic tubules of the male takes no part in the formation destined to furnish the ova and follicle cells

of the fully developed ovary. The question of the double character of the ovary clearly hinges on the interpretation of the neogenic zone as well as that of the portion underlying it. It is evident however, that in any event the morphological comparison of the medulla of the ovary and the testis is not very close in man.

For the converse of the ovarian-testis homology—the representation of the ovary in the testis—but little has been said. There is nothing in the normal adult testis that can be construed as characteristically ovarian, as this would of necessity lie outside the tunica albuginea. Janosik ('85) has described a late formation of large cells in the surface epithelium of the testis in the human fetus as an attempt to form follicles. Bühler ('06) mentions the occurrence of germ cells in the surface epithelium of fetal testes, as apparently does Coert ('90). It is clear that any proliferative activity in the covering epithelium of the testis after the formation of the germinal cords is on any interpretation very slight.

The present investigation was undertaken to determine the origin of the interstitial cells[2] of the ovary, so abundant in that organ in the cat. While the monograph upon the development of the ovary of the cat by von Winiwarter and Sainmont ('08) has rendered unnecessary a detailed presentation of many aspects of ovarian morphogenesis in this animal, a somewhat differing interpretation and different point of view appear to justify a brief consideration by me.

The study in so far as presented is a purely morphological one based upon 60 series of sections of ovaries from relatively late fetal to adult life, the periods particularly considered being therefore those of the fetal, post natal, and pre-sexual development. While several methods of fixation and staining were employed, as indicated in the foot-note,[3] depending upon what it was desired to bring out, the technique already described (Kingsbury

[2] Published as a separate article.

[3] The ovaries upon which the study is based are as given below. Only a portion of the animals were reared in the laboratory, the greater number were procured from householders and hence the data as to age is not as exact as it would otherwise have been. My observations confirm those of Sainmont and v. Winiwarter that there is a large variation in ovaries of the same age. Age is therefore

'11) was found particularly useful because of the easy differentiation of the cells containing 'lipoid' and so-called 'mitochondria' (representing undoubtedly lipoid in masked form). The mor-

a poor indicator of stage of development, and the seriation may be easily determined from the internal structure of the ovary itself.

NO.	STAGE AND LENGTH		PLANE	FIXER	STAIN
1	embryo	75 mm.	8 μ	Flemmings Fl.	iron hematox.
2	embryo	75 mm.	8 μ T.	Flemmings Fl.	iron hematox.
3	fetus	80 mm.	8 μ Longi.	Flemmings Fl.	iron hematox.
4	fetus	80 mm.	8 μ Trans.	Flemmings Fl.	iron hematox.
5	fetus	95 mm.	Trans.	Flemmings Fl.	iron hematox.
6	fetus	95 mm.		Flemmings Fl.	iron hematox.
7	fetus	95 mm.	Trans.	Flemmings Fl.	iron hematox.
8	fetus	95 mm.	Sag.	Flemmings El.	I. H. and Safr.
9	fetus	112 mm.	Long.	Zenker's Fl.	I. H.; H. and E.
10	3 to 4 day P.P.		Trans.	Zenker's Fl.	I. H.
11	3 to 4 day P.P.		Sag.	Zenker's Fl.	I. H.; Safr.
12	6 days P.P.		Sag.	Zenker's Fl.	I. H; H. and E.
13	Ca. 10 days P.P.		Trans.	Z(?)	H. and E.
14	8 days P.P.		Trans.	Z. + Mullers	Cu. H.
15	10 days P.P.		Trans.	Z.	I.H.
16	10 days P.P.		Trans.	Z.	I.H.
17	12 days P.P.		Trans.	Z. + M.	Cu.H.
18	14 days P.P.		Long.	Z. + M.	I.H.; Cu.H.
19	16 days P.P.		Trans.	Z. + M.	Cu.H.
20	Ca. 14 days		Trans.	Z.	I.H.
21	21 days P.P.		Trans.	Z.	I.H.
22	Ca. 4 wk.		Trans.	Flemmings Fl.	I.H.; Safr.
23	Ca. 4 wk.		Trans.	Flemmings Fl.	I.H.; Safr.
24	33 days P.P.		Trans.	Zenker's + Mullers; Z. + M.	Cu.H.
25	Ca. 5 wk.		Sag.	Cu. dichr, etc.	H. and E.
26			Sag.	Z.	I.H.
27			Sag.	Z.	I.H.
28			Trans.	Fl.	I.H.; Safr.
29			Trans.	Fl.	I.H.; Safr.; no stain
30	7 wk. P.P.		Trans.	Z.	I.H.; Cu.H.
31	7 wk. P.P.		Trans.	Z. + M.	Cu.H.
32			Trans.	Fl.	Safr.
33	10 wk. P.P.		Trans.	Z. + M.	Cu.H.
34	Ca. 10 wk.		Trans.	Z. + M.	Cu.H.
35	Ca. 3 mo.		Trans.	Fl.	I.H.; Safr.
36	Ca. 3 mo. (cystic)		Trans.	Fl.	
37	Ca. 4 mo.		Trans.	Fl.	I.H.; Safr.
38	half grown			Fl.	I.H.; Safr.
39				Z.	Cu.H.
40	approaching sexual maturity			Hermann's Fl.	No stain; Safr.
41			Trans.	Z. + M.; Benda's Fl.	Cu.H.; Benda's
42			Trans.	Z. + M.	Cu.H.; I.H.
43	young adult; virgin		Trans.	Picro-acetic	H. and orange
44	young adult; virgin		Trans.	Z.	I.H.
45	young adult; virgin		Trans.	Z.	H. and E.; H. and Pf.; I.H.
46	young adult; virgin		Trans.	Z.	I.H.; H. and E.
47	adult (young?) corp. lut.		Trans.	Z.	H. and E.
48	adult (young) corp. lut.		Trans.	Z.	H. and E.
49	adult		Trans.	Fl.	I.H.; Safr.
50	adult (injected)		Trans.	Alc.	H.
51	adult preg. (#132 a)		Trans.	Z. and M.	Cu.H.; I.H.
52	adult preg. (#E-1)		Trans.	Z.	I.H.; Cu.H.; H. and Pf.
53	adult preg. (#150 a)		Trans.	Z. + Pot. dichr.	H. and E.; I.H.; Cu.H.
54	adult preg. (fetus #73)		Trans.	Z.Z.	H. and E.
55	adult preg. (fetus)		Trans.	Fl.	I.H.; Safr.
56	adult (preg. near term)		Trans.	HgCl2.	I.H.; H. and Pf.; H. and E.
57	adult nursing		Trans.	HgCl2.	H. and E.; H.Pf.; I.H.; Elastin St.
58	adult (corp. lut.)		Trans.	Fl.	I.H.; Safr.
59	adult old (17 yr.)		Trans.	Fl.	Safr.; I.H.; no stain
60	(1 ovary pathol.)				

phological differentiation obtained by this technique is particularly serviceable. The dark blue interstitial cells and the epithelial structures a lighter blue contrasting strikingly with the golden brown of the stromal tissue and thus permitting them to be readily distinguished without high power examination, while at the same time a good fixation of cytoplasmic and nuclear detail was obtained.

The study began with a fetus of 75 mm. total length, which corresponds closely with the forty-day fetus of Sainmont. At this stage the morphology of the testis is fully established, as is also the general morphology of the ovary, which consists of primitive medulla and primitive cortex both composed of cordlike groups of epithelial or epitheloid cells as the parenchyma, with strands of stroma cells interspersed. The parenchyma of the primitive cortex is in broad connection with the surface epithelium and consists largely of genitoid cells with but few indifferent cells discernible. The strands of stroma cells are small and relatively insignificant. The parenchyma of the primitive medulla is made up of genitoid cells and indifferent cells the latter present in much greater amount as compared with the cortex. The genitoid cells resemble those of the primitive cortex markedly. While some of them lie free in the stroma, the majority are surrounded by indifferent cells, some few possessing an evident follicular epithelium. Certain of these cells have taken on the character of 'egg cells.'

The parenchyma of the primitive medulla and primitive cortex are broadly confluent with each other, the separation of the two being largely indicated by a more marked accumulation of stromal tissue in the intermediate zone, in the form of a trabecular meshwork of strands of spindle-shaped stroma cells, and blood vessels which have a general longitudinal direction in this region. These blood-vessels form convenient landmarks. The primitive cortex (as well as the definitive cortex later) is strikingly free from larger vessels.

At the outset it may be said that it is obvious that in the morphogenesis of such an organ as the ovary, its increase in size must be kept constantly in mind, in the analysis. The ovary of the 75-mm. embryo or fetus in which the structural components—

so-called medullary cords, egg cords, rete ovarii, and stroma—have already appeared, can nearly be contained in a single large Graafian follicle of the adult organ. An understanding of the morphological transformations that lead to the establishment of the adult organ is to be found only in a correct analysis of its growth. In the published descriptions of the development of the ovary the marked and rapid increase in size, it is felt by the writer, is frequently not adequately expressed or given due consideration. Thus, the so-called egg tubes of Pflüger are spoken of as 'down-growths' of the surface (germinal) epithelium, whereas they more exactly represent cell trails left behind in the advancement of the surface as the organ increases in size. This does not apply apparently to the earliest proliferative growth of the germinal epithelium in the indifferent period, in which apparently there is an actual displacement of the underlying tissue (Felix '11).

The medullary cords in similar manner represent the earliest proliferations of the mesothelium whose immediate activity apparently largely determines the early growth. Whether or not the mesothelium loses its connection with these early formed masses, the stroma growing between as a tunica albuginea primitiva as described by Sainmont, I cannot of course consider, since the stages studied do not include the early development. In the 75-mm. fetus a sharp separation into primitive medulla and cortex by a distinct stromal layer does not exist.

In the further prenatal growth of the ovary, as illustrated in the stages examined, the ovary more than doubles its diameters. This size increase is due to the double activity of the epithelial elements (ova and indifferent or follicle cells) and of the stroma. The surface mesothelium retains throughout its connection with the epithelial masses to which the term egg cords may be applied and which early assumed a more elongated form due to their greater separation by strands of stroma and the increase in size of the ovary due to their growth and that of the stroma as well. The seat of proliferation lies mainly peripherally in the primitive cortex and the growing zone becomes more superficial as development proceeds. Cell divisions also occur frequently in the sur-

face mesothelium, being apparently more abundant in the earlier stages in which the egg cords are relatively more broadly connected with it. It but shares with the underlying egg cords a common growth activity and in no sense can it be regarded as the sole direct center of proliferation from which the latter are formed. Indeed, at birth the mitoses are more abundant in the outer portions of the egg cords than in the surface epithelium with which they are connected. The synizesis figure of the germ cells forms a convenient landmark which serves to mark the centrifugal march of differentiation within the egg cords. Mitoses continue to occur within the peripheral portion of the egg cords until about two weeks after birth.

von Winiwarter and Sainmont have described the divisions of the oogonia as practically ceasing at the time of birth, marked multiplication appearing again in the twenty-first-day ovary and becoming permanently arrested soon thereafter. I have seen no evidence of such a periodicity in the oogonial divisions in the material employed by me, which, it must be confessed, was not as abundant as that studied by these authors. The advance of the 'wave of synizesis' appeared a fairly steady centrifugal progression. In the thirty-three-day ovary the superficially located germ cells of the cords were in the synizesis stage, all the more deeply placed cells being postsynizetic. In all subsequent stages the ova were definite oocytes and in the seven-weeks' ovary the definitive cortex with its primary or resisting follicles was differentiating out of the primitive cortex.

In its development the ovary of the cat, as far as concerns the growth and differentiation of the cells that—according to the writer's interpretation—are to become the definitive ova of the period of sexual maturity, conforms to the plan described by Felix for the human ovary, the egg cords being less distinct in the human ovary and the neogenic zone described by Felix less developed as a distinct zone in the cat. The distinctness of the neogenic zone destined to furnish the ova of the mature period would appear to be likewise evident earlier than in the cat. The stroma by its growth evidently plays an important part in the breaking up of the egg cords and the formation of the primary follicles of the

definitive cortex. In the deeper portions of the ovary marked growth activity of the stroma introduces a complexity not encountered in the typical zone of the primitive cortex. In the ovary before birth and for the first few days after birth the egg cords project deeply into the ovary, occupying a position which subsequently will become medulla. The egg cords are typically branched, presenting the characteristic appearance well known from previous published descriptions by several workers (figs. 8–9). In the deeper portions of the egg cords the ova are the most advanced in development and both types of cells—egg cell and follicle cell—may be recognized in this portion of the egg cord. The surrounding stroma is relatively denser in this middle zone of the ovary and the growth activity of the stroma appears particularly marked here. The strands of stroma cells run irregularly, some having a more radial, others a more tangential direction.

Because of the presence of egg cords in this zone, it is usually grouped as part of the primitive cortex. It might with equal propriety be regarded as part of the medulla, since medullary cords are also contained therein—that is, groupings of mainly indifferent cells with only an occasional definite ovum. This zone is in fact an intermediate zone and its peripheral portion persists as a boundary zone during the period of postnatal growth of the ovary.

In their deeper portions the egg cords become separated into cell groups containing one or more ova and in this way numerous follicles are formed. Their number appears to be added to by continued 'cutting off' of egg cells from the egg cords in the intermediate zone. In this zone of the postnatal ovary great complexity exists. It seems to represent the main line of advancing stromal growth following upon the peripheral (and superficial) zone of epithelial proliferation, perhaps associated with the beginning follicular differentiation.

In illustration of the more general features of the morphogenesis, figures 8 to 15 may be consulted. The distinctness of the cortex becomes progressively more marked until it assumes its definitive structure. This applies as well to the other zones of

the adult ovary. What has been spoken of as the intermediate zona is most distinct in the growing ovary in the postpartum period (fig. 10), losing its identity in the adolescent and adult period (figs. 14 and 15) as part of the zona parenchymatosa. The zone vascularis, on the contrary, can hardly be said to exist as such in the fetal ovary, and becomes more distinct as maturity is approached. Marginal growth, along the line where ovary and ovarian ligament join (represented by the white line of the adult human ovary) appears to play an important part in the assumption of the adult morphology. In the marginal zone growth continues apparently as long as the increase in size continues in the presexual period (approximately four months), the growth including the stroma as well as the parenchyma (elements of epithelial origin).

The zones of the ovary are but the expression of the mode of growth and type of blood supply, and hence possess no intrinsic or genetic significance such as the cortex and medulla of the suprarenal organ, for example, possess; but even so, the terminology of the regions of the mammalian ovary is not altogether satisfactory. The older terms, medulla and cortex, as well as the B.N.A. terms, zona vascularis and zona parenchymatosa are both employed and are both applied to the mature organ. The latter terms may be very satisfactorily employed in the description of the adult ovary but they are not so applicable in the developing structure due to the mode of growth. The terms introduced by Sainmont ('05) in his study of the development of the cat's ovary and subsequently adopted in the monograph by von Winiwarter and Sainmont ('08) are quite serviceable and are those which will be used here, with two or three modifications which render them more serviceable as expressions of the present writer's conception of the morphogenesis. The primitive cortex becomes the definitive cortex during development. This is a somewhat more restricted use of the term than that usually employed. It represents therefore only the outer portion of the definitive cortex in the more usual use, or the zona parenchymatosa. It is, however, as employed by His ('65) in the cat's ovary. The outer portion of the nucleus epithelio-stromalis centralis (of Sainmont) is the

intermediate zone described above. Into the composition of the zona parenchymatosa of the adult there goes, therefore, the definitive cortex, the intermediate zone and a portion of the epithelio-stromal nucleus. The zona vascularis is developed out of the basal nucleus (connective tissue) of the growing ovary extended at the expense of the epithelio-stromal nucleus, while the marginal growth of the ovary plays a part in establishing it. The primitive medulla would include the epithelio-stromal nucleus and the basal nucleus.

The developmental changes that take place in the deeper portions of the growing ovary, in the primitive medulla, are complex, and this is intensified by the desirability of determining, for theoretical reasons, the exact mode of growth and the morphological value at the different stages of the so-called medullary cords which have so frequently been regarded as the equivalents of the seminal tubules of the testis. The rete ovarii occupies a position in the cephalic portion of the medulla (fig. 9). Its tubules are, in the youngest embryo studied (75 mm.) easily distinguished, of definite cuboidal-columnar epithelium, with a distinct lumen. Subsequently, in older fetuses, the epithelium becomes more distinctly flattened and the rete character more accentuated (fig. 8). Whether the cell cords that form the structure are derived from the mesothelium at the cephalic end of the ovary, or are 'ingrowths' from the mesonephros, or are of double origin and nature, has not been made an object of investigation by me, and hence of course cannot be adequately considered at this time. The structure as observed in the growing organ suggests the last view. The rete furthermore undergoes a progressive change after birth and remains as a persistent structure in the adult ovary. It requires no very extensive study of the ovary of older fetal and new-born animals to determine that at least some of the medullary cords are connected with the rete ovarii, as has been described by others (von Winiwarter and Sainmont '08) and this requires therefore no extended description or comment at this point. Two other features of the medullary cords in the older embryos, the presence of fat-granules in the cells, and the occurrence of large cells within the cords, require brief discussion. Droplets of a

fatty ('lipoid') nature were found in the medullary cords of the 95 mm. and 112 mm. fetus, three to four-day kitten, and still demonstrable in the six-day kitten. They may have been present in the earlier stages examined, but the technique was not of a nature to demonstrate their presence easily. von Winiwarter and Sainmont found the fat globules appearing about forty-five days p.c. (i.e., 70 mm. length, Sainmont), and no longer present three days p.p. They reject the interpretation of Allen ('04) in the pig, that the presence of fat is indicative of degeneration of the medullary cords. They emphasize on the contrary the presence of fat as evidence of profound metabolic change taking place in the medullary cords at this time. This, perhaps, could hardly be questioned. It might be added, however, that one can hardly speak of a "disappearance of lipoid from the medullary cords," since the application of what I may term a 'mitochondrial technique' adduces evidence of the presence of fat in abundance in masked form in all the epithelial cells within the ovary—so-called medullary cords, follicle cells and ova—at all stages of their later growth, at least, appearing as free lipoid globules in the last, in the process of their vitellogenesis. Hence the question resolves itself into the reason for the existence of droplets of *free* lipoid in the epithelial cells (medullary cords) in the deeper portions of the ovaries. Inasmuch as I find evidence of dwindling and disappearance of these cell cords in the deeper portions of the ovary at about this time (after birth) I incline to the interpretation offered by Allen.

The 'large cells' present in the medullary cords and the surface epithelium have not been specially studied by me. They were encountered in the two youngest embryos and in the surface epithelium, particularly in the zone bordering the hilum, well along in postpartum stages. I have seen no reason for drawing a sharp line between these cells and obvious 'germ cells,' as I believe that all intergradations between them and the latter may be found. On the other hand, most of them, at least in the antepartum ovary, do not give rise to the definitive germ cells of the adult ovary, and hence are not primordial germ cells in the original sense ('Ureier,' of Waldeyer). It is unnecessary to

introduce a discussion of their interpretation and significance. Reference is simply made to the excellent discussion of von Winiwarter and Sainmont (p. 67). Those investigators who accept a definite cell-lineage for the germ cells, strongly suggested by the observations of Allen ('11), Rubaschkin ('09, '11) Wood, Dodds, and others, may interpret them as unproductive side lines of the germ-track or as a more or less temporary hypertrophy, for unknown reasons of 'indifferent cells' cells, as accepted by von Winiwarter and Sainmont, who believe that they subsequently return to normal size. Upon a purely physiologic or process interpretation, however, these cells might still be grouped with the germ cells as an expression of the oogenetic processes at work in the developing ovary.

The period of the postpartum growth leading up to the appearance of medullary follicles and their growth is a critical one in the theoretical interpretation of the morphology. The primitive cortex and medulla become accentuated and extended. The ovary in the first five weeks quite doubles its diameter. The stroma ovarii continues its obvious growth activity. The increase in the epithelioid cords and their morphological transformations furnish the characteristic feature of this period. They increase in bulk and while very irregular in contour often assume a more tubular form. Their cells increase in size and by their arrangement assume a form more characteristically epithelial, as about a potential lumen. Their nuclei lie more peripherally and the inner ends of the cells are more elongated, vacuolar and reticulate. Abundant 'mitochondrial' substance is present here. The structural appearance in such cases forces a comparison with the tubules of the testis, and the comparison becomes enhanced by the relation of the cell cords within the medulla of the ovary to the rete. The resemblance to the tubules of the undifferentiated embryonic testis, or more closely to the tubules of the cryptorchid is particularly striking. von Winiwarter and Sainmont ('08) have called attention to this resemblance of the medullary cords to the tubules of the testis, as the former ('00) had previously done in his paper on the development of the rabbit's ovary, and as several have done in a comparison of the ovarian medulla

with the testis. Such a comparison, to which the writer at first inclined, seems to him upon maturer consideration a superficial one. The elongated form, simulating a tubule is not the universal form of growth of these cell masses nor do they possess, when elongated, the morphology of the seminal tubules.[4] The form assumed is quite irregular and is obviously a factor of the growth of the epithelial cords and stromal strands in their mutual relation to one another in the growth of the ovary during this period.

The source of the cells that compose the cell cords and masses so prominent during the period of expansion is also important in this connection. Two modes of origin present themselves as possible: (1) A development by centrifugal growth of the primary medullary cords apparent in the embryo so that from them come all the epithelial cords inside the zone of the primitive cortex. This mode of growth would make the medullary structures a unit and strengthen the ovary-testis comparison. (2) That the cords and masses of epithelial cells within the medulla, while in many instances connected with the primary medullary cords and quite possibly grown out from these cell groups, are nevertheless derived in part from the indifferent cells contained in the egg cords of the embryo. The differences of the epithelial cells would be purely a matter of position and relation and not intrinsic or morphological.

It is this last view that seems to me undoubtedly the correct one. Small groups of epithelial cells are to be found at all stages of the postpartum growth in the central nucleus, the intermediate zone and, especially later, in the inner portion of the primitive cortex. They are formed by the breaking up of the inner portions of the egg cords into primary follicles. Many of these primary follicles and epithelial cell groups without an ovum enclosed come to lie within the medulla, in the epithelio-stromal nucleus where they subsequently grow and play a part in the development of the medullary follicles, presently to be described.

[4] Bremer, J. L. Amer. Jour. Anat., vol. 11, no. 4, May, 1911, pp. 393–416. Huber, G. Carl, and Curtis G. M. Anat. Rec., vol. 7, no. 6, June, 1913, pp. 207–220.

In connection with the comparison of the structures within the ovary to the testis tubules just considered, it may be said at this point that the cells are as strikingly follicle cells at this stage and place as subsequently in the development of the follicles of the adolescent and adult periods. This is particularly clearly shown in a comparison of such cells when a mitochondrial technique has been employed. The disposition of the mitochondrial granules in the inner ends of the cells in each instance serves to make the agreement more striking. The fact that these so-called medullary cords develop into follicles should leave no doubt of their ovarian character.

The medullary follicles. The development of the follicles within the medulla of the ovary of the kitten has been studied in detail by von Winiwarter and Sainmont, who apply the name of 'medullary follicles' and regard their appearance as marking a third stage in the development of the ovary. Their description, briefly stated, is as follows: At eight days postpartum the medullary cords are markedly elongated; at sixteen days they have increased in volume and the cells have hypertrophied and become more columnar in shape. This stage is the last one in which a connection of the medullary cords with Pflüger's egg cords exists. Ovules exist within the medullary cords, generally smaller than those of the primitive cortex. Small medullary cords are described in the zone bounding the primitive cortex and containing ovules which belong to Pflüger's egg cords. Such they interpret as ovules of Pflüger's egg cords which have become isolated with a medullary cord. At twenty-three days p.p., primordial medullary follicles, both uni- and pluri-ovular, are beginning their development. At thirty-five days, the next described stage, the medullary follicles have become voluminous structures. These they group as follicles in process of growth and follicles fully formed, the latter possessing an antrum comparable to the antrum of the definitive Graafian follicles of the adult period. The medullary follicles now undergo a peculiar degeneration during the next two or three weeks, so that, at sixty to sixty-five days p.p., all remains of the medullary follicles have completely disappeared, this degeneration being accomplished or accompa-

nied by an enucleation of the follicles by the ovarian stroma. The naked ova, so 'shelled out,' undergo a degeneration in the midst of the stroma. Not all ova degenerate through the destructive agency of the stroma, but undergo progressive degeneration within the medullary follicle. With the degeneration of the medullary follicles the indifferent cells and ova derived from the first proliferation of the germinal epithelium of the ovary entirely disappear.

An identical fate (i.e., degeneration) awaits the Graafian follicles derived from the egg tubes of Pflüger, or the second proliferation of the germinal epithelium.[5] Beginning about sixty to sixty-five days, Graafian follicles develop from the deeper portions of the cortical zone, the first ones being nearly always pluri-ovular, containing two or three or more ova. Very large follicles are thus formed, so that, about three and one-half or four months' post-partum, the ovary attains a large size, becoming subsequently reduced in size with the degeneration of these follicles and their absorption. At this same time, the remaining resting or primary follicles formed from the second proliferation have practically disappeared. A third proliferation[6] then furnishes the ova and follicle cells destined to develop into the Graafian follicles of the period of sexual maturity.

[5] von Winiwarter and Sainmont 1908, p. 85: "Nous nous proposons d'exposer dans le présent chapitre qu'un sort *identique* est reservé à tous les ovules et follicules de de Graaf qui dérivent des tubes de Pflüger ou cordons corticaux (seconde proliferation). Cette déchéance n'atteint pas tous les ovules au même degré de développement. Les uns, et ils sont majorité, ne dépassent pas le stade de follicule primordial. Les autres se transforment en folliclues de de Graaf plus ou moins volumineux. Neanmoins le résultat est le même: leur ensemble est voué à la mort. Deplus, la dégénérescence des follicules de de Graaf suit une marche très particulière, etablissant une transition manifeste et graduelle entre l'atrésie des follicules médullaires que nous avons décrite et l'atrésie typique, telle qu'elle a été étudiée dans l'ovaire adulte. C'est pourquoi nous faisons suivre la description des cordons médullaires par celles de l'évolution des cordons corticaux, afin de faire ressortir combien le développement de l'ovaire est continu et progressif."

[6] von Winiwarter and Sainmont, 1908, p. 89: "Il arrive un moment (vers $3\frac{1}{2}$ à 4 mois p. part.) où, pratiquement, tous les follicules primordiaux ont disparu. On ne voit plus alors sous l'épithélium de revêtement qu'une serie de cordons épithéliaux, représentant tout au moins en partie les anciennes cellules folliculeuses. Nous disons en partie, car, ainsi que nous le verrons ultérieurement, tandis que ces phénomènes régressifs se déroulent, l'épithélium de revêtement

I have sketched the general results of von Winiwarter and Sainmont because of the monographic character of the study made of the development of the ovary of the cat and the uniqueness of some of the results and interpretations offered. It is therefore with some hesitancy that I venture, from a study of material inferior in amount to theirs, to offer interpretations that differ in certain fundamental respects.

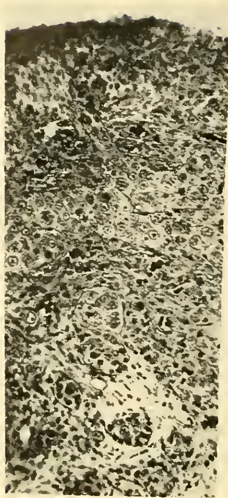
In as far as concerns the medullary follicles, the difference of interpretation has three aspects. In the first place, as has been fully indicated in the discussion of the medullary cords in the foregoing paragraphs, the writer is unable to accept the sharp distinction between the products of a "first and second proliferation," a distinction that plays so important a part in the interpretations of von Winiwarter and Sainmont. The distinction of medullary cord and sex cord seems to the writer more a matter of differentiation, location, and convenience for descriptive purposes rather than a sharp distinction of materials of morphologically different values. The follicles that begin their development during this period are thus but the earliest follicles which from position may appropriately be termed medullary follicles, it is true, but only for descriptive and topographical reasons. Their development, peculiarities and fate are closely involved in the growth processes taking place in the ovary at this time.

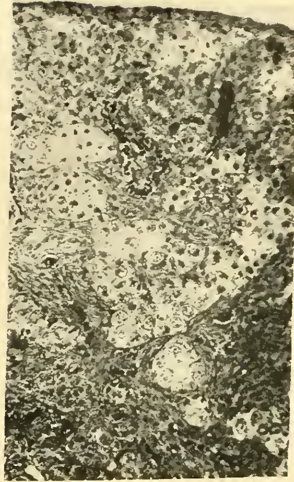
The second point of different interpretation involves the source of the ova and the early transformation of the inner portions of the 'egg cords.' As has been already stated, it has seemed to the writer clearly apparent from a comparison of the successive series of ovaries of advancing development, that going hand in hand with the peripheral growth of the egg cords there has been a central

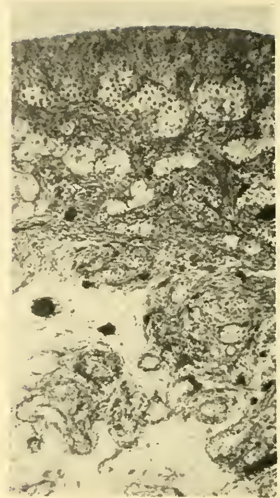
ne reste pas inactif. Une nouvelle prolifération (là troisième), celle des *invaginations épithéliales*, apparait et les colonnes cellulaircs qui les composent, traversent l'albuginée et se mêlent aux amas des cellules, folliculeuses. Comme ces deux formations sont constituèes de cellules épithéliales ordinaires, il est impossible morphologiquement de distinguer ce qui revient aux unes et aux autres. Toujours est-il que c'est à leurs dépens que se formeront la zone corticale définitive et les oeufs définitifs de l'adulte." (p. 260): "Ces invaginations, jointes aux cellules folliculeuses de la zone corticale primitive, aboutissent à la formation de la zone corticale définitive de l'ovaire, à laquelle, seule, sera réservée la production des oeufs définitifs. Son histoire appartient à un chapitre ultérieur."

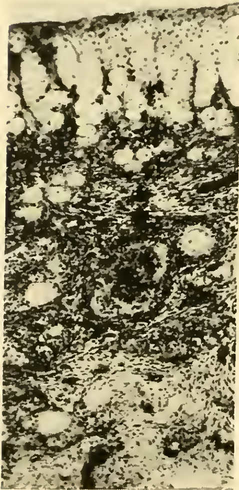
disassociation or breaking up of the egg cords in which the growth of the stroma has been largely instrumental. This leads to the isolation of the ova or groups of ova, and from these have come the ova which occur in the medullary follicles that begin their development in the third week after birth. In illustration of this interpretation there are submitted as text figures 1 to 6, six photographs of sections taken entirely at random and therefore not of illustrative value otherwise. In these the centrifugal oogenetic wave is indicated by the synizesis stage apparent in all save the first and last in which the ova are in the pre- and post-synizetic stages, respectively. From a comparison of these it will become apparent, I believe—if the growth of the ovary be kept in mind—that the large ova in the deeper portion which in the last two figures are in developing medullary follicles are derived from the progressive breaking up of the inner portions of the so-called egg-cords.

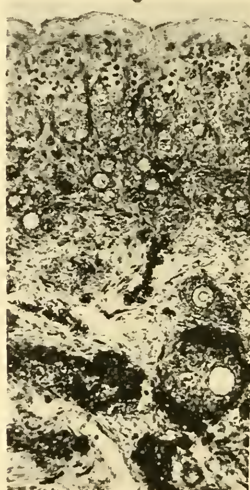
The third difference of interpretation of the medullary follicles concerns the meaning of the peculiarities of their form. The view of von Winiwarter and Sainmont, that the great irregularity and peculiar form of the follicles encountered in the ovary of the cat at this stage is due to the destructive activity of the ovarian stroma by penetrating and enucleating the follicle, has been noted above. The striking peculiarity of the follicles developing during this period was observed by myself before I had become aware of the work of von Winiwarter and Sainmont and they had been interpreted in quite the reverse direction, namely, as progressive rather than regressive pictures. Further examination and consideraation has but confirmed me in the interpretation. The medullary follicles began their progressive development early in the third week after birth, although a close limitation of the time of their appearance cannot be given. The numerous ova in the ovary within the zone of the primitive cortex possess well-marked follicular epithelium. Some of them are definitely within so-called medullary cords of elongated form, others within more irregular masses. A number lie free in the stroma, surrounded only by a single layer of follicle cells which may vary from flattened to columnar in shape. Such ova are particularly found in the peripheral











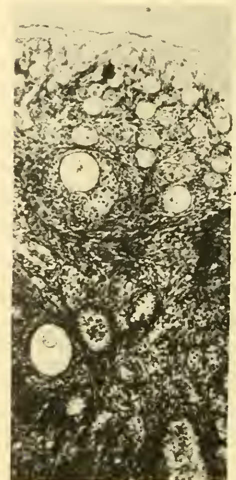


Fig. 1 80 mm. fetus. × 120.
Fig. 2 95 mm. fetus. × 110.
Fig. 3 Three to four day kitten. × 60.
Fig. 4 Twenty-one day kitten. × 60.
Fig. 5 Thirty-three day kitten. × 60.
Fig. 6 Five to six weeks kitten. × 60.

portion of the medulla and in the intermediate zone. Interspersed with these are cords or clusters of follicle cells whose connection with egg cells is not apparent. Some of these appear to come from the indifferent cells of the egg cord in its breaking up, or to have survived the degeneration of an egg cell which they once enveloped (fig. 16). Such epithelial structures with the surrounding stroma and the interstitial cells compose the epithelio-stromal nucleus of the two-week ovary. Marked growth of the masses and cords of follicular cells and of the stroma produces the ovary of two months. Very great irregularity is shown in the growth of the cords of follicle cells, and their relation to the ova is particularly interesting. A typical follicle formation of this period is shown in figures 24 and 25.

It is structures of this kind that have been interpreted by von Winiwarter and Sainmont as follicles in degeneration attended by penetration of the stroma and enucleation. Figures 16 to 25 are introduced in illustration of the reverse view. From the morphological relations there shown it would appear that the eggs are becoming enveloped by the growing follicle masses. Figure 16 shows two small egg cells of smaller size and close to each a cluster of follicle cells, doubtless derived from adjacent indifferent cells of the original egg cord. Each egg is invested by a layer of follicle cells of its own. By growth of the follicular cluster the ovum, in its epithelium, becomes partially surrounded (fig. 17, 18) and finally completely invested (figs. 19, 20) by the growing cell mass; and with the appearance of an antrum—indicated from the beginning—a Graafian follicle more or less irregular, is developed. Several ova may thus become included in a single follicle which may be of rather tubular form (fig. 23). The layer of follicle cells belonging to the ovum and immediately surrounding it may be traced well into the complete investment. The investing mass fuses with it and its identity is finally lost. It is indicated in figures 17 to 21.

Whether the investing follicular mass at the beginning is always or even usually distinct from the sheathing follicle cells is not easy to determine. In many instances, at least, it would appear that the investing mass is developed from one side of the

primary follicle or medullary cord and in the process of growth simply becomes wrapped around, enclosing ovum and its follicular epithelium of the opposite pole, as well as some of the stroma.

In support of the above interpretation and as opposed to the reverse one of regressive change by a process of enucleation, may be advanced: (1) the fact that in general the smaller egg cells are less enclosed; (2) that they lie generally more peripherally; (3) that the line of fusion of the investing epithelium may often be detected (figs. 19, 20, 22); (4) that the free egg cells possess in most instances at least a sheathing simple follicular epithelium; (5) that degenerating egg cells lying free in the stroma are but seldom encountered.

Accepting the interpretation above set forth, it can hardly be doubted that these follicles owe their irregular form relations to a compounding of the growth of the stroma and that of the follicular elements. It may be suggested that their peculiarities are to some extent due to an attempt at follicle formation in an ovary during rapid growth; or, put differently, the growth of follicular elements and ovarian stroma in lines at variance with each other prevents the free expansion and uniform growth so characteristic of the adult ovary, so that the growing follicular cords must grow around the ovum and hence come to invest it (figs. 24 and 25). The reverse, from the same point of view, might easily be conceived to occur and the follicular cells be stripped off as a result of the pressures and tensions of the stromal growth, and thus the ovum be left naked in the midst of the stroma, producing thus the effect of enucleation without attributing to the ovarian stroma a specific activity in the process. Indeed, from the figures of von Winiwarter and Sainmont, as well as my own observations, this appears frequently to be the case. What chemical correlations may likewise be involved cannot be estimated.

The follicular structures in the ovary at this period are of very varied form. In order to obtain a three-dimensional view of the relations, a model was made of a portion of an ovary (no. 26) in which follicular cords, follicles and ova inside the primitive cortex are illustrated. The ova shown are in all cases immediately surrounded by their simple follicular epithelium. Of two of the

follicles so shown, enlarged drawings are given in figures 29 and 30; figure 29 may be compared to the sectional view shown in figure 23 and figure 30 may be similarly compared with figure 18. Within the larger masses an ovum was contained completely enclosed. The picture that is afforded by the reconstruction supports, I believe, the evidence which the histology furnishes, namely, that in most instances the process illustrated by these peculiar follicle formations is one of inclusion rather than of exclusion of the ovum, in most instances at least.

Observations of these formations by earlier workers than von Winiwarter and Sainmont in the cat's ovary, or similar structures in the ovaries of other animals, appear to be lacking. von Kölliker, it is true, and Bühler in his earlier work, regarded the follicular epithelium as coming from the medullary cords and these in turn from the mesonephros, investing the egg cells (which were derived from the germinal epithelium) by growing around them, so that relations similar to those met with in the kitten's ovary may have been influential in forming their interpretations. But neither their description nor figures give us definite evidence.

The fate of these peculiar follicles is one of considerable importance in their interpretation. While many of these follicles evidently degenerate, the majority I believe remain as the large irregular, usually pluri-ovular follicles which are found in the ovaries of kittens before the onset of sexual maturity. Such follicles are shown in figure 13 and were figured by Sainmont (fig. 15) in his earlier paper, wherein they were not specifically discussed, although they were obviously thought of as derived from the "egg tubes of Pflüger." In the later and larger work with von Winiwarter, so often referred to in this paper, these follicles were described and regarded as developed out of the material of the second proliferation (Pflüger's egg tubes), succeeding as a second set the 'medullary follicles' formed from the medullary cords of the first proliferation which entirely degenerated. This interpretation is a necessary corollary to the interpretation given by them of the peculiar follicle formation with which we have just been dealing. Figures showing the irregularity and pluri-ovular character of these follicles are given (11 text figures, p. 90) but none

showing their development, so that it is somewhat difficult to judge of the evidence—aside from the interpretation of the peculiar medullary follicles of younger ovaries—that lead them to regard these as quite distinct from the follicles of the earlier period. Sainmont's figures (4 to 9) appear to me far from convincing. In their description their resemblance to the medullary follicles is pointed out and the similarity in their mode of 'degeneration.' They differ in the presence of a better developed theca and the relation and character of the interstitial cells.

As in their formative period—as I interpret it—these follicles are characterised by their great irregularity and the numerous ova usually contained. This appears in figure 13 of a single section, and more so in a model made from a section of the same ovary. In between these follicles occur numerous cords of cells (which were reproduced in part) and strands of interstitial cells (not shown in the model). The stroma forms a well defined theca for the follicle and it is there that the interstitial cells chiefly occur. Two of the irregular pluri-ovular follicles are drawn separately (figs. 31 and 32). Their irregularity is clearly apparent from these. The cords of cells are apparently vanishing structures—small groups of follicle cells derived from the medullary cords or of cortical origin. In these follicles several ova are contained.

The approach of sexual maturity is attended by a profound degeneration of the Graafian follicles formed during the pre-sexual period. In ovaries of this stage nearly all the larger Graafian follicles are found to be in some stage of aresia, as illustrated in one of the figures in my paper on the interstitial cells. Eight ovaries of this period were examined. The conclusion of von Winiwarter and Sainmont that the degenerations of this period involve also the primary follicles as well and that the ova of the sexual maturity are derived from a third 'down-growth' from the surface epithelium, renders this period one of marked importance. As far as the material studied by me goes, it has afforded no evidence of such a new formation of ova; in nearly all cases an abundance of resting or primary follicles in the cortex was found to be present, and it is believed that the primary follicles found in the

ovary during the adult period are formed in the differentiation of the cortical zone in the pre-sexual period. But one ovary (no. 39) was secured, clearly belonging to this period, in which the primary follicles had nearly completely disappeared, giving the picture described and figured by von Winiwarter and Sainmont. This was, however, regarded as a case of exceptionally profound degeneration, and showed no evidence of a renewed proliferation of oogonia from the surface epithelium. The margin of the ovary bordering the hilum long (up to three months, at least) remains a seat of proliferation and growth during the enlargement of the ovary in the pre-sexual period, and it is quite possible that in the second growth period (that immediately preceding sexual maturity) following the period of profound degeneration and corresponding diminution in volume, the marginal surface epithelium particularly resumes its proliferative activity with a resulting increase in the number of primary follicles. It is also quite possible that the profound degeneration and consequent third proliferation are related, and not constant but variable; only an extreme morphological interpretation would reject the possibility. The material, however, is insufficient for the settlement of these points. Neither the preliminary paper of von Winiwarter and Sainmont, nor the statements in their subsequent papers present the evidence in a form that seems to me fully convincing, and the promised chapter[7] upon the development of the third proliferation will be looked forward to with interest.

If it is a matter of deep-seated importance and not a mere expression of growth, a proliferation of ova just before sexual maturity will be found to occur in other mammals. It may be questioned, however, whether Rubaschkin ('12) is justified in regarding what he terms a 'third proliferation' from the surface epithelium of the guinea pig ovary, occurring *before* birth, as homologous with a (third) proliferation of ova just *preceding sexual maturity,* such as von Winiwarter and Sainmont describe for the cat.

As a result of the profound degenerations before the onset of sexual maturity there is left an ovary smaller but richer in stroma

[7] See quotation, from p. 260, in footnote 6, p. 363.

(fig. 14). The Graafian follicles that develop during the period of sexual maturity are almost always uni-ovular in the cat, are more regular and uniform in shape and the ova are characterized by a markedly different structural appearance as compared with the ova of the pre-sexual Graafian follicles as noted by von Winiwarter and Sainmont.

A very frequent accompaniment of the degeneration of the Graafian follicles of the pre-sexual period in kittens approaching sexual maturity, may be incidentally mentioned, namely, the occurrence of polar spindles, polar body formation and a form of degeneration of the ovum by fragmentation, simulating a parthenogenetic cleavage. It is unnecessary to consider here the question of their parthenogenetic nature. Similar conditions have been described in several forms. Bonnet has considered and rejected their parthenogenetic character. In the "Hertwig Handbuch," Waldeyer has discussed them and pronounced against their parthenogenetic significance, while Hertwig regards them as essentially a beginning parthenogenesis. In the cat they occur most abundantly in old kittens (ca. four months) in the large Graafian follicles that have begun degeneration as atresia folliculi. While no particular search for them has been made, four polar spindles and several fragmenting ova (fig. 28) were encountered in a single ovary and were found to occur in four ovaries of this period. In illustration of the typical and 'normal' appearance, figures 26 and 27 are submitted, showing a first polar body and second polar spindle (?) in the same ovum. That there is not a factor peculiar to the period of adolescence which determines the introduction of maturation, is evinced by the occurrence of the polar spindle formation in a degenerating follicle of an adult ovary. That the phenomenon is not simply an indication that the growth period had been completed and the follicle essentially mature when it enters upon atresia, is also shown by the occurrence of a polar spindle in an obviously young Graafian follicle. In most instances, however, the follicles in which polar spindle formation occurs are of large size. The constant correlation is that with degeneration of the follicle. There appears to be, therefore, some factor connected with atresia

folliculi that can, under certain unknown conditions, induce maturation.

In the period of sexual maturity the development of the Graafian follicles is quite variable and the morphology of the ovary fluctuates correspondingly. Figure 15 shows a typical section of the zones of the adult ovary, the zona parenchymatosa consisting of cortex and zone of Graafian follicles (epitheliostromal zone), and the zona vascularis.

GENERAL CONCLUSIONS

The results of this study of 60 series of cat ovaries may be summed up briefly at this point. From the stage with which the study began, when the ovarian character of the organ is clearly established (75 mm. fetus) to sexual maturity, the ovary increases in diameter approximately five times. The growth zone appears to be mostly peripheral, in and beneath the mesothelial covering epithelium that is the source of the ova and follicle cells. The greatest activity appears in the primitive cortex beneath the surface epithelium rather than in the latter, and growth continues here well into the postpartum period, as von Winiwarter and Sainmont have shown. Up to approximately the third week postpartum the surface epithelium and the underlying cell cords of mesothelial origin form a common mass, the latter being connected with the former. Subsequently (third week) they are separated by the appearance of a tunica albuginea.

At the margins of the ovary bordering the hilum the growth activity continues much longer (up to approximately three to four months) and along this bordering zone the surface epithelium retains its connection with the underlying egg and follicle cell groups.

The deeper masses of cells of mesothelial origin are the older and differentiation follows a centrifugal course save at the margin where younger stages in the oogenesis are to be encountered long after they have disappeared from the remainder of the ovary. In this growth the stroma plays an important part. The growth of the ovary may be said therefore to be mainly peripheral and marginal, and the differentiation centrifugal and marginal.

Accompanying the centrifugal wave of differentiation and growth there is a progressive advance in the state of development attained. Early in development (antepartum) large genitoid cells appear in the central portion mainly in the medullary cords. These disappear. The earliest follicles are central but do not attain large size of advanced development but degenerate. After birth (third week) come the irregular medullary follicles which, however, degenerate. An irregular centrifugal wave of degeneration might therefore be said to follow after the wave of differentiation.

The wave of differentiation and growth does not run through to completion but in the periphery proceeds much more slowly, leading, therefore, to the establishment of a cortex in which the follicles remain long in a resting stage, as the well-known primary follicles.

Degenerations affecting follicles of all stages of development occur at all periods of the life history as well. The small and immature ovary of the anti- and postpartum periods is obviously 'unable' to provide adequate blood supply for the follicles beginning development during these periods and hence the suggestion at once arises that therein is to be found the reason for the profound degenerations occurring in the pre-sexual period and after sexual maturity; that the processes, which in the adult ovary lead to the formation of the mature Graafian follicle, are operative from the beginning but continually fail, due to the absence of the necessary conditions (nutritive or otherwise) reaching progressively more advanced stages as development and growth proceed. The lack of proper vascular and nutritive conditions is doubtless the cause of many of the degenerations[8] (atresia folliculi; degenerations of primary follicles) particularly in the adult period. Inasmuch as it is extremely doubtful if the developmental factors are all intrinsic—that is, the growth and differentiation within the ovary independent of the rest of the organism—it is equally improbable that all the degenerations of the growth

[8] Compare the conclusions of Clark, from the study of injected human ovaries, immature and adult. J. G. Clark. Johns Hopkins Hospital Reports, vol. 9, 1901: pp. 593–676.

period can be explained as due simply to the intrinsic conditions of growth.

The relations of the zones that mark the morphogenesis of the cat's ovary may be illustrated by a series of schemata (text figure 7) wherein schema *A* indicates approximately the conditions in the 75 mm. fetus, schema *D*, the zones of the adult ovary. These schemata may also be directly compared with the photographs reproduced as figures 8 to 15. The nomenclature of the ovary has been briefly discussed (p. 357).

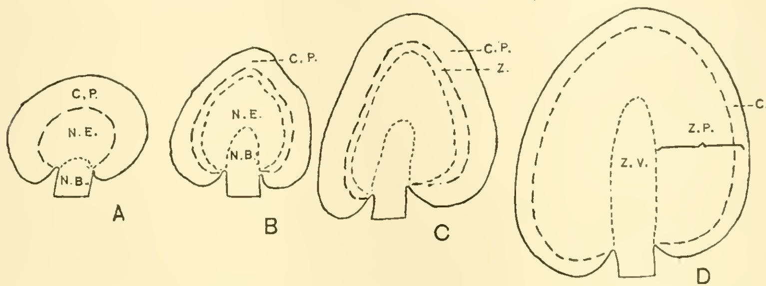


Fig. 7 Schema to illustrate the appearance of the zones in the morphogenesis of the cat's ovary. *C.P.*, primitive cortex; *C.*, cortex (definitive); *N.E.*, epithelio-stromal nucleus; *N.B.*, nucleus basalis; *Z.I.*, intermediate zone; *Z.P.*, zona parenchymatosa; *Z.V.*, zona vascularis.

In an attempted analysis of the morphogenesis of the mammalian ovary it would be necessary to compare the development in small, medium-sized and large animals in detail, in order thereby to eliminate the effects of size, and arrive if possible at the intrinsic aspects of the organogenesis. Such an analytical comparison of mammalian ovaries has hardly been done. The recent account of Felix of the development of the human ovary, as already sketched, indicates possibilities, and to it, along general lines, the development of the ovary of the cat appears to conform. The existence of 'medullary cords' and Pflüger's 'egg tubes' must be regarded as of secondary significance as but variations in the mode of growth. The neogenic zone of Felix obviously compares with the primitive cortex of the ovary of the cat. It is to be regretted that the description did not include the important period of development during childhood.

The development of the cat's ovary, according to my interpretation, affords no support for the view that the ovary proper is superimposed as a distinct growth on a vestigial testis represented by the rete ovarii and the medullary cords. The latter, while they present a superficial resemblance, histologically, particularly to the tubules of a cryptorchid testis, are nevertheless obviously ovarian. Their apparent testicular character is due to their form of growth. They contain ova and form follicles, while their cells clearly agree with the follicle cells in structure, and this is also clearly due to the processes occurring within them, which are themselves in part a function of their relations. There remains the fact of the existence of a rete ovarii, the undoubted homolog of the rete testis, particularly well developed in some animals, such as the cat, and connected with some of the so-called medullary cords which thus would appear clearly homologous with the tubuli contorti of the testis. The apparent dual structure of the ovary is but a part of the larger problem of the double development of the internal organs of reproduction in typical vertebrates, referred to at the beginning of the paper. The development of a rete and its connection with the parenchyma of the gonad in the female is but part of the tendency to develop the duct system of the male. The conclusions to which one is almost inevitable compelled is that of a deep-seated hermaphroditic tendency in the development of vertebrates, which finds expression in the double character of certain definite organs and structures. This but describes the morphogenetic pattern with a suggestion of its phylogenetic origin. Upon the analytical side, it might be affirmed that processes determining both male and female duct systems were present in development of each sex. If it were believed to put the problem on a better basis, it might be said that each sex is heterozygous in this respect, the factors determining the development of the male duct system becoming dominant in the course of development of the male and vice versa. The development of the ovary indicates strongly that the development and double character of the reproductive system must be clearly differentiated from that of sex itself. The development of the reproductive system is double—has two aspects: the establishment of the

fundamental plan of the system, and the underlying mode (metabolic attitude) that determines the direction taken—the "determination of sex." In the development of the human ovary and testis, for example, Felix has shown that in both sexes there is the same fundamental material which is worked over, so to speak, along one or the other lines of differentiation.

It was the suggestion, of Wilson I believe, that sex and the sexual characters might be differently determined, the latter by the heterochromosome. Suggesting in turn that the heterochromosome stands for the former; instead, it might be stated in closing as at the beginning of this paper, that the evidence indicates a quantitative rather than a qualitative sex difference, a different metabolic habit, degree or tendency that determines the result.

SUMMARY

1. In the development of the ovary of the cat, growth is mainly peripheral and marginal.

2. Differentiation therefore follows centrifugally.

3. The epithelial elements (parenchyma) occur in the form of cords.

4. Medullary cords and egg cords are not to be sharply distinguished.

5. The growth determines the appearance of fairly definite zones: (a) cortical, (b) intermediate, (c) epithelial stromal.

6. Degenerations occur throughout the period of growth and in the adult period.

7. In general, the degenerations follow a centrifugal course.

8. The stroma obviously plays an active and important part in ovarian growth.

9. The primitive cortex is interpreted as directly forming the definitive cortex containing the primary follicles.

10. No evidence was found of a new formation of ova just prior to sexual maturity.

11. Profound degeneration of the early formed Graafian follicles occurs, being most marked before the advent of sexual maturity.

12. Polar spindles, polar body formation and fragmentation (abnormal cleavage?) occurs particularly in the atresia folliculi preceding sexual maturity.

13. The Graafian follicles of the adult period are of a somewhat different type as compared with those of the growth period (presexual). Intergradation is, however, obvious.

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PLATE 1

EXPLANATION OF FIGURES

8 Transection of ovary, fetal kitten, 95 mm. (no. 5); rete ovarii, medullary cords (occupying central portion), egg cords, are shown. The 'synizetic wave' occupies approximately the middle of the zone of egg cords. Photograph. × 35.

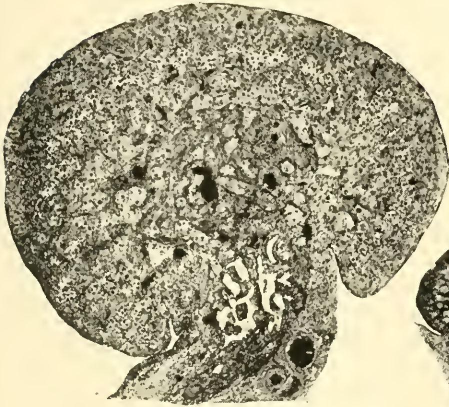
9 Longisection of ovary, kitten 3 to 4 days, P. P. (no. 11). The position of the rete in the cephalic portion (at the right) is illustrated. There are shown the branched egg cords occupying the primitive cortex and extending down into the epithelio-stromal zone, the basal connective tissue nucleus. Medullary cords (in the epithelio-stromal zone) are not shown at this magnification. Photograph. × 15.

10 Transection of ovary, kitten 14 days, P. P. (no. 20). The zones of the ovary appear more definite. Medullary follicles beginning. Photograph. × 20.

11 Transection of ovary, kitten, *Ca.* 4 weeks (no. 22). The medullary follicles developing. In the primitive cortex, the primary follicles are forming. Photograph. × 20.

12 Transection of ovary, kitten, 5 to 6 weeks (no. 24). The peculiar medullary follicles whose development is illustrated in figures 16 to 25 are here shown developing. The cortical egg cords are now dissolved into primary follicles, the primitive cortex becoming the definitive cortex. Photograph. × 15.

13 Transection of ovary, kitten, 3 months (no. 35). The irregular, pluri-ovular medullary follicles are now large. Their irregularity and pluri-ovular character are indicated. Compare figures 31 and 32. The cortex is occupied by primary follicles. Two sections of rete ovarii are shown in the center. Photograph. × 12.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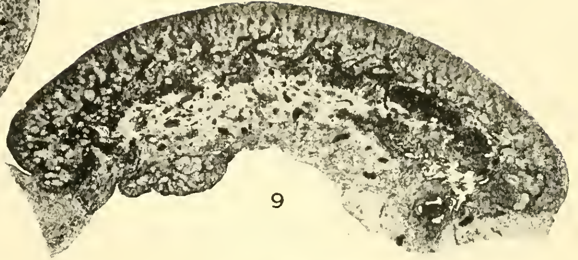
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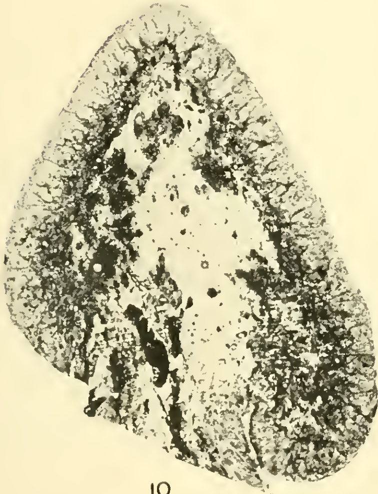
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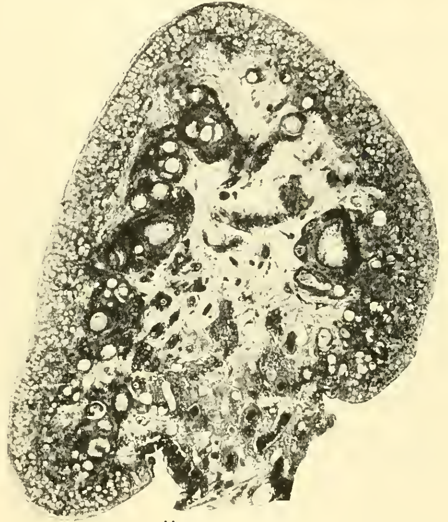
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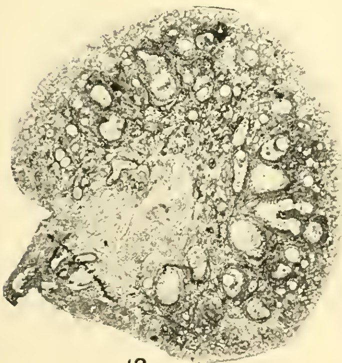
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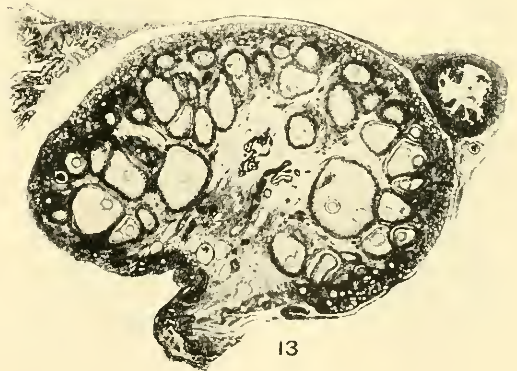
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PLATE 2

EXPLANATION OF FIGURES

14 Transection of ovary, young virgin adult cat (no. 45). The Graafian follicles of the presexual period have practically disappeared and those of the adult period are developing. The zona parenchymatosa is well indicated by the ovarian stroma. Photograph. × 15.

15 Section of ovary, adult cat (no. 51). The surface of a corpus luteum is cut. The zona parenchymatosa is occupied by the Graafian follicles in varying stages of development. The zone of primary follicles (cortex) is best indicated at the right and left. Photograph. × 12.

16 Two primary follicles with adjacent nests of follicle cells. From the intermediate zone of ovary no. 20 (14 day), showing 'stage 1' in the development of the irregular medullary follicles. Photograph. × 300.

17 Developing medullary follicles, 'stage 2.' Ovary no. 29. Photograph. × 250.

18 Developing medullary follicle, 'stage 3.' Ovary no. 29. Photograph. × 250. A nodule of stroma is included between the investing and sheathing follicle cells. Two groups of interstitial cells are seen at the right, between them a small cluster of follicle cells.

19 Medullary follicle (young), 'stage 4.' The line of junction of the investing follicle mass is shown at the lower right hand corner (arrow). Photograph. × 250.

20 Similar to figure 19. The line of junction may be seen (*j*). Note also a nodule of included stroma (*s*). Photograph. × 250.

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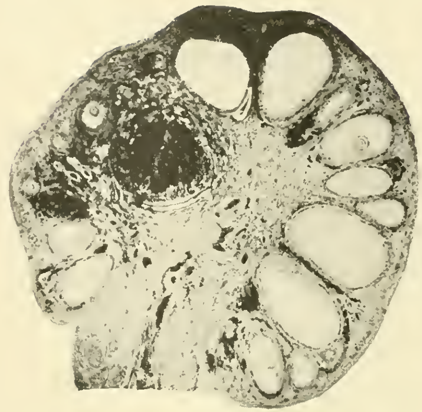
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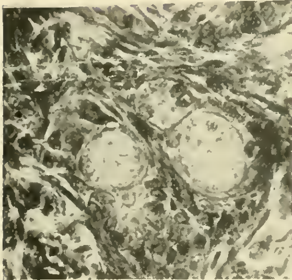
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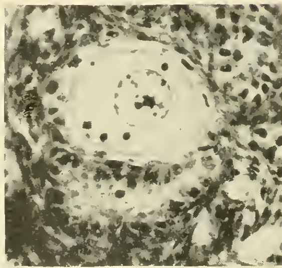
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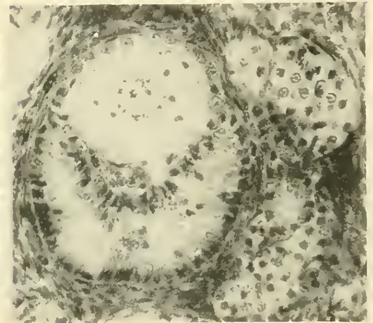
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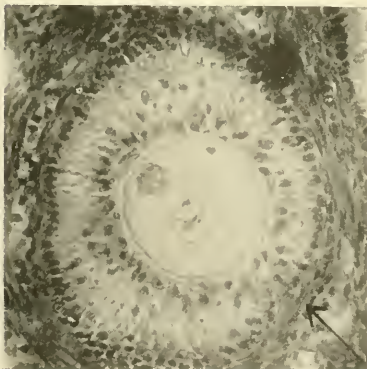
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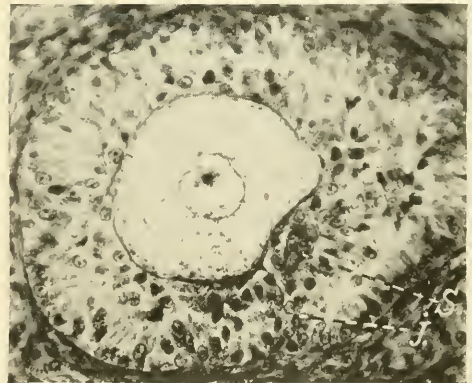
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PLATE 3

EXPLANATION OF FIGURES

21 Medullary follicle, illustrating the investment by the follicle mass. A group of stroma cells in the form of interstitial cells are being included (*i*). Photograph. × 250.

22 Irregular cumulus oophorus from a medullary follicle. The line of junction is still indicated (*j*). A small group of included stroma cells is also shown (*s*). Groups of interstitial cells lie external to the follicle (*i*). Photograph. × 250.

23 The development of medullary follicles. The elongated form of some of the follicle masses is illustrated. Four ova are related to the one shown, each with its peculiar sheathing follicular epithelium, two of them being cut through the nucleus. Photograph. × 60.

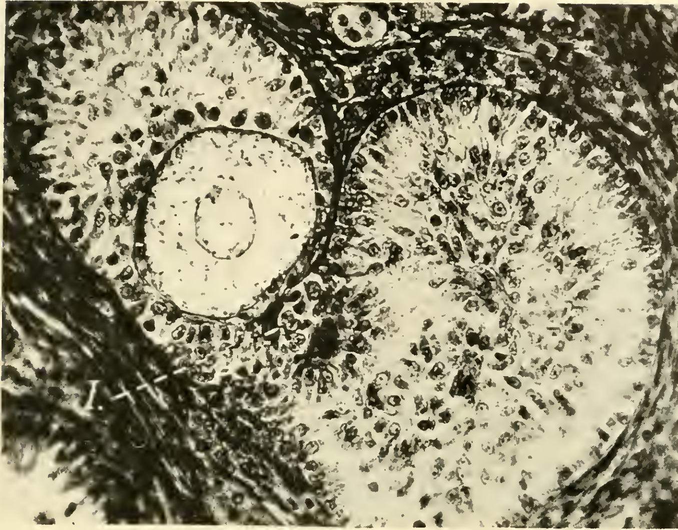
24 Two developing medullary follicles. Note the stromal investment of each. Groups of interstitial cells are shown interspersed. Photograph. × 100.

25 Two developing medullary follicles. Nodules of stroma are included between the investing and sheathing follicular epithelium. Photograph. × 100.

26 Mitotic spindle (polar spindle?) in a degenerating ovum. Ovary no. 39. Photograph. × 250.

27 Polar body. Degenerating ovum. From a section adjacent to that of figure 26. Photograph. × 250.

28 Ovum degenerating by fragmentation. A single nucleus is shown in the larger fragment. Two nuclei appear in the fragment immediately to the left of it. Photograph. × 250 (approx.).



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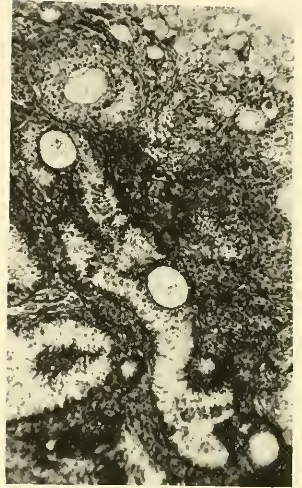

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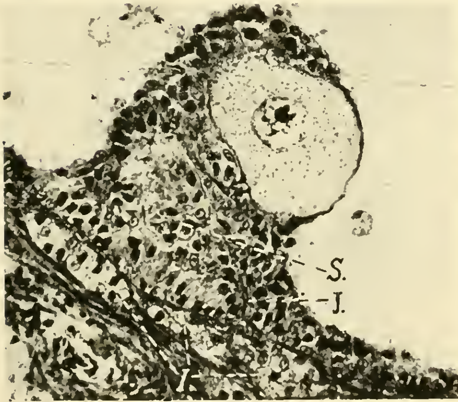

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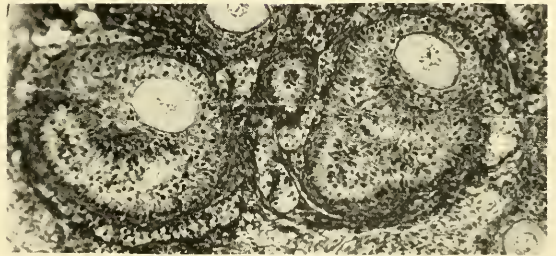

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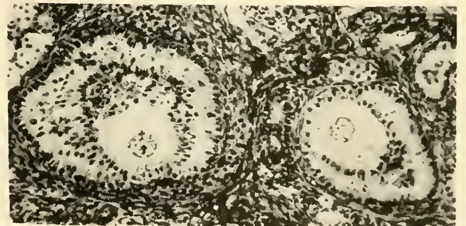

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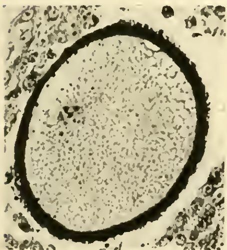

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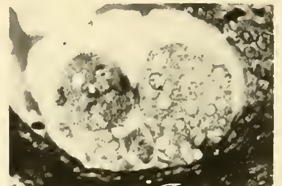


28

PLATE 4

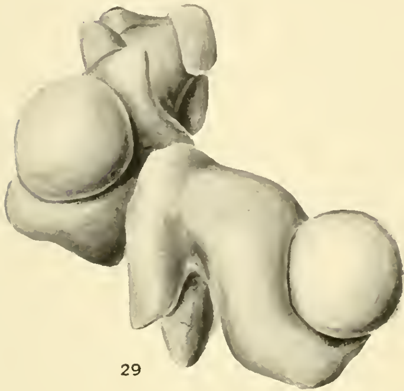
EXPLANATION OF FIGURES

29 Drawing of a single follicle grouping from a model of a segment of ovary no. 26. Two ova are shown each of which is within its own follicular epithelium. The irregularity of the 'investing' follicle mass is shown.

30 An ovum partially invested. From the model as in figure 29. A cordlike follicular mass lies adjacent to it.

31 Drawing of a single irregular pluri-ovular follicle from a model of a segment of ovary no. 35.

32 As in figure 31. The marked irregularity and cordlike extensions are shown.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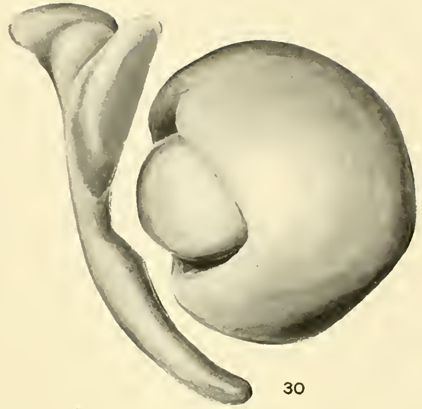
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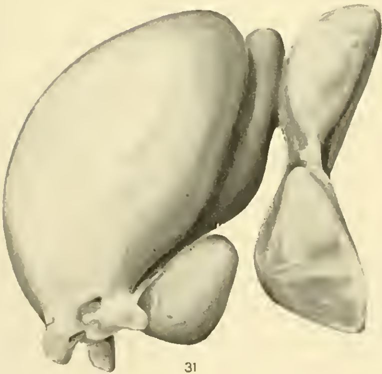
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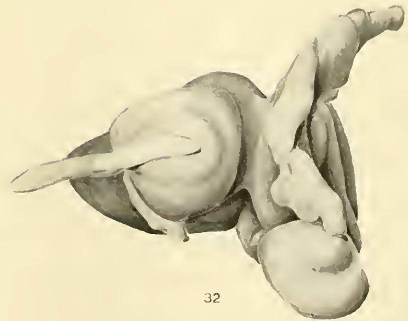
32

THE DEVELOPMENT OF THE CYTOPLASMIC CONSTITUENTS OF THE NERVE CELLS OF THE CHICK

I. MITOCHONDRIA AND NEUROFIBRILS

E. V. COWDRY

Associate in Anatomy, Johns Hopkins University

From the Anatomical Laboratory of the University of Chicago

FIVE PLATES

CONTENTS

Introduction 389
Literature 391
Material and methods 396
Mitochondria in early stages before the differentiation of neurofibrils: embryos of 0 to 14 somites 400
Mitochondria and neurofibrils in later stages: embryos of 15 to 33 somites 402
1. The stage of development at which the first neurofibrils are formed 402
2. Type of cell in which neurofibrils first develop 403
3. Region of cytoplasm in which neurofibrils originate 404
4. Comparison of mitochondria and neurofibrils in embryological series: embryos of 19 to 31 somites 405
Discussion 408
1. Foundation of the theory that neurofibrils are developed from mitochondria 408
2. Criticism of the theory 409
3. The origin of neurofibrils 414
Summary 416
Bibliography 417

INTRODUCTION

The attention which has been focussed, during the last four or five years, upon mitochondria as fundamental components of the cytoplasm of all cells has stimulated investigators in their attempts to determine the relation which these bodies bear to the activities of the cell and to the genesis of the cytoplasmic constituents. Thus we find that Meves ('07, p. 403) was the

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first to claim that they are transformed into neurofibrils. He brings forward the following generalization ('08, p. 845): that, with the specialization of the embryo into different organs and tissues, primitively similar cells assume special functions which find expression in characteristic structures or differentiations. All these products, no matter how heterogeneous they may be, arise through the metamorphosis of one and the same elementary plasma-constituent, the chondriosomes (mitochondria). Thus the different filamentous formations, such as the various fibrillar structures of epithelial cells, the protoplasmic filaments of epidermal cells, the fibrils of smooth and striated muscle, the neurofibrils, neuroglia threads and connective tissue fibrillae are, according to his conception, to be classified as products of chondriosomes.

Dr. Bensley pointed out to me certain facts which are inconsistent with this hypothesis of the transformation of mitochondria into neurofibrils. The far-reaching biological significance of this generalization of Meves, relating, as it does, to the fundamental properties of protoplasm; and the pressing need for further light upon the nature of the cytoplasmic constituents of animal cells, from the standpoint of both ontogenesis and phylogenesis, in order that investigations dealing with changes in cells under different functional conditions may be based on a secure foundation, have induced me to undertake this investigation.

I therefore decided to follow up my previous paper ('12 a) in which I determined the morphological independence of the mitochondria, chromidial substance, canalicular apparatus and neurofibrils in adult spinal ganglion cells of the pigeon, by further investigations the object of which would be (1) to determine as far as possible, the origin of the neurofibrils, chromidial substance and canalicular apparatus; (2) to study the morphological relations of these structures to each other in the course of development; and (3) to elucidate their functional significance. I have attempted throughout to give a full and accurate description of my findings, to state concisely their bearing upon the problems involved and to avoid transgressions from the realm of fact into the domain of theory, because we are at present hampered by a dearth of facts and a multitude of theories.

I am much indebted to Prof. R. R. Bensley and to Prof. C. Judson Herrick for their kind advice and invaluable criticism in connection with this work.

LITERATURE

In this connection we shall consider briefly the literature dealing with the mitochondria in early stages of development, the theory relating to the transformation of mitochondria into neurofibrils, the regional differentiation of neurofibrils, and finally certain general points of criticism which apply to the work upon the development of the cytoplasmic constituents of the nerve cells of the chick as a whole.

I know of no observations dealing with mitochondria in the ectodermal structures of chick embryos of less than fifteen hours incubation (Meves '08); but since the early stages have not been characterized by any data except the length of the period of incubation it is difficult to say how early observations have actually been made. Rubaschkin ('10) has described mitochondria in guinea pig embryos as early as the four cell stage. Moreover it has been shown that the mitochondria are distributed approximately equally on cell division to the two daughter cells, and that they are transmitted from one generation to another through the medium of the egg and in all probability of the sperm also. On account of considerations such as these the presumption is warranted that the mitochondria occurring in the nervous system of chick embryos are derived from those of the parental sex cells.

It has already been mentioned that Meves ('07, p. 403) was the first investigator to claim that mitochondria played a rôle in the formation of neurofibrils. He states subsequently ('08, p. 838), in the description of one of his figures, which was drawn from a preparation of the spinal cord of a chick embryo of three days and nine hours incubation and which shows a chain-like arrangement of the chondriokontes (mitochondria) in the cytoplasm of the neuroblasts, that he regards this illustration as evidence that these chains of chondriokontes produce the primitive neurofibrils, for he believes them to be the same threads which

are blackened by silver impregnation after Cajal. He adds that the completed neurofibrils (in the later stages of ontogenesis) cannot be stained by mitochondrial methods in the same way that the forerunners of the neurofibrils in the cells of the medullary tube, which stain by silver impregnation from the beginning of the third day, may be so demonstrated. He believes that there is a period in development when the neurofibrils may be stained by mitochondrial methods and also through silver impregnation.

Duesberg ('10, p. 612) refers to the researches of Meves and Hoven regarding the development of neurofibrils from mitochondria and remarks that the number of chondriosomes diminishes with the increase in the age of the embryo until in the adult nerve fiber none remain stainable by Benda's method. Meves, in a recent paper ('10 a, p. 655), apparently confirms this observation for he concludes that no chondriosomes remain, as such in adult spinal ganglion cells.

Hoven ('10) has furnished the most detailed observations in support of the mitochondrial origin of neurofibrils. He studied the formation of neurofibrils in the cells of the neural tube and spinal ganglia of chick embryos by the application of the Benda method and of Cajal's silver impregnation method. He found that the morphology and cytoplasmic arrangement of the chondriokontes, as demonstrated by Benda's method, bears a very striking resemblance to the appearance of the earliest neurofibrils demonstrable by silver impregnation in cells of the same stage of development. The chondriosomes at this period form a reticulum of undulating filaments (p. 475). Furthermore, in later stages the chondriokontes decrease in number as the neurofibrils increase in amount, and he asks the question if the chondriokontes do not give rise to the neurofibrils what becomes of them? He believes that the few which do persist in the adult nerve cell correspond to the internal reticular apparatus of Golgi, the Binnennetz of Kopsch, etc. As corroborative evidence he draws a close analogy between the formation of myofibrils, as indicated by Duesberg ('09 and '10), the transformation of chondriosomes into connective tissue fibrils (Meves '10) and the formation of neurofibrils from chondriosomes.

Marcora ('11, p. 952), so far as I have been able to determine, was the first investigator to criticise this conclusion and to emphasize the following differences between the chondriosomes and the primitive neurofibrils: (1) that the chondriokontes are short, thick, wavy filaments, never being continuous with the few chondriosomes in the axis cylinder; whereas the neurofibrils are fine, long, straight threads arranged near the greater process of the neuroblast and distinctly continuous with the numerous neurofibrils in the axis cylinder; and (2) that it is not possible to stain distinctively the neurofibrils with the mitochondrial dyes.

Furthermore, Duesberg ('12, p. 745) returns the verdict 'non proven' in the case of the neurofibrils, but maintains that he has incontestably demonstrated that myofibrils arise in like fashion from mitochondria.

The regional differentiation of neurofibrils in the chick will be considered under three headings:

(a) *The stage of development at which the first neurofibrils appear.* Besta ('04, quoted from Collin '06, p. 238) gives the degree of differentiation as that of sixty-five hours incubation; Cajal ('07, p. 178) fifty-six to sixty hours; Gerini ('08, p. 182) forty hours, and Hoven ('10, p. 441) 44 somites and seventy-six hours incubation. Other investigators working prior to these with more imperfect methods give the date even later.

(b) *Type of cell in which neurofibrils first develop.* Besta ('04, quoted from Collin '06, p. 238) and Gerini ('08, p. 182) conclude that the neurofibrils in the chick are formed in the bipolar cells of the outer layer of the neural tube; Cajal ('07, p. 178) in the apolar cells bordering the ventricle (i.e., in preneuroblastic cells); and Meves ('07, p. 403) and Hoven ('10, p. 478) assert that they are first differentiated in the cytoplasm of the neuroblasts.

(c) *Region of the cytoplasm in which neurofibrils originate.* Investigators are apparently agreed that the neurofibrils in the chick are first formed in a definite, restricted zone of the cytoplasm; for Besta ('04, quoted from Collin '06) claims that they are formed in the protoplasmic substance about the nuclei; Cajal ('07, p. 178) that they are differentiated in a network which arises in a fibrillogenous area in the distal portion of the

cell; Meves ('07, p. 838) that the chains of chondriokontes in the axis cylinder process and in the adjacent portion of the cytoplasm are converted into neurofibrils; Gerini ('08, p. 182) that they are formed at either pole of the nucleus from minute granules which stain deeply by Cajal's method; and, finally, Hoven ('10. p. 475) asserts that the neurofibrils are generated from a reticulum of mitochondrial filaments in the cytoplasm of the neuroblasts and ganglion cells.

Most of the work dealing with the development of the cytoplasmic constituents of the nerve cells of the chick seems to me to be open to criticism on the basis of the following considerations:

(1) *Point of view.* Goldschmidt's hypothesis ('09, p. 107) that mitochondria belong to the category of chromidial apparatus (Fauré-Frémiet '10, p. 483); Smirnow's conception ('07), amplified by Hoven ('10, p. 479), of the similarity of the mitochondria and the reticular apparatus; and, lastly, Meves generalization ('08, p. 845) that the fibrils in various types of cells are developed from mitochondria, forwarded as they were before definite proof was forthcoming, rendered the dissociation of the cytoplasmic constituents more difficult for investigators working on morphogenesis.

(2) *The confusion which has arisen by the application of many ill-chosen terms to a definite and concrete class of cell granulations called mitochondria by Benda in 1899.* Among these may be mentioned 'Chondriosomen' (granules), 'Chondriokonten' (thread-like granules), 'Chondriomiten' (granules distributed in rows) and 'Chondriom' (the cellular content of chrondiosomes). Meves has devised yet another series, 'Plastosomen,' 'Plastochondrien' and 'Plastoconten.' When one considers in addition the vast array of names applied in the older literature to mitochondria, such as for instance cytomicrosomes, interstitial granules, Flemming's fila, Altmann's bioblasts, fuchsinophile granules; although at the present day we recognize that each of them comprises also many granules which are not mitochondria, so that none of them may be regarded as synonyms for mitochondria, and also the fact that each term is modified more or less depending upon the na-

tionality of the investigator using it (cf. Chondriokonten, chondriocontes, condrioconti) it becomes at once evident that any additional terms only complicate the situation, especially when they have been devised to proclaim some functional interpretation. Moreover, we have no assurance that the slight differences implied by these terms will be appreciated by the majority of the investigators of the future as well as those of the present. It would be much safer and simpler to make use of the word 'mitochondria,' recognising that similar granules may assume different shapes under varying functional conditions, such as rate of multiplication, surface tension, and so forth, because by so doing we need not subscribe to any theoretical considerations respecting their significance.

(3) *The inadequacy of the technique employed.* Meves' generalization was apparently based, in the first instance, on the application of a slightly modified iron hematoxylin method of technique alone. The fact that Michaelis demonstrated in 1899 that mitochondria could be stained specifically by janus green, and that Bensley recognized the importance of this discovery, introduced it into histological technique and repeatedly called attention to it in his publications (1910 and again in 1911) has been completely ignored. It is true that we have not yet got a satisfactory dye for the neurofibrils and the canalicular apparatus, and that we cannot in fixed tissues even, demonstrate, in a constant fashion, the neurofibrils and the mitochondria specifically stained in the same cell. Thus we are frequently forced to piece together in our mind's eye the relations of the different cytoplasmic constituents to each other although we have to study them singly in separate preparations. Pending the discovery of vital dyes and synthetic methods all the available methods of technique must be employed so that the popular pitfall of supposing that one or two methods alone will tell the whole story may be avoided. Even when these precautions have been taken, the biologist should stop to consider what a multitude of factors associated with the structure of cells the microscope still fails to reveal to us.

(4) *The absence of controls.* The different stages in the embryological series have not as a rule been sufficienty accurately characterized. In some contributions the only criterion has been the number of hours of incubation, in others the length of the embryos alone, so that it is frequently difficult to tell what stage the investigator in question is describing. Furthermore, it has been overlooked that synthetic comparisons between the structures of cells which do not occur in the same parts of the nervous system of embryos of the same degree of differentiation are not valid, since all regions of the nervous system are never uniformly developed.

MATERIAL AND METHODS

The material consists solely of chick embryos and is in part summarised in table 1. The degree of specialization of the embryos was determined by a consideration of: (1) the number of mesodermic somites, (2) the period of incubation at 39°C., (3) the length (measured before removal from the egg) and (4) a comparison of their external morphology with the different stages figured and described in Duval's Atlas d'embrylogie and in Keibel and Abraham's Normentafel zur Entwicklungsgeschichte des Huhnes (Gallus domesticus). Reference was frequently made to Lillie's text-book on the development of the chick, and to Keibel's Normentafel zur Entwicklungsgeschichte des Kiebitzes.

The methods include the observation of living tissue, with and without the use of vital dyes; and of the impregnation and staining of fixed material, imbedded in paraffin and in celloidin. Each embryo was fixed, dehydrated and imbedded individually. The technical methods employed are given in table 2.

In the study of living tissue the portion of the nervous system under investigation was isolated in warm salt solut'on under the binocular microscope and immersed in the staining fluid. The stains were generally used in a concentration of 1 : 10,000 of 0.85 per cent sodium chloride solution. Preparations were examined from time to time until the desired intensity of coloration had been attained.

TABLE 1

Material

In the first vertical column the methods of technique are given. The embryo classified in the second is characterized by having the primitive fold, groove and pit alone differentiated; in that in the third the first intersomite groove is just appearing; while the relative differentiation of those in the succeeding columns is indicated by the number of mesodermic somites. The embryos are designated by their number in my collection. Thus, in the second and ensuing horizontal divisions the method of technique may be found and the numbers of the embryos prepared by it. All the embryos listed have been cut in complete series of sections.

METHOD			S3	9	10	12	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32
Meves' iron hematoxylin	386	386a	387	380	382	381				590		383	487	463	377	462	477	488	391	476	498	490	494	394	
Bensley's fuchsin methyl-green													384	378		379			390					393	
Benda's method														404			484	500	392	480					
Paton's method														468	465			461							445
																		460							
Cajal's method												594			526										
										600	591	593		509	525			560							
						598	589	588	599	595	585	547	542	493	522	545	503	507	511	510	508	513	549	512	517

TABLE 2
Methods

FOR	METHOD	FIXATION	STAIN
mitochondria	(1) Michaelis-Bensley	intravitam	janus green 1:10,000
	(2) Meves' 1908, p. 832	Flemming's fluid (modified)	iron hematoxylin with or without erythrosin
	(3) Benda's Hoven, 1910, p. 430	Flemming's fluid (modified)	alizarin crystal violet
	(4) Bensley's anilin fuchsin methyl green 1911, p. 309	acetic osmic bichromate mixture	anilin fuchsin with methyl green, toluidin blue, or methylene blue eosinate or methylene blue erythrosinate
	(5) Bensley's copper chrome hematoxylin, 1911, p. 310	acetic osmic bichromate mixture	hematoxylin
neurofibrils	(1) Paton's modification of Bielschowsky's method, 1907, p. 576	neutral formalin	silver nitrate
	(2) Cajal's formula I, 1907a, p. 215	silver nitrate	none
chromidial substance	(1)	Carnoy, etc.	toluidin blue and erythrosin
canalicular apparatus	(1) Kopsch, 1902	osmic acid	none
controls	(1)	formalin, neutral formalin, formalin acetic, chrome sublimate, sublimate acetic, sublimate formalin acetic, picro-sulphuric, osmic acid, nitric acid, etc.	toluidin blue erythrosin; hematoxylin; mitochondrial dyes, etc.

Embryos imbedded in paraffin were cut in serial sections by means of a Minot rotatory microtome. The great majority of the series were cut transversely; but others were made in coronal and sagittal planes so that the one-sided conceptions so likely to arise from the perusal of sections cut in a single direction, have been eliminated. All the material imbedded in paraffin was cut into sections 4 μ thick.

The celloidin method of imbedding was employed, in the case of several embryos, on account of the objections raised by certain authors (Cajal '07, p. 172, et al.) to paraffin. Complete series were made about 7 μ thick by the technique advocated by Maximow ('09, p. 184).

This investigation has been controlled as follows:

(1) The entire nervous system has been studied; but detailed comparisons have only been made between the results of different methods in the neural tube and the neural crest on the right side in the region of the sixth somite of embryos of the same degree of differentiation.

(2) The commoner artefacts due to fixation are shrinkage, distortion of form relations, and destruction, solution or change in the nucleus and cytoplasmic constituents. Shrinkage is dependent, to some degree, upon the water content, and since this probably varies in different regions we must look for unequal contraction. The diminution in the longitudinal axis was measured in the case of the mitochondrial and neurofibrillar fixatives; in the former it amounted to 23 per cent, in the latter to 19 per cent; (compare the size of the nuclei represented on plates 3 and 4). The finer form relations, as seen after fixations in vogue for mitochondria and neurofibrils, were controlled by comparison with the results obtained after a large number of fixations, especially adapted for their accurate delineation, and with living tissue teased out in warm physiological salt solution. It appears that the shape of the cells is but little altered by the fixations, and I am inclined to believe that, in the chick, the difficulty encountered in the observation of cell boundaries is in some measure due to shrinkage. Moreover, Harrison ('10, p. 793) states that the cells in the neural tube of frog embryos are perfectly

distinct, and ascribes the difficulty experienced by investigators in the demonstration of cell boundaries to inadequate technique. I have already ('12 a, p. 486) mentioned the precautions necessary in the fixation of the cytoplasmic components of adult nerve cells and the same apply without reservation to the embryo.

(3) The inevitable contraction, resulting from dehydration has been reduced by decreasing the period of immersion in the grades of alcohol to a few minutes only, and by the use of bergamot oil for clearing, which renders the use of absolute alcohol unnecessary.

(4) It is difficult to dissociate the effects of fixation and imbedding. The shrinkage due to imbedding in paraffin and celloidin was slight. I fail to see any very great or constant differences in the appearance of tissues treated by these two methods. In my experience the paraffin method does not produce any more artefacts than the celloidin method.

(5) Sectioning, if carefully carried out, does not produce serious artefacts (Cowdry '12 b, p. 491).

(6) The methods of impregnation and staining control each other, for the neurofibrils appear to be essentially similar in specimens prepared by Cajal's and Paton's technique, and the mitochondria in preparations made by the methods enumerated in table 2.

MITOCHONDRIA IN EARLY STAGES BEFORE THE DIFFERENTIATION OF NEUROFIBRILS: EMBRYOS OF 0 TO 14 SOMITES

Both filamentous and granular mitochondria undoubtedly exist in the nerve cells of the chick in these early stages, but the filamentous form is much more numerous. The illustrations (figs. 1 to 5) tend to minimize the uniformity in the morphology of the mitochondria on account of the fact that the long axes of the mitochondrial filaments are directed in all planes relative to the drawing and that they are cut in pieces of varying size at all angles during sectioning. Most of the mitochondria are of necessity oriented parallel to the long axis of the cells in which they occur. The true filamentous nature of the mitochondria is therefore obscured in sectioned tissues, particularly in sagittal series

where the sections are cut in most instances transversely to the length of the cells with the result that the mitochondria appear either uniformly granular or as short rods depending upon the thickness of the section in question. The preponderance of the filamentous form of mitochondria may be conclusively demonstrated in specimens stained with janus green intravitam.

The morphology and cytoplasmic arrangement of mitochondria are represented in figures 2, 3, and 1, which illustrate progressive stages in development. The specimens from which these figures were drawn were prepared by Meves' modification of the iron hematoxylin method so that the mitochondria appear a dark blue-black color and may be readily distinguished from the dark browinsh black yolk globules. The first (fig. 2) shows epithelial cells from near the middle line of the medullary plate of an embryo of twenty-four hours incubation at 39°C. in which the primitive groove fold and pit alone were differentiated. The mitochondria are seen in the form of filaments and granules distributed evenly throughout the cytoplasm, whereas the yolk spherules show a tendency to become grouped together in the distal, (i.e., remote from the membrana limitans interna) portions of the cells. The second (fig. 3) illustrates the appearance of epithelial and germinative cells from the neural fold in the region of the head process of an embryo of 3 somites and twenty-four hours incubation. The amount of yolk material is reduced and the mitochondria are far more abundant in the distal than they are in the proximal parts of the cells. I have not, however, been able to distinguish a similar heaping up of mitochondria in cells stained by janus green intravitam (fig. 23). The third drawing (fig. 1) is from the ventral half of the neural tube in the region of the tenth somite of an embryo of 12 somites and thirty-four hours incubation. The mitochondria are here distributed evenly in the cytoplasm and the yolk gloubules tend to be accumulated in the distal portions of the cells, just as in figure 2.

There are two points of considerable significance from the point of view of the theory of the transformation of mitochondria into neurofibrils. The first is that there is little, if any, variation in the morphology or staining reactions of mitochondria in these

early stages; and the second that these mitochondria in the cells of the neural tube apparently do not differ in any way, capable of detection by the methods of technique now in use, from the filamentous and granular mitochondria occurring in the structures derived from the other two germ layers (i.e., mesoderm and endoderm).

MITOCHONDRIA AND NEUROFIBRILS IN LATER STAGES: EMBRYOS OF 15 TO 33 SOMITES

1. The stage of development at which the first neurofibrils are formed

I find that neurofibrils are first formed in the nervous system of a chick embryo, the differentiation of which may be characterized as follows: Somites 15, length 5.8 mm., incubation at 39°C ± 40 hours (being slightly more advanced than Duval's embryo of 33 hours, fig. 268, p. 56). In such an embryo the neurofibrils are most abundant in the marginal neuroblasts in the hind-brain opposite the otic invagination (fig. 11). A few cells containing them may be seen in the midbrain (fig. 12); but in the forebrain the only indication of their presence is a more or less continuous network, blackened with silver nitrate, in the distal (remote from the membrana limitans interna) portions of the cells (figs. 10 and 12). Traces of them occur further caudad in the cells of the neural tube as far back as the fifth somite. There is a large area comprising the posterior portion of the midbrain and the anterior part of the hind-brain in which no neurofibrils are differentiated, even in the stages as far advanced as 20 somites.

Since the neurofibrillar methods of technique employed by me depend upon impregnation with silver nitrate, they are not sufficiently accurate to permit of complete enumeration of the cells in the different parts of the nervous system containing neurofibrils, and I have therefore been prevented from studying the regional differentiation of neurofibrils on a percentage basis and comparing my results with those obtained by Paton ('07) and Coghill ('09, etc.) who worked on fish and amphibian embryos respectively.

In general, the earliest neurofibrils appear to be formed in chick embryos in three chief localities: (1) in the hind-brain opposite the otic invagination (stages of 15 somites on); (2) in a center on either side of the extreme anterior end of the midbrain (stages of 18 somites and on); and (3) in the nuclei and root fibers of the cranial nerves (from stages of about 15 somites on). They are not differentiated until very much later (i.e., beyond 31 somites stages) in spinal ganglion cells.

2. Type of cell in which neurofibrils first develop

I have found neurofibrils in bipolar cells, in apolar cells, in marginal neuroblasts and in certain cells which occur within the cavity of the neural tube.

The neurofibrils in a typical bipolar cell are illustrated in figure 7. The cell is located on the right side of the hind-brain opposite the otic invagination of an embryo of 24 somites, length 7.5 mm., and sixty-five hours incubation, prepared by Cajal's silver nitrate method. The cytoplasm is stained a little bit darker yellow in the distal portion of the cell and the neurofibrils appear thicker and more continuous than in the proximal part.

The neurofibrils in apolar cells bordering the central canal are best shown in figure 22. This specimen was prepared by Paton's modification of the Bielschowsky silver impregnation method. The cells illustrated occur in the neural tube opposite the posterior part of the third somite of an embryo of 33 somites, length 8.3 mm., and seventy-four hours incubation. At *a* a continuous circumnuclear neurofibrillar network is seen in black against a dull red granular background. The appearance of such a neurofibrillar network in apolar cells is of distinctly rare occurrence. No networks of this nature were observed in embryos of 15 somites, which is the stage of development at which the neurofibrils first appear.

Marginal neuroblasts containing neurofibrils may be readily found in embryos of all stages intermediate between 15 and 32 somites, and they probably occur in further developed embryos also. Figure 11 shows the primitive neurofibrils in a marginal

neuroblast from the hind-brain on the right side opposite the otic invagination of an embryo of 15 somites, of 5.8 mm. in length and forty hours incubation prepared by Cajal's silver nitrate method. The neurofibrils occur throughout the cytoplasm which has assumed a rather dark yellowish tint. Figure 8 shows the neurofibrils in a cell of the same type in a more advanced embryo. Here the neurofibrils seem to be of finer diameter and more continuous, while the ground substance of the cytoplasm is of a distinctly lighter and more brilliant yellow tint.

Neurofibrils also occur in cells which seem to lie within the lumen of the neural tube, and in the processes of cells which extend along the ventricular border of the central canal. Figure 9 has been drawn from the left side of the hind-brain opposite the otic invagination of an embryo of 24 somites, length 7.5 mm. and sixty-five hours incubation treated by Cajal's silver nitrate method. The cell (a) is apparently within the cavity of the neural tube, and the process (b) of the cell (c) extends toward the membrana limitans interna, all three of which contain well differentiated neurofibrils. Figure 6, which represents a cell from the same region of the same embryo, shows a similar condition. Appearances such as these are distinctly rare, seem to be restricted to the region of the hind-brain and do not obtain in embryos during the early stages of neurofibrillar formation.

3. Region of the cytoplasm in which neurofibrils originate

Figures 10, 12 and 14 are from an embryo of 15 somites, length 5.8 mm. and incubation forty hours at 39°C., which represents the stage of development at which neurofibrils may first be distinguished. All of these three figures show that the neurofibrils are first formed in the immediate neighborhood of the nuclei. The fact that these primitive fibrils are in the form of a network is best illustrated at *a* figure 14. In the cells containing neurofibrils the distal region of the cytoplasm (i.e., that near the membrana limitans externa) stains a darker yellowish brown color than does the more proximal part, on the opposite side of the nuclei, whereas in cells without neurofibrils the cytoplasm

stains evenly throughout. It is in this distal, highly colored, portion of the cytoplasm that the neurofibrils are later first completely differentiated. It should be noted, in passing, that certain portions of the cells of the adjacent myoblasts likewise stain a similar dark yellowish brown color with silver nitrate.

4. Comparison of mitochondria and neurofibrils in embryological series: embryos of 19 to 31 somites

It has already been mentioned that we have not at present a technique at our disposal by means of which the mitochondria and neurofibrils may be stained differentially in the same cell in a constant and trustworthy fashion. For this reason a synthesis must be resorted to. With this in mind I have endeavored to build up a synthetic picture of mitochondria and neurofibrils as they appear in cells of increasing degrees of differentiation. Two series of embryos extending from stages of 19 to 31 somites, each member of a series differing from the preceding by the acquisition of a single mesodermic somite, were employed, one being prepared by Meves' iron hematoxylin method for mitochondria (figs. 15 a, etc.) the other by Cajal's silver nitrate method for neurofibrils (figs. 15 b, etc.). Four additional methods of technique were used for control, namely, janus green intravitam, Bensley's anilin fuchsin methyl green method, the Benda method and Paton's modification of the Bielschowsky silver impregnation technique. All embryos were cut in complete serial transverse sections. In making the comparisons morphological equivalents (of approximately the same area and thickness, 4 μ) alone were used, or, in other words, cells occurring on the right side of the neural tube opposite the sixth somite in embryos of the same degree of differentiation. The two chief parallel series, which are in part represented in plates 3 and 4, afford a fairly secure basis for the comparison of the form, distribution, staining reactions and relative amount of mitochondria and neurofibrils in developing nerve cells.

a. Form. The shape of mitochondria is, to some extent, determined by the configuration of the cells containing them. Thus

in the rounded neuroblasts, in apolar cells, and in the irregular shaped cells within the lumen of the neural tube they are either in the form of granules or of straight or slightly curved rods with rounded ends; whereas, in the bipolar cells they are longer and straighter. I have never observed any network formation or fusion of mitochondria in fixed preparations or in specimens stained with janus green intravitam, although I have searched carefully for it in the region of the axone and axone hillock. It should again be emphasized that the illustrations tend to minimize the uniformity in the morphology of the mitochondria on account of the fact that their long axes are directed in all planes relative to the drawing and that in sectioning they have been cut at all angles.

The mitochondria show no variation in their morphology of which abundant examples may not be seen in neighboring structures derived from mesoderm and endoderm. Neither do they differ in shape from the mitochondria occurring in the neural tube before the formation of any neurofibrils.

The appearance of neurofibrils is fairly constant in strictly homologous cells of these four types. They are apparently continuous, the mitochondria discontinuous: they seem to form a reticulum, whereas the mitochondria never lost their individuality; and finally the neurofibrils are of very fine diameter and irregular outlines, in spite of the fact that the tendency of impregnation methods is by precipitation, and in other ways, to add to the size of the structures demonstrated.

b. Distribution. The mitochondria, like the neurofibrils, are generally arranged parallel to the long axis of the cell, as in the case of the neuroblasts, the bipolar and epithelial cells. Occasionally the neurofibrils are arranged in a whorl-like concentric fashion about the nucleus, but the mitochondria are not similarly oriented. Both neurofibrils and mitochondria occur in all parts of the cytoplasm. In some specimens, stained with iron hematoxylin, the mitochondria gather more thickly in the peripheral parts of the cell, although such an accumulation is not apparent in cells stained with janus green intravitam (figs. 23 and 24).

c. Staining reactions. No variations or change could be detected in the results of fixation or in the staining reactions of mitochondria coincident with the development of neurofibrils. Their degree of solubility in acetic acid is apparently constant, and throughout the series of preparations (which of course is much more complete than it was possible to represent on the plates) they appear a dark blue black color, which is the same in figure 15 a, at a stage when no neurofibrils are differentiated, as it is in figure 20 a after a large nunber of neurofibrils have been formed. Moreover, the staining reactions of mitochondria in the neural tube of this series of preparations, running parallel as they do to the differentiation of neurofibrils, do not differ, in any appreciable way, from those of mitochondria in other neighboring tissues of the same embryos. They are likewise similar to those of mitochondria occurring in the neural tube before the differentiation of any neurofibrils. The only variation in the staining reactions of the neural tube in this series seems to be in the case of the background and to be dependent upon the length of differentiation in iron alum and the duration of the subsequent washing in tap water.

It is very difficult for me to determine whether a change in the reaction of neurofibrils to silver nitrate during the course of development actually exists in my preparations. The variations in color which I have observed may be due to a host of possible factors among which the duration and temperature of impregnation, the presence or absence of light, and so forth, may be mentioned. I have absolutely no reliable evidence, therefore, of any change in the chemical composition of neurofibrils from the time that they are first laid down to the most highly specialized embryos in my series.

d. Relative amount of mitochondria and neurofibrils.[1] As I have already stated, the figures on plates 3 and 4 constitute the essential features of a comparison of the results yielded by Meves' iron hematoxylin method and by Cajal's silver nitrate technique

[1] The relatively smaller size of the nuclei in figures 15 a to 20 a is indicative of the fact, already mentioned (p. 399), that the shrinkage is greater in the mitochondrial preparations than it is in the neurofibrillar ones.

respectively. In both cases, drawings have only been made from sections of the neural tube on the right side opposite the sixth somite, and the degree of specialization of the embryos from which the figures on plate 3 were drawn corresponds severally with that of those represented on plate 4, figure 15 a to figure 15 b, and so on. The preparations show that in progressive stages in the differentiation of the cells of the neural tube there is no decrease in the amount of mitochondria parallel to the increase in neurofibrillar material.

DISCUSSION

1. Foundation of the theory that the neurofibrils are developed from mitochondria

This hypothesis is based to some extent upon the following statements, which have been advanced, in whole or in part, by the investigators named.

a. That the neurofibrils increase in amount as the mitochondria decrease until finally the adult condition is attained in which the neurofibrils are completely differentiated and the mitochondria absent. (Duesberg '10, p. 512; Hoven '10, p. 478; and Meves '10 a, p. 655).

b. That microchemical transitions exist between mitochondria and neurofibrils, since the primitive neurofibrils may first be stained by mitochondrial methods, then by both mitochondrial and neurofibrillar methods and, finally, by the various neurofibrillar methods of technique alone (Meves '08, p. 838; Hoven '10, p. 478, etc.).

c. That morphological transitions also exist between mitochondria and neurofibrils: according to Meves ('08, p. 838) chains of mitochondria are transformed into neurofibrils; according to Hoven ('10, p. 475) the mitochondria form a reticulum from which the neurofibrils are differentiated.

d. That the development of the myofibrils, connective tissue fibrils and the fibrils in epithelial cells support this theory since they, in a similar fashion, are developed from mitochondria. This constitutes the argument from analogy (Benda '99; Meves '07; Duesberg '10; Meves '10; Firket '11 and Duesberg '12).

2. Criticism of the theory

The material and methods upon which the statements are based. In many cases the embryological series have been made up of embryos of different types of animals. It is a well-known fact that similar portions of the nervous system of different animals are often not differentiated at the same rate, so that comparisons made in a heterogeneous series are not valid. I know of no investigator who has clearly and concisely described either the extent or the composition of his series of embryos. The criteria used for the characterization of the different developmental stages have been lamentably deficient. Every biologist knows that embryos of apparently the same period of incubation may differ widely in their degree of differentiation. This is particularly true in the case of eggs which have been bought from dealers and about which we consequently know but little.

Complete series, cut in transverse, sagittal and coronal planes, have not, as a rule been studied; and care has not been taken, in forming a synthetic picture of the cytoplasmic constituents, to draw material only from strictly homologous cells (Herrick '09) of the same degree of differentiation. Few methods of technique have as a rule been taken advantage of, and, in some instances, conclusions have been deduced solely from the application of a single method. In the majority of investigations no reference is made to the use of subsidiary methods designed for the control of the finer form relations, and resort has never been made to vital dyes.

The statements. (1) My own observations are utterly at variance with the first argument, for I find that there is no decrease in mitochondria running parallel to the formation of neurofibrils. Moreover the statement that mitochondria are absent in the adult condition is wholly unwarranted in view of the fact that several investigators have unquestionably demonstrated mitochondria in adult nerve cells. Altmann, as far back as 1890, described and figured them in spinal ganglion cells of the frog (fig. 2), and in the Purkinje cells of the cerebellum of the cat (fig. 3) as well as in the brain wall and in the medullary tube of cat

embryos (figs. 1 and 2). Held also has demonstrated mitochondria by both the Altmann method ('97, figs. 1 and 2) and the iron hematoxylin technique ('97 a, fig. 10) in adult nerve cells. In addition I, myself, have shown (1912 a and 1912 b) that mitochondria are present in large numbers in adult spinal ganglion cells of the pigeon.

(2) The second statement postulates the existence of three distinct phases in the developing neurofibril, each of which is characterized by certain microchemical properties. In the first stage the primitive neurofibrils may, it is said, be stained by mitochondrial methods, in the second by both mitochondrial and neurofibrillar methods and in the third by the various neurofibrillar methods of technique alone.

I find that neurofibrils in chick embryos may be stained by such mitochondrial methods as the iron hematoxylin method of Meves (fig. 4), Benda's method, the anilin fuchsin methylene blue erythrosinate (fig. 27) and the anilin fuchsin toluidin blue (figs. 21, 26 and 25) methods of Bensley; but the staining is not specific and seems to depend upon the degree of differentiation. A comparison of figures 21, 26 and 25 will show that this is the case with respect to the last mentioned method. These three figures have been drawn from neighboring sections of the same embryo, mounted on the same slide and prepared by Bensley's anilin fuchsin toluidin blue method. In the first (fig. 21) the differentiation is practically nil, the mitochondria staining exactly the same color as the neurofibrils; in the second (fig. 26) it has been carried a little further with the result that the neurofibrils have lost their bright crimson color and have assumed a dull red shade; while in the last (fig. 25) the decolorization has been carried to an extreme so that the neurofibrils have lost all of the acid fuchsin and have become stained with the differentiator, toluidin blue. It is to be noted that, in these progressive stages of differentiation, the initial affinity of the neurofibrils for an acid dye (acid fuchsin) in which they resembled mitochondria, is gradually changed to an affinity for a basic dye (toluidin blue), while the intensity of the coloration of the mitochondria with the acid fuchsin remains unaltered. Furthermore, the fact that

this coloration of the neurofibrils by mitochondrial dyes is marked in adult cells (Cowdry '12 b, figs. 1, 2, 5, 7 and 13) should be taken into consideration before regarding it to be indicative of transitions between mitochondria and the primitive neurofibrils.

Let us now consider the statement that the primitive neurofibrils may be stained by both the mitochondrial and the neurofibrillar methods (i.e., the second phase). The completeness of the demonstration of mitochondria by the iron hematoxylin method depends upon the presence in the fixative of chromic acid, osmic acid and acetic acid, in suitable amounts, and on the mordanting action of iron alum; while their complete absence in the neurofibrillar preparations is due to the unmodified action of silver nitrate. The neurofibrils seem to have a specific affinity for silver nitrate, upon which all silver impregnation methods depend. So that it is extremely unlikely, especially in the absence of direct evidence, that so widely divergent methods stain the same thing, namely, the primitive neurofibrils, for if the primitive neurofibrils may be stained by both methods, they must of necessity partake of the microchemical properties of both mitochondria and neurofibrils which are, to some extent, mutually incompatible.

Finally, the neurofibrils are said to enter on a third phase in their history characterized by the loss of their affinities for mitochondrial dyes. I have nevertheless failed to find any conclusive evidence that the neurofibrils change their chemical composition after their first formation. My failure may be due to the unstandardized condition of the neurofibrillar methods of technique which still prevails. In any case the burden of supplying the evidence rests with those who make the statement.

If neurofibrils are formed by a chemical transformation of mitochondrial substance into neurofibrillar material, one would expect to find variations in the effects of fixation and in the staining properties of mitochondria during the formation of neurofibrils. I have shown that the exact converse obtains. Both the solubility of mitochondria in acetic acid and their staining reactions in the cells of the neural tube in which neurofibrils are being formed are remarkably uniform and constant. More-

over these properties apparently differ in no wise from those of mitochondria in the neural tube before the formation of neurofibrils or from the mitochondria in other embryonic cells.

It is evident therefore that the facts do not justify the statement that microchemical transitions exist between mitochondria and neurofibrils.

(3) With respect to the evidence for morphological transitions between mitochondria and neurofibrils I would state that I have failed to confirm Meves' contention that chains of mitochondria are transformed into neurofibrils. Mitochondria are sometimes oriented end to end and one may often observe very long filamentous mitochondria, like those represented in figure 4, for instance. It is however a very far cry from either a linear arrangement of mitochondria or from long filamentous mitochondria to neurofibrils. This is manifested, among other things, by the fact, already mentioned, that there is nothing peculiarly distinctive about the morphology or the arrangement of mitochondria in the cells of the neural tube during neurofibrillar formation: they are alike indistinguishable, on the basis of their morphology and distribution, from the mitochondria in the neural tube in stages prior to the differentiation of neurofibrils, and from the mitochondria occurring in other embryonic tissues both before and contemporaneous with the development of neurofibrils. So that on the ground of morphology and cytoplasmic arrangement of mitochondria there is just as much evidence for the formation of neurofibrils in structures derived from mesoderm and endoderm as there is in the case of the neural tube.

Since Hoven's own figures do not show a reticulum, but rather an interlacing of independent mitochondrial filaments, and since I have been unable to discover a reticulum composed of mitochondria in any of my preparations, the presumption is warranted that the mitochondria do not lose their individuality by coalescing to form a network.

The conclusion is likewise justified that the facts, so far as we know them do not support the statement that morphological transitions occur between mitochondria and neurofibrils.

(4) The argument from analogy is based on the development of myofibrils, connective tissue fibrils and epidermal fibrils from mitochondria according to the following observers.

Benda ('99) and Meves ('07) were among the first to come to the conclusion that mitochondria become changed into myofibrils. Duesberg wrote an excellent paper in 1910 in which he supported their conclusion. In a subsequent contribution ('12, p. 745) he states that while the two above named works (those of Meves and Hoven) render the plastochondrial (mitochondrial) origin of collagenous fibrils and of nerve fibrils only probable, the study of the fate of plastosomes (mitochondria) in myoblasts permits their rôle in the formation of myofibrils to be shown unquestionably. I do not, however, feel ready to accept Duesberg's evidence until I have satisfied myself that the similarity which he found in the staining reactions of mitochondria and myofibrils by the Benda method is indicative of like chemical composition. It is necessary for us to bear in mind that all structures which stain alike by a single method are not necessarily of the same nature. This similarity in staining is the only evidence which he brings forward to prove the existence of microchemical transitions between mitochondria and myofibrils. It remains to be proved whether it is sufficient or not.

Meves ('10) has studied the origin of connective tissue fibrils in the tendons of chick embryos. The technique employed consisted of fixation in his modification of Flemming's fluid, staining with iron hematoxylin for mitochondria and of counterstaining the fibrils with acid fuchsin. He found that the fibrils first appear in the peripheral portions of the cells where the mitochondria are abundant and have become elongated, and claimed to have established a quantitative relation between the increase in the number of fibrils and the decrease in mitochondria. He concluded that the connective tissue fibrils are formed by a modification of mitochondria. Since he has failed to show a gradation between the black stained mitochondria and the brilliant red colored fibrils, he assumes (p. 164) that there is a time when the mitochondria change their chemical character so that they cannot be stained by either iron hematoxylin or acid fuchsin, that

it is during this stage that they become oriented end to end and fuse to form neurofibrils, and finally, that the fibrils change for a second time their chemical composition so that they acquire a marked affinity for collagen staining dyes.

The work of Firket ('11) on the formation of epidermal fibrils in the cells of the beak and feathers of chick embryos, and that of Arnold ('12) on the origin of fibrils in malignant tumors is likewise open to criticism owing to their failure to demonstrate transitions between mitochondria and the fibrils, and on account of their tacit supposition that structures which stain by the same method of technique are of similar chemical composition (Bensley '10).

3. *The origin of neurofibrils*

In attacking the problem of the origin of neurofibrils it is necessary to bear in mind all the possible factors, which, by any stretch of the imagination, may be involved. These should be carefully studied and eliminated one by one from consideration; but in so doing, it is absolutely essential to remember that the cell maintains its existence by virtue of the interaction of these same factors, and that any attempt to dissociate and analyse them is artificial. The nucleus, chromidial substance, canalicular apparatus and the ground substance ought therefore to be considered in addition to mitochondria.

I have already criticised the theory of the mitochondrial origin of neurofibrils and this discussion has been carried sufficiently far to show that the mitochondria should not be selected as being the sole agents in the formation of neurofibrils. It is a very different thing to conclude that neurofibrils are differentiated by an actual transformation of mitochondria than it is to entertain the possibility that mitochondria may, in some way, be associated with the histogenesis of neurofibrils; for I am not a great believer in the feasibility of attempts to dissociate cytoplasmic activities.

Changes in the nucleus prior to and contemporaneous with the development of neurofibrils have been described by Gerini ('08,

p. 182). He finds that the nucleoli in the peripheral cells of the ventrolateral portion of the cord of chick embryos assume a bipolar arrangement; that many minute granules, staining darkly by Cajal's method, appear at either pole of the nucleus; and that the first neurofibrils arise about the periphery of these granules. I, also, have found that the first indications of neurofibrils appear in a part of the cytoplasm which stains especially darkly by Cajal's method.

The Nissl substance may apparently be excluded from the discussion, since, according to Marcora ('11, p. 946), it first appears in the spinal ganglion cells of chick embryos of about six days' incubation. Collin fixes the time even later for he states ('06, p. 259) that it begins to manifest itself after about ten days' incubation. Moreover, I have failed to demonstrate this material in the nervous system of chick embryos of from 15 to 20 somites, which is the stage during which neurofibrillation begins.

The canalicular apparatus may, likewise, be eliminated. Marcora ('11, fig. 22) has demonstrated it in the cells of the neural tube of a duck embryo of three days' incubation. Repeated efforts, on my part, have not given any indication of its presence in the earliest stages of the formation of neurofibrils, although indications of it possibly occur in figures 21, 25 and 26, where a system of clear, unstained canals may be seen in the cytoplasm.

It is my opinion that further investigation will show that both the canalicular apparatus and the chromidial substance appear much earlier in development than has hitherto been supposed, for they and the neurofibrils are, to some extent at least, indicative of the same thing, namely, the functional maturity of the cell. Furthermore, the tendency in the past has been for investigators to trace back these constituents of the cytoplasm into earlier and earlier stages of development with each advance in technique, and our present technique is by no means perfect.

It is impossible to rule out, in a similar fashion, the ground substance from consideration because it is, like the mitochondria, inseparably connected with all stages in the life of the cell. The neurofibrils are therefore, in all probability, developed from the ground substance, or from formed elements within it as yet un-

known, in response to physiological demands incident at the time of their differentiation. The darker staining of the perinuclear cytoplasm with silver nitrate (figs. 10, 12 and 14) in early neurofibrillar stages strongly indicates the possibility that the nucleus either contributes substance or participates in some way in the formation of neurofibrils.

SUMMARY

1. The neurofibrils are first formed in developing chick embryos as a differentiation of the ground substance (in the majority of cases of the peripheral neuroblasts) at a stage of development characterized by possessing 15 somites, being about 5.8 mm. in length and having been incubated for forty hours at 39°C. ± (p. 402).

2. In the early phases of the development of the chick the earliest neurofibrils are formed in three chief localities: (1) in the hind-brain opposite the otic invaginations (stages of 15 somites on); (2) in the nuclei and root fibers of the cranial nerves (from stages of about 15 somites on); and (3) in a center on either side of the extreme anterior end of the midbrain (stages of 18 somites on) (p. 403).

3. There is no evidence that mitochondria are transformed into neurofibrils. The facts are these: (1) that there is no decrease in the amount of mitochondria in development parallel to the increase in the neurofibrils; (2) that the mitochondria do not show, either by a variation in their morphology, staining reactions, or in any other fashion, capable of detection by our present methods of technique, indications of being transformed into material of different chemical composition; and (3) that during the first part, at least (embryos of somites 15 to 32) of the period of formation of neurofibrils, the mitochondria in the neural tube retain the similarity in their morphology and staining reactions to the mitochondria occurring in the structures derived from the other two germ layers (i.e., mesoderm and endoderm) which they possessed before any neurofibrils became differentiated (pp. 405–408).

4. Mitochondria are present in the early stages in the differentiation of the nerve cells of the chick (embryos somites 0 to 32) and there is ample evidence that they occur throughout cytomorphosis. The neurofibrils, on the other hand, are only present in later stages (embryos of 15 somites on) in evident adaptation to functional demands. Mitochondria may therefore be regarded as cytoplasmic elements of a generalized nature, not participating in so specialized a cell function as the development of neurofibrils; while the neurofibrils are to be looked upon as indicative of the differentiation of the cells in which they are found (p. 403).

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EXPLANATION OF PLATES

All the illustrations have been drawn by Mr. A. B. Streedain, artist to the Department of Anatomy of the University of Chicago, to whom I wish to acknowledge my thanks. Preparations of nerve cells of developing chick embryos alone have been used. Camera lucida, Zeiss apochromatic objective 1.5 mm. and compensating ocular 6 were employed for all except figures 21 and 23–27, of which figures 21 and 25–27 were drawn with objective 1.5 mm. and ocular 8, figure 23 with objective 2 mm. and ocular 4 and figure 24 with objective 2 mm. and ocular 6. They have not been reduced in reproduction so that their magnification as they now appear on the plates is as follows: for figures 21 and 25–27, 2200 diameters; for figure 23, 820 diameters and 1050 diameters for figure 24, for all the others 1500 diameters. All sections were cut 4 μ in thickness. Only specimens from transverse sections have been figured. The colors have invariably been put in by daylight so that the uncertainty and variability involved in artificial illumination has been avoided.

ABBREVIATIONS

m.l.int., membrana limitans interna
m.l.ext., membrana limitans externa
r.ant., radix anterior
g.sp., ganglion spinale
s., mesodermic somite

PLATE 1

EXPLANTION OF FIGURES

This plate illustrates, among other things, the behavior of mitochondria in early stages of development. All the specimens figured have been prepared by Meves' modification of the iron hematoxylin method so that the mitochondria appear as black, filamentous or granular structures against a gray background.

1 Epithelial cells from the ventral half of the neural tube in the region of the tenth somite in an embryo of 12 somites, thirty-four hours incubation (p. 401).

2 Epithelial cells from near the middle line of the medullary plate of an embryo of twenty-four hours incubation at 39°C., in which the primitive groove, fold and pit alone were differentiated. The mitochondria appear as granules and straight or wavy filaments of a black color. They occur in all parts of the cytoplasm and may readily be distinguished from the black spherical yolk granules (p. 401).

3 Epithelial and germinative cells from the neural fold in the region of the head process of an embryo of 3 somites, twenty-four hours incubation. The mitochondria are distributed throughout the cytoplasm but show a tendency to become accumulated in the distal parts of the cell. Only a few spherical, jet-black yolk granules may be distinguished at this stage (p. 401).

4 Radix anterior (*r.ant.*) with the neighboring portion of the neural tube, which have become slightly separated from each other during the preparation. Taken from opposite the tenth somite on the right side of an embryo of 31 somites, seventy hours incubation. In the radix anterior mitochondria and neurofibrils may be made out together; the former, rods or wavy filaments of different lengths, the latter, threads of finer diameter and of a lighter color. The mitochondria in the cytoplasm of the neuroblast (a) are shorter, curved more closely packed together and do not fuse together to form a network (p. 410).

5 From the neural tube opposite the tenth somite on the right side of an embryo of 26 somites, and fifty-eight hours incubation. The cell outlines are indistinguishable. The chromosomes in a dividing germinative cell are shown at (a) and may be differentiated from the filamentous mitochondria by their characteristic arrangement. The mitochondria are somewhat larger than in the preceding figures (p. 401).

1

2

3

4

5

A.B. Streedain del.

PLATE 2

EXPLANATION OF FIGURES

All the preparations illustrated on this plate have been drawn from specimens prepared by Cajal's silver nitrate method (formula I). Figures 6 to 9 show the different types of cells in which neurofibrils are formed; figures 10, 11, 12 and 14 the appearance of the earliest neurofibrils to become differentiated (i.e., in an embryo of 15 somites) and figure 13 the neurofibrils passing out from the neural tube among the cells of the myoblast in a later stage. Unfortunately the preparations could not be illustrated in their original colors on account of the cost of reproduction.

6 From the right side of the hind-brain, opposite the otic invagination, of an embryo of 24 somites, length 7.5 mm. and sixty-five hours incubation. This figure shows a cell sending out a process, containing neurofibrils, which pierces the membrana limitans interna (*m.l.int.*) and extends for some distance within the cavity of the neural tube (p. 404).

7 Bipolar cell from the same region of the same embryo. The neurofibrils are black. The membrana limitans interna (*m.l.int.*) and externa (*m. l. ext.*) are shown for orientation (p. 403).

8 Marginal neuroblast from the same region of the same embryo. The neurofibrils seem to form a more or less continuous network (p. 403).

9 From the same region of the same embryo but on the left side. The cell (a) is apparently within the cavity of the neural tube and the process (b) of the cell (c) extends toward the neural tube. All three contain neurofibrils (p. 404).

10 Cells from the right side of the forebrain of an embryo of 15 somites, length 5.8 mm., incubation forty hours at 39°C, being slightly further advanced than Duval's embryo of thirty-three hours, (fig. 268, p. 56). The darkly staining portion of the middle cell affixed to the distal pole of the nucleus contains within it a blackened network which probably represents the first stage in the histogenesis of the neurofibrils (p. 402).

11 More completely formed neurofibrils occurring in a marginal neuroblast in the hind-brain on the right side, opposite the otic invagination of the same embryo (p. 402).

12 Cells from the right side of the mid-brain of the same embryo showing a stage in the formation of neurofibrils similar to that represented in figure 10 (p. 402).

13 Neural tube with the adjacent myoblast in the region of the sixth somite on the right side of an embryo of 29 somites, length 8.1 mm. and incubation sixty-three hours. The neurofibrils are seen winding in and out among the mesoblastic cells (p. 411).

14 Cells from the same region of the same embryo as figure 10 showing, rather more satisfactorily than figures 10 and 12, the darkly staining distal part of the cytoplasm in which the neurofibrillar network is formed (p. 404).

m.l.int.
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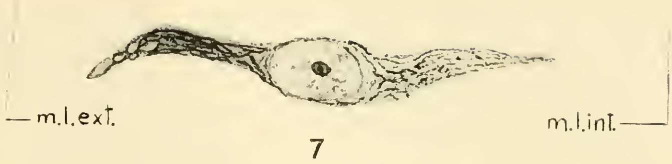
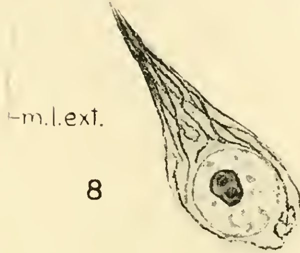

m.l.ext.
m.l.int.
7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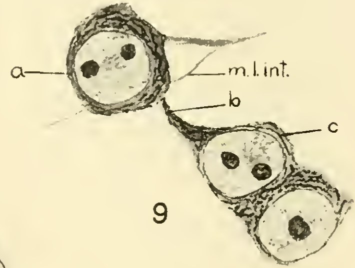
a
m.l.int.
b
c
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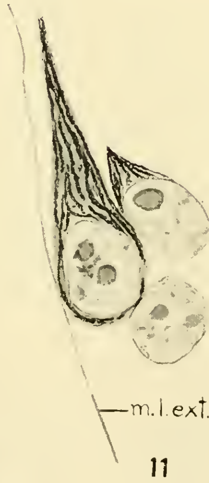
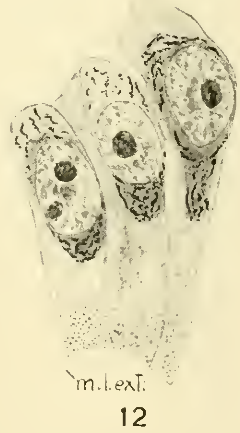
m.l.ext.
10


m.l.ext.
11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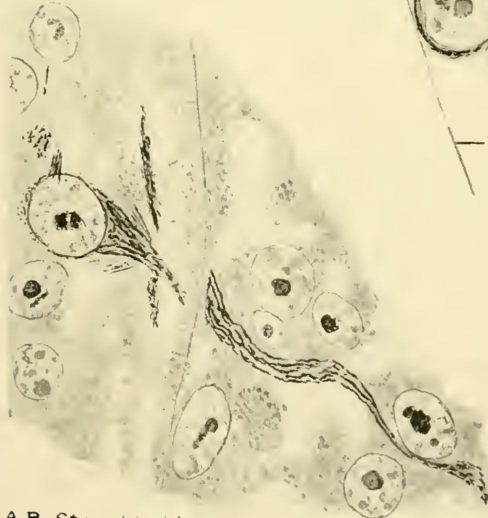
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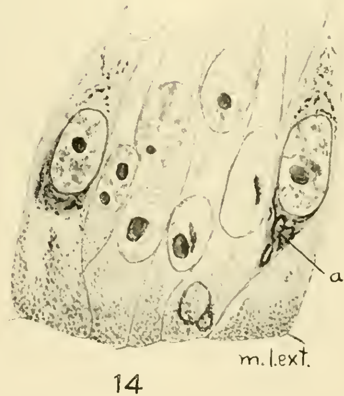
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m.l.ext.
14

PLATE 3

EXPLANATION OF FIGURES

Plates 3 and 4 constitute the essential features of a comparison of the results yielded by Meves' iron hematoxylin method and by Cajal's silver nitrate technique respectively. In both cases drawings have only been made from sections of the neural tube on the right side opposite the sixth somite and the degree of differentiation of the embryos from which the figures of plate 3 were drawn corresponds severally with that of those represented on plate 4 (fig. 15 a to fig. 15 b and so on). The drawings have been arranged so that the membrana limitans externa is to the left hand side of the observer. A collation is therefore justified since morphologic equivalents of approximately the same area and thickness (4μ) are only being used. This comparison of homologous equivalents in increasing stages of development prepared by these two methods, the one showing mitochondria, the other neurofibrils, indicates: (1) That there is no decrease in the amount of mitochondria parallel to the increase in neurofibrils. (2) That the mitochondria do not show, either by a variation in their morphology, staining reactions, or in any other way, capable of being detected by our present-day methods, indications of being transformed into material of radically different chemical composition (pp. 407 and 416).

15 a From the right side of the neural tube in the region of the sixth somite of an embryo of 17 somites, length 5.5 mm. and incubation forty-five hours. The mitochondria are filamentous, their irregular shape, as seen in the drawings, depends upon the fact that their long axes are directed in all planes relative to the drawing and that they are cut in pieces of many sizes, at all angles during sectioning. There is a remarkable variation in the color of the back-ground, for in two cells, (a) and (b), the cytoplasm remains practically unstained, although there is apparently no other difference between them and the neighboring cells (p. 408).

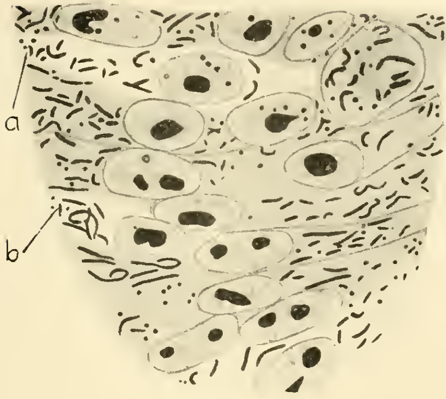
16 a Same region, embryo of 20 somites, sixty-two hours incubation. There is no change in the mitochondria.

17 a Same region, embryo of 22 somites, fifty-two hours incubation. The section has been cut rather obliquely. There is no change in the mitochondria.

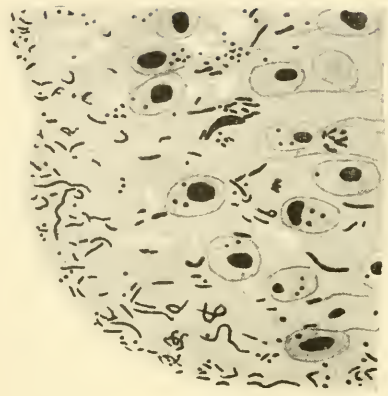
18 a Same region, embryo of 24 somites, length 7.0 mm. and sixty hours incubation. The section is even more oblique. The mitochondria show no change.

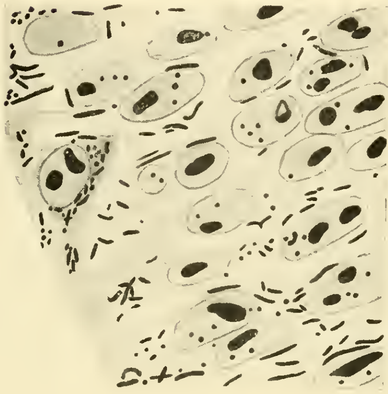
19 a Same region, embryo of 28 somites, sixty-four hours incubation. No change in mitochondria.

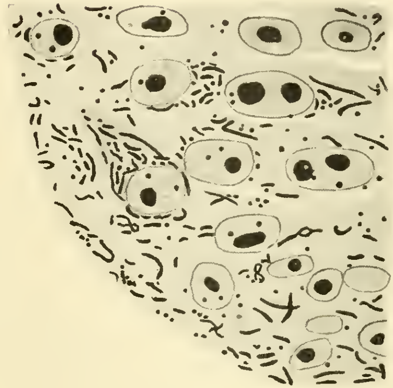
20 a Same region, embryo of 31 somites, seventy hours incubation. There is still no change in the mitochondria.

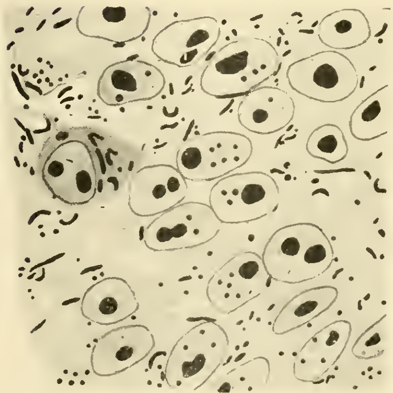


15 a


18 a


16 a


19 a


A B. Streedain del.

17 a

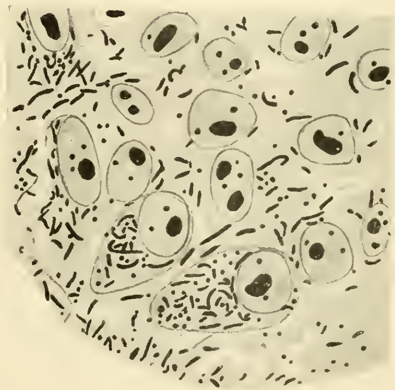

20 a

PLATE 4

EXPLANATION OF FIGURES

This plate logically belongs to the preceding (plate 3) in connection with which it has been described. All the figures are from preparations made by Cajal's silver nitrate method.

15 b A portion of the neural tube from opposite the sixth somite on the right side of an embryo of 17 somites, 44 hours and 15 minutes incubation. No neurofibrils are formed, but the cytoplasm in the distal portions of the cells (a), (b) and (c) is stained especially darkly with the silver nitrate.

16 b Same region, embryo of 20 somites, length 7.4 mm., 88 hours, and 20 minutes incubation. The neurofibrils have appeared, in a single cell, and are distinctly stained.

17 b Same region, embryo of 22 somites. Considerable increase in neurofibrillar material.

18 b Same region, embryo of 24 somites, length 7.5 mm., 65 hours incubation. Shows some neurofibrils passing through the membrana limitans externa into the myoblast.

19 b Same region, embryo of 28 somites, length 6.8 mm., 63 hours incubation. Neurofibrillar material increased.

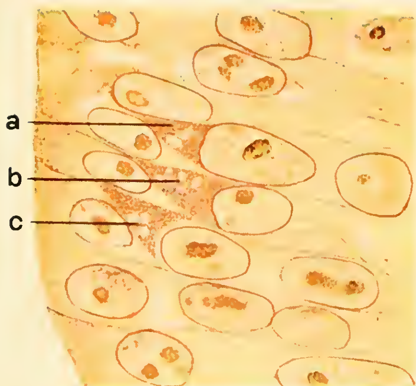
20 b Same region, embryo of 31 somites, length 7.3 mm., 62 hours incubation. Shows a great increase in the number of neurofibrils, many of which now run parallel to the long axis of the neural tube.

PLATE 5

EXPLANATION OF FIGURES

Figures 21, 26 and 25 represent progressive stages of differentiation in Bensley's anilin fuchsin toluidin blue method. These figures have been drawn from neighboring sections of the same embryo, mounted on the same slide. In the first (fig. 21) the differentiation is practically nil, the mitochondria staining exactly the same color as the neurofibrils; in the second (fig. 26) it has been carried a little further with the result that the neurofibrils have lost their bright crimson color and have assumed a dull red shade; while in the last (fig. 25) the decolorization has been carried to an extreme so that the neurofibrils have lost all of the acid fuchsin and have become stained with the differentiator, toluidin blue. It is to be noted that in these progressive stages of differentiation the initial affinity of the neurofibrils for an acid dye (acid fuchsin), in which they resembled mitochondria, is gradually changed to an affinity for a basic dye (toluidin blue), while the intensity of the coloration of the mitochondria remains unaltered. Conclusions based upon the apparent similarity in the staining reactions of mitochondria and the primitive neurofibrils should therefore be received with caution (p. 410 and 416).

21 Cell and accumulation of neurofibrils from the ventral portion of the neural tube of an embryo, length from cervical flexure to tail flexure 8.4 mm., 100 hours incubation, corresponding closely in degree of differentiation to Duval's embryo of 96 hours. Stained by Bensley's anilin fuchsin toluidin blue method



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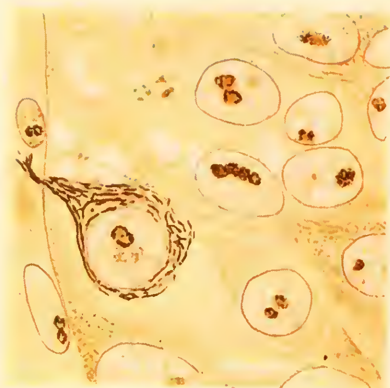
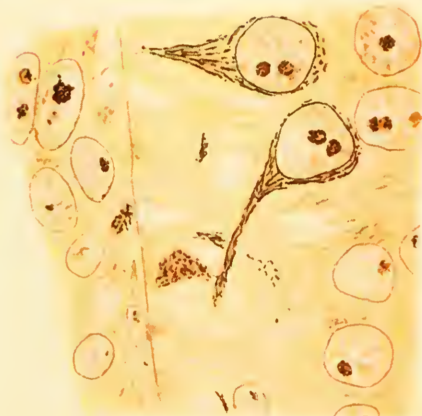


18 b

16 b



19 b

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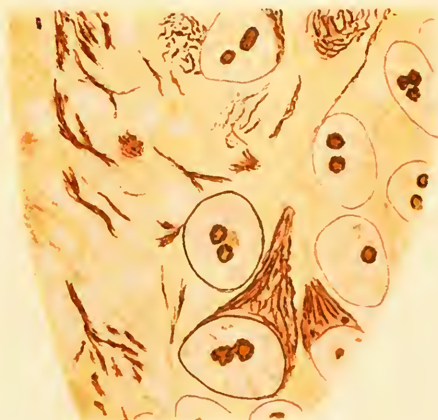
17 b



20 b

with but slight differentiation, the neurofibrils and the mitochondria staining exactly the same color. The clear, uncolored spaces within the cell may constitute the canalicular apparatus, because it is demonstrated, in a similar manner, by this technique applied to adult spinal ganglion cells (Cowdry, '12 a, fig. 1).

22 Apolar cells bordering the central canal of the neural tube opposite the posterior part of the third somite from an embryo of 33 somites, length 8.3 mm. and 74 hours incubation, prepared by Paton's modification of the Bielschowsky method. The circumnuclear neurofibrillar network are shown in black (p. 403); compare this with Cajal 1907, figures 1 and 2.

23 Pear-shaped neuroblast isolated from the neural tube opposite the tenth somite on the right side of an embryo of 25 somites, length 7.45 mm., stained intravitam in a 1:10,000 solution of janus green in 0.85 per cent sodium chloride solution. The magnification is less, so that the granular mitochondria, shown in green, seem smaller than in the other illustrations (p. 401).

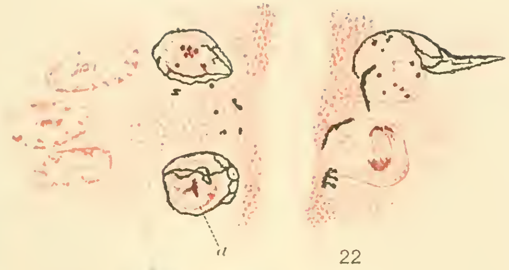
24 Cells from the same region of the same embryo stained in the same way, the sole difference being the substitution of ocular 6 in place of compensating ocular 4 in making the drawing. Mitochondria bluish green. The cell walls are quite distinct (p. 401).

25 Taken from the dorsal portion of the neural tube of the same embryo as figure 21, prepared by the same method and mounted on the same slide. The process from the cell (a) is closely applied to that from the cell (b), both piercing the membrana limitans externa together. The differentiation has been carried to an extreme so that the neurofibrils have completely lost all shade of acid fuchsin and have assumed the blue color of the differentiator, toluidin blue, whereas the mitochondria still retain their original bright crimson coloration. The neurofibrils and the mitochondria are seen side by side in the processes of both cells differentially stained. The clear spaces in the cell (b) may, as in figure 21, represent the canalicular apparatus (p. 410).

26 Section from the same embryo, slide and method, which was also taken from the dorsal part of the neural tube. The process from the cell (a) pierces the membrana limitans externa and extends among the spinal ganglion cells (*g. sp.*). The differentiation is here intermediate between that represented in figures 21 and 25. The neurofibrils have consequently lost their bright crimson coloration (fig 21) and have become a brownish red color so that they may readily be distinguished from the mitochondria which are in the interfibrillar substance in the cell process. A few clear, tortuous, uncolored spaces are seen in the spinal ganglion cell (*g.sp.*) which are possibly the canalicular apparatus (p. 410).

27 A portion of the radix anterior from an embryo, length from the cervical flexure to the tail flexure 8.4 mm., period of incubation 100 hours, corresponding closely in degree of differentiation to Duval's embryo of 96 hours, which has been stained by Bensley's anilin fuchsin methylene blue erythrosinate method. The sheath cells (a) are shown and the mitochondria within them. The neurofibrils appear a yellowish brown color and a few bright crimson mitochondria are visible in the interfibrillar substance (p. 410).

21



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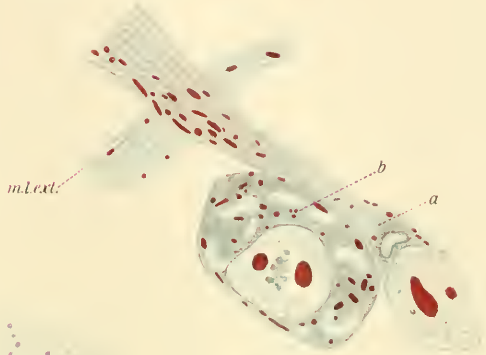

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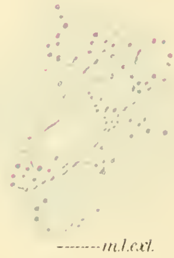

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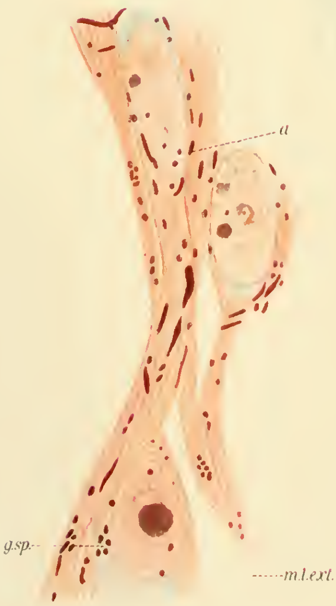

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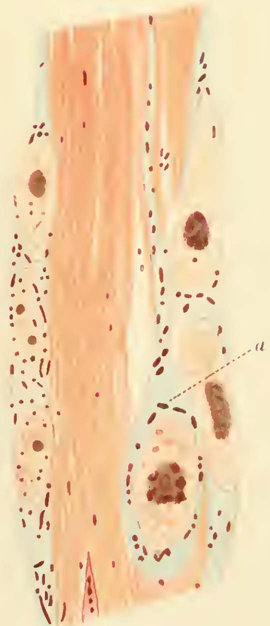

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A. B. Streedain del.

Werner & Winter, Frankfurt a/M.

FEEDING EXPERIMENTS ON TADPOLES

II. A FURTHER CONTRIBUTION TO THE KNOWLEDGE OF ORGANS WITH INTERNAL SECRETION

J. F. GUDERNATSCH

Department of Anatomy, Cornell University Medical College, New York
(From the Department of Histology and Embryology, University of Munich. Director: Prof. S. Mollier)

TWO DOUBLE PLATES

Certain mammalian glands with a so-called internal secretion, when given as food, can enact a decided influence on the growth and differentiation of amphibian embryos. This was shown by experiments that were carried out during the summer of 1911 on tadpoles of Rana temporaria and Rana esculenta.

During the spring of 1912 these experiments were repeated and at the same time varied to such an extent that no doubt as to their results remained. Although the experiments of the two seasons revealed many precise data, there is still a great number of obscure features regarding the definite action of internally secreting glands when fed to the tadpoles. While the influence on growth and differentiation resulting from feeding some of the glands was striking, others exerted no marked effects. The action of the latter, in part or entirely, may not be concerned with those most important physiological processes in the embryo, namely, growth and differentiation. They might play their chief rôle, then, in the household of the postembryonic organism. Or, since taken from mammals, some of these internally secreting glands, if at all connected with embryonic development, may fail to reveal this influence, when fed to amphibian embryos. Their action must finally be studied in experiments on higher vertebrates.

The experiments in 1912 were performed on tadpoles of Rana temporaria, Rana esculenta, Bufo vulgaris and Triton alpestris.

The eggs of these species were collected from ponds in the vicinity of Munich and reared in the laboratory. They were kept in large aquaria and after hatching were transferred to smaller dishes, each dish containing about 100 individuals. After the larvae had grown for some time and seemed to be crowded in the dishes they were separated into smaller groups. To avoid possible errors, a great number of individuals was always used for each kind of experiment. All in all, over 20,000 tadpoles were treated.

The eggs from different localities were kept separate and, as far as possible, eggs apparently coming from the same mother were grouped in one set and used for a special experiment. Whenever the eggs necessary for one experiment could not all be supplied by a single female I endeavored to select eggs coming from the same locality, and apparently in the same stages of development. For example, set III probably contained eggs from three mothers, but the time of hatching, April 7 and 8, and their sizes, 10 to 11.5 mm at the beginning of the experiment, show them to be in similar stages of development. Again, for other experiments in which a large number of animals was required, as in sets VIII to XI over 4000, several sets in apparently the same stage of development were thoroughly mixed and then divided into smaller groups. In these last sets the tadpoles hatched between April 10 and 13 and a month later, when the feeding began, their sizes varied from 13 to 18 mm; this difference is not greater than one would encounter in eggs from a single individual.

The water was changed at least once a day. While standing, its temperature varied with the room temperature, a process that corresponds to natural conditions. However, even in a room with apparently uniform temperature, the water will not show exactly the same temperature in all the dishes, as Barfurth has pointed out. To overcome any unequal influence of light, air and temperature resulting from the position of the dishes, they were shifted several times a day so as to progress in a certain order. With this precaution there were no detectable differences of temperature in the dishes assigned to one set. There was sometimes a slight difference of temperature between the dishes

used for the different experiments, but this was of no importance, so long as the tadpoles used for one experiment were kept under uniform conditions.

As during the previous year, the food in small pieces was placed in the water and there voraciously taken by the animals, with the exception of pancreas which never seemed to excite their appetite very greatly.

Last year the tadpoles had their natural food previous to the experiments, this year in all but one experiment no other food was received except the one chosen for the studies. In 1911 after the feeding began the animals were kept exclusively on a one-gland diet; in 1912 these experiments were repeated with part of the animals while in addition a mixed, either two-animal tissues or gland-plant diet, was introduced.

The following were used as foods: thyroid, adrenal, liver, spleen, hypophysis, brain, pancreas and muscle from the horse, testicle, ovary and thymus from cattle, and as vegetable food Elodea canadensis and Ceratophyllum demersum.

It would not have been possible to perform such an extensive series of experiments but for the courtesy and generosity of Prof. S. Mollier, in whose laboratory in Munich the experimental part of this paper was carried out. It is with pleasure that I express my best thanks for the many kindnesses shown me during my stay in his laboratory.

Experiment I

Rana temporaria, Set I. Figure 1, *a* to *zf*. Hatched April 4 to 7, 1912. Feeding began April 13, 1912. Original size 10.2 to 12 mm.

The diary reads as follows:

April 4 The feeding begins.
April 22 Thyroid-fed have hind legs forming.
April 24 Thyroid-fed have fore legs forming.
April 25 Thyroid-fed begin to die off.
April 29 Last thyroid-fed animals dead. Thymus- liver- spleen- and muscle-fed tadpoles are larger than the other groups.
April 30 Muscle, liver and spleen ones show hind leg buds.
May 4 Adrenal cortex, adrenal medulla and ovary have hind leg buds faintly noticeable.

TABLE 1

Measurements in millimeters during the experiment

DATE	CONTROL	MUSCLE	MUSCLE-THYROID	LIVER	LIVER-THYROID	SPLEEN	SPLEEN-THYROID	BRAIN	BRAIN-THYROID	ADRENAL CORTEX	ADRENAL CORTEX-THYROID	ADRENAL MEDULLA	ADRENAL MEDULLA-THYROID	OVARY	OVARY-THYROID	THYMUS	THYROID
April 13......								10.5-12.0 mm.									
April 29......	12.0-15.0	17.0-24.0		18.0-26.0		18.0-24.0				14.0-20.0		14.0-18.0		14.0-19.0		18.0-24.0	10.0-11.0
May 8......	14.0-18[1]	18.0-25.0		20.0-29.0		19.0-28.0				14.0-23.0		14.0-22.0		14.0-21.0		19.0-25.0	Dead
	5.0- 6.0	7.0- 9.0		7.0-11.0		7.0 -9.0				4.0- 8.0		5.0- 8.0		5.0- 8.0		7.0- 9.0	Apr. 29 (Completely metamorphosed)
	9.0-12.0	11.0-16.0		15.0-18.0		12.0-17.0				10.0-15.0		9.0-14.0		9.0-13.0		11.0-16.0	
	3.0- 4.0	4.0- 6.0		5.0- 6.0		4.0- 5.0				3.0- 5.0		3.0- 5.0		3.0- 5.0		4.0- 5.0	
May 15.....	14.0-19.0	18.0-27.0		20.0-30.0		19.0-30.0		Feeding		15.0-25.0		15.0-25.0		15.0-24.0		20.0-28.0	
	5.0- 7.0	7.0-10.0		8.0-11.0		7.0-10.0		started		5.0- 9.0		5.0-10.0		6.0- 9.0		7.0-10.0	
	9.0-13.0	11.0-17.0		14.0-19.0		12.0-20.0		May 10		10.0-16.0		10.0-15.0		9.0-16.0		12.0-18.0	
	3.0- 4.0	4.0- 6.5		5.0- 7.0		4.0- 6.0				3.0- 5.0		3.5- 5.0		3.0- 5.5		4.0- 5.5	
	From now on fed on algae																
May 24.....	15.0-24.0	19.0-31.0		20.0-32.0		19.0-31.0		16.0-24.0		15.0-28.0		15.0-28.0		16.0-28.0		21.0-31.0	
	5.0- 9.0	7.0-11.0		7.5-11.0		7.0-11.0		5.0- 9.5		5.0-10.0		5.0-10.5		6.0-10.0		7.5-10.0	
	9.5-16.0	12.0-20.0		14.0-20.0		12.5-20.0		10.0-16.0		10.0-19.0		9.0-17.5		10.0-18.0		13.0-21.0	
	3.0- 5.0	4.0- 7.0		5.0- 7.0		4.0- 6.0		3.0- 5.5		3.0- 6.0		3.5- 6.0		3.5- 6.0		4.5- 6.0	
May 31.....	15.5-25.0	19.0-32.0		21.0-32.0		19.0-32.0		16.0-26.5		15.5-29.5		15.0-28.5		16.0-28.5		21.0-31.5	
	5.0- 9.5	7.0-11.5		7.5-11.5		7.0-11.0		5.0- 9.5		5.5-10.5		5.0-10.5		6.0-10.0		7.5-10.5	
	9.5-16.0	12.0-20.5		14.0-20.5		12.5-21.5		10.0-17.5		10.0-19.0		9.0-18.0		10.0-18.5		13.0-21.5	
	3.0- 5.5	4.0- 7.0		5.0- 7.0		4.0- 6.5		3.0- 5.5		3.0- 6.5		3.5- 6.0		4.0- 6.5		4.5- 6.0	
June 11.....	16.0-26.0	19.5-33.0		21.5-33.0		19.0-33.0		16.0-29.0		16.0-31.0		16.0-29.5		16.0-29 5		21.0-32.0	
	5.5-10.0	7.0-11.5		7.5-11.5		7.0-11.5		5.0-10.0		6.0-11.0		5.5-11.0		6.0-10.0		7.5-11.0	
	10.0-16.0	14.0-21.5		14.0-20.5		12.5-22.0		10.0-19.0		10.0-20.5		10.0-19.0		10.0-19.5		13.0-21.5	
	3.0- 5.5	4.0- 7.0		5.0- 7.0		4.5- 7.0		3.0- 5.5		4.0- 7.0		3.5- 6.0		4.0- 6.5		4.5- 6.5	
June 21.....	16.0-27.5	20.5-37.0	18.5-28.0	21.5-35.0	18.0-28.0	19.0-33.0	11.0-24.0	16.0-32.0	15.0-24.5	16.0-32.5	13.0-21.0	16.0-32.5	15.0-26.0	16.0-31.5	16.0-23.0	22.0-32.5	
	5.5-10.5	7.5-12.5	6.5- 9.0	8.0-12.0	6.5- 9.0	7.0-11.5	4.0- 8.0	6.0-11.5	5.0- 7.0	6.0-12.0	5.0- 8.5	6.0-11.5	5.5- 9.0	6.0-11.0	5.5- 7.5	8.5-11.5	
	10.0-17.0	15.0-25.0	12.0-19.0	15.0-23.0	11.5-19.0	12.5-22.0	7.0-16.0	10.0-20.5	10.0-17.5	10.0-21.5	8.0-17.5	10.0-21.0	8.5-17.0	10.0-20.5	10.0-16.5	13.5-21.5	
	3.5- 6.0	4.5- 7.5	3.5- 6.0	4.5- 7.0	4.0- 5.5	4 5- 7.0	3.5- 4.5	3.0- 6.5	3.0- 5.0	4.0- 7.5	3.5- 4.5	4.0- 7.0	4.5- 5.0	4.0- 7.0	4.0- 5.0	5.0- 7.0	
July 6......			16.0-19.0		15.0-18.0		9.0-17.0		10.0-17.0		9.5-16.0		10.0-17.5		10.0-17.0		
			6.0- 8.0		5.5- 8.0		4.5- 7.0		4.0- 6.5		4.0- 6.0		4.5- 6.5		4.5- 6.0		
			9.0-11.0		8.0-10.0		4.0-10.0		6.0-11.0		5.5-10.0		5.5-11.0		6.0-11.0		
			2.5- 4.0		3.0- 4.0		2.5- 4.0		2.5- 4.0		2.5- 3.5		3.0- 4.0		2.5- 4.0		
July 11.....	18.0-29.0	24.0-38.0		22.0-32.0		21.0-33.5		19.0-33.0		19.0-33.0		19.0-32.5		22.0-34.0		22.0-33.0	
	7.0-11.0	9.0-11.5		8.0-11.5		7.0-11.5		6.5-12.0		7.0-13.0		7.0-11.5		8.5-11.5		8.5-11.5	
	11.0-18.0	15.0-26.5		15.0-23.0		14.0-22.0		11.5-21.0		12.0-20.0		12.0-21.0		13.5-23.5		13.5-22.0	
	4.0- 6.5	4.5- 7.5		4.5- 7.0		4.5- 7.0		4.0- 7.5		4.0- 8.0		4.0- 7.0		5.0- 7.0		5.0- 7.5	

[1] When four measurements are given, the first refers to the entire length, second to length of body, third to length of tail, fourth to breadth of body.

May 14 To this time the controls have been starving, in order to test the possible influence of hunger on development. They show no signs of advancement, but are far behind in size. From now on they are fed on Elodea canadensis.

A group originally intended for a hypophysis diet also starved up to this day, and from now on is fed on brain.

The liver-fed ones have a greenish color, the thymus-fed animals are deep black.

May 24 The smallest individuals in all sets begin to die off. The spleen-fed ones become very black, the bigger ones on adrenal cortex become very light, lighter than on adrenal medulla.

June 12 The first liver-fed ones show hind legs; this is 49 days later than the time at which the thyroid-fed ones attained the same condition.

June 14 Hind legs appear in adrenal-cortex-fed tadpoles. Their body assumes a peculiar triangular shape (fig. 7 *b*), very pointed at the anterior end and broad at the posterior. They are all becoming light, while all the adrenal-medulla-fed ones are dark.

June 17 A number of each group, 70 to 80, which have no hind legs except the small buds, are placed on thyroid diet.

June 19 The light color of the adrenal-thyroid tadpoles is disappearing.

June 20 Those liver-fed ones that have put out fore legs since June 12 shorten their tails.

The liver-thyroid animals develop hind legs.

June 21 Adrenal medulla-thyroid and muscle-thyroid tadpoles develop hind legs. The former are extremely active.

June 22 Liver-thyroid and muscle-thyroid ones assume a frog shape.

June 23 Spleen-thyroid and adrenal cortex-thyroid ones develop hind legs. The first liver-fed frog leaves the water.

June 24 Spleen-thyroid and adrenal cortex-thyroid animals assume frog shape. Brain-thyroid ones develop hind legs. Adrenal cortex tadpoles are extremely light greenish-yellow.

Brain-fed ones assume somewhat the triangular adrenal cortex shape described on June 14.

June 25 Liver-thyroid and adrenal medulla-thyroid animals show fore legs.

June 26 Spleen-thyroid-fed ones show fore legs.

Ovary-thyroid and brain-thyroid tadpoles assume frog shape, the latter are very active.

June 27 Muscle-thyroid animals show fore legs.

June 29 Adrenal cortex-thyroid and brain-thyroid ones show fore legs.

June 30 Ovary-thyroid ones show fore legs.

July 6 All the thyroid-fed ones are fixed, since many of them die.

July 7 Brain-fed tadpoles show hind leg buds.

July 11 Muscle-fed ones have the best developed hind legs, next the ovary and adrenal-cortex ones which have short but very strong legs, then adrenal-medulla, brain and spleen.
The color of the animals on the different diets is: control, brown; muscle, dark brown; liver, dark greenish; spleen, dark; adrenal cortex, very light brown; adrenal medulla, dark brown; ovary, yellowish; thymus, dark; brain, brown.
July 19 Muscle-fed ones begin metamorphosing.
July 20 Ovary-fed ones begin metamorphosing.
Thymus-fed have no signs of legs, spleen-fed only small buds.

In this experiment the thyroid treatment proved to be as effective as in the experiments performed in 1911. Tadpoles that had been fed on no other food than thyroid grew hind legs 9 days after the feeding began and fore legs only 2 days later. Normally several weeks would have elapsed between the appearance of the hind and the fore extremities. When these thyroid-fed tadpoles put out their anterior limbs and began to shorten their tail, they were 18 to 20 days old, calculating from the date of hatching. Normally they would take from 10 to 12 weeks to complete their metamorphosis.

The tadpoles fed on ordinary meat, muscle (fig. 1 *d*, 1 *n*), showed distinct hind legs 44 days and fore legs 71 days later than the thyroid-fed ones. The first individual in the muscle-fed group to complete its metamorphosis was approximately 104 days old.

Liver-fed tadpoles (fig. 1 *a*, 1 *i*, 1 *x*) show the best growth, although their growth is only slightly better than that of the spleen and thymus sets. Next to the thyroid group the liver-fed tadpoles also showed most rapid progress in differentiation. They grew hind legs 40 days and fore legs 49 days later than the thyroid group. The first liver-fed specimen to complete its metamorphosis was approximately 78 days old.

The spleen- (fig. 1 *b*, 1 *l*) and thymus- (fig. 1 *c*) fed tadpoles show almost parallel courses in growth and differentiation. During the first weeks of the experiments the spleen group ran a little ahead of the thymus specimens. The members of both groups became extremely dark in color during the course of the feedings. At the approximate age of 104 days, when the experi-

ment was discontinued, none in either group had hind legs out, although spleen-fed ones showed leg buds.

The three groups of tadpoles fed on adrenal cortex (fig. 1 *f*, 1 *r*), adrenal medulla (fig. 1 *g*, 1 *t*) and ovary (fig. 1 *e*, 1 *p*) were during the first weeks of growth behind the muscle, liver, spleen and thymus groups. Later, however, they grew more rapidly. Some of those fed on ovary actually reached the average size of the faster groups and completed their metamorphosis at the approximate age of 105 days.

The adrenal cortex tadpoles were somewhat faster in development and grew better than the adrenal medulla ones. The former budded hind legs on the 71st and the latter on the 81st day. At the conclusion of the experiment the adrenal cortex ones had strong well developed legs, while the legs of the medulla-fed individuals were still short and drawn close to the body.

In previous experiments, in which adrenal cortex and medulla had not been given separately, it was seen that the adrenal-fed tadpoles became extremely light in color after 3 or 4 weeks of feeding. The pigment cells were found to be completely contracted. The suggestion was made "that the extract from the chromaffine cells of the medulla which dissolved in the water caused the pigment cells to contract" and "former experiments with adrenalin would warrant such a suggestion." In the present experiments, however, separate sets of tadpoles were fed on cortex and medulla respectively. After 5 weeks' feeding those fed on adrenal cortex became much lighter than those fed on adrenal medulla or any other food. This difference in color became more evident as the experiment proceeded, until the cortex-fed tadpoles had an extremely light, greenish-yellow tint. Thus the above suggestion is doubtless incorrect, at least in these experiments, yet the true cause of the light pigmentation is still obscure.

These cortex-fed tadpoles also differed from the other groups in still another feature, namely, the peculiar triangular shape (fig. 7 *b*) of their bodies described above. A few of the brain-fed tadpoles somewhat approached this condition.

The chief purpose of feeding the set I on different foods was to study the influence of the thyroid treatment, after the ani-

mals had fed for sometime on various other tissues. The tadpoles were about 10 weeks old, when the thyroid was given. They were fed only four times. This feeding caused the same acceleration in differentiation that was noticed in former experiments. Within 3 to 7 days the reaction to the treatment became evident.

There were differences in the rapidity of this reaction among the different sets of tadpoles, as may be seen from the diary, but whether or not these differences are significant as to the value of the food given before the thyroid treatment, remains an open question.

The liver-thyroid-fed tadpoles (fig. 1 *k*) were the first ones to develop hind and fore legs. This fact might easily be explained on the ground that the liver-fed group was the most advanced in the entire series (May 8, June 1, June 12, and June 23, in the diary). Yet why those fed, for instance, on spleen, the slowest lot in the series, having no legs even on July 19, and those fed on adrenal medulla which were at the start of the thyroid treatment smaller than the liver-fed ones, should react to the thyroid stimulus as fast as the liver group, cannot be explained on the above basis suggested for the liver-fed group.

On the other hand, the adrenal cortex group though of about the same size are 10 days ahead of the adrenal medulla group in differentiation. Yet after thyroid application they do not react with as great speed as the latter, and fall behind in the series.

Throughout the experiment it was observed that muscle-liver- and spleen-fed tadpoles belonged to one class as far as their growth was concerned, while those fed on adrenal cortex, adrenal medulla and ovary formed another class. As soon, however, as the thyroid factor was introduced this classification was disturbed. Thus the previous feeding may influence to some degree the mode of reaction to the thyroid stimulus. No variety of previous feeding can, however, completely prevent this reaction for any length of time. After a shorter or longer interval (in this experiment the difference was only from 3 to 7 days) every tadpole, as far as macroscopic features are concerned, will respond to the thyroid stimulus, no matter what kind of food had been previously given.

To show the different degrees of response to the thyroid food in the various groups, table 2 may be added. While it does not reveal, as said before, any important points as to the respective value of the different foods given before the thyroid diet, it shows some striking changes in the subsequent positions in the series of the various groups, especially those fed on adrenal medulla, spleen and adrenal cortex.

TABLE 2

AVERAGE SIZE AT THE START OF THE THYROID DIET	AVERAGE SIZE 7 DAYS LATER	PERCENTAGE LOSS IN LENGTH		TIME OF REACTION
muscle	muscle	adr. med,	12.761	liver (fig. 1 *k*)
liver	liver	liver,	14.777	adr. med. (fig. 1 *u*, 1 *d*
spleen	adrenal med.	brain,	15.053	muscle (fig. 1 *o*, 1 *za*)
adrenal cortex	brain	muscle,	15.454	spleen (fig. 1 *m*, 1 *z*)
adrenal medulla	ovary	ovary,	16.129	adr. cor. (fig. 1 *s*, 1 *zc*)
brain	spleen	adr. cor.,	28.421	brain (fig. 1 *w*, 1 *ze*)
ovary	adrenal cortex	spleen,	32.692	ovary (fig. 1 *q*, 1 *zb*)

Experiment II

Rana temporaria, Set IV. Figures 4, *a* to *p*. The tadpoles hatched on April 5 to 6, and the feedings began April 13, 1912. Original length of the specimens was 12 to 12.5 mm.

The diary records of the experiment are given below:

April 13 The feeding was started.

April 16 Those fed on thymus and spleen have become larger than the others.

April 17 Thyroid-fed tadpoles have become considerably smaller than thymus-fed ones and the thymus ones are getting dark.

April 18 Thymus-fed tadpoles are much darker than the others, spleen-fed specimens are also getting dark. Thyroid ones now show frog-like bodies.

April 19 Thymus-fed individuals are almost black, spleen-fed very dark, muscle-fed much lighter, thyroid-fed darker than muscle, but lighter than thymus and spleen. Thyroid ones have distinct frog shape, their hind legs are beginning to bud (14 days old).

One set of thymus-fed individuals is now changed to a thyroid diet, thymus-thyroid I.

April 20 The resorption of the tail in thyroid-fed individuals is distinctly noticeable. The specimens all lie on the bottom of the dish and appear frail, taking no more food, while the others in the experiment swim actively.

TABLE 3

Measurements in millimeters during the experiment

DATE	CONTROL	MUSCLE	MUSCLE-THYROID	SPLEEN	SPLEEN-THYROID	THYMUS	THYMUS-THYROID						THYROID
							I	II	III	IV	V	VI	
Apr. 13..	12-12.5 mm.												
Apr. 17..		14.0		15.0		15.0							13.0
Apr. 20	length												
	14.0-15.0	18.0-19.0		18.0-20.0		18.0-20.0	18.0-20.0						12.5-13.0
	breadth												
	3.0	4.5- 5.0		4.5- 5.0		4.0- 5.0	4.0- 5.0						2.5- 3.0
Apr. 22							14.0-17.0						12.0-12.5
							4.0						2.0- 3.0
Apr. 29	14.0-15.0	18.0-20.0		18.0-21.0		18.0-21.0	8.0-10.0						10.0-12.0
	3.0	4.0- 5.0		4.0- 5.0		4.0- 5.0	3.0						2.0- 3.0
May 6	14.0-15[1]	18.0-21.0		18.0-22.0		18.0-23.0		18.0-23.0					
	5.0- 6.0	6.0- 8.0		6.0- 9.0		6.0- 9.0		6.0- 9.0					
	9.0	11.0-13.0		12.0-14.0		13.0-15.0		13.0-15.0					
	3.0- 3.5	4.0- 5.0		4.0- 5.0		4.0- 5.0		4.0- 5.0					
May 13	14.0-15.0	18.0-25.0		18.0-23.0		18.0-24.0		10.0-15.0	18.0-24.0				
	5.0- 6.0	6.0-10.0		6.0- 9.0		6.0-10.0		4.0- 6.0	6.0-10.0				
	9.0	11.0-17.0		12.0-15.0		13.0-16.0		4.0- 8.0	13.0-16.0				
	3.0- 3.5	4.0- 6.0		4.0- 5.0		4.0- 6.0		3.0- 4.0	4.0- 6.0				
	from now on fed on algae												
May 20	15.0-22.0	17.0-28.0		18.0-23.0		18.0-26.0		7.0-12.0	10.5-20.0				
	5.0- 8.0	6.0-10.0		6.0- 9.0		6.0-10.0		4.0- 5.5	4.5- 7.0				
	8.0-14.0	13.0-19.0		12.0-15.0		13.0-17.0		3.5- 7.0	6.0-13.0				
	3.0- 5.0	3.5- 6.0		4.0- 5.5		4.0- 6.0		2.5- 4.0	3.0- 4.0				
May 22						18.0-27.0			8.5-19.0	18.0-27.0			
						6.0-10.0			4.5- 7.0	6.0-10.0			
						13.0-17.0			3.5-12.0	13.0-17.0			
						4.0- 6.0			3.0- 4.0	4.0- 6.0			

May 21								7.0 11.5	8.0 17.0			
								4.0- 5.5	4.5- 6.5			
								3.0- 6.0	2.5-12.0			
								2.5- 3.0	3.0- 4.0			
May 27	15.0-24.0	18.0-28.5		18.0-24.5		18.0-28.0		7.0-11.5	7.5-17.0	14.0-26.0		
	5.0- 9.0	6.0-10.0		6.0- 9.0		6.0-10.0		4.0- 5.5	4.5- 6.5	6.0-10.0		
	8.0-15.5	13.0-19.0		12.0-16.0		13.0-18.0		3.0- 6.0	2.5-12.0	9.5-16.0		
	3.0- 5.0	3.5- 6.0		3.0- 5.5		4.0- 6.0		2.5- 3.0	3.0- 4.0	3.5- 6.0		
May 31								7.0-11.5	7.5-16.0	12.0-21.0		
								4.0- 5.0	4.5- 6.0	5.0- 8.0		
								3.0- 6.0	3.5-10.0	6.0-14.5		
								2.5- 3.0	3.0- 4.0	3.0- 4.5		
June 5	15.5-25.0	18.0-29.0		18.0-25.0		18.0-30.0			7.0-15.0	9.0-16.0	18.0-30.0	
	6.0-10.0	6.0-10.0		6.0- 9.0		6.0-10.5			3.5- 5.0	4.5- 6.0	6.0-10.5	
	9.0-15.5	13.0-19.0		12.0-16.0		13.0-20.0			3.5-10.0	3.5-10.0	13.0-20.0	
	3.0- 5.5	3.5- 6.0		3.0- 5.5		4.0- 6.0			2.5- 3.5	3.0- 4.0	4.0- 6.0	
June 12										11.0-15.5	15.0-21.0	
										5.0- 6.0	5.5- 7.5	
										5.0-10.0	10.0-14.0	
										3.0- 4.0	3.5- 4.5	
June 17			18.0-29.0		18.0-26.5					9.0-15.0	9.0-17.0	18. -30.0
			6.0-10.0		6.5-10.0					5.0- 6.0	5.0- 6.5	6.0-10.5
			13.0-19.0		12.0-17.0					5.0- 9.0	4.5-11.5	13.0-20.0
			3.5- 6.0		4.0- 6.0					3.0- 4.0	3.0- 4.0	4.0- 6.0
June 24	23.0-25.0	19.0-29.0	13.5-19.0	18.0-28.5	15.0-19.0	18.0-30.0						16.0-22.0
	9.5-10.0	7.0-10.0	6.0- 7.0	6.5-10.0	5.0- 6.0	6.0-10.5						5.5- 7.5
	13.5-15.5	13.0-19.0	7.0-12.5	12.0-18.0	8.5-13.0	13.0-20.0						10.5-14.5
	5.5- 6.0	4.5- 6.0	4.0- 4.5	4.0- 6.0	3.5- 4.0	4.5- 6.0						3.5- 5.0
July 11										one alive		
	23.0-25.0	21.5-31.0		18.0-30.0		18.0-31.0				15.5		11.0
	9.5-10.0	7.5-11.0		7.0-10.5		6.0-11.5				6.5		5.5
	13.5-15.5	14.0-20.0		12.0-20.0		13.0-20.0				9.0		5.5
	6.0	4.5- 7.0		4.0- 6.5		4.5- 8.0				4.0		4.0

[1] When four measurements are given, the first refers to the entire length, second to length of body, third to length of tail, fourth to breadth of body.

April 21 The thyroid individuals are budding fore legs, the left one first;[1] 16 days old.

April 22 Thyroid ones begin to die.

Only 3 days after the thymus-fed ones were changed to thyroid the influence is noticeable. They have become reduced in size, take on frog shape and bud hind legs.

April 23 Fore legs budding after a treatment of only four days. Thymus-thyroid I do not seem as frail as animals fed on thyroid alone.

April 25 Thyroid-fed animals do not develop their limbs any further, nor do they reduce their tails much further since last observed; progress has ceased.

April 29 The last thyroid and thymus-thyroid I fed animals die.

May 6 A second group of thymus-fed animals is changed to thyroid, thymus-thyroid II.

May 9 Thymus-thyroid II have hind legs appearing and the frog shape is noticeable.

May 10 Thymus-thyroid II have fore legs budding out, the left one first.

May 13 A third group of thymus-fed animals is changed to thyroid, thymus-thyroid III.

May 15 The control animals have not been fed so far. They do not show any sign of differentiation and very little growth. From now they are fed on Elodea canadensis.

May 18 All thymus-thyroid II have both fore legs.

Thymus-thyroid III have hind leg buds.

May 22 All thymus-thyroid III have their hind legs well developed.

Other thymus-fed animals changed to thyroid, thymus-thyroid IV.

May 28 Thymus-thyroid III have fore legs.

Thymus-thyroid IV have hind legs.

June 2 Thymus-thyroid IV have fore legs.

June 5 Thymus-thyroid II begin to die.

June 6 Some muscle-fed tadpoles have hind legs beginning.

June 7 Another group of thymus-fed animals put on a thyroid diet, thymus-thyroid V.

June 12 Thymus-thyroid V have hind legs beginning and the frog shape faintly noticeable.

June 17 Again a group of thymus-fed tadpoles changed to thyroid diet, thymus-thyroid VI.

Part of muscle and spleen fed animals put on a thyroid diet, muscle-thyroid and spleen-thyroid.

June 18 Last thymus-thyroid II dies.

The members of the thymus-thyroid V group have fore leg buds.

[1] Barfurth states that in 80% of the tadpoles the right fore leg appears first. I have always observed the opposite.

June 19 All thymus-thyroid V die, thyroid had been placed in the water every day.
June 20 Thymus-thyroid VI have hind leg buds.
June 21 Muscle-thyroid have hind leg buds.
June 24 Spleen-thyroid have hind leg buds.
June 25 Thymus-thyroid VI and muscle-thyroid groups have fore leg buds.
June 26 Spleen-fed tadpoles have hind legs.
June 27 Spleen-thyroid group have beginning fore legs.
June 8 Thymus group have hind leg buds.
June 29 Control tadpoles have hind leg buds.
July 3 Last animal of the thymus-thyroid III group dies.
July 6 Last individuals of the muscle-thyroid and spleen-thyroid animals are preserved.
July 10 The remaining thymus-thyroid VI are preserved.
July 14 Last one of the thymus-thyroid IV group dies.
July 20 The experiment is discontinued.
The muscle-fed tadpoles are best differentiated.

Since Experiment III was performed along the same lines as Experiment II, it may be well now to give the data of Experiment III and discuss the two groups together.

Experiment III

Rana temporaria, Set III. Figure 3, *a* to *n*. Contained apparently three lots of eggs mixed; they hatched April 7 to 8 and the feedings began April 13, 1912. The original lengths of the tadpoles were from 10 to 11.5 mm.

The experimental data are as follows:

April 13 Food is placed in the dishes for the first time.
April 15 Food is first taken by the animals.
April 18 Thymus-fed specimens become darker in color.
April 20 Some of the muscle- and thymus-fed tadpoles show hind leg buds.
May 6 Part of the thymus-fed animals changed to thyroid diet, thymus-thyroid I.
May 10 All individuals of the thymus-thyroid I group have hind legs and the frog shape is noticeable.
May 13 Thymus-thyroid I group have fore legs budding, the left one first. They lie on their backs and breathe rapidly.
A second group of thymus-fed animals is put on thyroid, thymus-thyroid II.
May 15 The control has not yet been fed. It remains far behind the other groups in size and shows no signs of differentiation. From now on the control group is fed on Elodea canadensis.

TABLE 4

Measurements in millimeters during the experiment

DATE	CONTROL	MUSCLE	MUSCLE-THYROID	THYMUS	THYMUS-THYROID				
					I	II	III	IV	V
April 13..	10.0–11.5								
Apr. 20	12.0–13.0	14.0–16.0	length	15.0–18.0					
		3.5– 4.0	breadth	3.5– 4.0					
May 6	12.0–14.0[1]	15.0–21.0		15.0–21.0	15.0–21.0				
	3.0– 4.0	5.0– 7.0		5.0– 7.0	5.0– 7.0				
	9.0–10.0	12.0–14.0		12.0–15.0	12.0–15.0				
	2.5– 3.0	3.0– 4.0		3.0– 4.0	3.0– 4.0				
May 13	12.0–15.0	15.0–22.0		15.0–22.0	9.0–14.0				
	3.0– 5.0	5.0– 8.0		5.0– 8.0	4.0– 6.0				
	9.0–10.0	12.0–15.0		12.0–15.0	3.0– 9.0				
	3.0– 4.0	3.0– 5.0		3.0– 5.0	3.0– 4.0				
May 15	from now on fed on algae				9.0–12.0	15.0–22.0			
					3.0– 5.0	5.0– 8.0			
					3.0– 7.5	12.0–15.0			
					2.5– 3.5	3.0– 5.0			
May 18					7.0–12.0	13.0–19.5			
					3.0– 5.0	4.5– 7.0			
					3.0– 7.5	8.5–14.0			
					2.5– 3.5	3.0– 4.0			
May 20	12.0–20.0	16.0–23.0		15.0–22.0	7.0–11.0	9.0–18.0			
	3.0– 8.0	5.0– 8.0		5.0– 8.0	3.0– 5.0	4.0– 6.0			
	9.0–13.0	9.5–15.5		9.0–14.0	3.0– 7.0	4.5–12.0			
	3.0– 4.5	3.0– 5.0		3.0– 5.0	2.5– 3.5	3.0– 4.0			
May 22					7.0–11.0	8.0–16.5	15.0–21.0		
					3.0– 5.0	4.0– 6.0	5.0– 7.5		
					3.0– 7.0	3.5–11.0	9.0–14.0		
					2.5– 3.5	3.0– 4.0	3.0– 4.5		

Date									
May 27	14.0–22.0	16.5–25.0		15.0–24.0	7.0–11.0	8.0–16.5	14.0–21.0		
	5.0– 8.5	5.0– 9.0		5.5– 9.5	3.0– 5.0	4.0– 6.0	5.0– 7.5		
	9.0–14.0	11.0–17.0		9.0–16.0	3.0– 6.0	3.5–11.0	7.5–13.5		
	3.0– 4.5	3.0– 5.0		3.0– 5.0	2.5– 3.5	3.0– 4.0	3.0– 4.5		
May 31					6.5–10.0	7.0–14.0	11.0–19.5		
					3.0– 5.0	3.5– 5.5	4.5– 6.5		
					2.0– 5.0	3.5– 9.0	6.0–13.0		
					2.5– 3.0	2.5– 3.5	3.0– 4.0		
June 5	14.0–23.0	16.5–27.0		15.0–25.5	6.5–10.0	8.0–14.0	9.0–18.0	15.0–25.5	
	5.0– 9.0	5.0– 9.5		5.5– 9.5	3.0– 5.0	4.0– 5.5	4.5– 6.0	5.5– 9.5	
	9.0–14.5	11.0–19.0		9.0–17.5	2.0– 5.0	3.5– 9.0	4.0–12.0	9.0–17.5	
	3.0– 4.5	3.0– 5.0		3.0– 5.0	2.5– 3.0	2.5– 4.0	3.0– 4.0	3.0– 5.0	
June 12		Short ones die				11.0–14.0	9.0–15.0	12.5–22.0	
						4.5– 5.5	4.5– 5.5	5.0– 8.0	
						3.5– 8.5	3.5–10.5	7.5–14.5	
						3.0– 3.5	3.0– 4.0	3.0– 4.5	
June 17	17.0–23.0	18.0–27.0	18.0–27.0	16.0–26.0		11.0–14.0	9.0–15.0	8.0–19.0	16.0–26.0
	7.0– 9.0	6.0–10.0	6.0–10.0	6.0–10.0		4.5– 5.5	4.5– 5.5	3.5– 5.5	6.0–10.0
	10.0–14.5	12.5–19.0	12.5–19.0	10.0–17.5		3.5– 8.5	3.5–10.5	5.0–14.5	10.0–17.5
	4.0– 5.0	4.0– 5.5	4.0– 5.5	3.5– 5.5		3.0– 3.5	3.0– 4.0	3.0– 4.0	3.5– 5.5
June 24	21.0–23.0	20.0–28.0	16.0–21.0	17.0–27.0			9.0–15.0		14.0–20.0
	8.5– 9.0	6.5–10.0	5.5– 7.0	6.0–10.0			4.5– 5.5		5.5– 7.0
	12.0–14.5	13.5–19.0	11.5–14.0	11.0–17.5			3.5–10.5		8.5–13.0
	4.5– 5.5	4.0– 5.5	3.5– 5.0	3.5– 6.0			3.0– 4.0		3.5– 4.5
July 11	21.0–24.0	21.0–29.0		19.0–31.5			14.0–15.5		8.0–12.0
	8.5– 9.0	6.5–10.5		7.0–11.0			5.0– 6.5		5.5– 6.5
	12.5–15.0	14.5–19.0		12.0–20.5			9.0– 9.5		2.5– 5.5
	4.5– 6.0	4.0– 5.5		3.5– 6.0			4.0		2.5– 3.5

[1] When four measurements are given, the first refers to the entire length, second to length of body, third to length of tail, fourth to breadth of body.

May 18 The thymus-thyroid I animals are hardly able to swim; when disturbed they move for a few seconds with convulsive jerks, then drop again to the bottom of the dish.

May 19 The thymus-thyroid II group (fed thyroid three times only, May 13, 14 and 17) have hind legs and the frog shape becomes noticeable.

May 22 A third group of thymus-fed animals put on thyroid, thymus-thyroid III.

May 27 Thymus-thyroid II have fore leg buds.

May 28 Thymus-thyroid III (fed four times on thyroid, May 22, 23, 25 and 26) have hind leg buds.

June 2 Thymus-thyroid III have fore leg buds.

June 7 A fourth group of the thymus-fed tadpoles are put on thyroid, thymus-thyroid IV.

June 9 The last specimens of the thymus-thyroid I group are dying.

June 12 The thymus-thyroid IV animals have hind leg buds and the frog-shape is noticeable.

June 17 A fifth group of the thymus fed animals is changed to a thyroid diet, thymus-thyroid V.

Also some of the muscle-fed ones are put on thyroid.

June 18 The thymus-thyroid IV animals have fore legs, but these specimens are dying.

June 20 Last of the thymus-thyroid II are dying.

June 21 Thymus-thyroid V have hind legs.

June 25 Thymus-thyroid IV have fore legs.

June 26 The muscle-thyroid animals have hind legs.

June 30 The muscle-thyroid animals have fore legs.

July 1 A group of thymus-fed specimens put on a liver diet.

July 4 Some of the thymus-thyroid III group that have been kept on vegetable food since June 5 seem to recover from the thyroid influence.

July 7 The last thymus-thyroid V are dying.

July 14 Muscle-fed tadpoles grow hind legs.

July 20 The experiment is discontinued, and only the muscle-fed animals have extremities.

Experiments II and III were performed for the purpose of determining whether tadpoles of different ages would react to the thyroid diet in similar or different ways. For this purpose a great number of tadpoles were kept on a thymus diet and groups of these were changed to a thyroid diet at various times. Table 5 shows the time of reaction to the thyroid stimulus in the different groups. The number of days in the first columns indicates the respective ages of the animals at the start of the thyroid feed-

TABLE 5

SET IV		SET III	
14 days	hind 3 days fore 4 days		
30 days	hind 4 days fore 6 days	28 days	hind 4 days fore 7 days
37 days	hind 6 days fore 15 days	35 days	hind 6 days fore 14 days
46 days	hind 6 days fore 11 days	44 days	hind 6 days fore 10 days
62 days	hind 5 days fore 11 days	60 days	hind 5 days fore 11 days
72 days	hind 3 days fore 8 days	70 days	hind 4 days fore 8 days

ings. The figures in the other columns give the number of days required by each set in developing hind and fore legs.

The similarity of the results in the two series is striking. There is a gradual decrease in the rapidity of action of the thyroid influence up to the age of about 5 weeks followed by a steady increase after this time. Whether or not this is a general rule must be decided by further experiments. It is also difficult to give a satisfactory explanation for this phenomenon, yet the following suggestion may be advanced. The young tadpoles were very quickly affected by the thyroid feedings because the previous thymus diet had not acted long enough to delay or counterbalance the thyroid stimulus. Older animals had enough thymus *agens* accumulated to retard the thyroid action. Still older tadpoles were more nearly approaching the normal time of metamorphosis and may thus have been ripe to respond to an accelerating stimulus. The last argument, however, seems especially weak, since it introduces a new factor in the action of thymus diet; namely, that if it is prolonged, it loses the retarding influence and thus is less able to counteract the thyroid. This factor has not yet been demonstrated; thus no conclusions as to the rapidity of the thyroid influence on different age tadpoles can be based upon it.

It has been shown, however, in the experiments of 1911 that thymus will affect older tadpoles less than younger ones. Another point may also be mentioned without attempting to explain the situation. Table 6 shows that old thymus-fed tadpoles, when put on a thyroid diet, respond more quickly to the thyroid stimulus than do tadpoles of the same age which have been fed on other substances. One should have expected just the contrary, judging especially from Experiment VI in 1911, in which liver-thyroid-fed tadpoles developed their fore legs 3 days earlier than thymus-thyroid-fed ones.

TABLE 6

	SET IV: 72 DAYS OLD			SET III: 70 DAYS OLD	
	Thymus-thyroid	Muscle-thyroid	Spleen-thyroid	Thymus-thyroid	Muscle-thyroid
Hind legs in	3 days	4 days	7 days	4 days	9 days
Fore legs in	8 days	8 days	10 days	8 days	13 days

In Experiments II and III those tadpoles (fig. 3 *c* to *h*, 3 *k*, 3 *m*, 3 *n*; fig. 4 *e* to *i*, 4 *l*, 4 *n* to *p*) that were transferred from thymus to thyroid diets were fed on the latter gland only three or four times. When they had developed their fore legs water-plants were placed in the dishes. It had been noticed that thyroid-fed tadpoles would die very soon after putting out fore legs. In these experiments those that were placed in dishes containing plants, although they were never seen to feed on the plants, could be kept alive for some time, 20 to 53 days, while those specimens remaining in water in which thyroid was placed longer than was absolutely necessary for developing the extremities died within 10 to 12 days after the beginning of the thyroid diet. Those living longer did not develop any further than those dying early, but remained stationary except for a further reduction in size, especially of the tail.

Table 7 gives the length of time that the different thymus-thyroid-fed sets were kept alive in the two experiments. Tadpoles in Experiment III were about 2 days younger than those in Experiment IV, when the feeding began.

TABLE 7

SET IV		SET III	
Age at the time thyroid was given	Days living after thyroid treatment	Age at the time thyroid was given	Days living after thyroid treatment
14 days	10 days (no plants)		
30 days	43 days	28 days	33 days
37 days	51 days	35 days	38 days
46 days	53 days	44 days	50 days
62 days	12 days (no plants)	60 days	11 days (no plants)
72 days	23 days	70 days	20 days

A glance at table 5 will show that the tadpoles of 37 and 46 (or 35 and 44) days showed the slowest reaction to the thyroid treatment. The possible reasons for this behavior were discussed above.

Experiment IV

Rana temporaria, Set II. Figure 2, *a* to *n*. 960 tadpoles hatched on April 6 to 7, 1912, and fed on muscle until June 3. The experiment started on June 5.

This experiment confirmed the results of the two previous ones. Tadpoles (fig. 2, *a*) which had lived for 50 days on an ordinary meat diet were later affected by the thyroid stimulus as greatly as animals fed only on a thyroid diet. The main object of this experiment, however, was to ascertain how long the thyroid *agens* had to be applied before results were noticeable, and furthermore, whether or not it would be possible to counteract the thyroid influence after one or more days feeding. A great number of tadpoles were reared on muscle and at the age of 50 days were divided into groups of 80 individuals each. The different groups were then fed on thyroid from 1 to 5 days and after this treatment five of the groups were put on a vegetable diet (Elodea canadensis) (fig. 2, *f* to *i*) and 5 others on muscle diet (fig. 2, *c* to *e*).

One group (fig. 2, *b*) was allowed to starve to test whether hunger following a long period of feeding (over 6 weeks) would bring about a quicker differentiation.

Still another group (fig. 2, *l*) was kept in water in which small pieces of thyroid gland were placed, but so arranged that the

TABLE 8

Measurements in millimeters and diary notes

DATE	CONTROL UNFED	PLANTS AFTER A THYROID DIET OF					MUSCLE AFTER A THYROID DIET OF					THYROID EMULSION
		1 day	2 days	3 days	4 days	5 days	1 day	2 days	3 days	4 days	5 days	
June 5.					14.0–22.0 mm[1]							
					4.5– 9.0 mm							
					9.0–15.0 mm							
					3.5– 5.5 mm							
					After a few days' feeding the shorter individuals die							
June 10..					Hind legs							
June 11..			Hind legs									
June 12	14.5–22.0	16.0–22.0	9.5–19.0	11.0–19.0	15.0–20.0	14.0–17.0	17.0–22.0	15.0	17.0–19.0	11.0–18.0	15.5–19.0	14.0–22.0
	4.5– 9.0	5.0– 8.5	4.5– 6.5	4.5– 6.5	5.0– 7.0	5.0– 6.5	6.0– 8.5	6.5	6.0– 7.0	5.5– 7.0	5.5– 7.0	4.5– 9.0
	9.0–15.0	10.5–14.0	4.0–12.5	6.0–13.5	8.0–13.5	7.5–11.5	6.5–15.0	8.5	11.0–12.5	4.5–11.5	9.0–13.0	9.0–14.0
	3.0– 5.5	3.0– 5.0	3.0– 4.0	3.0– 4.0	3.0– 4.5	3.0– 4.5	4.0– 5.0	4.0	4.0– 4.5	4.0– 4.5	3.0– 4.0	3.0– 5.0
		Frog-shape faintly noticeable						Only 1 living	About 80% have died			
					Frog-shape noticeable							
June 13										Fore legs		Hind legs frog shape
June 14			Fore legs					Last one dead				
June 15..					Fore legs							

June 18	16.0–24.0	16.0–22.0	8.0–16.0	10.5–18.0	9.5–19.0	10.0–17.0	17.5–28.0		17.0–18.0	9.5–12.5	9.0–18.0	12.0–17.0
	5.5– 9.5	5.0– 8.5	4.5– 6.5	4.5– 6.5	4.0– 7.0	4.5– 6.5	7.0– 9.5		6.0– 6.5	5.5– 6.0	5.0– 6.0	4.5– 6.0
	11.0–16.0	10.5–14.0	1.5–10.0	6.0–12.0	5.0–13.0	5.0–10.5	10.5–18.5		11.0–11.5	3.5– 7.0	2.5–12.0	7.5–11.0
	3.0– 5.5	3.0– 5.0	3.0– 4.0	2.5– 4.0	3.0– 4.5	3.0– 4.0	4.0– 6.0		4.0	3.0– 3.5	3.0– 3.5	3.0– 4.0
June 19		Hind legs	A great number die				About 10% living		About 10% living	4% living		11.0–13.0
												4.5– 5.5
												6.5– 8.0
												3.0– 3.5
												All dead
June 25	18.0–27.0	17.5–25.0	15.0–18.0	9.0–16.0	11.0–19.0	10.0–14.0	18.0–31.0		18.0–19.5	9.0–11.5	10.0–17.0	
	6.5–10.0	6.0– 9.5	5.0– 6.0	3.5–5.5	4.5– 7.0	4.0– 6.0	7.0–11.0		6.0– 7.5	4.0– 6.0	4.5– 5.5	
	11.5–17.0	11.0–16.0	10.0–12.0	5.5–10.5	6.5–12.0	5.0– 9.0	11.0–20.0		12.0–12.5	3.0– 7.0	5.5–11.5	
	3.5– 6.0	3.5– 6.0	3.0– 4.0	2.5– 3.5	3.0– 4.5	3.0– 3.5	4.0– 6.5		4.5	3.0– 3.5	3.0– 3.5	
July 2.						Last one dead						
July 4...									1 living	Last ones dead		
July 5...	Hind legs											
July 8...			Last ones dead									
July 11	18.0–30.0	19.0–27.0			16.0–21.0		19.0–33.0		24.0			
	6.5–11.5	7.0–10.0			6.5– 7.5		8.0–10.5		8.5			
	11.5–18.5	12.0–17.0			9.5–14.0		11.0–22.5		15.5			
	3.5– 6.5	4.0– 6.0			3.0– 4.5		5.0– 6.5		5.0			
July 15		5 living			1 living		4 living; fore legs		1 living			

[1] When four measurements are given, the first refers to the entire length, second to length of body, third to length of tail, fourth to breadth of body.

animals could not feed on it. The gland was placed in a small glass vial closed with gauze and allowed to stand upright, the open end being beneath the surface of the water. Thus no thyroid particles could get out into the water, merely an extract or emulsion of thyroid constituents which diffused from the vial into the dish. Animals kept in these dishes developed their hind legs 8 days after the beginning of the experiment and the frog shape became noticeable about the same time. Six days later they all died without having grown fore legs, but their size on that day, June 19, shows that they were completely under the thyroid influence. Therefore the product of the thyroid which caused the rapid differentiation in all the previous experiments must be soluble in water.

The control animals which were kept starving after the 50 days feeding did not grow hind legs until July 5, considerably later than the thyroid treated ones. Thus the results after thyroid application are different from those Barfurth reports in his "Der Hunger als förderndes Prinzip in der Natur," and cannot be attributed to starvation of the tadpoles. Among the ten main groups of the experiment the thyroid influence manifested itself in different ways. As a general result it may be stated that a thyroid application of only 24 hours sufficed to show decided effects (fig. 2 *c*, 2 *f*), and that a treatment of more than 24 hours (2 to 5 days) gave the typical results described in all previous experiments.

In the five groups that received vegetable food following the thyroid dose and in the five groups that were fed again on muscle, a great number of individuals, 50 to 60 per cent, mainly the shorter ones, died within 2 or 3 days after the first feeding. This accords with observations in other cases where thyroid was given after previous feeding, but is in striking contrast with the happenings in experiments where thyroid food was applied from the start. In the latter experiments very few animals were lost during the first days. It remains to be answered why the application of thyroid should affect tadpoles that had not been previously fed on other diets less harmfully than those previously fed on various tissues.

The tadpoles which again received muscle after the thyroid treatment reacted more strongly to the stimulus than those put on a vegetable diet. They all grew hind legs within from 1 to 5 days, the frog shape became noticeable very soon, and they died in great numbers, so that 7 days after the beginning of the experiment only 20 per cent were alive. The animals treated from 3 to 5 days grew fore legs on the 8th day, those treated 2 days were all dead on the 9th day; this group was affected most strongly, which may be a mere coincidence. The groups fed only one day grew fore legs on the 42d day, at this time only four of this group were alive, only one of the 3 day group, and none of the 4 and 5 day groups, the last ones having died on the 29th day of the experiment. That is, out of 400 individuals only 4 in the 1 day group and 1 in the 3 day group were able to survive the thyroid shock. The table of growth on page 450 shows that these surviving individuals actually begin to grow again. These 4 animals of the 1 day group seem to have absorbed a very small quantity of the thyroid *agens*. They stop their precocious differentiation very soon and do not grow fore legs until July 17, which is 34 days later than the 3 and 5 day groups.

The second set of tadpoles, those put on a vegetable diet after a thyroid treatment, reacted in a similar fashion to that just described, except perhaps as stated above, they were somewhat less affected. This might suggest that a meat free diet may help to counteract thyroid influences. The 3 and 5 day groups grow hind legs on the 5th day of the experiment, the 2 day group on the 6th day, and the 1 day group not until the 14th day. Fore legs appear in the 3, 4 and 5 day groups on the 10th day, in the 2 day group on the 9th day, in the 1 day group none had appeared on the 46th day, when the experiment was discontinued. While many animals died during the first few days, some were able to keep alive. On the 27th day the last ones of the 5 day group died, and on the 33d day all of the 2 and 3 day groups were dead. There were five of the 1 day group and one of the 4 day group alive on the 42d day. These, as the table of growth of page 450 shows, start again to grow.

TABLE 9

Measurements in millimeters and diary notes

DATE	CONTROL UNFED	AQUARIUM	BLOOD	PANCREAS	PANCREAS EMULSION	SPLEEN	SPLEEN EMULSION	LIVER	LIVER EMULSION	TESTICLE	TESTICLE EMULSION	OVARY	OVARY EMULSION	ADRENAL	ADRENAL EMULSION	THYMUS	THYMUS EMULSION	THYROID	THYROID EMULSION
May 8									13–	18 mm.[1]									
									4–	6 mm.									
									8	12 mm.									
									3–	4 mm.									
May 12	Many animals die in the emulsions																	Last thyroid feeding	
May 13																		Hind legs noticeable	
May 15	13.0–17.0	13.0–21.0	13.0–18.0	13–17		13–19		13.0–19.0	13.0–15.0	13.0–18.0		13.0–18.0		13.0–18.5	13.0–15.0	13.0–19.0	13 0–18.0	10.0–15.0	12.0–16.0
	4 0– 6.0	5.0– 8.0	4.0– 6.0	4– 6		4– 7		4.0– 7.0	4.0– 6.0	3.0– 4.5		4.0– 6.0		4.0– 6.5	4.0– 6.0	4.0– 7.0	4.0– 6.0	4.0– 5.0	4.0– 6.0
	8.0–12.0	8.0–14.0	8.0–12.0	8–12		8–12		8.0–12.0	8.0–10.0	8.0–12.0		8.0–12.0		8.0–12.0	8.0–11.0	8.0–12.0	8.0–12.0	5.0–10.0	6.0–10 0
	3.0– 4.0	3.0– 5.0	3.0– 4.0	3–4		3–4		3.0– 4.0	3.0– 4.0	3.0– 4.0		3.0– 4.0		3.0– 4.5	3.0– 4.0	3.0– 4.0	3.0– 4.0	3.0– 4.0	3.0– 4 0
				Do not appear to take food, many dying															
May 18																		7.0–14.0	10.5–15.0
																		3.5– 4.5	3.5– 5.5
																		4.5–10.0	7.0–10.0
																		2.5– 3.0	2.5–3.5
																		Left fore leg	
May 20																		7.0–14.0	7.5–14.0
																		3.5– 4.5	3.5– 5.5
																		4.5–10.0	4.0– 9.0
																		2.5–3.0	2.5– 3.5
																		Transferred into aerated water	
May 22	13.0–17.0	17.0–27.0	13.0–21.0	13.0–21.0		14.0–21.0	14.5–24.0	13.0–23.0	14.0–20.0	13.0–22.0	14.0–23.0	13.0–22.0	14.0–22.0	13.0–22.0	13.5–21.5	13.0–23.0	13.0–21.0	7.0–12.0	7.5–14.0
	4.0– 6.0	6.5–11.0	4.5– 7.0	4.5– 8.0		5.0– 8.0	5.0– 8.5	4.5– 9.0	4.5– 7.0	4.0– 8.0	5.0– 9.0	5.0– 9.0	5.0– 8.5	4.5– 9.0	4.5– 8.0	5.0– 9.0	4.0– 8.0	3.5– 4.5	3.5– 5.5
	8.0–12.0	11.0–17.0	8.0–14.0	10.0–14.5		9.0–13.5	8.5–16.0	9.0–14.0	9.5–14.0	8.0–14.0	8.5–14.0	9.0–14.0	9.0–14.0	8.5–14.0	8.5–13.5	8.0–14.0	8.0–14.0	3.5– 9.0	4.0– 9.0
	3.0– 4.0	4.0– 6.0	3.0–4.0	3.0– 5.0		3.0– 5.0	3.0– 5.0	3.0– 5.5	3.0– 4.0	3.0– 5.0	3.0– 5.0	3.0– 5.0	3.0– 5.0	3.0– 5.0	3.0– 5.0	3.0– 5.5	3.0– 5.0	2.5– 3.0	2.5– 3 5
				Only 3 living															
May 24	Many of the short individuals begin to die																		
May 26																		Fore legs	
May 31	14.0–18.0	17.0–29.0	13.0–24.0	16.0–21.0		14.0–23.0	15.0–25.0	13.0–25.0	19.0–21.0	13.0–23.0	14.0–24.0	16.0–23.0	14.0–22.0	13.0–23.0	15.0–25.0	13.0–24.5	13.0–22.0	6.0–12.0	6.0–13.0
	5.0– 6.0	6.5–11.0	5.0– 8.0	6.0– 8.0		5.0– 9.0	5.0– 9.0	5.0–10.0	7.0– 8.0	4.0– 9.0	5.0–10.0	5.0– 9.0	5.0– 8.5	4.5–10.0	6.0– 9.0	5.0– 9.0	4.0– 8.0	2.5– 5.0	3.0– 5.0
	8.0–13.0	11.0–18.0	8.5–16.0	10.0–14.5		9.0–14.5	9.0–16.0	9.0–15.0	12.5–14.0	8.0–15.0	8.5–16.0	10.0–15.0	9.0–14.0	8.5–14.0	9.5–16.5	8.0–15.5	8.0–15.0	2.0– 8.0	2.5– 9.0
	3.0– 4.0	4.0– 6.0	3.0– 5.0	4.0– 5.0		3.0– 5.0	3.0– 5.5	3.0– 6.0	4.0– 4.5	3.0– 5.5	3.0– 5.0	3.0– 6.0	3.0– 5.5	3.0– 6.0	3.0– 5.0	3.0– 5.5	3.0– 5.0	2.0– 3.0	2.5– 3.0

June 1....																		Last ones dead
June 12	Died	15.5–29.0	14.5–28.0	21.0		14.0–26.0	15.5–27.5	16.0–27.0	22.0–26.5	14.0–26.5	15.0–26.5	17.0–26.5	14.5–27.5	14.0–24.0	15.5–29.0	14.0–25.5	14.0–26.0	
	June 7th.	5.5–10.5	5.0–10.0	7.0		5.0– 9.5	5.5– 9.5	5.0–10.5	8.0– 9.5	5.0–10.0	5.5–10.0	5.5–10.0	5.5– 9.5	5.0– 9.0	5.5 –10.0	5.5– 9.5	5.0– 9.5	
		10.0–19.0	9.0–18.0	14.0		9.0–17.0	10.0–18.0	11.0–17.0	14.0–17.0	8.0–16.5	9.5–16.5	10.0–16.5	9.0–17.5	9.0–15.5	10.0–19.0	9.0–16.5	9.0–16.5	
		4.0– 6.0	3.0– 5.0	4.5		3.0– 5.5	3.0– 5.5	3.5– 6.5	4.5– 5.5	3.0– 5.5	3.5– 6.0	3.0– 6.0	3.5– 5.5	3.5– 6.5	3.5– 6.5	3.5– 5.5	3.5– 6.5	
June 19...		Hind legs																
June 20...			Hind legs					Hind legs				Hind legs						
June 24		15.5–30.0	17.0–31.5	21.5		16.0–27.5	17.5–30.0	17.0–30.0	25.0–27.5	16.5–32.0	15.5–28.0	17.0–31.0	16.5–30.5	18.0–28.0	19.0–32.0	15.0–27.0	18.0–30.0	
		5.5–11.0	6.0–11.0	7.0		6.0–10.0	6.0–10.0	6.0–11.0	8.0– 9.5	6.0–11.0	5.5–10.5	6.0–10.5	5.5–10.0	7.0–12.0	7.0–10.5	5.5– 9.5	7.0–10.0	
		10.0–19.0	11.0–20.5	14.5		10.0–18.0	11.5–20.0	11.0–20.0	17.0–18.0	10.5–21.0	10.0–17.5	11.0–20.5	11.5–20.5	11.0–16.0	12.0–21.5	9.5–17.5	11.0–20.0	
		4.0– 6.0	4.0– 6.5	4.5		3.5– 6.0	4.5– 6.0	4.5– 6.5	5.0– 5.5	4.0– 6.5	3.5– 6.0	4.0– 7.0	3.5– 6.5	5.0– 8.0	4.0– 6.5	3.5– 5.5	4.0– 6.5	
June 26...										Hind legs				Hind legs				
June 28...																Hind leg buds		
July 5....			Fore legs					Fore legs										
July 8..			First metamorphosis	Dead														
July 11.		18.0–30.0	17.0–31.5			16.0–31.5		17.0–30.0		16.5–33.5		20.0–33.5		18.0–34.0		19.0–31.0		
		7.0–11.0	6.0–11.0			6.0–11.0		6.0–11.0		6.0–11.5		7.0–11.0		7.0–12.0		7.0–11.0		
		11.0–19.0	11.0–20.5			10.0–20.5		11.0–20.0		10.5–22.0		13.0–22.5		11.0–22.0		12.0–20.0		
		4.5– 6.0	4.0– 6.5			3.5– 6.5		4.5– 6.5		4.0– 7.5		4.0– 7.0		5.0– 8.0		4.0– 6.5		
July 12.								First metamorphosis										
July 14...												Fore legs						
July 18.						Hind leg buds												
July 19...										Fore legs								
July 21												First metamorphosis						
July 26.										First metamorphosis								

[1] When four measurements are given, the first refers to the entire length, second to length of body, third to length of tail, fourth to breadth of body.

Experiment V

Rana temporaria, Set VIII to XI, voluntarily mixed. These hatched April 10 to 13 and the experiment started May 8, 1912.

In addition to the organs used in the previous experiments, pancreas and testicle were given as food in this experiment. Each organ was given in two forms; with one group of tadpoles a small piece of the tissue was placed in the water; to a second group the food was given in a crushed form, a piece of the tissue being squeezed between the fingers, until it was broken into minute particles. This was done for two reasons. First to allow juices that might form an active part of the glands to ooze out into the water as quickly as possible, second to give the weaker individuals a chance to find food particles. In almost every experiment it was seen that a number of the smallest individuals did not grow and after a time began to die. It seems, however, that this is not due to an inability to obtain food, but because they were less fit to live and were simply weeded out in the early stages of development.

The tadpoles fed on the crushed glands developed in essentially the same manner as the others, thus after several weeks both sets were placed in one dish and fed alike.

One set of animals was fed on clotted blood. They did best of all. A control set was fed on Elodea canadensis and another was starved.

All of the tadpoles were about 4 weeks old at the beginning of the experiment and had not been fed. When starved the animals eat the dead ones, if these are not removed, but their rate of growth can by no means compare with that of tadpoles receiving plenty of food.

The thyroid-fed tadpoles showed the same rapid development as observed in previous experiments. They could not be kept alive longer than 23 days, although after 10 days they were put in water through which a constant current of air was passed. The groups fed on other tissues also showed the usual rate of growth. Those individuals fed on spleen, liver and thymus ran rather parallel courses and progressed somewhat faster during the

early days of the experiment than the tadpoles fed on testicle, ovary and adrenal. The latter three groups also ran similar courses. The set fed on plants showed a rather rapid growth, about equal to that of the spleen, liver and thymus groups. The tadpoles in these groups reached on the average a length of 30 to 31 mm at the time of metamorphosis, while the specimens in the slower growing groups reached a length of 33 to 34 mm before they metamorphosed. This fact might lead one to conclude that the materials fed to the faster groups, vegetable, blood, spleen, liver and thymus, contain an *agens* which causes a more rapid differentiation than the foods given to the second groups, testicle, ovary, adrenal. This, however, is not the case, since in the first group the spleen- and thymus-fed tadpoles exhibited no signs of an approaching metamorphosis at a time when the tadpoles of the second group had begun to leave the water.

Attention should be called to the fact that in this experiment as in the previous ones of 1912 the spleen- and thymus-fed groups (spleen had not been fed in 1911) showed almost parallel courses of development, neither reaching the stage of metamorphosis when the other groups had. Whether or not this indicates an influence of the spleen, or perhaps of all lymphatic organs, similar to that of the thymus in retarding differentiation I am at present unable to decide.

The tadpoles intended for a pancreas diet did not feed freely and most of them died early.

The starved animals did not differentiate beyond their original stage and began to die about 4 weeks after the beginning of the experiment.

The pigmentation exhibited by the animals was very much the same as in previous experiments. The thymus-fed individuals, however, did not become as dark as usual. Those fed on blood and plants became very dark.

Experiment VI

Rana temporaria, Set V to VII. Figure 5, *a* to *e*. Probably three sets mixed. They hatched April 6 to 8 and were not fed until May 24, when the experiment began.

During the early part of the season hypophysis was not available, so that no experiments with it could be carried out simultaneously with the other. Therefore this late experiment of hypophysis feeding is not of great significance. It is reported here to show the rates of growth of tadpoles fed on glandular and others on the nervous parts of the hypophysis.

TABLE 10

Measurements in millimeters, and diary

DATE	CONTROL VEGETABLE	HYPOPHYSIS GLANDULAR	HYPOPHYSIS NERVOUS
May 24	19.0–21.0[1]	17.0–26.0	17.0–26.0
	7.0– 8.0	6.0– 9.0	6.0– 9.0
	11.0–13.0	10.5–16.0	10.5–17.0
	3.5– 4.5	4.0– 6.0	4.0– 6.0
May 31	19.0–22.0	17.0–26.5	17.0–28.0
	7.0– 8.5	6.5–10.0	6.0–10.0
	11.0–13.5	10.5–18.0	11.0–18.5
	4.0– 5.0	4.0– 6.0	4.0– 6.0
June 7	19.0–22.5	17.0–27.5	17.0–29.0
	7.0– 8.5	6.5–10.0	6.0–10.0
	11.0–14.0	11.0–18.5	11.0–19.0
	4.0– 5.0	4.0– 6.0	4.0– 6.0
June 24	23.0–25.0	17.5–30.5	17.0–30.5
	8.0– 9.5	6.5–11.5	6.0–11.0
	15.0–15.5	11.0–19.0	11.0–19.5
	5.0– 6.0	4.5– 7.0	4.0– 7.0
June 26		Hind legs	
		Extremely voluminous bodies.	
June 30			One individual grows faster than the others and develops hind legs
			32.0
			11.0
			21.0
			7.0
July 11	25.0–27.0	19.5–30.5	20.0–32.0, 34
	9.0–10.0	7.0–11.5	7.5–11.0, 11
	16.0–17.0	12.5–19.0	12.5–21.5, 23
	5.0– 6.0	4.5– 7.0	4.5– 6.5, 7
		Short, but very strong extremities. Begin to die	Only one, the biggest, has hind legs, but still drawn close to the body

[1] When four measurements are given, the first refers to the entire length, second to length of body, third to length of tail, fourth to breadth of body.

The difference between the rates of growth of the two sets is not marked, there may be a little faster growth in the set fed on neural hypophysis. The tadpoles fed on glandular hypophysis (fig. 5 *b*, 5 *d*) show decidedly better differentiation and gradually become less pigmented than those fed on neural hypophysis (fig. 5 *e*).

Experiment VII

Bufo vulgaris, Set I. Figure 8, *a* to *d*. Hatched April 10 to 20, 1912, and the feeding began May 6.

The experimental data are as follows:

May 5 The feeding began.
May 11 All the thyroid-fed specimens have hind legs and the frog shape is noticeable. They were fed only four times.
May 18 Thyroid-fed animals have developed fore legs.
May 22 Algae were placed in with the thyroid-fed tadpoles.
June 18 Last thyroid-fed ones die.
June 20 Thymus-fed ones have hind leg buds.
June 24 Control animals have hind leg buds.
July 11 The muscle-fed tadpoles have the best differentiated hind legs.
July 14 Muscle-fed animals grow fore legs.
July 20 The first metamorphosis in muscle-fed group. The control still have very small hind leg buds. Thymus-fed animals have their hind legs still drawn close to the body.

This experiment introduced a new species into the study, Bufo vulgaris, yet the results, as one might have expected, are essentially the same. The thyroid-fed group (fig. 8, *d*) showed their usual precocious differentiation, their decrease in size is not as marked as in the Rana species. The thymus-fed group (fig. 8, *c*) did not grow much faster than the muscle-fed ones (fig. 8, *b*) and towards the end of the experiment they actually fell behind in size, yet they showed little, if any, differentiation at the time of the first metamorphosis of the muscle-fed tadpoles. A considerable number of the thymus group died when they reached a length of about 21 mm (status thymicus, thymus death).

TABLE 11

Measurements in millimeters

DATE	CONTROL VEGETABLE	MUSCLE	THYMUS	THYROID
May 6	13–16 mm.[1] 5– 7 mm. 7– 9 mm. 3– 5 mm.			
May 13	14.0–16.0 5.0– 7.0 7.0– 9.0 3.0– 5.0	15.0–17.0 6.0– 8.0 8.0–10.0 4.0– 5.0	14.0–17.0 6.0– 8.0 8.0–10.0 4.0– 5.0	13.0–15.5 5.0– 7.0 7.0– 9.0 3.0– 4.0 Legs 0.8
May 15				11.5–15.5 5.0– 6.5 5.0– 9.0 3.0– 4.0
May 18				6.5–15.5 4.0– 6.5 2.5– 9.0 3.0– 4.0
May 20				11.0–15.5 4.5– 6.5 6.5– 9.0 3.0– 4.0
May 22	14.0–19.0 5.5– 8.0 7.5–11.0 3.5– 5.0	16.0–19.0 7.0– 9.0 9.0–11.0 4.0– 5.0	14.0–20.0 6.0– 9.5 8.0–11.5 4.0– 6.0	11.0–15.5 4.5– 6.5 5.0– 9.0 3.0– 4.0
May 30	14.0–20.0 5.5– 8.0 7.5–12.0 4.0– 5.0	16.0–20.0 7.0– 9.0 9.0–11.5 4.0– 5.0	14.0–20.0 6.5– 9.5 8.5–11.5 4.0– 6.0	10.0–15.0 4.5– 6.5 5.0– 9.0 3.0– 4.0
June 6	14.0–20.0 5.5– 8.0 7.5–12.0 4.0– 5.0	16.0–20.0 7.0– 9.0 9.0–11.5 4.0– 5.0	14.0–21.0 7.0– 9.5 8.5–11.5 4.0– 6.0	10.0–14.5 4.5– 6.0 5.0– 8.5 3.0– 4.0
June 18	16.0–20.0 6.5– 8.5 8.0–12.0 4.0– 5.5	16.0–20.0 7.0– 9.0 9.0–11.5 4.0– 5.0	14.0–21.5 7.0– 9.0 8.5–12.0 4.0– 6.0	10.0–13.0 4.5– 6.0 4.0– 7.0 3.0– 4.0
June 25	17.0–20.5 6.5– 8.5 9.0–12.0 4.0– 5.5	16.0–21.0 7.0– 9.0 10.0–12.0 4.0– 5.5	17.5–22.0 7.0– 9.5 10.5–12.5 4.0– 6.5	
July 11	18.0–21.0 6.5– 9.0 10.0–12.0 4.5– 6.0	18.0–24.5 7.5–10.5 10.5–13.0 4.0– 6.0	19.0–22.5 8.0– 9.5 11.0–13.0 4.5– 6.5	

[1] When four measurements are given the first refers to the entire length, second to length of body, third to length of tail, fourth to breadth of body.

The next three experiments to be described were performed for the purpose of studying the influence of a mixed diet on growth and development. One kind of food was given one day and another the next. Previously the animals had been fed for a considerable length of time on one kind of tissue and were then transferred to another.

Experiments VIII and IX consider a thyroid-thymus diet only. The tadpoles of Experiment VIII had been fed on muscle before the thyroid-thymus treatment began, while those of Experiment IX had starved. It may be well at present to record the notes

TABLE 12

Measurements in millimeters and diary notes

Date	Measurements and notes
June 5	21.0–28.0 8.5–10.5 13.0–19.0 4.5– 6.0
June 10	Hind legs appear
June 12	20.0–24.0 7.0– 8.0 12.0–16.0 4.5– 5.5 The frog shape is noticeable
June 18	14.0–24.0 5.5– 8.0 9.0–16.0 3.5– 5.0 Very active; legs are very thin (fig. 2 *m*), but much better developed than in thyroid (Experiment IV, fig. 4 *p*). Length of the hind legs 2.5 to 3.5 mm; the abdomen is bloated.
June 19	About 50% die
June 25	14.0–22.0 5.5– 7.5 8.5–14.5 3.5– 5.0 Fore legs appear 10 days later than in the all-thyroid group (Experiment IV).
July 7	14.0–20.0 5.5– 7.5 8.5–12.5 3.5– 4.5 Last ones preserved

from these experiments and later discuss them in connection with Experiment X, in which several kinds of foods in various combinations were applied.

TABLE 13

Measurements in millimeters and diary notes

	CONTROL (VEGETABLE)	THYMUS-THYROID
June 5	14.0–22.0 5.0– 8.0 9.0–14.0 3.0– 4.0	
June 12	15.0–25.0 5.5– 8.5 10.0–17.0 3.5– 4.5	14.0–21.0 5.0– 7.0 9.0–14.0 3.0– 4.0
June 14		Hind legs appear Many die
June 18	15.5–26.0 5.5– 9.0 10.5–17.0 3.5– 5.0	14.0–20.0 5.0– 7.0 9.0–13.0 3.0– 4.0 Fore legs appear Very active The abdomen is bloated
June 24	16.0–26.0 5.5– 9.0 10.5–17.5 3.5– 5.5	14.0–18.5 5.0– 6.0 9.0–11.5 3.0– 4.0
July 8	Hind leg buds	
July 13		Last one dies

Experiment VIII

Rana temporaria, Set II. Figure 2, *k*, 2, *m*. Hatched April 6 to 7, 1912 (Experiment IV), and fed on muscle until June 3. The largest individuals of Set II were selected for this experiment. The feeding of thyroid-thymus began on June 5.

Experiment IX

Rana temporaria, Sets V to VII. Figure 6, *a* to *d*. Probably three sets mixed. Hatched April 6 to 8, 1912, and not fed until June 5, when the experiment was started.

Experiment X

Bufo vulgaris, Set III. Figure 9, *a* to *r*. Brought into the laboratory May 21, 1912, size 18 to 23 mm, age unknown. Experiment started June 5. Thirteen groups of tadpoles, 150 individuals in each, were given food as indicated below; one group was starved.

A part of this experiment corresponds to Experiment IV in which several groups of tadpoles of Rana temporaria were fed on thyroid for from 1 to 5 days respectively and afterwards put on a vegetable diet. The Bufo tadpoles reacted very quickly to the thyroid stimulus, but when the thyroid feeding was stopped, they seemed to overcome the thyroid influence more readily than any other tadpoles. The groups fed on thyroid from 3 to 5 days (fig. 9, *b* to *d*) developed hind legs 5 days after the first feeding, showing as in other experiments, that a 3 day feeding of thyroid gland suffices to give the typical results. The 2 day group budded the hind extremities on the 7th day and the 1 day group on the 11th day. These intervals approach close to those observed in Experiment IV, which were 5, 6 and 14 days. The anterior extremities appear in the 5 day group 14 days after the first feeding (in Experiment IV on the 10th day) and this group begins to undergo metamorphosis on the 18th day. In the other, 1 to 4 day groups, the after-treatment with vegetable food seems to check the hastened differentiation following the intake of thyroid tissue. They finally do not go much faster than tadpoles which had not received thyroid. It might thus appear as if the Bufo tadpoles had a stronger resistancy against the thyroid stimulus than the Rana larvae. This point is not entirely clear, however, since the former had not received any food before the thyroid feedings began, while the latter had previously lived for 50 days on muscle. As has been stated above, any meat diet before or after the thyroid treatment is apt to render the animals more susceptible to the thyroid stimulus than does starvation.

The thyroid treated Bufo tadpoles do not reduce their size so much as the Rana tadpoles, as before mentioned under Experiment VII.

TABLE 14

Measurements in millimeters and diary

DATE	CONTROL UNFED	TESTICLE	PLANTS AFTER THYROID DIET OF					THYROID				THYMUS		HYPOPHYSIS VEGETABLE
			1 day	2 days	3 days	4 days	5 days	Thymus	Muscle	Hypophysis	Vegetable	Hypophysis	Vegetable	
June 5							18–23 mm.[1] 7–9 mm. 10–14 mm. 4–6 mm.							
June 10							Hind legs							
June 12	18.0–23.0 7.0–9.0 10.0–14.0 4.0–6.0	18.0–23.0 7.0–9.0 10.0–14.0 4.0–6.0	18.0–21.0 7.0–9.0 10.0–12.0 4.0–5.5	18.0–22.0 7.0–9.0 10.0–13.0 4.0–5.5 Hind legs	18.0–22.5 7.0–9.0 10.0–14.0 4.0–5.5	18.0–22.5 7.0–9.0 10.0–14.0 4.0–5.5 Toad-shape', faintly visible	18.0–21.0 7.0–9.0 10.0–13.0 4.0–5.5 distinct	18.0–22.0 7.0–9.0 10.0–14.0 4.0–5.5 Toad-shape noticeable	18.0–23.0 7.0–9.0 10.0–14.0 4.0–5.5	18.0–22.5 7.0–9.0 10.0–14.0 4.0–5.5	18.0–23.0 7.0–9.0 10.0–14.0 4.0–5.0	18.5–24.5 7.0–9.0 10.0–15.5 4.0–6.0	18.0–23.0 7.0–9.0 10.0–14.0 4.0–6.0	18.0–23.0 7.0–9.0 10.0–14.0 4.0–6.0
June 16			Hind legs											
June 18	18.0–23.0 7.0–9.0 10.0–14.0 4.0–6.0	18.0–23.0 7.0–9.0 10.0–14.0 4.0–6.0	17.0–21.5 7.0–9.0 10.0–12.5 4.0–5.5	17.0–21.5 7.0–9.0 10.0–12.5 4.0–5.5	17.0–21.5 7.0–9.0 10.0–12.0 4.0–5.5	17.0–22.5 7.0–9.0 10.0–14.0 4.0–5.5	17.0–21.0 7.0–8.5 10.0–13.0 4.0–5.0	17.0–21.0 7.0–9.0 10.0–13.0 4.0–5.0	16.5–21.5 6.0–8.5 10.0–13.0 4.0–5.0	17.5–22.0 7.0–9.0 10.0–14.0 4.0–5.0	17.5–22.0 7.0–9.0 10.0–13.0 4.0–5.0	18.5–24.5 7.0–9.0 10.0–15.5 4.0–6.0	18.0–23.0 7.0–9.0 10.0–14.0 4.0–6.0	18.0–23.0 7.0–9.0 10.0–14.0 4.0–6.0
June 19					Toad-shape distinct		Fore legs	Many dying						
June 20				Toad-shape								A few have hind leg buds		
June 21			Toad-shape											Hind leg buds
June 23							First metamorphosis; typical toad, no tail; length 6.5 breadth 3						Hind leg buds	

June 24		Hind leg buds												
June 25	18.5–23.0	18.0–24.0	17.5–22.0	17.0–22.0	17.0–22.0	17.0–22.0	17.0–21.0	15.5–20.0	15.0–20.5	15.0–21.0	16.0–20.5	20.0–24.5	19.0–24.0	20.0–24.0
	7.0– 9.0	7.0– 9.5	7.5– 9.0	7.0– 9.0	7.0– 9.0	7.0– 9.0	7.0– 8.5	6.0– 8.0	5.5– 8.0	5.5– 8.0	6.0– 8.0	7.0– 9.5	7.0–10.0	7.0– 9.5
	10.0–14.0	10.0–14.5	10.0–13.0	10.0–13.0	10.0–13.0	10.0–14.0	10.0–13.0	9.5–12.0	8.5–12.0	9.5–13.5	9.5–12.5	10.0–15.0	12.0–14.0	12.0–14.5
	4.0– 6.0	4.0– 6.0	4.0– 6.0	4.0– 6.0	4.0– 5.5	4.0– 5.5	4.0– 5.0	4.0– 4.5	3.5– 5.0	3.5– 4.5	4.0– 4.5	4.0– 6.0	4.0– 6.0	4.0– 6.0
	Hind leg buds		Leg buds 2.0		Legs 2.5	Legs 3.5	Legs 3.5 Toes good	Legs 3.5-4 Toes very good	Toes very good	Legs 3–4				
June 26										Fore legs				Hind legs
June 27									Fore legs			Hind legs		
June 29		Hind legs	Seem to recover from the thyroid influence					Fore legs					Hind legs	
July 1										First metamorphoses				
July 2									First metamorphosis					
July 5								First metamorphosis						
July 7														Fore legs
July 10		Fore legs											Fore legs	
July 11	18.5–23.0	18.0–24.0	18.5–22.0	18.0–22.5	17.5–22.0	17.0–22.5	17.0–21.0	10.0	11.0–18.0	14.0–18.0	16.0–18.0	20.0–25.0	20.0–25.0	20.0–25.0
	7.0– 9.0	7.0– 9.5	7.5– 9.0	7.0– 9.0	7.0– 9.0	7.0– 9.0	7.0– 8.5	7.0	5.5– 6.5	5.5– 7.0	5.5– 7.0	7.0–10.0	7.0–10.0	7.5–10.5
	10.0–14.0	10.0–14.5	11.0–13.5	11.0–13.5	10.5–13.0	10.0–14.0	10.0–13.0	3.0	5.5–11.5	8.5–11.0	8.5–11.0	10.0–15.0	13.0–15.0	12.5–14.5
	4.0– 6.0	4.0– 6.0	4.0– 6.0	4.0– 6.0	4.0– 5.5	4.0– 5.5	4.0– 5.0	3.5	3.5– 4.5	3.5– 4.5	3.5– 4.5	4.0– 6.0	4.0– 6.0	4.0– 6.0
July 16														First metamorphosis
July 18		First metamorphosis						Last metamorphosis					First metamorphosis	
July 22									Last metamorphosis					
July 23										Last metamorphosis				

[1] When four measurements are given, the first refers to the entire length, second to length of body, third to length of tail, fourth to breadth of body.

The main interest of Experiment X lies in the results following the mixed feedings of two kinds of animal tissues or animal tissue and plants.

It will be seen from the notes that, whenever thyroid tissue was one of the two foods it exerted an accelerating influence on development. The four groups fed on thyroid-thymus (fig. 9 *h*), thyroid-muscle (fig. 9 *f*, 9 *n*), thyroid-hypophysis (fig. 9 *i*, 9 *p*) and thyroid-plants (fig. 9 *g*, 9 *q*) developed their hind legs on the 5th day. The fore legs appeared in the third and fourth groups on the 21st day, in the second group on the 22d day and in the 1st group on the 24th day and the tadpoles of the several groups began to metamorphose in the same order. These facts indicate that the thymus was best able to counteract the thyroid, the hypophysis and plants least. It is peculiar, however, that the order of the last individual metamorphoses is exactly the reverse, the thyroid-thymus group completing the change first, the thyroid-plant group last. It is doubtful whether this has any connection with the fact that the greatest reduction in size was in the first group and gradually less down to the fourth.

We may now compare these thyroid-thymus results with those of Experiments VIII and IX. They are essentially alike, the thymus always being able to check the thyroid influence to some degree, but unable to suppress it entirely (fig. 2 *k*, 2 *m*, 6 *b*, 6 *d*, 9 *h*).

The remaining three groups, thymus-hypophysis (fig. 9 *i*, 9 *p*), thymus-plants (fig. 9 *k*, 9 *q*) and hypophysis-plants run much slower than the corresponding thyroid groups. Their hind legs appear from 16 to 19 days later than in the thyroid combination feedings.

The hypophysis-plant group leads the three, the thymus-plant group is a few days behind. This corresponds to the thyroid groups, where the hypophysis was less able to counteract the thyroid acceleration than was the thymus. In the thymus-hypophysis group (9 *l*, 9 *r*) two retarding factors combine, therefore the tadpoles fed on this diet never develop fore legs nor do they begin to metamorphose. Yet they are very big healthy specimens.

It is thus clear that, whenever thyroid forms a part of the diet, a precocious differentiation sets in; when thymus is added (thymus-thyroid, thymus-hypophysis, thymus-plants), the opposite influence is noticed.

One group (9 *m*) of Bufo tadpoles was starved to test again whether hunger might cause differentiation, but no differentiation was noted even 5 weeks after the start of the experiment.

Finally another group was fed on testicle. The testicle diet had not been fully tested before. The development of these tadpoles did not differ from the control and this group can therefore serve for a further comparison with the groups given a mixed diet.

Experiments were also begun on Triton alpestris and on Bufo vulgaris tadpoles which had a part of their tails amputated, but the time was too short to carry them out completely.

Feedings were started with Triton alpestris larvae on June 24. On July 12 the outer gills of the thyroid-fed ones had disappeared and the fore legs were well differentiated. The thymus-fed specimens still possessed their outer gills and had only small buds of their fore legs. The animals treated with other foods showed intermediate conditions.

Bufo vulgaris tadpoles of 19 to 23 mm in length, tails 10 to 14 mm. long, had 5 mm of their tails amputated on June 24. The next day separate groups were started on thyroid, thymus, muscle and vegetable diets. The thyroid group developed hind legs on July 3 (8th day) and fore legs on July 12 (17th day). The thymus and muscle groups developed hind legs on July 9 (14th day). Their measurements are given in table 15, page 468.

The thymus-fed ones had grown considerably by July 14 and regained their former average length, since they had almost completely regenerated their tails. The other groups had grown and regenerated much less, the thyroid least of all, while in turn it showed the fastest differentiation. These observations correspond with those of 1911. *Romeis* has studied extensively the

TABLE 15

	CONTROL VEGETABLE	THYROID	THYMUS	MUSCLE
	mm			
June 24	19.0–23.0	(14–19 mm after amputation)		
	7.0– 9.0			
	10.0–14.0	(5– 9 mm after amputation)		
	3.5– 5.5			
July 14	13.5–18.0	11.0–14.0	20.0–22.0	15.0–18.5
	7.0– 9.5	5.0– 6.5	9.0–10.5	7.5– 9.5
	6.0– 9.0	6.0– 8.0	11.0–12.0	7.5– 9.0
	4.0– 5.0	2.5– 4.0	5.0– 6.5	4.5– 5.5

influence of different diets on the regeneration of the tail in Rana esculenta tadpoles.

The experiments of 1911 and 1912 leave no doubt that the mammalian thyroid gland contains an *agens* which, when the gland is given as food, calls forth a rapid differentiation in a developing vertebrate organism. This differentiation may be brought about at any stage of development, before maturity is reached. The differentiation may therefore be highly precocious in cases where the treatment is begun on extremely young animals. Experiment I showed that tadpoles could be brought to the point of metamorphosis within 18 days after hatching, while normally they would require 10 to 12 weeks to reach such a stage. The results of this premature metamorphosis are perfect frogs of minute size, pygmy frogs, as the figures show (fig. 4 *o*, 4 *p* and others). The thyroid influence is very decided and there is no escape from it for any tadpole given a thyroid diet. All of the individuals, even if thousands be employed as was the case in several experiments, will react almost immediately to the thyroid stimulus, so that certain changes in their structure may be observed after 24 hours, when only one application of thyroid has been made. They will all react simultaneously so that any individual differences in development will become unobservable.

The experiments further bring out the fact that the time of reaction to the thyroid stimulus varies to a certain degree with the

kind of food that had been given before the thyroid diet was started. There is also a difference in response between previously starved and fed tadpoles. The experiments with mixed diets showed that the accelerating influence of the thyroid could be checked to some extent and the rapid differentiation more or less retarded. However, there is no complete counteraction against the thyroid stimulus, so that sooner or later any tadpole, receiving thyroid after other or mixed with other diets, must respond to its influence.

Experiments were carried out to determine the least amount of thyroid food necessary to produce the typical reactions, and also to determine whether or not the tadpoles could recover from the thyroid shock if afterwards put on other food. A feeding of only 24 hours—that is to say, the thyroid was kept in the dish for about 24 hours, though the animals did not feed on it continuously—sufficed to cause a hastened differentiation. A feeding for three days was enough to give the fastest rate of differentiation, a rate that could not be increased by longer feedings.

When thyroid is applied too rapidly, the animals usually die very soon after the appearance of their fore limbs and the simultaneous reduction of their tails. By careful feedings at rather long intervals and in all not more than four times, the animals may be kept alive for several weeks. They will not undergo, however, any further changes, except perhaps a continued reduction of their tails, nor will they ever feed again. In 1911 I succeeded in bringing some tadpoles to a complete absorption of their tails and these thyroid frogs were kept alive on wet sand for from 2 to 4 days. In 1912 some Bufo tadpoles almost completely absorbed their tails under the thyroid treatment (fig. 9 *e*, 9 *n*), but could not be kept alive for more than 24 hours.

A recovery from the thyroid influence is extremely rare. Only 5 individuals out of 400 in one experiment and 6 in another were able to survive, and, although, they were never seen to feed, began slowly to grow again after a standstill of several weeks.

Not one of the many thousand tadpoles fed on thymus in the spring of 1912 could be brought to metamorphosis during the 15 weeks in which the animals were under observation, while tadpoles of the same set, though fed on other substances, metamorphosed before that time (fig. 4 *o*, *p*, liver). In some experiments the thymus fed tadpoles never succeeded in growing their hind extremities before the controls completely metamorphosed. Yet the thymus tadpoles would grow very rapidly, especially during the first weeks of the experiments.

The tadpoles feed on spleen behaved in very much the same way as the thymus-fed ones, though they always were somewhat ahead of the latter and did not counteract the thyroid feedings so strongly. Of the great number of spleen fed tadpoles also not one could be caused to metamorphose.

We must therefore conclude that the thymus and to some extent the spleen also, and probably the lymphatic organs, when given as food, will cause a rapid growth followed by a rather late differentiation or none at all..

These experiments with various foods, except thyroid, thymus and spleen, and the investigations of other workers show that the animals must reach a certain constant minimum size before the final metamorphosis can begin. There is on the other hand a constant assimilation of food and a gradual increase in body size up to a constant maximum, beyond which a normal animal may not pass without the beginning of the final differentiation and metamorphosis.

In the thyroid-fed tadpoles there is differentiation without growth, in the thymus- (and spleen-) fed tadpoles growth without differentiation. The experiments, therefore, emphasize the fact that in development we deal with two entirely separate factors, the factor of growth and the factor of differentiation.

The two naturally work simultaneously; but differentiation is not the result of growth, otherwise there could be no thyroid differ-

entiation without growth. Nor must growth necessarily be followed by differentiation, as seen in the growing thymus tadpoles.

One might say that ordinary foods which are being assimilated bring with them the two factors of growth and differentiation. Then the assumption is necessary that the thyroid food lacks the power of causing growth, while the thymus and spleen lack the power of causing differentiation. But we can hardly assume that all the various kinds of foods an animal may take in, with the only exceptions of thyroid and thymus, contain these two factors in the proper proportions.

The factors for growth and differentiation can only be located within the organism itself.

We face the following propositions:

1. The thyroid has the power to excite differentiation, but it lacks the power to cause growth.

The thyroid calls forth differentiation, whether the animals be small or large, and without regard to the standard minimum size, necessary for the final change. It must possess an *agens* for stimulating differentiation which other foods do not possess. That the thyroid also possesses a power which prevents growth is not evident. The suppression of growth may merely be incidental, for rapid differentiation does not allow growth.

2. The thymus has the power to stimulate growth, but lacks the power to excite differentiation.

It has been stated above that tadpoles feeding on any food (except thyroid) reach a maximum size, and when this maximum is reached, differentiation begins independently. The thymus-fed tadpoles reach this maximum size and differentiation should set in of itself, even if the thymus lacked the necessary stimulus. But differentiation does not begin; therefore the thymus (and spleen) must exert an influence not possessed by the other foods which suppresses differentiation. That the thymus possesses the power to stimulate growth, is again not so evident, since the thymus growth may be merely the normal result of the intake of food. The thymus growth is rather rapid, but this may be attributed to the better nutritive qualities of the thymus tissue. The thymus-fed tadpoles may also grow beyond the normal as brought out in

1911, yet this again may be merely incidental. There is no beginning of differentiation in thymus-fed tadpoles which would check a further growth.

We can only say with reasonable safety:

1. The thyroid possesses a quality that stimulates differentiation, not contained in any other food used.[2]

2. The thymus (and spleen) possess a quality that suppresses differentiation not contained in any other food used.

Thus the thyroid and thymus must produce, or at least contain, agents which, when passed into developing organisms, in the one case stimulate, in the other suppress, differentiation. The production of such substances to be thrown into the circulation characterises the thyroid and thymus as glands with a positive internal secretion. That these two types of tissues may also be capable of performing the reverse action, viz. the elimination of certain substances from the circulation, as has been assumed especially for the thyroid, is neither demonstrated nor denied by these experiments.

The other glands used in the studies may or may not contain either the accelerating or the depressing power. However, the macroscopic differences in the rate of growth and differentiation of tadpoles fed on such glands and of the control animals are only slight and might easily be attributed to differences in the nutritive values of the various foods. The feeding experiments on tadpoles are, therefore, not likely to reveal these factors, if at all present in other glands, in a striking degree. Their study must be left to experiments of a different kind.

[2] By 'differentiation' is meant merely the macroscopic changes, hind, forelimbs, metamorphosis. The microscopic differentiation will be discussed in a later paper.

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All illustrations taken from living tadpoles. All figures in natural size except figures 10, *a* to *i* and 11, *a* to *f*.

PLATE 1

EXPLANATION OF FIGURES

1, a to ze Rana temporaria I. Experiment I. *a*, liver; *b*, spleen; *c*, thymus; *d*, muscle; *e*, ovary; *f*, adrenal cortex; *g*, adrenal medulla; *h*, brain; June 6, 1912. *i*, liver; *k*, liver-thyroid; *l*, spleen; *m*, spleen-thyroid; *n*, muscle; *o*, muscle-thyroid; *p*, ovary; *q*, ovary-thyroid; *r*, adrenal cortex; *s*, adrenal cortex-thyroid; *t*, adrenal medulla; *u*, adrenal medulla-thyroid; *v*, brain; *w*, brain-thyroid; June 26, 1912. In *k*, *m*, *o*, *q*, *s*, *u*, *w*, thyroid feeding had been started on June 17, 1912. *x*, liver; *y*, brain; *z*, spleen-thyroid; *za*, muscle-thyroid; *zb*, ovary-thyroid; *ze*, adrenal cortex-thyroid; *zd*, adrenal medulla-thyroid; *ze*, brain-thyroid, July 6, 1912. In *z* to *ze*, thyroid feeding had been started on June 17, 1912.

2, a to n Rana temporaria II. Experiment IV. *a*, original size, June 4, 1912. *b*, control; *c*, thyroid-muscle, thyroid given 1 day; *d*, thyroid-muscle, thyroid given 3 days; *e*, thyroid-muscle, thyroid given 5 days; June 17, 1912. *f*, thyroid-plants; thyroid given 1 day; *g*, thyroid-plants, thyroid given 2 days; *h*, thyroid-plants, thyroid given 4 days; *i*, thyroid-plants, thyroid given 5 days; *k*, thyroid-thymus; *l*, thyroid emulsion; June 17, 1912. *m*, thyroid-thymus, *n*, thyroid-muscle; thryoid given 4 days; June 26.

3, a to n. Rana temporaria III. Experiment III. *a*, thymus; *b*, muscle; *c*, thymus-thyroid I; *d*, thymus-thyroid II; *e*, thymus-thyroid III; June 1, 1912. *f*, thymus-thyroid II; *g*, thymus-thyroid III; *h*, thymus-thyroid IV; June 17, 1912. *i*, thymus; *k*, thymus-thyroid V; *l*, muscle; *m*, muscle-thyroid; June 26, 1912. *n*, muscle-thyroid; July 6, 1912.

5, a to e Rana temporaria V to VII. Experiment VI. *a*, control; *b*, glandular hypophysis; June 26. *c*, control; *d*, glandular; *e*, neural hypophysis; July 6, 1912.

6, a to d Rana temporaria V to VII. Experiment IX. *a*, control; *b*, thymus-thyroid; June 17, 1912. *c*, control; *d*, thymus-thyroid; June 26, 1912.

1 b
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k
m
6 c
5 d

PLATE 2

EXPLANATION OF FIGURES

4, a to p Rana temporaria IV. Experiment II. *a*, control; *b*, thymus; *c*, spleen; *d*, muscle (thyroid under 4, *o* and *p*); *e*, thymus-thyroid II; *f*, thymus-thyroid III; *g*, thymus-thyroid IV; June 1, 1912. *h*, thymus-thyroid IV; *i*, thymus-thyroid V; June 17, 1912. *k*, muscle; *l*, muscle-thyroid; *m*, spleen; *n*, spleen-thyroid; June 26, 1912. *o* to *p*, 1 frog fed on liver, metamorphosing on July 12, and 5 pigmy frogs, fed on thyroid metamorphosing on April 22, 81 days earlier. *o*, dorsal; *p*, ventral view.

7, a to *b* Rana temporaria VIII to XI. Experiment V. *a*, spleen; *b*, adrenal; July 6, 1912.

8, a to d Bufo vulgaris I. Experiment VII. *a*, control; *b*, muscle; *c*, thymus; *d*, thyroid; June 6, 1912.

9, a to r Bufo vulgaris III. Experiment X. *a*, original size; June 5, 1912. *b*, control; *c*, thyroid-plants, thyroid given 1 day; *d*, thyroid-plants, thyroid given 3 days; *e*, thyroid-plants, thyroid given 5 days; June 26, 1912. *f*, thyroid-muscle; *g*, thyroid-plants (alternately); *h*, thyroid-thymus; *i*, thyroid-hypophysis; *k*, thymus-plants; *l*, thymus-hypophysis; June 26, 1912. *m*, control; *n*, thyroid-muscle; *o*, thyroid-plants; *p*, thyroid-hypophysis; *q*, thymus-plants; *r*, thymus-hypophysis; July 6, 1912.

10, a to i Rana temporaria IV. Experiment II; compare figure 4, *a* to *p*. Thyroid-fed frogs, 16 to 18 days old, at the time of metamorphosis, figure 4, *l*.

11, a to f. Tails of tadpoles fed on different substances, to show pigmentation; figure 4, *l*. *a* thymus; *b*, liver; *c*, spleen; *d*, muscle; *e*, adrenal cortex; *f*, adrenal medulla.

4 d

4 e 4 f 4 g 4 h 4 i

4 k 4 l 4 o

4 m 4 n

5 b

9 a 9 c 9 d 9 e

9 f 9 g 9 h 9 k

9 n 9 o

9 p 9 q

10 b 10 c 10 d 10 e

10 g 10 h

11 b 11 c 11 d

ORIGIN AND EARLY HISTORY OF THE PRIMORDIAL GERM-CELLS IN THE CHICK

CHARLES H. SWIFT

From the Hull Laboratory of Anatomy, University of Chicago

FIFTEEN FIGURES

CONTENTS

Introduction and review of the literature.................................... 483
Material and methods.................................... 489
Origin and history of the primordial germ-cells.................................... 491
1. Structure of the germ-cells.................................... 491
2. Migration of the germ-cells.................................... 496
3. Origin of the germ-cells.................................... 510
Summary and conclusions.................................... 514
Bibliography.................................... 515

INTRODUCTION AND REVIEW OF THE LITERATURE

There are several theories held as to the origin of primordial germ-cells. The theory that they arise from a differentiated portion of the coelomic epithelium over the Wolffian body has priority and should be mentioned first. Waldeyer ('70) was the first to advance this idea as to an origin from a so-called germinal epithelium. His opinion advanced in the work "Eierstock und Ei," remained unchallenged for a long time. It has had a number of supporters, and even at the present day, the theory that the primordial germ-cells arise in the germinal epithelium, is the only one found in a majority of text-books. That this theory should have a long life is only natural. The early investigators did not possess the finished methods or perfected instruments of today and so were placed at a serious disadvantage when working with the younger stages. Also, as we now know, the germ cells in the earlier stages of development, are not grouped, but may be widely scattered, and for this reason, if for no other, they

would be much more difficult of demonstration than when collected into a relatively circumscribed area like the germinal epithelium or developing gonad. In the older embryos, however, they were able to see the typical primordial germ-cells in the modified coelomic epithelium, and so naturally thought that they arose by a series of transformations from the epithelial cells. Semon ('87) claimed to have seen a number of cells undergoing this change of form, but since that time no other investigator has ever been able to see any intermediate stages between the cells of the germinal epithelium and the primordial germ-cells. D'Hollander ('04) described in the chick embryo of ten days, however, the production of oogonia from a superficial indifferent layer of cells—another term for germinal epithelium. According to d'Hollander, the germinal epithelium gives rise to epithelial buds, which grow down into the ovarian stroma. From these epithelial, germinative buds, by a process of differentiation, the oogonia and primordial follicular cells arise. This origin of oogonia at such a late period and from the germinal epithelium must be secondary to the production of primordial germ-cells, which, according to all authorities, make their appearance in the gonad at a much earlier period of development than ten days.

Another view as to the origin of primordial germ-cells may be called the gonotome theory. The supporters of this theory agree with the advocates of the germinal epithelium origin in so far that the primordial germ-cells are found at one time in the modified coelomic epithelium. They, however, claim that the primordial germ-cells do not arise in the germinal epithelium, but from a portion of the segmental mesoderm, and reach the epithelium at a later period either by migration or by tissue growth. Rückert ('88) advanced this idea, and called that portion of the segmental mesoderm, from which he supposed they took origin, the gonotome. That certain of the primordial germ-cells are, at times, found in the region of the segmental mesoderm there can be no doubt, but, their occurrence there can be explained in another and better way than by supposing that they take origin there. This theory has had few advocates, and is of importance now, mainly because of the historical interest attached to it.

The supporters of the third theory, that of early segregation, agree with the exponents of the other two, that the primordial germ-cells are at one time in their history found in the germinal epithelium. They do not, however, believe that they arise in situ, nor from the gonotome, but that they have a much longer history dating back to an early embryonic stage. It may be possible, at some future time, to trace the primordial germ-cells back to the very early divisions of the segmenting egg, if not to the fertilized egg itself. In vertebrates, the work of Eigenmann ('97), demonstrated an origin of primordial germ-cells within five divisions of the fertilized egg; that is, he found that the youngest germ-cells possessed a size equivalent to blastomeres of the fifth cleavage. If this last is correct, that the germ-cells have an independent existence from a very early period, and are not differentiated from somatic cells, then the idea of germ-plasm continuity has received valuable support. This idea of early and extraregional origin is borne out by all recent research, and is becoming more firmly established with each succeeding year.

The names of Hoffmann ('93) Eigenmann ('97), Nussbaum ('01) and Beard ('04) are associated with the early years of this early segregation theory of germ-cell origin.

Hoffman ('93) was the first to bring forward any evidence which cast doubt on the theory of origin of primordial germ-cells from the germinal epithelium. He employed in his work twelve species of birds, in three of which, Haematopus ostralegus, Sterna paradisea, and Gallinula chloropus, there was sufficient evidence brought out to prove that some, if not all the primordial germ-cells, did not originate in the modified coelomic epithelium. In the three species mentioned above, he found at the proper time, numbers of primordial ova in the germinal epithelium. But, in addition he found cells—supposedly primordial ova, because of their resemblance to those found later in the germinal epithelium—in embryos of 23 somites. An embryo of 23 somites does not possess the so-called germinal epithelium, the coelomic epithelium over the Wolffian body not having been modified at this age, yet in these he found primordial germ-cells far removed from the site of the future sex-gland, in the splanchnic

plate of mesoderm, in the region between splanchnic mesoderm and entoderm and in the entoderm.

Eigenmann ('97) was one of the first to prove that primordial germ-cells may have an extra-regional origin. His work on the viviparous teleost Cymatogaster showed that the primordial germ-cells in that form, have an origin from cells having the size of fifth cleavage blastomeres.

Beard ('04) discovered in Raja batis that the primordial germ-cells have an origin from a group of cells in the anterior part of the blastoderm, beneath the embryonic anlage, and that these cells then migrate in towards the region of the future sex-glands along a very definite path, through the hypoblast of the gut and the line between hypoblast and unsegmented splanchnic mesoderm. These early extra-embryonic germ-cells, which he called megaspheres, differ in no particular, except size, from the germ-cells, which are found in the older embryos.

Nussbaum ('01), Rubaschkin ('07), and von Berenberg-Gossler ('12), in addition to Hoffmann ('93) have investigated the primordial germ-cells in bird embryos. The first three worked with the chick and were able to find in embryos of 22 to 23 somites typical germ-cells in the entoderm and splanchnic mesoderm lateral to the coelomic angle, some of which in older embryos passed into the germinal epithelium. The 22-somite embryo is the youngest in which primordial germ-cells have been found in the chick.

Allen ('06), ('09), ('09), investigated this question of primordial germ-cell origin in Chrysemys, Amia and Lepidosteus and discovered that the primordial germ-cells in these forms had an entodermal origin, in the case of Chrysemys from the entoderm near the margin of the area pellucida in a zone extending on either side from the anterior extremity of the pronephros to a point behind the embryo. The primordial germ-cells of Lepidosteus have a not very different origin, while in Amia they were shown to come from the entoderm of the roof and margin of the floor of the sub-germinal cavity.

Allen has shown that in these forms there is a definite migration. In the youngest embryos the primordial germ-cells have

an amoeboid form and a lateral position in the hypoblast; in older stages these cells still have an amoeboid form, but have a more median position, which they have attained by a centripetal movement along the hypoblast. In the still older embryos these cells are found to have lost their amoeboid character and to be in the entoderm under the notochord or in the mesoderm in that immediate neighborhood. Thence, according to the evidence of the still older embryos, they pass into the mesentery and finally assume a position which corresponds to that of the future sex-gland.

Woods ('02), working on Acanthias, found that the primordial germ-cells were first evident in the entoderm and in the periblast and thence migrated to the sex-gland anlage.

Jarvis ('08), in Phrynosoma, found that the germ-cells appeared first in the entoderm of the vascular area of the blastoderm, where they were cephalad, caudad, and laterad to the embryo. Then by a definite migration path they reached the germinal anlage.

Dodds ('10), investigating the question of germ origin and history in the teleost, Lophius, was able to recognize the primordial germ-cells in the primitive entoblast, when the blastoderm had not quite half covered the yolk. He, however, believed that they were set apart at a time earlier still in embryonic history.

Finally, as far as vertebrates are concerned, Fuss ('11), in a human embryo of four weeks, with about 33 somites, demonstrated extra-regional primordial germ-cells in the mesentery directly under the coelomic epithelium.

Among invertebrates, in many phyla, the primordial germ-cells have been observed at a distance from the site of the future sex-gland, that is, in an extra-regional position, and in several cases have been traced back to their true origin. Indeed, Balbiani's ('85) work with Chironomus was one of the very earliest in which a definite origin was proven for the primordial germ-cells. He found that they originated from cells differentiated very early in the history of the segmenting egg.

The most remarkable known case of early differentiation of the primordial germ-cells is that of Ascaris, in which Boveri

('92) was able to follow them back directly through all the cleavage stages to the two-cell stage.

Haecker ('97), in Cyclops, and Hegner ('09), in some Chrysomelid beetles, were able also to trace the primordial germ-cells back to early segmentation stages.

It may be said, then, that in many groups of vertebrates, as well as invertebrates, all the primordial germ-cells do not originate in a differentiated portion of the coelomic epithelium called the germinal epithelium. In many of the cases cited the exact origin of the primordial germ-cells has not been found, but even in these it has been definitely established that the germ-cells arise in the early history of the embryo long before the appearance of a germinal epithelium. So, with the evidence at hand, it would seem that Nussbaum was correct when in 1880 he formulated the hypothesis that "the sexual cells do not come from any cells that have given up their embryonic character or have gone into building any part of the body, nor do sexual cells ever go into body formation."

The bird groups still offer an excellent opportunity for research into the history of the primordial germ-cells, for, notwithstanding the amount of work which has been done by Nussbaum, Hoffmann, Rubaschkin, von Berenberg-Gossler, and others, the origin of the primordial germ-cells still remains unrevealed. The stage of 22 or 23 somites is the youngest in which germ-cells have been described among birds.

In carrying out this research I have received constant aid and advice from Prof. R. R. Bensley. I wish to thank him for this, as well as for a training which has made possible whatever in my results may be considered of value. I also wish to thank Dr. E. V. Cowdry and Dr. G. W. Bartelmez for their assistance as well as kindness in allowing me to use their excellent preparations. I am also indebted to Mr. A. B. Streedain for the drawings which illustrate this article.

MATERIAL AND METHODS

In the selection of a fixative for the germ-cells, the yolk material, mitochondria, and attraction-sphere, in addition to the nucleus and general cytoplasm, have to be taken into consideration. The same fixing fluid cannot be relied upon in all stages, but in order to meet the changing cell content, a variation is necessary. For instance, the yolk-laden germ-cell of the very young embryos requires very different treatment from the almost yolk-free cell of the later stages, if equally good results in both cases are desired. So that in the case of embryos over 20 somites in development, in which the germ-cells contain only a moderate amount of the primary vitellus, a fixing fluid containing osmic acid may be used to advantage. Osmic acid resembles potassium bi-chromate, with which it is frequently combined, in being an excellent cytoplasmic preservative. It has its drawbacks, however, in being a poor penetrant, amd in blackening the yolk of the germ-cells. This last property of osmic acid is a decided disadvantage in the early stages, in which the cytoplasm of the germ-cells is stuffed with yolk spheres, and an advantage in the case of the older embryos, in which the yolk is reduced in amount, since the blackening of the vitellus facilitates identification.

Fixatives such as Benda's, Meves' modification of Flemming's fluid, and Bensley's acetic-osmic-bichromate mixture, all of which contain osmic acid, were found to be best adapted for use in the case of older embryos, in which there is little yolk material remaining. They preserve such cytoplasmic structures as mitochondria and the attraction-sphere excellently, and, thanks to their osmic acid content, bring out the yolk spheres plainly. However, in the younger embryos, from the primitive streak stage to 20 somites, they were found to be not nearly so efficient. In these stages the yolk content of the germ-cells is so great that the value of any fixative containing osmic acid is seriously diminished. In these young embryos a mixture of equal parts of 5 per cent trichloracetic acid and 5 per cent sublimate was found to be excellent. The mitochondria are not preserved by this

method, but the obscuring of the nucleus and cytoplasm by the large, blackened yolk spheres is avoided.

An ideal fixing fluid should not only be an excellent cytoplasmic preservative but should also have the ability to penetrate rapidly. Unfortunately osmic acid and potassium bichromate are lacking in this last quality. Acetic acid is frequently combined with them because of its penetrating powers. However, too much acetic acid is worse than none at all, since cytoplasm and its contained organs, like mitochondria, are very sensitive to this agent. For this reason Zenker's fluid is of no value in the preservation of mitochondria. The acetic-osmic-bichromate mixture of Bensley contains these fixing agents in about the proper proportions, and yet even this leaves something to be desired. It is of value as a fixative only when small pieces are used. It really preserves, as in the natural state, only the outer surface of a piece of tissue. In the center of a block of any size the mitochondria are found to be globular, instead of rod-like, or even to have disappeared altogether. The value of the acetic-osmic-bichromate mixture depends upon all the elements in it. They do not all penetrate with equal speed; the acetic acid is much the more rapid in this regard. Near the periphery of a piece of tissue they act in unison, as is intended, but the acetic acid soon forges ahead and acts in an injurious manner upon the cytoplasm and especially upon the mitochondria,

The following staining methods were employed. Following the Benda fixaton the Benda staining method was used. This procedure is the one commonly employed for demonstrating mitochondria. It was first published by Benda in 1901.

Following Bensley's acetic-osmic-bichromate mixture two general staining methods were used, namely, the anilin acid fuchsin-methyl green method and Bensley's copper-chrome-hematoxylin technique. There were, however, several modifications of the first. In place of methyl green as a counterstain, toluidin blue or Wright's blood stain may be substituted. Bensley ('11) described these methods in detail, so there is no need to recapitulate here.

All these procedures are reliable and a beautiful result is usually obtained, especially at the periphery of a section.

Following Meves' modification of Flemming's fluid the iron-hematoxylin stain was employed. This method was found to be more reliable in the moderately young embryos—15 somites to 25 somites—than the acetic-osmic-bichromate method.

After the 5 per cent trichloracetic acid and 5 per cent sublimate fixation iron-hematoxylin and acid fuchsin gave excellent results. The cytoplasm is well preserved and the attraction-sphere especially so. The centrosomes were more frequently seen after this procedure than after any other.

In all cases where embryos of relatively advanced age were employed, the anterior body wall, amnion and viscera were removed, thus exposing directly to the fixative the Wolffian body and gonad. All sections were cut 4 micra thick.

Table 1 will show at a glance the number of embryos employed, their age, together with the methods used in fixation and staining.

ORIGIN AND HISTORY OF THE PRIMORDIAL GERM-CELLS

1. Structure of the germ-cells

It will be better to describe the form and structure of the primordial germ-cells in the chick before proceeding to a description of their origin and history. In this way the necessity of describing them with each stage of development will be avoided. There is all the more reason for this since more than one portrayal of them would be a repetition. The change in the germ-cells, from their origin to the time when they pass into the indifferent gonad, is limited to one or two structures. These variations will be taken up at the stage of development at which they appear most evident.

Size and shape. The most noticeable attributes of these primordial germ-cells, the characters, which first attract attention in examining a section containing them, are their size and shape. Isolated in the general mesenchymal tissue they appear immense (figs. 2 and 8). Their size, which is much greater than that of

TABLE 1

Method		Primitive streak	Somites 3	s. 6	s. 9	s. 10	s. 12	s. 16	s. 17	s. 18	s. 19	s. 20	s. 21	s. 22	s. 23	s. 24	s. 25	s. 26	s. 27	s. 28	s. 29	s. 30	s. 31	s. 32	s. 33	Days 4½
Meves' fixation and Meves' iron hematoxylin stain		3	1	1	1	1	1	1	2	2	2	1	1	1	1	1	1	1	1	1	1	1	1			
Bensley's acetic-osmic-bichromate fixation	Bensley's acid fuchsin and methyl green stain																							1	2	
	Bensley's acid fuchsin and Wright's stain.																									2
	Bensley's copper-chrome-hematoxylin stain																									2
Benda's method and Benda s stain													1			1	1	1	1							
Trichlor acetic acid (5%) and corrosive sublimate (5%). Iron hematoxylin and acid fuchsin stain		1	1																							

the neighboring cells, is due to an increase in volume of both nucleus and cytoplasm. Their shape also is different from that of the surrounding cells. The latter are frequently stellate, although they may be round, oval, flattened, or elongated, while the germ-cells are always round or oval (figs. 2, 6, and 10). The germ-cells have an average diameter of about 16 micra. They may have a diameter as small as 14 micra, while in exceptional cases this may reach 20 or even 22 micra.

Nucleus. The large nucleus, which has a diameter of 8 to 12 micra, is spherical and vesicular, and is nearly always eccentrically placed (figs. 7, 8 and 13). It is surrounded by a definite nuclear membrane. On comparing the nuclei of the primordial germ-cells, with those of the neighboring cells, in addition to the disparity in size, it is noticed that the germ-cell nuclei are clearer. The germ-cell nuclei seem to contain about the same amount of chromatin as the nuclei of neighboring cells, but owing to the increase in size of the former it appears greatly reduced in amount. The germ-cell nucleus, at one pole, is separated from the cell membrane by a thin layer of cytoplasm, while the attraction sphere generally occupies the other pole, where a wider expanse of cytoplasm separates the nucleus from the membrane. The clear appearance of the nucleus of the germ-cell is not confined to the chick alone but is common to many species.

The chromatin within the nucleus generally has a definite arrangement, being grouped in two masses (figs. 2, 4, and 8). This holds in nearly all stages except the youngest where the chromatin has the appearance of a reticulum. The chromatin in the two masses, mentioned above, appears in the granular form. The two masses themselves may be quite distinct, being separated by a wide clear interval, or they may approach each other closely. In some cases they are connected by granular threads. This definite arrangement of the chromatin does not appear after one fixation only, but can be observed after acetic-osmic-bichromate, equal parts of 5 per cent trichloracetic acid and 5 per cent sublimate as well as Meves' modification of Flemming's fluid.

The attraction-sphere. The attraction sphere is the most characteristic organ of the primordial germ-cell in the chick, and,

oddly enough, its importance has been overlooked by all workers except Rubaschkin ('08) and von Berenberg-Gossler ('12). It is present in all stages, and, from the origin of the germ-cell to its entrance into the indifferent gonad, does not undergo any change. It appears as a condensed or flattened sphere of cytoplasm resting usually on the nuclear membrane, on that side of the nucleus, which is the farthest removed from the cell membrane (figs. 2, 5 and 9). It may rest on the nuclear membrane directly, in which case, it has a concavity towards the nucleus, or it may be separated from the nucleus by some distance. It has an average diameter of 3 to 4 micra, but in some cases where it is flattened against the nucleus, its long axis may measure 6 micra. At times it is very discrete and seems to be surrounded by a definite membrane. The cytoplasm immediately around it appears less dense than the rest, so that the attraction-sphere seems to be situated in a vacuole. In all stages and with all stains the sphere is prominent and unless obscured by yolk spheres is easily seen.

The centrosomes are best seen after trichloracetic acid fixation and iron-hematoxylin staining, when they appear as black dots. With this fixation cytoplasmic radiations may be observed radiating out from the attraction sphere.

Attraction-spheres are, of course, not confined to the primordial germ-cells, but in the other cells they are not nearly so large, distinct or constant as in the germ-cells. Because of its size, discreteness and constancy, it serves as the best criterion for identification of germ-cells in the chick.

Yolk. Yolk material is a very characteristic constituent of the primordial germ-cells in the chick. Its abundance in the young stages has been recorded by Nussbaum ('01), Rubaschkin ('08) and von Berenberg-Gossler ('12). The very young germ-cells, just after their origin, are simply loaded with the yolk which has the form of spheres; the cytoplasm is so crowded that practically nothing but yolk can be seen (figs. 12 and 14). Most abundant in the younger germ-cells, it gradually decreases in amount as the embryo increases in age. In embryos having about 20 somites and thereafter, there is a great variation in the quantity of yolk in the germ-cells. In some embryos the germ-

cells may all have no yolk, while in others, some will have yolk while the remainder will have none at all.

In the embryos of moderate age—20 to 26 somites—there is evidence that the yolk is undergoing digestion, as is shown by the fact that in the same primordial germ-cell fixed with osmic acid some of the yolk spheres will be intensely black while others will be brown or yellowish. The yolk spheres which are not black in these cells are frequently surrounded by a clear ring.

In the embryos of about 20 to 26 somites, the yolk spheres reach their greatest size (fig. 7). In one case one had a diameter of 8 micra. In the younger germ-cells the yolk spheres are smaller having an average diameter of 2 to 3 micra. In embryos with 33 somites to four and one-half days the yolk content varies a great deal; usually there is only a small quantity present, which is confined to 5 to 10 small spheres, which usually show signs of digestion.

In the very young primordial germ-cells the yolk is scattered evenly throughout the germ-cell, usually entirely surrounding the nucleus, which may have a central rather than an eccentric position. As the germ-cell grows older the yolk diminishes in amount and retreats to one side of the nucleus, usually the cell pole which is occupied by the attraction-sphere (figs. 7 and 9). As the yolk decreases still more in amount it is confined to the region between the attraction-sphere and the cell membrane. At this stage the yolk spheres are apt to be arranged in a horse-shoe shaped manner, with the concavity turned towards the attraction-sphere (fig. 6).

The yolk remains in the primordial germ-cells long after it has disappeared from the tissue cells. The presence of the yolk in the germ-cells is probably related to the absence of mitoses in these cells.

Mitochondria. The mitochondria in the primordial germ-cells of the chick are not at all characteristic. They resemble the mitochondria of the somatic cells (figs. 5, 8 and 11), and at the same time retain the same appearance, whether in the primitive streak stage or four and one-half day chick. They are seen usually to be in the form of short rods of a length varying from 1 to

3 micra. These rod-like mitochondria do not as a rule have a homogenous structure, but are beaded or granular rods; that is, beads or granules closely approximated and arranged in a row or rod. At the same time in all stages discrete granules may be present, but they are never seen to the exclusion of the rod form unless the fixation has been faulty.

This is at variance with the work of Rubaschkin ('10) and Tschaschin ('10), who found, the former in mammals and the latter in birds, that the primordial germ-cells are characterized by granular mitochondria, while those of the somatic cells are rodlike in character.

Size of the primordial germ-cell, nucleus and attraction-sphere. The size of the germ-cell varies little, if any, from its origin, to its entrance into the indifferent gonad. The average diameter of nearly every one is found between the figures 14 and 18 micra. The majority approach the larger figures.

The nucleus undergoes a little variation in size. That of the germ-cells of the primitive streak stage to 9 somites has a diameter of 8 to 10 micra. In germ-cells of embryos possessing more than 12 somites the nuclear diameter varies between 10 to 12 micra.

The attraction-sphere has a constant long diameter of 3 to 4 micra. This relatively constant size of the primordial germ-cell, and of its organs, the nucleus and attraction-sphere, together with the retention of yolk, after its disappearance from the somatic cells, may be taken as an evidence that the germ-cells do not divide during their migration history.

2. *Migration of the germ-cells*

In investigating the history of the primordial germ-cells of the chick it was found to be expedient to commence the study on older embryos. The advantages of this method of study are obvious; the older embryos are larger and therefore much easier to handle than the younger ones, and in addition the germ-cells have been observed down to embryos of about 22 somites and described and studied by several able investigators—notably Hoffman ('93), Nussbaum ('01), Rubaschkin ('07), Tschaschin ('10) and von Berenberg-Gossler ('12). Therefore, in begin-

ning with these older forms, one can become familiar with the germ-cells in regions where they have been seen and studied before, and acquire a more perfect technique, before following them back through an unknown migration to an equally unknown and obscure origin.

In an embryo of four and one-half days incubation the germinal epithelium is found to be developed along the medial surface of the Wolffian body. It is composed of cells which differ from those of the general coelomic epithelium in being cuboidal or columnar. Owing to the greater height of these cells a ridge-like appearance is produced on the medial surface of the Wolffian body, which may simulate a developing gonad. The gonad itself is forming only at the level of the anterior half of the Wolffian body, where stroma is beginning to appear beneath the germinal epithelium. The developing mesentery, springing from between the Wolffian bodies, has attained a length easily appreciated by the unaided eye.

Scattered here and there in the stroma of the developing gonad, in the root of the mesentery, both in the mesenchymal tissue and in the coelomic epithelium near the coelomic angle, and in the germinal epithelium, clothing the gonad, are certain cells which are sharply marked off from the neighboring cells. They are the primordial germ-cells which have previously been described in this article, and which have been noticed and studied at this stage and this region by all investigators of germ-cell history in the chick since the publication of Waldeyer's "Eierstock und Ei."

By far the greater number of the primordial germ-cells in the four and one-half day embryo are found in the gonad, and in the root of the mesentery near the coelomic angle, but some are to be seen in other situations, namely, in the mesentery at a distance from its point of attachment to the body, in the wall of the gut, in the mesenchyme behind the aorta and in front of the notochord and even an occasional one dorsal and lateral to the notochord.

The germinal epithelium in the chick of four and one-half days of incubation is well developed. It consists of a strip of cuboidal to columnar cells, without any basement membrane, which on

either side merge into the flatter cells of the general coelomic epithelium. Scattered among the germinal epithelial cells an ocasional primordial germ-cell is to be seen. In observing these cells the question naturally arises as to whether they have arisen in situ or have reached that position through a migration after having originated elsewhere. Waldeyer ('70), it will be remembered, formulated, the former idea. The researches of Hoffman ('93), Nussbaum ('01), Rubaschkin ('07), and others have cast rather serious doubts upon the correctness of this theory. In fact it may be said to have been disproven as regards some of the germ-cells. The fact that typical germ-cells are found in the root of the mesentery and in the coelomic epithelium before the differentiation of a portion of the coelomic epithelium into germinal epithelium certainly is proof enough that some of them at least do not arise there. However, the origin of some of the germ-cells from the germinal epithelium has never been positively dispproven. Semon ('87) in supporting Waldeyer's ('70) idea, claims to have seen transitions between cells of the germinal epithelium and primordial germ-cells. In that case, in these chick embryos fixed after an incubation of four and one-half days, one would expect to see certain of the epithelial cells with the large attraction-sphere which is so constant and characteristic of the primordial germ-cells. Also, it would naturally be expected that some of them should possess yolk granules, and the large vesicular nucleus and typical chromatin arrangement, while yet possessing the moderate size and cuboidal shape, which is typical of the epithelial cells. On the contrary, however, there seem to be no cells present which could in any way be classed as intermediate between the germinal epithelial cells and the true primordial germ-cells. They are all either germ-cells or epithelial cells, and, as far as the four and one-half day chick is concerned, one is forced to the conclusion, either that the germ-cells in the germinal epithelium do not arise in situ but reach that position through tissue growth or migration, or, if any have arisen there through a transformation, it occurred at an earlier stage. All recent investigation seems to favor the former alternative.

In chick embryos possessing 31 to 33 pairs of somites, the gonad has not commenced to develop, nor has the germinal epithelium been differentiated, as yet, from the coelomic epithelium. At this stage the primordial germ-cells are found in the root of the forming mesentery, in the coelomic epithelium, in the neighborhood of the coelomic angle, and, in the medial and posterior

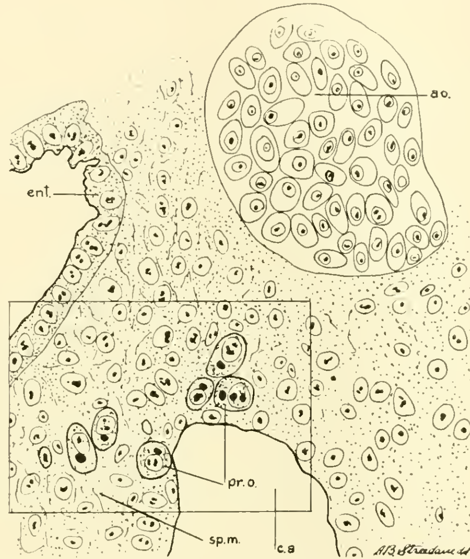


Fig. 1 Portion of a transverse section through the twenty-second somite of a 33 somite embryo; same embryo as figure 2, for which this figure is the key-plate. Bensley's acetic-osmic-bichromate fixation and Bensley's anilin acid fuchsin-Wright's stain. × 120. This figure is intended to give an idea as to the usual position of the primordial germ-cells in the 33 somite chick. *pr.o.*, primordial germ-cells; *ent.*, entoderm; *sp.m.*, splanchnic plate of mesoderm; *ao.*, aorta; *c.a.* coelomic angle.

region of the splanchnic plate of mesoderm, just where it is passing into the developing mesentery (fig. 1). The vast majority of the germ-cells are found in these regions, but an occasional one is located elsewhere; far out in the splanchnic mesoderm, and at times in a more dorsal situation in the loose tissue around the aortae.

The primordial germ-cells, at the stage of 31 to 33 somites, are frequenty found in groups of three or four, and are typical germ-cells in all respects (fig. 2). Only two facts relating to them are worthy of special attention. The yolk material found in them is subject to great variation and is undergoing some change, which is evidenced by the varying reaction to the osmic acid and stain. In some germ-cells the yolk is black, in others brown or yellow, while in others it is stained by the dye used rather than the fixing agent. In some cases all these changes are shown in a single germ-cell. The yolk material is probably being used up in cell metabolism, or being transported elsewhere.

The amoeboid appearance of the primordial germ-cells at this stage is also worthy of mention. This property was not evident in the germ-cells of the four and one-half day chick, owing to the fact that active migration had ceased. However, the germ-cells at 31 to 33 somites are highly amoeboid. Nearly all possess an oval shape, while many are drawn out into a tapering process, which extends between the somatic cells. This process is single and is usually on the side of the cell opposite to the attraction-sphere.

Embryos with 29 and 26 pairs of somites may be classed together, since there is no difference worthy of mention as far as the position of the germ-cells is concerned. In these embryos of this age the primordial germ-cells are found in the splanchnic mesoderm at no great distance from the point where splanchnopleure and somatopleure become continuous, that is, near the coelomic angle. In the embryo possessing 29 somites 110 germ-cells were counted. Of this number the vast majority (98) were found to be in the splanchnic mesoderm while only 12 were counted in the somatopleuric side of the angle of the coelom.

The primordial germ-cells in both these embryos are present either in the mesodermal portion of the splanchnopleure or between the mesoderm and entoderm. In not a single instance is one to be seen in the entoderm. The germ-cells are usually found singly, although, as was mentioned before, they may occur in groups of two to four. They are all situated behind the level of the 20th mesoblastic somite.

The next embryo to be studied possesses 25 pairs of somites and an approximate age of forty-four hours.

Down to this point the situation in which the primordial germ-cells are found agrees with that given by all who have done any investigation on this subject. Hoffman ('93) and, in fact, all who have worked upon the subject of primordial germ-cell history in the chick, have described the indifferent gonad and the presence of the sex-cells in the splanchnic mesoderm. No one,

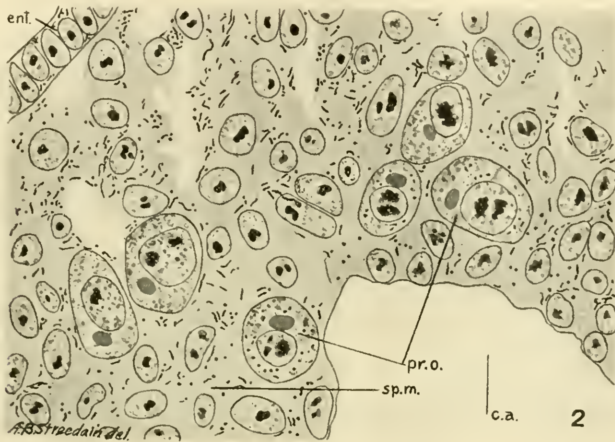


Fig. 2 Portion of a transverse section through the twenty-second somite of a 33 somite embryo; this figure is drawn from the same embryo as figure 1. Bensley's acetic-osmic-bichromate fixation and Bensley's anilin acid fuchsin-Wright's stain. × 787. This figure shows the position, form and grouping of the primordial germ-cells in the 33 somite chick embryo. *pr.o.*, primordial germ-cells; *ent.*, entoderm; *sp.m.*, splanchnic plate of mesoderm; *c.a.*, coelomic angle.

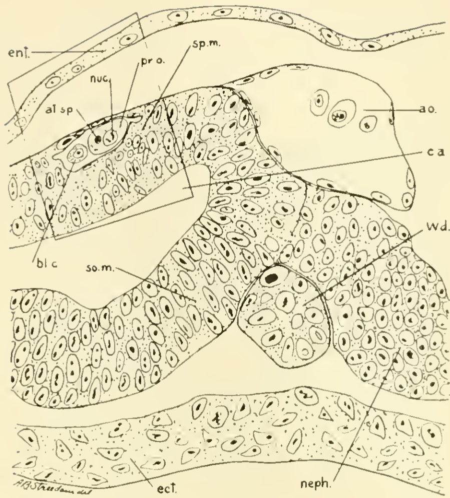
however, has been able to trace them back to a stage earlier than that of 22 somites. This inability to find them in younger embryos is surprising but nevertheless can be explained. The students of germ-cell history and migration in the chick have, no doubt, had constantly in mind the work of Beard, Allen, Woods and Jarvis, who investigated this question in forms, in which a definite migration occurred through the entoderm or splanchnic mesoderm. They have expected this same kind of migration to

occur in the chick, and, therefore, have studied with care the entoderm and splanchnic mesoderm, and in so doing have neglected the key to the situation, the blood vessels.

The embryo with 25 somites may be called a transition stage, for the primordial germ-cells are present both in the blood vessels (figs. 3 and 4) and in the splanchnic mesoderm. The embryo with 21 somites may also be placed in this category. Were it not for the presence of primordial germ-cells in the blood vessels, this embryo with 25 somites could be passed over rapidly, because the position of those in the tissues coincides exactly with their situation in the older embryos. That is to say, they are found in the splanchnic mesoderm near its junction with the somatic mesoderm at the coelomic angle.

In the blood vessels the primordial germ-cells are found in two situations, in the small vessels of the medial portion of the splanchnic mesoderm (figs. 3 and 4) caudad to the 19th somite in certain vessels of the head (fig. 5).

The primordial germ-cells found in the latter situation—that is, in the vessels of the head region of this 25 somite embryo—are remarkably disposed. Twelve embryos of about this age—between 22 and 29 somites—were examined, yet in only one, that possessing 25 somites, were the germ-cells massed. And, indeed, in no embryo studied was a like condition encountered. The arrangement, then, of the germ-cells is probably abnormal, but nevertheless, merits a description. The germ cells are present in the radicles of the anterior cardinal veins (fig. 5). In this region, as was mentioned before, the germ cells are not found singly as in the vessels of the splanchnic mesoderm, but in masses or lumps. These masses are four in number; a large group on either side of the forebrain and two smaller groups. These collections of germ-cells seem to entirely occlude the vessels in which they are contained and remind one of emboli (fig. 5). Mixed in with the germ-cells are the smaller blood cells (fig. 5). In one of the large groups four of the germ-cells are dividing; this presence of mitotic figures is worthy of notice, since in only one primordial germ-cell (fig. 10), these excepted, was division observed, from their origin until they pass into the gonad. To



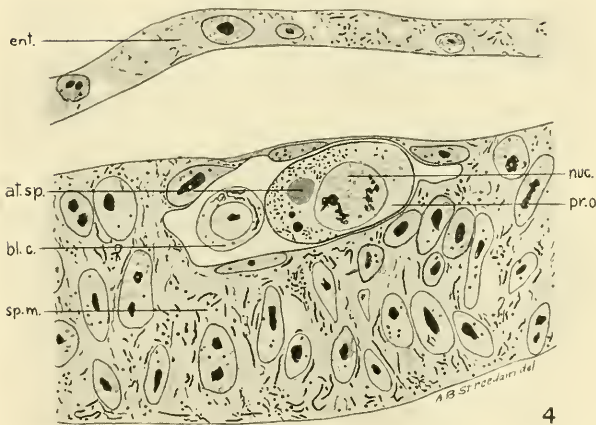


Fig. 3 Portion of a transverse section through the twentieth somite of a 25 somite embryo; same embryo as figure 4, for which this figure is the key-plate. Meves' fixation and Meves' iron-hematoxylin stain. × 120. *pro.*, primordial germ-cell in small blood vessel of splanchnic mesoderm; *nuc.*, nucleus of primordial germ-cell; *at.sp.*, attraction-sphere of primordial germ-cell; *bl.c.*, blood cell in blood vessel; *sp.m.*, splanchnic layer of mesoderm; *so.m.*, somatic layer of mesoderm; *ao.*, aorta; *ent.*, entoderm; *ect.*, ectoderm; *Wd.*, Wolffian duct; *neph.*, nephrotome.

Fig. 4 Portion of a transverse section through the twentieth somite of a 25 somite embryo; this figure is drawn from the same embryo as figure 3 and is a higher magnification of the marked off region. Meves' fixation and Meves' iron-hematoxylin stain. × 1125. *pr.o.*, primordial germ-cell; *nuc.*, nucleus of germ-cell; *at.sp.*, attraction-sphere of germ-cell containing a centrosome; *bl.c.*, blood-cell; *ent.*, entoderm; *sp.m.*, splanchnic mesoderm.

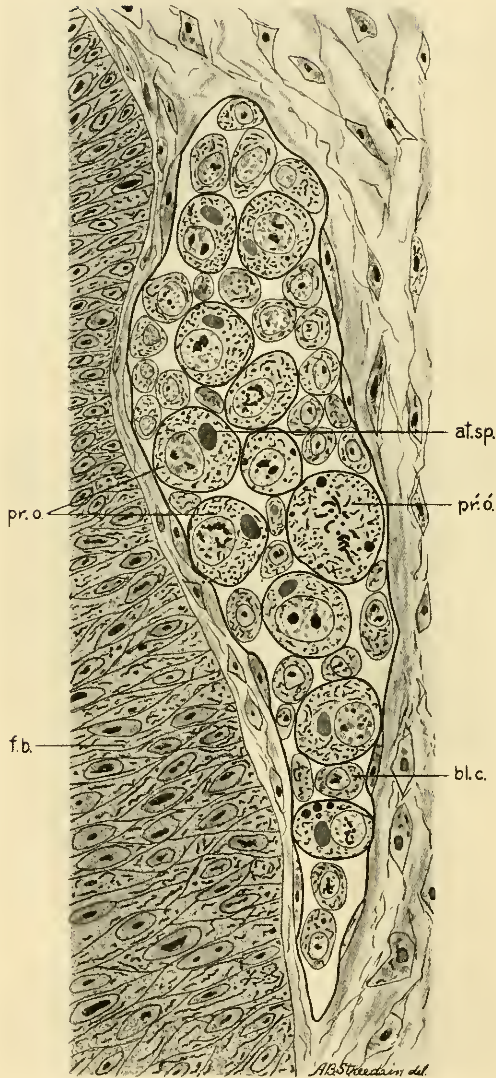


Fig. 5 Portion of a transverse section through the head region of an embryo with 25 somites. Meves' fixation and Meves' iron-hematoxylin stain. × 787. *pr.o.*, primordial germ-cell; *pr.o'.*, primordial germ-cell dividing; *at.sp.*, attraction-sphere of germ-cell; *bl.c.*, blood cell; *f.b.*, fore-brain.

repeat, then, this massing of the primordial germ-cells in the vessels is something out of the ordinary for no evidence of it is to be seen in either older or younger embryos.

The largest mass (fig. 5) in proximity to the fore brain contains 35 germ-cells and extends through a dozen 4 micra sections.

In the former situation in this embryo—that is in the splanchnic mesoderm—there are 56 germ-cells in all. These are distributed as follows—48 in the tissues and 8 in the vessels. The cells in the blood vessels are in some cases larger than average caliber of the vessel so that its walls are pushed out noticeably on either side (fig. 4).

As regards the number of primordial germ-cells found in the embryos, in which they are present in the blood vessels, it may be said here that the figures given are only approximately correct. This applies to embryos with 25 somites, and especially to those which are younger, for in the latter, as will be seen, the germ-cells are found in the larger blood vessels, and the loss due to staining and dehydrating is greater than in the case of the older embryos, in which the primordial germ-cells are present in the smaller vessels only.

That the large cells within the blood vessels are primordial germ-cells, and that they pass out into the tissues, there can be no doubt. In the first place, they are identical in appearance with the germ-cells described in the splanchnic mesoderm of older embryos (compare figs. 2 and 4). This similarity is best observed in the embryo of 25 somites and the next younger one studied, which possesses 21 somites. In these two embryos, which have been called transition stages, because the primordial germ-cells are in the vessels as well as in the tissues, in neighboring sections germ-cells may be observed in both situations (figs. 7, 8, 9 and 11). Thus, it will be noted that the germ-cells in both situations have the same size and shape (figs. 8 and 9). The mitochondria are similar, nor is there any nuclear difference. Cells within and without the vessels have the same amount of yolk material and the same conspicuous attraction-sphere (figs. 8 and 9).

Add to this similarity the facts (1) that, in embryos younger than 21 somites these large characteristics cells are all in the vessels; (2) that in embryos with 21 and 25 somites respectively they are in the tissues as well as in the vessels (figs. 4, 6, 7, 8 and 10); and (3) that, in embryos older than this they are found in the tissues of the splanchnic mesoderm alone; so that there can be no question as to the fact that the large, characteristic cells in the blood vessels are primordial germ-cells and are identical with the germ-cells found in the splanchnic mesoderm.

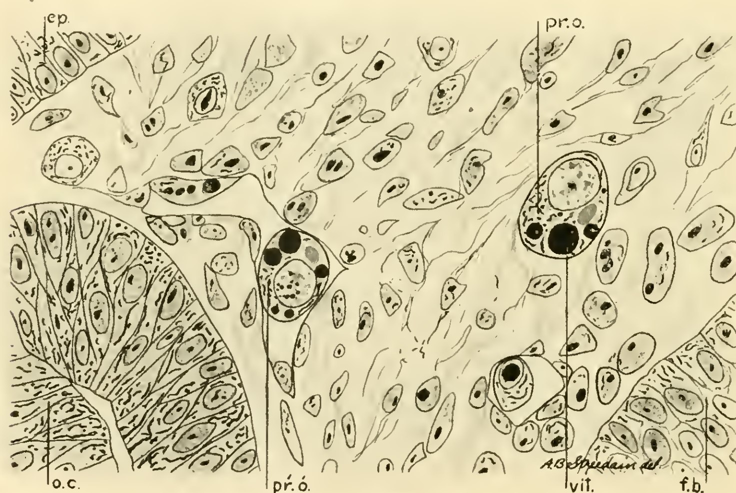


Fig. 6 Portion of a transverse section through the head region of a 21 somite embryo. Meves' fixation and Meves' iron-hematoxylin stain. × 787. *pr.o.*, primordial germ-cell near fore-brain; *pr'.o'.*, primordial germ-cell in a blood vessel near optic cup; *vit.*, yolk sphere; *f.b.*, fore-brain; *o.c.*, optic cup; *ep.*, epidermis.

In the embryo with 21 mesoblastic somites there are present altogether 73 primordial germ-cells. Of these, 13 are outside the blood-vessels, nearly all in the splanchnic mesoderm, and 60 are contained within vessels throughout the embryos and area vasculosa. Of these 60, in the vessels of the splanchnic mesoderm, there are 30, while in vessels of the head region 25 are to be seen (fig. 6). The distribution of the germ-cells in the splanchnic mesoderm is of interest in that all except two or three are caudad

to the 18th somite. This applies to cells both in and without the vessels. In the splanchnic mesoderm region of this embryo two cells are present which are partly within and partly without the vessel (fig. 11).

In regard to the embryos with 25 and 21 somites several interesting facts should be recapitulated. In both, germ-cells are both within and without the blood vessels. In the younger embryo the cells in the vessels are more numerous, while in the older embryo this state of affairs is reversed. Also, let it be remembered that all investigators hitherto have failed to find the germ-cells in chick embryos with less than 22 somites.

In one series of an embryo with 19 mesoblastic somites there are present in all 82 primordial germ-cells. The distribution of these is markedly different from anything described hitherto. All, with the exception of 6, are in the blood-vessels. They are found in the large (fig. 13) as well as small channels both of the area vasculosa and embryo proper. They are present in the heart and aortae as well as in the capillaries. They are never found in groups and in only one case could two be seen in a single field.

The germ-cells in this embryo as well as those still younger are characterized by an immense amount of yolk (figs. 12 and 13). This yolk material consists of spheres, having a diameter of 2–4 micra, and is massed principally in that portion of the cell in which is located the attraction-sphere. However, this yolk is not confined only to the pole of the cell occupied by the attraction-sphere, but usually surrounds the nucleus completely (fig. 12). This yolk is so abundant, that in preparations fixed with a fluid containing osmic acid, the other cell contents are seriously masked.

In embryos of this age the primordial germ-cells possess, in nearly all cases, amoeboid processes.

The stages of 16, 12 and 10 somites respectively may be passed over rapidly since they present nothing essentially different from the 19 somite embryo as far as either the distribution or form of the germ-cells is concerned. Thus, the latter are found in the area vasculosa and in the developing vascular structures of the embryo proper. In the embryo possessing 12 somites, one germ-

cell in particular is found in the heart. It must have moved in through the vessels by its own amoeboid properties, since the heart, in an embryo of this age, has not as yet begun to propel the blood. In fact this cell has a marked amoeboid appearance.

In embryos with 9 and 6 somites respectively the primordial germ-cells are all anterior and antero-lateral in respect to the embryo proper. Most of the germ-cells are present in the space between germ-wall entoderm and mesoderm. The few present in the mesodermal spaces have reached that position by migration from the former position. Some are present in the space between ectoderm and mesoderm; they have either passed through the mesoderm from the space between entoderm and mesoderm or have been left in that position by the outgrowth of mesoderm.

These germ-cells at this age have the large attraction-sphere so often referred to, as well as the abundant yolk. The nucleus is not so large as in older germ-cells and the chromatin does not exist in the two definite masses. Instead it is more evenly scattered throughout the nucleus and in some is reticular in appearance.

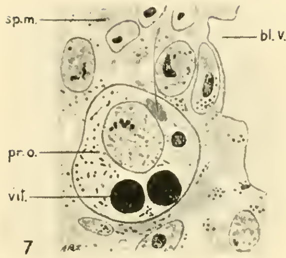
The germ-cells are very conspicuous, as, either isolated or in groups of two or three, they lie in the space between entoderm and mesoderm.

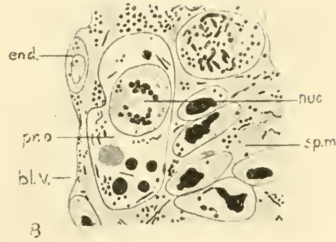
Fig. 7 Portion of a transverse section through a 21 somite chick embryo at the level of the 20th somite. Meves' fixation and Meves' iron-hematoxylin stain. × 1125. *pr.o.*, primordial germ-cell; *vit.*, sphere of vitellus or yolk material; *sp.m.*, splanchnic mesoderm; *bl.v.*, lumen of blood vessel.

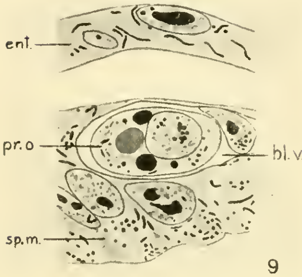
Fig. 8 Portion of a transverse section through a 21 somite chick at the level of of the 18th somite. Meves' fixation and Meves' iron-hematoxylin stain. × 1125. *pr.o.*, primordial germ-cell; *nuc.*, nucleus of primordial germ-cell; *sp.m.*, splanchnic mesoderm; *bl.v.*, lumen of blood vessel; *end.*, nucleus of endothelial cell.

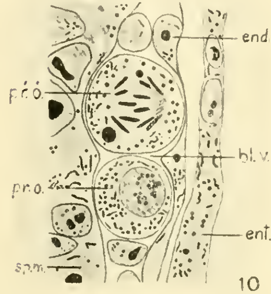
Fig. 9 Portion of a transverse section through a 21 somite chick embryo at the level of the 21st somite. Meves' fixation and Meves' iron-hematoxylin stain. × 1125. *pr.o.*, primordial germ-cell; *sp.m.*, splanchnic mesoderm; *bl.v.*, lumen of blood vessel; *ent.*, entoderm.

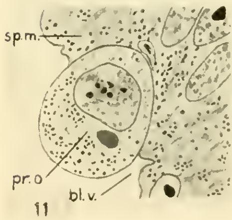
Fig. 10 Portion of a transverse section through a 21 somite chick embryo at the level of the 19th somite. Meves' fixation and Meves' iron-hematoxylin stain. × 1125. *pr.o.*, primordial germ-cell; *pr.'o.'*, primordial germ-cell dividing; *sp.m.*, splanchnic mesoderm; *bl.v.*, lumen of blood vessel; *end.*, nucleus of endothelial cell; *ent.*, entoderm.











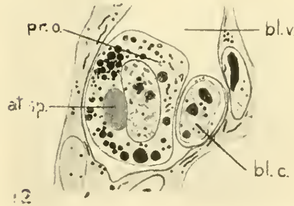


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Fig. 11 Portion of transverse section through a 21 somite chick embryo. Meves' fixation and Meves' iron-hematoxylin stain. × 1125. *pr.o.*, primordial germ-cell; *sp.m.*, splanchnic mesoderm; *bl.v.*, lumen of blood vessel.

Fig. 12 Portion of a transverse section through a 19 somite chick embryo. Meves' fixation and Meves' iron-hematoxylin stain. × 1125. *pr.o.*, primordial germ-cell; *bl.c.*, blood cell; *bl.v.*, lumen of blood vessel.

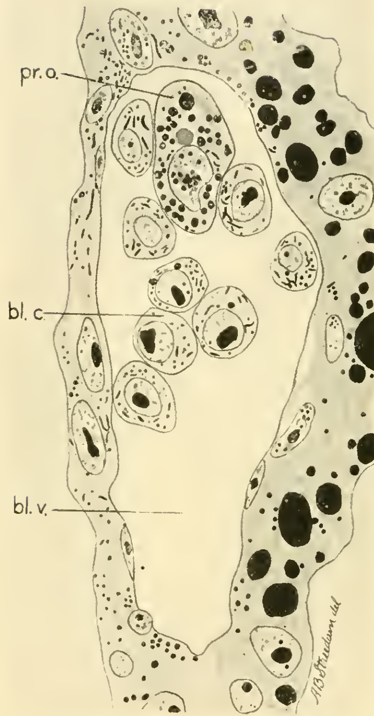

Fig. 13 Portion of a transverse section through a 19 somite chick embryo. Meves' fixation and Meves' iron-hematoxylin stain. × 1125. *pr.o.*, primordial germ-cell; *at.sp.*, attraction-sphere of germ-cell; *bl.c.*, blood cell; *bl.v.*, lumen of blood vessel.

3. Origin of the germ-cells

Dantschakoff ('08) has described certain cells in the chick embryo, which arise anterior and lateral to the primitive streak and young embryo. They originate, she states, from the margin of the germ-wall entoderm. They begin to appear during the primitive streak stage and continue to be produced until the embryo has 3 pairs of somites. They are large, have a large round nucleus and an abundance of yolk, and are especially characterized by their amoeboid properties. Because of their amoeboid

tendencies she called them entodermal wander-cells. They appear at first in groups, between the germ wall entoderm and ectoderm, anterior to the forming embryo, before mesodermal tissue has grown out into that region. They then enter the mesoderm and forming blood-vessels, and, either by their own amoeboid powers, or aided by the vascular circulation penetrate into every part of the area vasculosa and embryo. Although for some time in the vascular system yet they are not blood cells as is evidenced by their appearance, origin and fate. Some of these entodermal wander-cells enter the embryonic tissues, leaving the blood-vessels by their power of diapedesis, and degenerate. Others undergo a like fate in the blood-vessels themselves. The remainder disappear from the blood-vessels and by the time the embryo has 22 somites all have gone. Although the fate of those which do not degenerate is unknown, yet Mlle. Dantschakoff believes that they have no share in tissue formation.

These entodermal wander-cells of Dantschakoff are in reality the primordial germ-cells of the chick. There is a close agreement as far as origin is concerned, for I also find that the germ-cells originate from certain cells of the germ-wall entoderm near its junction with the area pellucida. I find also that they are produced during the primitive streak stage and in the embryo possessing at least 3 somites.

These germ-cells arise in a region of the germ-wall anterior to the forming embryo and also antero-lateral. This region, just at the junction of area pellucida and germ-wall, has roughly the shape of a crescent, the concavity being turned towards the embryo; the horns extend caudalward on either side (fig. 15). In this crescent shaped region many of the germ-wall entodermal cells resemble the germ-cells (fig. 14). They have the round nucleus, great quantity of yolk and the large conspicuous attraction-sphere.

That these particular germ-wall entodermal cells are producing the primordial germ-cells, is proven by the fact that in the primitive streak stage, before the appearance of mesoderm anterior to the embryo, the germ-cells are grouped in the space between entoderm and ectoderm in this immediate neighborhood; the

germ-cells cytologically are very similar to the cells of the germ-wall near its border (fig. 14); mitoses are also seen in these cells (fig. 14).

When the mesoderm reaches this region anterior to the embryo, which it does relatively late, the germ-cells enter the mesoderm and the forming blood-vessels.

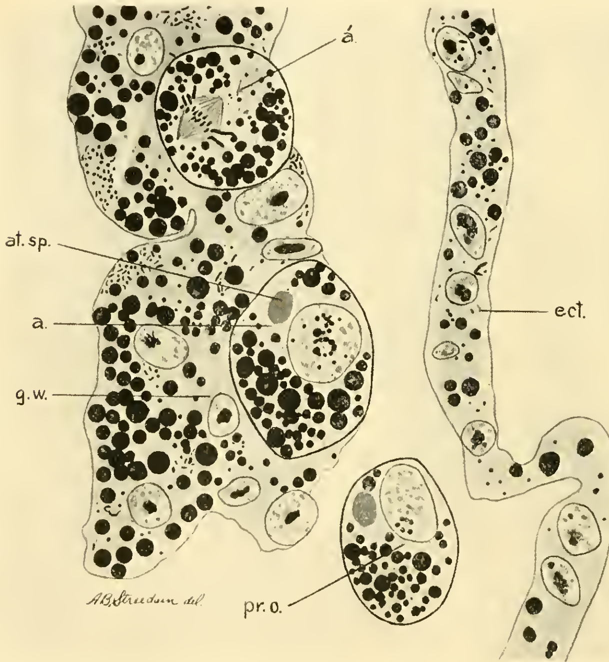

Fig. 14 Portion of a transverse section through an embryo of primitive streak stage development. Meves' fixation and Meves' iron-hematoxylin stain. × 1125. *pr.o.*, primordial germ-cell; *a.*, cell of germ-wall, which resembles primordial germ-cell; *a'.*, cell of germ-wall dividing; *at.sp.*, attraction-sphere of germ-wall cell; *g.w.*, germ-wall entoderm; *ect.*, ectoderm.

As regards the history of the entodermal wander-cells in the blood there is also a close agreement with that of the germ-cells. However, a majority of the germ-cells do not degenerate in the blood stream or in the tissues. Only a few of the germ-cells leave the vessels before a stage of 21 somites is reached and only a few degenerate.

The statement made by Dantschakoff, that the entodermal wander-cells leave the blood entirely by the time the embryo has 22 somites, is of interest in view of the fact that at that stage the

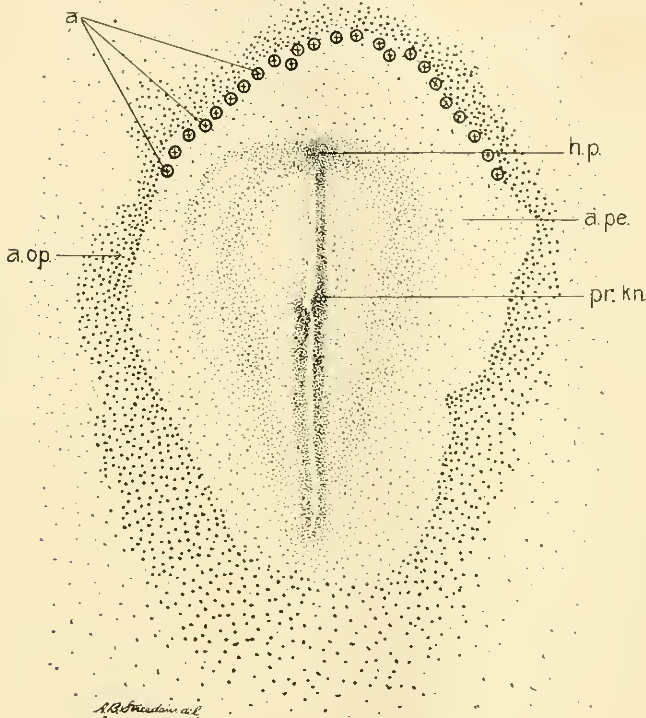

Fig. 15 Surface view of a primitive streak stage of a chick embryo, viewed by transmitted light (semi-diagrammatic). Zeiss ocular 2, Zeiss objective A_3. This figure is intended to show the point at which the primordial germ-cells arise. The circles *a*, at the junction of germ wall and area pellucida, indicate this region. *a.*, region in which the primordial germ-cells arise; *h.p.*, head process; *pr.kn.*, primitive knot; *a.op.*, area opaca; *a.pe.*, area pellucida.

germ-cells are leaving the vessels of the splanchnic mesoderm and passing into the tissues of the splanchnic mesoderm near the coelomic angle.

SUMMARY AND CONCLUSIONS

1. The primordial germ-cells arise anterior and antero-lateral to the embryo in a specialized region of germ-wall entoderm just at the margin of the area pellucida. This region has roughly the shape of a crescent and the germ-cells arise during the primitive streak stage and until the embryo has about 3 somites. The concavity of this crescent is towards the embryo and the horns extend caudalward on either side.

2. Owing to the late appearance of mesoderm in this region, the primordial germ-cells at first are in the space between entoderm and ectoderm. Later, thanks to their amoeboid power, they enter the mesoderm and the forming blood vessels of the mesoderm.

3. They are at first carried by their own movement, and later by that of the blood to all parts of the embryo and vascular area. They remain generally distributed in this way until the embryo has about 20 somites.

4. In embryos with about 20 to 22 pairs of somites, the primordial germ-cells, while generally distributed throughout the embryo in the blood-vessels, are becoming relatively more numerous in the vessels of the splanchnic mesoderm. This increase in the number of the germ-cells in the vessels of the splanchic mesoderm may be in part only apparent, that is, a degeneration of some may have occurred elsewhere, or, it may be a real increase due to some influence, probably of a chemotactic nature, exerted in the region of the future gonad. At this period the great majority of the cells are found in the vessels, but a few, chiefly in the splanchnic mesoderm, are present in the tissues. In some cases they are present in the wall of the vessel, as if fixed in the act of leaving the vessel for the tissues (fig. 11).

5. In embryos with about 23 to 25 pairs of somites the majority of the primordial germ-cells are found in the mesodermal tissue of the splanchnic mesoderm near the angle of the coelom. A few cells are still present in the blood-vessels; at the 25th somite stage, in one embryo, in addition to the few found in the vessels of the splanchnic mesoderm, four masses of germ-cells are in the

vessels of the head. This is an abnormal condition, since the germ-cells in all the other embryos studied, are never grouped in this way. The embryo with 25 somites is the oldest in which germ-cells are found in the vessels.

The embryo with about 22 to 23 segments is the youngest in which the primordial germ-cells have hitherto been described in the bird. Nussbaum ('80) and Keibel and Abraham ('00) in the chick, and Hoffmann ('93) in Gallinula, Sterna and Haematopus described the germ-cells at 23 somites in the splanchnopleure.

6. In embryos possessing about 26 to 29 somites the primordial germ-cells are found in the splanchnic mesoderm near the radix mesenterii.

7. In embryos with 30 to 33 somites the primordial germ-cells are in the radix mesenterii and coelomic epithelium on both sides of the coelomic angle. They remain in this position until the formation of the gonad begins when they gradually pass into that organ.

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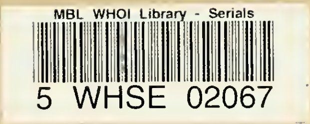
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