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Dissertation

THE BEHAVIOR OF ADRENOCORTICAL TRANSPLANTS IN THE RAT UNDER THE INFLUENCE OF INGESTED SODIUM AND POTASSIUM SALTS

by

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Vous enfin, délégués des nations étrangères, qui êtes venus de si loin donner une preuve de sympathie à la France, vous m'apportez la joie la plus profonde que puisse éprouver un homme qui croit invinciblement que la science et la paix triompheront de l'ignorance et de la guerre, que les peuples s'entendront, non pour détruire, mais pour édifier, et que l'avenir appartiendra à ceux qui auront le plus fait pour l'humanité souffrante.

Louis Pasteur

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I. THE PRESENT STATUS OF ADRENAL TRANSPLANTATION

1. Introduction

Adrenal transplantation, like the grafting of any other tissue, may serve two purposes which actually are not strictly separate since one leads to the other: the transplantation may be an experimental or a therapeutic one.

Ever since Canalis' performance of the first adrenal autografting in 1887 in animals and Jabulay's attempt in 1897 to employ heterotransplants in the treatment of cortical insufficiency, a vast amount of work has been done with adrenal transplantation and a definite increase of knowledge has been derived from it even if there has been little to no conspicuous advance in its therapeutic application.

The therapeutic application will always remain the ultimate goal of any transplantation research. This goal has to be kept in mind in spite of set-backs and of a justified scepticism as to the results obtained. Nor should this goal be forgotten in an era of chemotherapy with synthetic substitutional agents which represent a true and great advance. However, the administration of exogenous synthetic or extract material in endocrine deficiency states is far from ideal, even if the preparations now available become less expensive. The brilliant successes

of insulin and cortical preparations should in no way be belittled, but it should be pointed out that they definitely are two-edged weapons, require continued administration and supervision and still entail considerable expense and inconvenience. The reestablishment of a permanently functioning gland that could meet the needs of the patient's bodily economy would be close to the ideal state, the normal gland. The outspoken unsuitability of pancreatic tissue for any kind of grafting and the lack of therapeutic success in the case of adrenal transplantation have shattered the hopes of the profession for ever attaining the goal of successful therapeutic application. This, however, does not justify the abandonment of transplantation research. On the contrary, it will, now more than ever, mean that the whole field of endocrine transplantation will have to be revised and put on a sound physiological basis.

The older literature about organ transplantation in general and with reference to the adrenals has been reviewed by Knauer (180).*

The therapeutic transplantation of adrenal tissue will be considered separately in Appendix D.

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* The numbers refer to the bibliography at the end of the dissertation.

2. Experimental adrenal transplantation

Since the turn of the century experimental pathologists have shown a great interest in adrenal transplantation as a means of investigating the etiology of tumors. Inspired by Grawitz's and Cohnheim's ideas about the genesis of neoplasms in general and adrenal tumors in particular, it was attempted to produce new adrenal growths by stalked transplants in dogs, cats, and rabbits (105, 106, 108, 283) and by free transplantation in rabbits, guinea pigs, and rats (324, 178, 219, 258, 259). This was done in order to reproduce a situation which was believed to exist in a tumor-bearing organism.

At this point a general remark about free and stalked grafts should be made.

The stalked grafts as they were frequently performed in dogs, rabbits, and cats (75, 105-108, 225, 283) are not true transplants. They possess their intact vascular supply as do the frequently employed skin flaps in plastic surgery. The 'transplants' usually were placed into the kidneys. This type of procedure is merely a dislocation, a pseudotransplantation. It is not surprising that such pseudotransplants have a good chance for lasting survival and functioning of cortex and medulla.

It occurs now and then in the process of nephrectomy for whatever reason it is performed (acute suppuration of the

kidney, chronic pyelonephritis, pyonephrosis, infected hydronephrosis, tuberculosis, neoplasms) that an adrenal dislocation and fixation somewhere else is necessary in order to separate the adrenal from the kidney and preserve the former. However, under normal topographic-anatomic conditions such a displacement is not necessary in nephrectomy.

Adrenal pseudotransplantation shall not be considered in this paper unless specifically mentioned. Likewise, procedures such as that of leaving the gland in its normal bed after demedullating it or ligating its blood supply (358) will not be discussed. Furthermore, some work (4) is so inadequate in planning and execution that it will not be included.

The topic of this dissertation is the free adrenal transplant , that is, the completely separated adrenal tissue, freed from its surroundings, without intact vascular supply, and implanted in a new environment of the same or of a different organism. Such a transplant has to carry a double load. (a) It must adapt itself to the new environment, reestablish the nutritional supply, regenerate to a normal morphologic and functional condition and regain its link in metabolic chains. (b) At the same time, it has to provide the specific secretions essential for the functional integrity of the organism. It is obvious that this heavy task will frequently not be fulfilled by a transplant but will result in complete exhaustion of the grafted tissue. The factors

facilitating or opposing a successful transplantation, therefore, deserve attention and discussion.

However, before such a discussion is undertaken, the use of adrenal transplantation as a technique for physiological investigations of various kinds should be mentioned. Many investigators, particularly Wyman, Ingle and their coworkers, have used adrenal grafts in order to explore cortical and medullary function. Others were primarily interested in general problems of tissue reactions, growth and regeneration.

The following problems were approached by this method: the relation of the adrenal to the susceptibility of the organism to histamine (337,344, 345, 353), anaphylactic shock (339), resistance (162), blood volume (343), blood NPN and urea (357), blood sugar level (349, 356), temperature regulation (342, 348), arterial lesions (187), spontaneous activity (242), mineral metabolism (247), early sex development (220), the mechanism of vascular responses to adrenalin and of adrenalin intoxication (346, 350, 354), adrenalin-induced lens opacities (312).

Moreover, the host-donor tissue compatibility and the differentials of individuality, organs, and species (191-194) were extensively investigated by means of transplantation of adrenal tissue among others. The influence of ultracentrifugation upon specialized tissues and their viability and

and functional capacity as grafts were also investigated with adrenal transplantation (77, 78).

3. Factors influencing the fate of adrenal transplants

Much of the work done with adrenal transplantation is not conclusive because the methods employed do not meet critical analysis as to their optimal suitability for transplantation. Moreover, various investigators differ widely concerning the criteria of successful grafting. In many cases, a graft which looked somewhat like a normal gland was called a "take", or a survival of the animal for a few weeks or even days has sometimes been considered a proof that the transplant took and functioned. Conclusions of this kind are, of course, fallacious.

It is obvious that no method of grafting with observation of all possible precautions can be expected to be completely successful. This holds true even for tissues of rather low specialization and is particularly true for complicated, fragile, highly differentiated endocrine tissue.

(1) Operative skill and technique, strict asepsis, most careful handling of the transplanted tissue, optimal postoperative care, - all of these factors in relation to autoplastic subcutaneous transplantation in the rat will be described below under "Methods".

One or two-stage operative methods involving adrenalectomy

and auto- or homotransplantation have been employed successfully. If a two-stage procedure is done (e.g. unilateral adrenalectomy and transplantation in the first session and removal of the remaining adrenal at the second stage), not too long a period of time should elapse before the final operation. If one waits too long, the remaining gland will undergo hypertrophy and suppress any attempt of the transplant to take. With a waiting period of one or several months (178), not much success can be expected.

(2) <u>Type and state of the tissue to be transplanted</u>. It has been mentioned already that the differentiation of the tissue has a bearing on the chances of taking. The transfusion of blood (essentially a transplantation of the tissue, blood) has a great expectancy for success with a minimum sacrifice of tissue elements. Skin and cartilage can be transplanted far better than endocrine tissue. The transplantation of epidermis is an example of one of the most successful grafting procedures.

The health and freshness of material (for homotransplantation) is essential. Tissue stored at low temperature soon loses its capacity for regeneration. Autopsy material for direct transplantation or for preliminary tissue culture has to be obtained without delay and under strict aseptic precautions.

As for the adrenal, the presence of adrenocapsular cells and auto- or homotransplantation have been employed successfully. If a two-stage procedure is done (e.g. unilateral adrenalectomy and transplantation in the first session and removal of the remaining adrenal at the second stage), not too long a period of time should elapse before the final operation. If one waits too long, the remaining gland will undergo hypertrophy and suppress any attempt of the transplant to take. With a waiting period of one or several months (178), not much success can be expected.

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As for the adrenal, the presence of adrenomedullar cells in the grafted tissue is imperative (148). The transplantation of the capsule alone gives as good results as that of the entire gland (146). Decapsulated transplants have little if any chance to take (356). The enucleated portion (medulla, reticularis and fasciculata) does not regenerate (146).

As a related consideration, the well-established life cycle of the cortical cell (129, 260, 362, 367) should be stressed. The number of mitotic figures in the marginal portion, and the presence of phagocytic, macrophagic, granulocytic, lymphocytic and monocytic elements in the inner part of the cortex suggest the development of the cortical cells in or near the capsule , with a subsequent gradual inward movement, eventual physiological destruction in the innermost zone and removal of the debris by the scavenger cells. The "light" and "dark" cells of earlier investigators proved to be early stages of degenerating cells.

This inward-growth of indifferent capsular cells, the continuity of cortical cell types, and a life cycle of about 20-23 days have been traced in various species of animals by means of trypan blue injections. The speed of this inward movement with the final disposal of the senescent cells reflect the demands for cortical hormones imposed by body needs. Toxins and poisons in not too large doses have been shown to accelerate the formation of cells from the parent layer and the destruction in the inner zone.

It has to be emphasized that this development of the cortical cell from the capsule with subsequent migration towards the center takes place in the auto- and homotransplanted cortex (22, 314) just as it does in the normal gland.

The gradual inward differentiation of cortical cells makes the conventional division into zona glomerulosa, fasciculata and reticularis more a matter of convenience as to locating general cortical areas than of definite structural and functional significance. Recent histochemical studies (26, 27) led to a differentiation into presecretory, secretory, postsecretory and senescent zones in the cortex of the cat which do not coincide with the zoning in glomerulosa, fasciculata and reticularis. The hormone production is thought to be confined to the osmophil lipid vacuoles of the spongiocytes in the outer fasciculata. The hypothesis has been advanced (291) that the secretions of the outer cortical layer may undergo chemical changes, a "maturation", while passing down the cortical sinusoids toward the medulla.

(3) Size of transplant. As is implied in the definition of a free transplant , the tissue lacks proper nutrition after being grafted until an adequate blood supply is reestablished. During this time the transplanted tissue must live by processes of osmosis and diffusion. It is obvious that the chances for nutritive media to reach and supply a tissue, decrease with an increase of bulk of the tissue which has to

be penetrated . If a whole adrenal gland is grafted, the diffusion to the center is apparently not adequate and the highly differentiated inner cortical and medullary cells will not survive the vascular interruption. Moreover, the elimination of metabolic waste products of the cells will be impaired and may, conceivably, produce an additional damage to the tissue. Since the central parts of a graft die very quickly, it is of advantage to employ small amounts of tissue, fragments, halved glands, or spread-out cells from tissue cultures in order to facilitate an optimal nutrition.

The nutritional difficulties in transplants of whole gland may, at least in part, account for the failure of obtaining medullary takes. The frequency of persistence of medullary cells is greatly enhanced when the surrounding cortical layers are teased away before transplantation (314).

It is conceivable that the grafting of much surrounding connective and fatty tissue together with the glandular body may do more harm than good. It might be that the destruction and incorporation of this surrounding tissue with the accompanying cellular, reticulo-endothelial, and inflammatory reactions may damage or impede the proper graft. This is of little or no significance in the transplantations reported below by the author since the amount of periadrenal structures is not very voluminous; but it may have some importance in larger animals or with tissues other than the adrenal.

(4) Age of the graft or the donor. The age of the grafted tissue and with it the degree of a general cellular differentiation have a profound influence upon the chances for taking and of lasting preservation of the transplant. It is a well-established empirical fact that the younger the tissue or the donor the better the takes. In homotransplantation, embryonic tissues or those from fetuses or newborn organisms are by far superior to adult tissues (196, 258, 259).

The fetal cortex differs histologically from the adult cortex. Involutionary changes, a physiological degeneration, together with shrinking of the gland take place at birth with a subsequent change from the eosinophilic fetal to the basophilic adult type of cortex and the disappearance of the X zone. It may be that the withdrawal of the female sex hormone initiates these changes which eventually and gradually bring about the adult form of the cortical organ.

The recognition of the growth capacity of embryonal or fetal cortical tissue led to attempts to utilize the potentialities of the young tissue for experimental and therapeutic transplantation (see Appendix D, case 31). It is felt that the grafting of fetal structures is more promising and more likely to furnish a matrix for successful regenerative processes than is adult cortical tissue. The mitotic index has been shown to be greatest in embryonal cortices; it decreases with advancing age (32).

Various minced embryonic rat tissues have been transplanted into young rats, in whom the brain was alleged a particularly favorable site (331). A greater number of successful takes was obtained than with adult tissue. Among the successful transplants were a thyroid and a pituitary. Willis (331) regards this as a prospect for a possible application in surgery. It would not be too difficult to procure material: fresh sterile fetuses or embryos from clean miscarriages, ectopic pregnancies or from therapeutic abortions may be used as a source of tissue. Judged from the results of these experiments in the rat, tissues obtained from human embryos of about 6 to 12 weeks of age may be expected to give more successful results than those of older fetuses.

May (cited in 128) succeeded with embryonic thyroid, parathyroid and pituitary homotransplants while Parodi (cited in 40, 95) used embryonal cortical tissue for homotransplantation with subsequent survival and proliferation. Successful adrenal homotransplantation will be dealt with below.

(5) Age of the host in homotransplants. The fact that young animals in general afford to transplants a better chance to take than do older hosts may be due to the more developed defense mechanisms and "differentials" in the latter group. This will be discussed in point 7 of this chapter.

(6) Site of transplantation. All conceivable tissues and organs have been chosen as beds for cortical transplants,

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(6) Site of transplantation. All conceivable tissues and organs have been chosen as beds for cortical transplants,

and different groups of workers have recommended their sites as most suitable for transplantation. For all practical purposes it is essential that no damage is done to an important organ by implanting another tissue and traumatizing the former. The choice of the brain as the site of adrenal transplantation, for instance, is prohibitive.

The vascular conditions at the place of grafting are of significance for an adequate nutrition of the transplant before a direct new vascularization can be established. The vascularity of the bed should not be excessive, however, because a hematoma might choke the grafted tissue. This is demonstrated by the vascular state of the ovaries during estrus which interferes with transplantation while this site otherwise yields excellent results with adrenal transplants (231).

Furthermore, the proper site should guarantee the absence of undue pressure and strangulation by the surrounding tissue. It should also provide an enclosure which would eliminate the possibility of movement.

Last but not least, an easy access to the site of transplantation facilitates the operative procedures and thus prevents shock and other complications.

The author felt that the subcutaneous tissues of the rat fulfilled the requirements to a large degree and helped to

facilitate the establishment of a circulation to the grafted tissue.

Direct vascular anastomoses between graft and the circulation of the host have been attempted without success. v. Haberer (106) tried to perform a direct vascular connection between an implanted adrenal and the circulatory system of human corpses. He admitted that his technique did not lead to satisfactory results. Other workers (cited in 95) attempted to maintain a direct vascular connection to the graft but also failed since the transplants necrotized.

Failures in obtaining takes of whole glands have been attributed in part to irritating properties of the degenerating medulla leading to massive necrosis. Consequently, some workers were led to the practice of demedullating the adrenal before transplanting it. Several investigators noticed a peculiar and marked sensitivity of the subcutaneous and muscular tissues of the guinea pig to medullary tissue with the formation of edema and hemorrhage (81). Whether or not this was due to a local vasoconstriction with subsequent ischemia and reduced vascularization induced by the adrenalin content of the graft remained controversial; it also was suggested that some unknown substance present only in certain species might be inimical to the grafted tissue.

(7) <u>Genetic relationship in homo- and heterotransplantation</u>.

Aside from sensitization and allergic phenomena which may

arise from any foreign protein introduced into an organism and which may occur in transplantation, the degree of the genetic relationship between host and donor and its association with a more or less violent host reaction to the graft deserves attention. The closer this genetic relationship the smaller are the chances for a cytologic reaction to occur in the host tissues. Transplants in general, therefore, are more successful the closer the relationship between donor and recipient is. It has been shown that adrenal homotransplants are constantly more successful in maternal tissues than in the tissues of non-related rats (258). The studies of Loeb and his coworkers (191-194), employing transplantation of various tissues within and across species borders, led to the assumption of socalled differentials, factors which account for the compatibility and reactions between host and transplanted tissues.

Loeb pointed out that in young animals the defense mechanisms are not yet fully developed, and the differentials not yet coined, as is indicated by the less severe cytological reactions between recipient and donor tissue.

It could be shown that inbreeding of rats through successive brother-sister matings does not lead to a distinct diminution of the intensity of the reaction against homotransplanted tissue, That means, that in addition to organ specificities and individual tissue differentials there are individuality arise from any foreign protein introduced into an organism and which may occur in transplantation, the degree of the genetic relationship between host and donor and its association with a more or less violent host reaction to the graft deserves attention. The closer this genetic relationship the smaller are the chances for a cytologic reaction to occur in the host tissues. Transplants in general, therefore, are more successful the closer the relationship between donor and recipient is. It has been shown that adrenal homotransplants are constantly more successful in maternal tissues than in the tissues of non-related rats (256). The studies of Loeb and his coworkers (191-194), employing transplantation of various tissues within and across species borders, led to the assumption of socalled differentials, factors which account for the compatibility and reactions between host and transplanted tissues.

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The lymphocytic elements seem to be capable of detecting fine degrees of dissimilarity between donor and graft and, according to these workers, account for and initiate the antagonistic host reactions.

Thus the role of the genetic relationship in transplantation with its indirect influence on survival and function of the graft appears to emphasize the importance of the choice of the donor.

The significance of blood groups in relation to compatibility between donor and recipient tissues is still an unsettled question. Various workers in the field of therapeutic transplantation (see Appendix D) chose donors with a blood group compatible to that of the recipient whenever possible. On the other hand, Burhoe's work (cited in 153) seems to indicate that the blood grouping of donor and host does not influence the viability of certain tissue transplants.

It has been attempted to attenuate and modify the inherent organismal and tissue differences by a preliminary adaptation of the donor tissue to those of the host by first growing them in vitro (tissue culture) before the actual transplantation. Stone and coworkers (284-288) have done the most extensive work in this field. They made the endocrine tissues to be grafted thrive in the alien chemical environment of the new

host by means of planting them in tissue cultures on media containing serum and plasma of the future host for a period of two to four weeks. The proper tissue culture technique, such as the employment of small tissue fragments to guarantee an intimate contact with the medium, or the frequent change of media, seemed to be imperative. This adaptation to the recipient's blood elements to avoid foreign protein and antagonistic reactions was followed by the transplantation of thyroid and parathyroid tissue into dogs. The authors report successes and consider this procedure a great promise for a progress in transplantation. Their favorite site of transplantation is the loose tissue of the axillae or groins. They also tried parathyroid and thyroid homografting in humans after tissue culture preparation with apparently good results. However, they were not successful with adrenal tissue after attempting to separate cortical from medullary cells in tissue culture and then grafting them as homotransplants into dogs. The application of adrenal tissue from cultures to humans will be dealt with in Appendix D (see case 33 and 34).

The tissue culture method as an adaptation previous to transplantation has been employed with more or less success with different endocrine and nonendocrine tissues, e.g. for pituitary homografts in rats (124).

Lux et al. (200) concluded from their experiments with

adrenal homografts after tissue culture that this method does not insure survival of the graft and that it is rather doubtful that biologic differentials existing between host and donor may be modified during tissue culture. In addition to their evidence that the use of host or foreign sera in the cultures did not decide success or failure, they showed in animals which succumbed that reactions did occur regardless of the sera employed. Thus, tissue culture had not made the grafts compatible with the biologic characteristics of the host. These workers ascribe any success in transplantation to a definite compatibility between graft and host which existed long before the tissue culture rather than to an artificial "acclimatization".

Hoffmeister (130) transplanted many endocrine and non-endocrine tissues after attempting to inhibit the histiocytic apparatus of the reticulo-endothelial system with trypan blue. The experiments resulted in failures. Nor did his efforts to "prepare" the receiver with the serum of the donor or the donor with the serum of the receiver lead to success.

All of the tissue culture experiments show a property of adrenal tissue which seems to be of great importance. Mammalian tissue is known to survive and grow in tissue cultures for indefinite periods of time. Embryonic tissues show an enormous rate of growth. Since this is true for non-endocrine and endocrine structures - e.g. Lux et al. showed that new-

born adrenal tissue of the rat grows exceedingly well in vitro - it appears that these cells have an inherent power of growth which cannot be related to regulatory pituitary influences. This basic capability of growth seems to account for the survival of cortical cells and their function in the hypophysectomized organism despite the well-recognized and understood adrenal atrophy. Could there be any way to stimulate and enhance this original power of the cell?

(8) The physiological "need". The socalled "law of deficiency" or "Halsted's law" expresses an empirical experience of Halsted (110, 111) and other workers. They found that parathyroid autotransplants could not be successful unless one created an adequate physiological deficit of the particular secretion. They already recognized that an endocrine hyperfunction, that is, an excess of what is required by the organism, cannot be induced by grafting ("supertransplantation") since the non-deficient organism cannot maintain endocrine tissue in addition to its own of the same type.

This claim, i.e. that there be a need for an endocrine secretion before a take of a corresponding organ could be obtained, was not unchallenged. Shambaugh (270) showed that large amounts of parathormone or viosterol did not inhibit the survival of parathyroid autografts. The same author also achieved successful autotransplants regardless of the amount of parathyroid tissue removed before transplantation. In these

cases a physiological need was apparently not a requisite for the grafts' survival. Stilling (282) claimed to have found some preserved cortical tissue from an autoplastic graft done three years earlier. The rabbit possessed one intact gland!

One might also consider numerous other examples as exceptions to the law of deficiency. Takes were obtained in two-stage transplantation procedures*(41, 80, 164, 174, 189, 229) even if the second stage was performed weeks or months after the first; in other words, in spite of the presence of intact cortical tissue for a considerable time and during the most critical period for the graft. The same exception is evident in those animals in which the administration of maintenance doses of cortin for a few days did not prevent the regeneration of adrenal transplants (138, 150, 153, 200). Turner (314) observed some cortical proliferation in homoplastic transplants to the eyes of non-adrenalectomized rats. Other workers (258, 259) supposedly succeeded with adrenal homografts in non-adrenalectomized or unilaterally adrenalectomized hosts; the grafted tissue was obtained from a young donor. Many examples could be cited (e.g. 78, 87, 164, 183) where cortical takes were found despite the presence of hypertrophied accessory tissue. Several such cases in the author's series belong to this category and will be cited below.

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* 1st stage: unilateral adrenalectomy and transplantation;
2nd stage: removal of the remaining gland some time later.

Thus, it seemed that the concept of a deficiency as the responsible factor determining and limiting the growth of endocrine transplants, often did not exactly agree with the actual experiences, although it did have a certain general validity. The situation remained more or less obscure until the more recent elucidation of the pituitary-endorgan relationship. Although the inter-endocrine relations are still far from being fully comprehended, the whole problem has been put on a sounder basis. It is to be hoped that the quantitative aspects of this relationship will be known soon and will enable the investigator to judge the chances of successful cortical transplantation and its limitations in terms of available or administrable units of corticotropin.*

The experience (352) that neither autoplastic nor homoplastic cortical grafts are successful in hypophysectomized rats but that the excision of the pituitary results in atrophy of the intact adrenals, led to the present concept:

It is not a "need" for or "deficiency" of a secretion but the available amount of an anterior pituitary principle which determines the integrity of an endocrine organ, the growth of transplants, and the power of regeneration. In the case of the adrenal cortex, the hypophysial corticotropin maintains a certain amount of cortical tissue, whether it is the normal

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* The term corticotropin as used by the author does not apply to certain much-debated preparations of various investigators (unless particularly mentioned) but designates a pituitary principle with a specific stimulating effect on the adrenal cortex.

set of organs in the intact animal or accessory structures and transplanted tissue in the adrenalectomized organism. Usually, the simultaneous presence of intact glands and additional transplanted tissue is more than the available amount of corticotropin can master. The hypophysial secretion apparently prefers the already established cortical tissue, namely, the one or two intact glands, or remnants or accessories, if present. A knowledge of the exact mechanism of this relation between pituitary principle and the cortical end-organ, i.e. of the underlying metabolic processes, is still lacking.

The only way to bring about an increased capacity of the organism for cortical tissue would be to increase the amount of available corticotropin by means of stimulating the pituitary in some way, or by administering exogenous corticotropic material. Turner (313) was successful in increasing the percentage of incorporated adrenal homografts, their size, and the degree of regeneration, by pituitary homotransplants to the host rats.

The fact that the maintenance of normal and transplanted cortical tissue is governed by the same mechanism is demonstrated after hypophysectomy: just as the intact glands undergo atrophic changes, so is regression of established autografts observed (352).

However, the cortical atrophy in hypophysial deprivation

is not an absolute one. Since hypophysectomy is not immediately fatal whereas adrenalectomy is, the atrophic cortex of the hypophysectomized animal must function at least to a certain extent, as evidenced by the survival of hypophysectomized animals. Moreover, it has been shown that atrophic cortices obtained from hypophysectomized rats do grow as healthy homografts in adrenalectomized animals with intact pituitary (352).

Returning to the original question regarding the factors influencing cortical transplants, it can be said, then, that the functional capacity of the pituitary via its corticotropic principle governs maintenance, growth and regeneration of cortical tissue, be it in situ, an accessory body or a transplant. This seems to be true for all endocrine tissues. The amount of available corticotropin thus constitutes the limiting factor which prevents supertransplantation. It has been shown that transplants of new-born adrenal tissue into intact rats do not survive (148).

Considering the exceptions to Halsted's law mentioned above, this concept of pituitary-endorgan relationship permits the following conclusion. If transplanted cells persist despite the presence of normal adrenal tissue, or if the administration of endorgan hormone does not inhibit transplant regeneration, or if hypertrophy of accessories takes place in spite of surviving grafts, that must imply: some of the cortical tissue does not function at its optimal metabolic capacity; it

does not utilize the full amount of its pituitary metabolic stimulant. Thus, the extra amount of the latter can take care of additional cortical tissue which possibly exists at a subnormal functional level, too. The administration of exogenous cortical hormone, though depressing pituitary activity, might not inhibit the corticotropin output enough to bring about complete failure of the transplants. The subnormal functional level of the tissues concerned must not necessarily represent an irreversible damage but rather a reduced "hibernating" functional state. This leads to the author's conception that there is a "dormant" functional capacity of accessory structures and transplanted cells which makes it possible for them to change from a deficient subnormal to an adequate normal activity, provided the corresponding pituitary stimulation is not lacking.

The question has been brought up whether there might be more corticotropin in the organism than is needed to maintain the normal glands (314). Although this question cannot be answered at present, such an assumption is not imperative. Available evidence suggests a deficient functional level of tissue in excess of what is expected, thus indicating the limited amount of corticotropic material.

Despite the immensely important influence of the pituitary on the function of cortical tissue, it should not be forgotten that cortical cells have a certain inherent and independent does not utilize the full amount of its pituitary metabolic stimulant. Thus, the extra amount of the latter can take care of additional cortical tissue which possibly exists at a subnormal functional level, too. The administration of exogenous cortical hormone, through depressing pituitary activity, might not inhibit the corticotropin output enough to bring about complete failure of the transplants. The subnormal functional level of the tissues concerned must not necessarily represent an irreversible damage but rather a reduced "hibernating" functional state. This leads to the author's conception that there is a "dormant" functional capacity of accessory structures and transplanted cells which makes it possible for them to change from a deficient subnormal to an adequate normal activity, provided the corresponding pituitary stimulation is not lacking.

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Despite the immensely important influence of the pituitary on the function of cortical tissue, it should not be forgotten that cortical cells have a certain inherent and independent functional capacity as is shown in the hypophysectomized animal and in tissue cultures (discussed above).

The recognition of the extent and limitations of the pituitary's influence on cortical growth afford one more conclusion. It has not infrequently been suggested that a cortical graft might stimulate the defective or undeveloped main glands of a patient and initiate their normal development or help to regain their former functional capacity (74, 130, 239, 287). If the pituitary-cortical concept is correct, such a stimulation cannot possibly take place. Indeed, the evidence indicates that the depressive effect of the newly introduced cortical tissue upon the anterior pituitary may actually harm the already subfunctional main cortices.

It may be stated at this point that attempts to stimulate transplants have been reported. The results obtained, however, are entirely inconclusive. The daily administration of arsenic (178) has been said to have a beneficial effect on the growth of autoplastic adrenal transplants. But the small number of experimental animals and the admittedly incomplete electrocoagulation of the remaining gland of the sole survivor nullify the above conclusion. Diphtheria toxin (!) and arsenic in form of Fowler's solution were employed in the hope of influencing adrenal homografts (258). No success was obtained. Platinum chloride was claimed by the same group to exert a stimulating effect on adrenal homografts in the kidney of rats.

The renal hyperemia or the hypertension derived from renal lesions was considered to be of significance as a possible mechanism of this stimulation.

4. Adrenal autotransplantation

Autoplastic adrenal transplantation has been performed by numerous workers in the rat, guinea pig, rabbit, dog, cat, frog, with or without deprivation of the organism of all remaining cortical tissue.

The bulk of the work with transplantation as well as the closely related investigations on adrenal insufficiency have been done in the rat. For technical and economic reasons the rat is very well qualified as a general laboratory animal. It displays a marked similarity to man since relations between mammals having the same food habits tend to be close. This permits a limited translation of experiences with this animal into terms of human physiology. As Donaldson (76) says: "It is not intended to convey the notion that the rat is a bewitched prince or that man is an overgrown rat".

Despite its general suitability for experimental investigations, the rat has one property which does not make it an ideal subject for adrenocortical research, namely, the frequent occurrence of accessory cortical tissue. This point will be considered below in greater detail. It should be kept in mind, that this very property constitutes a handicap for

judging the completeness of the absence of cortical tissue, or the functional capacity of transplanted organs. This disadvantage is definitely realized by the author. Notwithstanding this disadvantage, rats were used in this study for the following technical reasons: the size of the animal quarters, the availability of this type of animal, the low expense of its maintenance. Moreover, the desire to correlate our results with the work already done made it advisable to use routine animals and techniques.

Sites employed by others for adrenal transplants include: kidney, omentum, ovary, scrotum, testis, liver, spleen, pituitary, thyroid, brain, eye, ear, lymph sac. In addition, adrenal transplants have been administered by the following routes: subcutaneous, intramuscular (abdominal, dorsal muscles), intraperitoneal, intravenous (splenic v.), and subdural.

The following papers report definitely unsuccessful results with adrenal transplantation in various animals and sites: 29-31, 33, 41, 52, 59, 61, 133, 134, 169, 210, 271, 281, 289, 358, 360.

A great number of positive results in rats, dogs, guinea pigs, and rabbits with subcutaneous or muscular tissues, ear, kidney, ovary, testis, pituitary, eye, spleen, and mesentery as the sites of implantation have been reported: 22, 40, 41, 59, 77, 78, 80, 87, 137, 138, 144-146, 148, 162-166, 174, 178, 183, 189, 229-231, 236 (?), 242, 264 (?), 265(?), 337, 339,

340, 342-350, 352-355, 356, 357. Others are mentioned elsewhere in this paper. These "successful" results have to be viewed with great reserve. There is no doubt in the author's mind that their number would dwindle considerably if a closer inspection of the techniques used and the results obtained would be possible. Of course, this does not mean that we doubt the possibility of "takes" of autoplastic adrenocortical transplants. But it should be emphasized here, that the frequently inadequate techniques employed, the small number of animals used, the short periods of observation, the rather indiscriminate reliance on macroscopic appearance, the lack of histological inspection, the paucity of adequate autopsies, and the wide difference in opinion as to what a successful transplant is, make it difficult if not impossible to accept all the claims which have been made. It is far beyond the scope of this paper to state all the objections to these and other reports. Rather it is much more profitable to summarize the benefits of these reports. This has been done in part in the preceding chapter and shall be pursued further in the course of the paper. It must be understood that not all of these reports are subject to the criticisms noted above. Many of them are distinguished by a clear and unbiased approach, by skilled pursuit and sound evaluation of this difficult and intricate problem. Certain pertinent results and observations can be selected from the literature.

Interesting surgical techniques have been used by some

340, 342-350, 352-355, 356, 357. Others are mentioned elsewhere in this paper. These "successful" results have to be viewed with great reserve. There is no doubt in the author's mind that their number would dwindle considerably if a closer inspection of the techniques used and the results obtained would be possible. Of course, this does not mean that we doubt the possibility of "takes" of autoplastic adrenocortical transplants. But it should be emphasized here, that the frequently inadequate techniques employed, the small number of animals used, the short periods of observation, the rather indiscriminate reliance on macroscopic appearance, the lack of histological inspection, the paucity of adequate autopsies, and the wide difference in opinion as to what a successful transplant is, make it difficult if not impossible to accept all the claims which have been made. It is far beyond the scope of this paper to state all the objections to these and other reports. Rather it is much more profitable to summarize the benefits of these reports. This has been done in part in the preceding chapter and shall be pursued further in the course of the paper. It must be understood that not all of these reports are subject to the criticisms noted above. Many of them are distinguished by a clear and unbiased approach, by skilled pursuit and sound evaluation of this difficult and intricate problem. Certain pertinent results and observations can be selected from the literature.

Interesting surgical techniques have been used by some investigators with success. The kidney with its adrenal was excised and anastomosed to the carotid artery of the dog with the ureter leading to the outside; the kidney was removed from this subcutaneous site some time later, as was the other intact adrenal gland. Takes were obtained in seven dogs (189). A "sandwiching" of the mobilized ovary and the adrenal gland in the dog was performed followed three weeks later by a complete separation of the adrenal neuromuscular supply together with a free transplantation of the adrenal-ovarian complex to the lower abdomen (80). The first stage of this method is actually a stalked, and not a free transplantation. However, it has to be considered a free transplant since this preservation of a stalk was only a temporary measure and was used to facilitate the subsequent free transplantation. Successful results were obtained in 8 dogs.

The ovaries have been employed as a site for implantation by many workers (Ingle and his group, and others). The failure to obtain successful takes during estrus (231) demonstrates very well the deleterious effect of excessive vascularity as has been mentioned above. The increased ovarian activity of this phase resulted in marked hemorrhages during and after operation. Death of the animals in whom takes were not obtained occurred 14-32 days after operation. The successes obtained resulted from transplantation during the "interval" as indicated by vaginal smears. Such transplantation to the ovary in rats

was consistently successful (137) whereas grafting to the spleen was only slightly less successful.

Wyman (341) obtained successful intramuscular autografts in 95% of a large series of rats. This high figure was reached under optimal conditions and with all doubtful cases discarded. All of the animals with successful transplants gained weight, possessed an abundant amount of abdominal fat, showed normal blood sugar levels, and lacked chromaffine tissue. Kroc (183) grafted adrenal tissue to the ear of rats and found that the availability of salt licks helped to prolong survival in the grafted animals. 40% of his survivors died after the removal of the graft, but lived longer than adrenalectomized rats. The relatively low mortality and the development of accessory cortical tissue after removal of the grafts indicates the existence of more accessory tissue than could be assumed from the mortality rate following simple adrenalectomy. His highest rate of takes was 52% (males without salt licks). That is far below Wyman's figure of 95%. The discrepancy may be due to the lower temperature of the ear, and a difference in the vascularity of this site.

Grafts as such do not survive. Degeneration of medulla and cortex except for a narrow band of the capsule and zona glomerulosa takes place within 24-48 hours (146). The subsequent regeneration from this marginal region begins apparently on the third day after operation and leads to complete restoration

within 5 or 6 weeks (148). The regenerated cortical mass may even exceed the size of the original graft. No significant regeneration is observed when one adrenal is left intact. The gradual regain of cortical functional capacity is shown by the inactivity of the transplanted rats for two weeks after operation with a subsequent return to normal activity levels (145).

The recognition of cortical layers in transplants has been a matter of controversy. In grafts to the ovaries, no distinct zones but rather closely packed cells near the capsule, and spongiocytes in more distant layers were observed (148). Other workers have reported a fairly normal histological picture with certain variations which did not permit a classification in definite layers (242, 265). Another investigator, however, claimed that in intramuscular transplants three distinct cortical layers could be recognized (162-166). A morphological arrangement similar to the normal cortex was also observed in homotransplants of adrenal tissue (228, 314).

An amazing vitality of cortical cells was demonstrated by Dornfeld's experiments (77, 78). His results show that our emphasis on speedy transplantation and most careful handling of the transplant, though undoubtedly beneficial, are merely precautionary measures as is asepsis, but that cortical cells can stand fairly rough treatment and yet retain full vitality. He subjected cortical tissue to the enormous ultracentrifugal force of 400,000 times gravity for 30 minutes and left the

centrifuged tissue in Locke-Lewis solution for three hours previous to grafting. This procedure, despite the production of a marked cytological displacement, did not hinder the subsequent abundant cortical regeneration just as it did not impede the growth of cancer cells, embryonic rat tissues, pituitary cells, etc.

Dornfeld facilitated the survival of the operated animals by means of providing a high salt diet (Locke-Lewis solution instead of drinking water). This has been done by other investigators, too. Eversole et al. (87) transplanted adrenal tissue into various organs and gave the operated animals 1% NaCl solution for 7-10 days after transplantation. Higgins and Ingle (128) placed their homografted rats on a high salt diet.

The type of cellular proliferation in the regenerating cortical transplant has attracted the attention of several investigators. Baker and Baillif (22) showed an active mitotic cortical regeneration in their intramuscular grafts, with the greatest mitotic activity from the 2nd to the 7th day after transplantation. They observed the completion of regeneration within a month and assumed that the transplant began to function within a few days after implantation. Jaffe (162-166) who observed regeneration of grafts within 3-4 weeks and obtained takes sometimes of the size of the normal adult adrenal, observed a proliferation in the regenerating grafts

of guinea pigs which was largely amitotic.

We are not prepared to make any definite statement on this question since our histological methods did not facilitate the investigation of mitotic activity. Special stains and the arrest of mitoses with colchicine are necessary in order to obtain conclusive results on the degree of mitotic activity in the transplanted tissues. From the literature it appears that mitotic division plays an essential part in the regenerative processes.

5. Adrenal homotransplantation

Homoplastic adrenal transplantation has been performed in various species including the rat, rabbit, guinea pig, mouse and frog. Subcutaneous and muscular tissues, the peritoneal cavity, and eye, ear, ovary, testis, thyroid, kidney, liver, spleen, bone marrow, brain, sciatic nerve, and lymph sac have been employed as sites of transplantation.

Reports of unsuccessful homografts include 31, 41, 81, 99, 131, 133, 164, 187, 196, 201, 210, 235.

Successful homografts were reported in 1 (?), 41, 126, 128, 130 (citation), 150, 153, 183, 200, 207, 219, 223, 228, 236 (?), 242, 258, 259, 265 (?)(higher incidence of homoplastic than of autoplastic takes!!), 313-315, 347, 351-353, 355. The "successful" results have to be considered with reservation for the same reasons as described in the chapter on autotransplantation.

Some pertinent observations of various investigators should be recorded. Homografts to the ovaries of rats (150, 153, 223) showed that transplantation across strain borders did not prove successful whereas takes were obtained within the same strain, with better results when the animals were closely related, and best when they were siblings.

An interesting but rather doubtful report (219) of homotransplants to the kidneys of rabbits records that no proliferation could be observed from very young embryonal adrenal tissue. Older fetal adrenals or the organs of the newborn, however, took. In one case the transplant supposedly initiated the invasive , infiltrating, metastasizing growth of a renal parenchymal tumor. And all of that with the recipient's adrenals intact! That result was considered as evidence for the Cohnheim and Ribbert concepts of tumor genesis. It is obvious that one cannot but doubt this startling observation.

Other workers (258, 259) who also homografted to the kidney, claimed to have obtained takes which attained "tumor proportions , sometimes exceeding the size of adult adrenal glands; and that again in non-adrenalectomized or unilaterally adrenalectomized rats!

One investigator (228) reported 84% successful grafts with the adrenal tissue obtained from young donors and transplanted into the brain of adrenalectomized rats.

Homotransplantation of adrenal tissue into the anterior chamber of the eye was favored by Turner and his associates (313, 315). This site permits a direct observation and offers favorable nutritional conditions. Although these workers obtained some takes in the intact hosts (rats), the bilateral adrenalectomy or the introduction of pituitary homotransplants facilitated the regeneration and persistence of cortical cells.

Successful subcutaneous transplants in rats were obtained by Lux et al. (200) who subjected the cortical tissue to culture in vitro prior to transplantation. They grafted 30-40 fragments to the groin and observed subsequent fusion.

Higgins and Ingle's (126, 128) subcutaneous grafts were placed in the groin close to the femoral vein. They found that adult tissues were invariably incompatible with the host tissues. However, the use of adrenals obtained from newborn rats yielded positive results. The authors conclude that the undeveloped differentials of newborn rat tissue still may permit a certain adaptibility of graft to host tissues. In a series of 21 attempted transplants to unrelated adult rats, 9 took; in another series they reported the survival and functioning of 24 grafts out of 40. The older the donor the less was the chance for success.

Wyman and tum Suden (347) obtained 100% success in a series of 4 animals with intramuscular transplants (litter-

mate tissue); these animals had 1, 3, 4, and 4 takes. The grafting of auto- and homoplastic tissue to the same animal was also successful, and showed that the presence of an autograft apparently did not inhibit homotransplants. It was concluded that the organism does not seem to exhibit a preference for auto- or homografts when both are offered. These authors did not find a correlation between the age at transplantation (above 45 days) and the growth of a transplant or an accessory. A take is established within 4 months; later on little if any further transplant growth occurs. They found that the female rat regenerates over twice as much as the male. Despite considerable variation and regardless of the number of takes, the total amount of regenerated cortical tissue was fairly constant. Wyman and tum Suden also obtained successful intramuscular homotransplants between non-sibling rats in 32% of males and in 25% of females (355). This report corrected an earlier statement based on a smaller series of animals (351) in which a far greater sex difference was found. These experimenters (355) found a greater incidence of takes in the males when gonadectomy was performed simultaneously with adrenalectomy and transplantation than in non-gonadectomized control rats. No change in the incidence was observed when the castration was done 2-3 months before the transplantation; and likewise, no significant change occurred in female rats when simultaneously gonadectomized.

6. Adrenal heterotransplantation

Little has to be said about heterografts. A variety of species and different sites for adrenal transplants have been tried. These attempts, however, were invariably unsuccessful (99, 201, 347). With one "exception": Aouslender (18) transplanted multiple adrenal fragments from oxen or cats into the subcutaneous tissues of dogs, and obtained an amazing number of "takes", apparently even without adrenalectomizing the recipients. One of her conclusions is that adrenal cortex under those conditions "takes perfectly well". However, she admits that the grafts eventually become completely replaced by connective tissue. Such a conclusion together with the obvious lack of knowledge about the pioneer work which had already been done at that time by American investigators, makes this piece of work hardly worthy of consideration.

7. The functional capacity of adrenal transplants

Numerous attempts have been made to show the adequacy of function of successfully transplanted adrenal tissue.

Essentially normal conditions were observed relative to general health of the grafted animals, growth (78) and gain of weight which might even exceed the normal range (242), blood pressure (80), reproduction (78), estrous cycle (207), blood sugar (80, 356), NPN and urea nitrogen (80, 357), blood volume (343),

histological picture; absence of degeneration or exhaustion atrophy as long as 276 days after transplantation (164), mineral intake (247), work capacity and voluntary activity (137, 144, 145, 165, 242), resistance to cold and heat (138, 189, 342, 348), resistance to diphtheria toxin (337), resistance to typhoid vaccine (162), resistance to distemper in dogs (80).

However, several workers were able to show that in spite of unimpaired health, apparently normal activity, resistance and biochemical values, inadequacies of the established adrenal grafts can be revealed under certain conditions. Examples of a diminished functional capacity of cortical transplants are: the increased sensitivity to phenobarbital (80), the slightly decreased blood and plasma volume (80), the unusually low arterio-venous difference (O_2) (80, 189), the lack of normal protection and water elimination in experimental water intoxication (87); [even fragments in situ were, despite their regeneration, not so efficient as the intact gland but far better than any type of graft. However, animals maintained with cortin showed an essentially normal protection against water intoxication.] the impaired hemodiluting capacity following intraperitoneal glucose injection (341), the response of the transplanted organism to an acute emergency such as histamine poisoning or anaphylactic shock (138, 339, 353), the increased susceptibility to adrenalin (354). It has

to be admitted that the disturbances in histamine poisoning and anaphylactic shock and the adrenalin susceptibility may in part be due to the lack of medullary tissue.

Several investigators removed established cortical grafts from the site of transplantation in order to show that this cortical tissue was responsible for the survival of the grafted animal. Reports of removal of autografts from rats, dogs, and rabbits with subsequent death of the animal include 40, 41, 77, 78, 80, 145, 174, 229-231. The survival periods after removal ranged from two to 23 days. Reports of removal of homografts from rats and rabbits with subsequent death of the animal include 41, 126, 128, 200, 207. These animals died within 21 days after removal.

From the reports in the literature and some observations of the author it appears that the grafts' capacity to maintain life, growth, and an apparently normal behavior may be deceiving and may lead to the conclusion that the transplant substitutes completely for the hormone production of the intact glands. That this is not the case has been suggested by the above examples.

The maintenance of life, for instance, is by itself not a suitable criterion for the functional capacity of a graft. Similar criticism applies to other apparently normal conditions used as criteria in the grafted animal.

Ingle and coworkers (152) noted that there is a wide

discrepancy between the minimal amounts of cortin necessary for maintaining life and those required for the replacement of all adrenocortical functions.

From the evidence cited above the following conclusion may be drawn: heterotopic transplants, similar to cortical tissue in its normal place, seem to have a remarkable "margin of safety", a feature common to various endocrine structures. Thus, a small cortical fragment, regardless of whether it is left in situ or transplanted, whether it is an accessory body or a free transplant, may be able to maintain the organism in an apparently normal condition for some time at least.

This property is of great importance for the understanding of chronic cortical insufficiency, be it due to disease or exhaustion of accessory or grafted tissue. A case report of an Addison patient (60) showed that in absence of any macroscopic cortical tissue, small microscopic cortical islands apparently maintained the patient's life for 35 years before a final exhaustion of this tissue (believed to be the result of a faulty embryonic development) led to death. Small cortical fragments left in situ in the experimental animal led to indefinite survival even without loss of weight (230, 231). The manifestation of insufficiency in man or animal with latent cortical deficiency indicates that the cortical tissue has finally become depleted and exhausted. That this margin of safety is a rather large one can be seen from the fact

that even small cortical rests in caseous tuberculous adrenals may function adequately under conditions free from stress without the appearance of symptoms of insufficiency. (304). Any form of stress, however, will rapidly bring about the syndrome of insufficiency with circulatory collapse due to exhaustion of the available cortical tissue and the lack of hormone reserves. The similarity between clinical cases and the states of latent and chronic insufficiency in experimental animals is obvious.

It is exceedingly difficult to judge the viability, durability, and efficiency of a transplant because of (a) the margin of safety, (b) the experience of various workers that several body functions appear to be normal in the grafted animal and that stress conditions are necessary to reveal certain instabilities and deficiencies, and (c) the lack of specific criteria. Little progress can be expected until more will be known about adrenal physiology and biochemistry and about various aspects of transplantation. As to the latter, there is practically nothing known concerning the fate of cortical transplants after a long period of time, whether its involutionary changes in senility are coordinated with the general ageing of the organism or whether the fate of the graft follows other paths. The short observation periods of many workers do not for the most part even permit a clear decision whether the graft had actually taken or whether it

just was tolerated as a foreign body. In the author's experience, it appears that it is not necessary to assume that the body would launch a violent counterattack against a graft which finally will not take. It seems, on the contrary, that the tolerance for autografted tissue, at least in the rat, is rather great and that absorption processes may go on for a long time. As a result an observer may harbor false hopes for the viability of the graft.

It would be most desirable to have a criterion of cortical function comparable to the basal metabolic rate as an indicator of thyroid activity. Since such a standard is not available and since certain biochemical examinations could not be undertaken for technical reasons, the author had to rely on the criteria of functional capacity of takes that are listed below. It is realized that any criteria known at present are not completely satisfactory if employed alone, and even if several are employed, they do not necessarily give adequate information about the functional efficiency of a graft.

(a) Proof of absence of accessory or remnant tissue. At autopsy of the animals with established grafts, a search for accessory bodies or suspicious structures must be done as carefully as possible and in doubtful cases histological examination is imperative. It is quite clear, however, that in order to exclude accessory tissue with complete certainty, a most exhaustive microscopic investigation using

serial sections of every millimeter of the retroperitoneal tissues is necessary. The author is sure that he has not overlooked any gross accessory or remnant cortical tissue and that in several instances doubtful and minute amounts of such tissue could be identified or excluded. However, it is not claimed that the presence of microscopic islands could be proved or disproved in all cases. The observations of many investigators and the author's material indicate that adrenalectomized rats may contain cortical reserves which influence the survival of the animals or the fate of the grafts.

(b) The microscopic appearance of the takes permits a fairly reliable judgement of whether a graft has taken at all.

However, one should be well aware of the fact that any translation of morphologic observations into functional terms is a dubious practice. The color, vascularization, consistency, and imbedding of a graft indicate its incorporation, but nothing else. The author did not see any advantage in grading the takes according to size, and gross and microscopic appearance. The histological examination, though clarifying many uncertainties as to the morphologic integrity of the grafts, does not provide direct information about the functional capacity of the transplanted tissue.

(c) The behavior, survival, and growth of the animals indicate, provided no other cortical tissue is present, that the graft is able to produce at least a certain minimal

amount of hormone. This information, however, is not adequate for judging how close to the lower limit of the margin of safety the animal exists. Only stress, such as discontinuation of the supportive salt treatment, or infection, may reveal whether the available cortex is capable of maintaining the upward trend in the animal's development under unfavorable conditions. The fact that in so many rats of this study not even a temporary loss of body weight could be observed after operation shows that the regenerative processes in these particular animals keep pace with absorption, degeneration, and disintegration and that reserve forces which permit an undisturbed and essentially normal continuation of the animal's development are quickly mobilized.

(d) The events following removal of the takes permit a judgment of the functional ability of the incorporated cortical tissues, provided the absence of other cortical structures can be shown. The ensuing death clearly indicates that the organism is not able to survive without the secretions of cortical tissue. A careful technique of the removal of the graft is necessary to exclude shock as a possible cause of death. Moreover, the varying survival times of rats deprived of their cortices permit certain conclusions as to the success of the organism in mobilizing its very last reserves (microscopic accessories). If animals deprived of their grafts still survive, it can be assumed with reasonable certainty that the

grafts were not fully adequate functionally. Accessory tissue must have been in a state where it already had developed sufficiently to take over cortical functions in part or in full, or it was about to do so. In other words, enough corticotropin was available for accessory structures even in the presence of the grafts. It can be assumed that, because of its decreased function or quantitative reduction, the graft does not utilize all available corticotropin, the excess of which is used for accessory cortical tissue.

II. PRESENTATION OF THE PROBLEM OF THIS INVESTIGATION

In order to investigate factors, other than those discussed above (chapter I, 3), which might facilitate or impede the incorporation and regeneration of adrenal transplants, we decided to employ a group of substances with a definite and well-known effect on the state of cortical insufficiency. As yet no one seems to have attempted to learn whether the administration of sodium or potassium has any influence on adrenal grafts. Dr. Leland C. Wyman kindly suggested the idea of oral administration of sodium chloride or potassium chloride solutions to albino rats with autoplastic adrenal transplants in order to observe the effect on the incidence of incorporation and on the character of the grafts.

The intimate relationship of these ions to adrenocortical function and their importance in the prevention and treatment of cortical insufficiency made this study all the more desirable since one would be led to either of the following conclusions.

(1) If essential differences should be found between the sodium chloride, potassium chloride, and control groups, it could be concluded that changes in the electrolyte balance exert a direct or indirect influence on the mechanism of incorporation and regeneration. Such an influence might afford an explanation of the results of electrolyte administration

which hitherto have been interpreted as sequences of mere substitution. Moreover, it would yield broad prospects for experimental and therapeutic application.

It could be that the administration of sodium chloride might impede the growth of transplants by decreasing the cortin requirement of the organism with a subsequent diminished corticotropin output of the anterior pituitary. On the other hand, potassium chloride might favor the growth of transplants by increasing the need for cortical hormone through the toxic properties of KCl and thus initiating a larger corticotropin output which would benefit the graft. One might conclude, then, that an altered demand for cortical hormone consequent to the administration of salt solutions influences the pituitary-adrenal balance, which forms the basis of adrenal function and has taken the place of the old Halsted law of a "physiological need".

If the reverse should occur - favorable effect of NaCl, unfavorable effect of KCl on growth of transplants - it might be inferred that the action of the electrolytes on the more or less insufficient organism brings about changes in the internal environment which in turn facilitate or hinder the growth of the transplants.

(2) If no significant differences should be found, it might be concluded that these electrolytes in the concentrations employed have no influence on incorporation and

regeneration of cortical grafts and that these processes take place irrespective of the concentration and distribution of sodium and potassium in the body fluids.

Before presenting methods, results and conclusions of this study, a detailed discussion of electrolyte distribution, water balance, and kidney function in relation to the adrenal cortex is essential for the understanding of this aspect of adrenal physiology and its relationship to the stated problem.

III. THE RELATION OF THE ADRENAL CORTEX TO ELECTROLYTE AND WATER BALANCE AND RENAL FUNCTION

1. Sodium and potassium in body fluids

The sodium ion (96) is the chief base radical of the extracellular fluids. (The chloride ion is the main extracellular acid radical.) Sodium is fixed in the extracellular site and its concentration largely controls the degree of cellular hydration. Water does not enter or leave cellular structures unless there is a change in the extracellular sodium concentration. A decrease in the extracellular sodium contents leads to cellular hydration: the extracellular compartment becomes hypotonic to the intracellular compartment; water is taken up by the cells at the expense of the circulating and interstitial fluids. Thus, cellular hydration is associated with anhydremia and extracellular dehydration. This shift is corrected by the administration of sodium salt. It is obvious that the amount of retained sodium must largely determine the extracellular fluid volume. A relatively constant extracellular sodium concentration is guarded by the normal kidney by means of tubular reabsorption or rejection of sodium. Hypertonic NaCl solution is an efficient diuretic (more than isotonic NaCl) due to increased glomerular filtration and decreased tubular reabsorption. Sodium is pharmacologically inert; it does not seem to have any specific effect on tissues. Its function is apparently purely osmotic in character.

The potassium ion (88,96,172) is the chief base radical of the intracellular compartment. (Phosphate is the main intracellular acid radical.) As a building stone of cellular structures, potassium is necessary for growth. The potassium enables the cellular fluid to be isotonic with the extracellular compartment. Under normal conditions its extracellular concentration is low. Potassium stays at the intracellular site because no other ion can get in to take the place of the potassium, and the anions with which potassium cannot leave the cells. While the cell boundaries are impermeable to sodium and non-monovalent anions, they are freely permeable to potassium and monovalent anions. This property will be seen to be of major importance in the mechanism of cortical insufficiency and related disturbances. Potassium is readily absorbed from the intestinal tract or from the parenteral site of administration. Since it diffuses into all body water it is rapidly taken up by the tissues. The normal renal tubules reject very efficiently any excess amount of potassium. This tubular rejection and the subsequent excretion of K which is accompanied by an appreciable amount of water are the basis for the diuretic action of potassium in any concentration.

Potassium plays a role in the transmission of the nerve impulses and in cardiac and skeletal muscle function.*

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* It is of interest to note that the muscular weakness in myasthenia gravis is associated with an increased potassium content of the affected muscles (62). A similar disturbance in cortical insufficiency will be dealt with below.

Potassium exhibits a synergistic action with adrenalin; it acts directly on the adrenal medulla. This cation is an antagonist to insulin. All action of the potassium ion are antagonized and inhibited by calcium.

An excessive loss of NaCl and water leads to a shift of potassium from the cells into the plasma. Such an increase in the level of plasma potassium takes place in traumatic, anaphylactic, hyperthermic and histamine shock, hemorrhage, cortical insufficiency, intraperitoneal glucose administration, and in intestinal obstruction or fistulas. The increased extracellular potassium can be interpreted as an attempt to build up the blood volume at the expense of the cellular fluids. This increase, however, is highly toxic to the organism as is seen from the depressant effects on muscular, nervous, and cardiac activity (influencing impulse conduction as well as muscle contractility).

Potassium and sodium are mutually replacable (73). Though an increased sodium or potassium intake can bring about a corresponding temporary increase in potassium or sodium excretion respectively, it cannot lead to a serious depletion of either of these ions since the healthy kidney protects the organism effectively from the toxic influences of repeated and massive administration of the chlorides of sodium and potassium (179). The partial replacement of potassium by

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The changes of the total base (mainly Na and K) run parallel to those of water: base retention leads to water retention, while water accumulation is accompanied by storage of base.

2. Sodium deficiency

Many symptoms of experimental sodium deficiency simulate those of cortical insufficiency and result in altered fluid distribution and clinical dehydration. In order to bring about such a Na deficiency, diets moderately or extremely low in sodium, and intraperitoneal administration of glucose have been employed in experimental animals (12, 54, 67, 168, 226, 227, 241, 273). The depletion of the extracellular cation must lead to a water loss in order to maintain a normal extracellular concentration. The result is a negative water balance with the intracellular volume above normal. Sodium excretion under the experimental conditions is rapid and potassium is retained. The retention of potassium represents an attempt to compensate for sodium in the neutralization of metabolic acids excreted in the urine in order to maintain a normal acid-base balance. The water consumption of these animals is decreased. Other symptoms include deficient growth, loss of weight, severe ocular disturbances with

ensuing blindness, reproductive disturbances, decreased resistance to infections, deficient utilization of protein and negative nitrogen balance. The condition can be corrected by the administration of cortin.

Clinical hyponatremic conditions other than cortical insufficiency (including abnormal fluid losses, surgical intervention, organic lesions, etc.) have been described (38, 43, 44, 267). Adrenal preparations and salt therapy have yielded encouraging results in some cases.

3. Toxicity of potassium

The toxicity of potassium has already been mentioned. It is observed in the intact animal and in the perfused isolated heart where it leads to diastolic standstill. Continuous intravenous infusion or duodenal administration of potassium result in complete disorganization of the ventricular complex with a final diastolic cardiac arrest. From these experiments a specific effect of potassium on cardiac automaticity and conductivity has been concluded (333, 334). It is of interest that cardiac glucosides protect normal animals against potassium poisoning, lower the potassium level, and prolong the life of adrenalectomized animals; in these respects they resemble cortical hormone (363).

The production of gross symptoms of cortical insufficiency in the normal animal by means of sublethal doses of KCl led

to the hypothesis that the increased potassium concentration in insufficiency is the basic cause for the syndrome (364, 366). This concept which will be dealt with below has received considerable attention and comment. Serious doubt was cast on it when intravenous administration of KCl, though lethal, did not produce symptoms of insufficiency (263).

4. Potassium and cortical insufficiency

The abrupt increase of the potassium concentration of extracellular fluids in adrenal insufficiency together with a decrease in the renal excretion of this ion is the most conspicuous feature of the potassium disturbance in this condition. The muscular potassium concentration is also increased and is accompanied by an increased intracellular water content (39). The potassium of heart muscle may show increases which, however, are not so striking as those in skeletal muscle and are not associated with an increase in intracellular water. Liver and kidney have not been shown to exhibit striking electrolyte and water changes. Marenzi (205), however, observed a decrease in liver and heart potassium which would indicate that the increase in the amount of serum potassium which stays in circulation without being excreted, is achieved at the expense of liver and heart tissues. The bodies of insufficient animals show a definite increase in potassium whereas the sodium contents are slightly decreased (112).

The accumulation of potassium in the organism may be explained by

(a) a failure of the kidney to excrete potassium adequately;

(b) a hepatic failure to deposit potassium during synthesis of glycogen as is done under normal conditions, at least in small amounts (39);

(c) a failure of the muscles to take up more potassium against an already high concentration of this ion;

(d) a lack of fixation of potassium by the tissues.

The accumulation of potassium in insufficiency is accompanied by a low blood sugar concentration. In the normal animal, on the other hand, the blood sugar level and the potassium concentration usually vary together in the same direction (39).

The decreased ability of the organism to dispose of potassium accounts for the increased susceptibility of the insufficient animal or patient to exogenous potassium administration (8, 222, 328). A high potassium content in the diet accelerates the onset and aggravates the symptoms of cortical insufficiency. The precipitation of crises is frequently preceded by an increased potassium concentration in the red blood cells. A striking correlation between plasma potassium concentration and auricular fibrillation has been observed (222). The administration of 0.1% KCl to adrenalectomized rats led to severe diuresis, negative water balance, and death (93).

These toxic symptoms and the rapid collapse can be prevented to some extent by an adequate amount of sodium in the diet. A simple restriction of the potassium content in the diet together with an adequate salt intake is of great importance in the treatment of Addison's disease. It prevents crises and reduces the hormone requirements (329). The administration of cortical hormones protects the insufficient organism very efficiently from potassium poisoning. The resistance to potassium may actually be raised above normal.

The increased extracellular concentration of potassium has been considered a possible cause for many of the symptoms of insufficiency, or even the basic cause of the insufficiency syndrome (310, 311, 364-366). However, observations have been presented which - without belittling the importance of the potassium disturbance in cortical insufficiency - make it unlikely that we are dealing here with the causative factor (66, 112, 177, 274). No definite serum potassium concentration is toxic. There is no constant correlation between the potassium level and the severity of the symptoms of insufficiency. Death may occur even without a marked elevation of potassium. The role of potassium as a cause of the symptoms of cortical insufficiency has been disproved at least insofar as the changes of blood sugar and tissue glycogen are concerned.

5. The kidney and cortical insufficiency

(References: 24, 113, 115, 257, 303, 317)

The lavish sodium excretion of the insufficient organism accompanied by loss of chloride and water leads to fluid withdrawal from the extracellular compartment into the cells of the tissues, thus initiating an osmotic imbalance. Not enough sodium is reabsorbed to maintain a normal osmotic pressure of the extracellular fluid. The latter becomes hypotonic to the intracellular compartment, a fact which favors a water shift into the cells with subsequent anhydremia, hemoconcentration, and reduced circulatory volume. This is shown by the increased values for plasma proteins, hematocrit, red count, hemoglobin, and oxygen capacity, and by the decreased plasma volume, blood pressure and rate of blood flow.

This migration of extracellular fluid into the cells together with a fluid shift from intravascular to extravascular spaces and the increased urine excretion is reversed in the recovery phase of the insufficient organism under treatment. The disappearance of symptoms is accompanied by the mobilization and return of tissue fluids and electrolytes to extracellular spaces and fluids. This shift may take place in spite of low Na and Cl levels (294). On the other hand, severe insufficiency with hemoconcentration, dehydration, and circulatory collapse can occur without significant Na, Cl, and water loss, and serum Na, Cl, and K changes (296).

The symptoms of insufficiency do not necessarily run parallel with the degree of sodium depletion. As a matter of fact, marmots and opossums even retain NaCl after adrenalectomy, yet they develop symptoms of insufficiency.

In adrenalectomized rats on a life maintaining diet a urinary excretory rate five times that of the normal control was observed (24), whereas the untreated animals showed a normal or decreased excretory rate with death ensuing. Oliguria may occur in the terminal stages of Addison's disease. A low ratio of water intake to urine output is a constant finding in insufficiency.

Although no consistent pathological findings in the kidneys of the insufficient organism have been recorded, degenerative changes in the proximal tubules are encountered often enough to suggest these tubules as the primary site of the disturbance. It is possible that many of the electrolyte and water changes together with their secondary symptoms may be referable to this deranged tubular reabsorption. However, it might be that the renal disturbance is a manifestation of a primary deficiency somewhere else, since the composition of the blood definitely influences the excretory activity of the tubule. For example, an excess of sodium in the blood is followed by a diminished reabsorption of this ion.

The functional disturbances of the kidneys in the state of cortical insufficiency are:

failure of the tubules to maintain electrolyte gradients between plasma and urine, i.e. failure to reabsorb sodium from the glomerular filtrate and to excrete potassium and phosphate; increased tubular reabsorption of potassium; decreased glomerular filtration rate due to the loss of Na and Cl; (the capacity for tubular reabsorption of glucose is also impaired);

the relatively increased diuresis in spite of a diminished food and water intake leading to a negative water balance in cortical insufficiency;

the impaired nitrogen-concentrating power of the kidney as reflected in the subsequent increase of the urea nitrogen and NPN levels of the blood; the cortical secretions apparently stimulate nitrogen excretion as evidenced by the nitrogen retention and decreased ammonia excretion in cortical insufficiency. This disturbance is probably renal in origin and is due, at least in part, to decreased renal blood flow;

retention of sulfate and phosphate, decreased urea clearance, and reduction of the renal blood flow may occur.

Slices of kidney tissue obtained from insufficient animals show a lower rate of oxygen uptake, a significant reduction of the respiratory rate, a decreased rate of deamination of amino acids, and defective oxidative processes (257). It may well be that the renal disturbance in insufficiency will find

an explanation in an underlying failure in the energy-yielding processes of this organ.

Treatment with salt or desoxycorticosterone (DCA) influence renal activity but do not restore its function completely. However, the administration of extract and particularly of the amorphous fraction bring about the restoration of a normal renal excretory and reabsorptive activity as far as the electrolytes in question are concerned.

6. Multiple electrolyte disturbances; acidosis; water distribution; cardiovascular disturbances; membrane permeability; diagnostic tests in adrenocortical insufficiency

(a) It appears that the electrolyte disturbances in adrenal insufficiency, from whatever primary cause they may arise, cannot be expressed in terms of sodium and potassium alone. One group of investigators (247) employing the appetite method (multiple mineral choice) showed that not only sodium and chloride, but also potassium, calcium, magnesium, lactate and phosphate ions are lost after adrenalectomy, a fact which indicates that the cortical hormones regulate not only the sodium metabolism but also that of most of the electrolytes found in the normal blood serum. The same investigators showed that the ratio K:Na is very important and that if enough sodium and other blood plasma ions are given to the insufficient animal, much potassium can be administered

without harm and even with beneficial results. The observation of a negative balance of Na, Cl, N_2, P, K, Ca, Mg in the insufficient animal (256) led Rubin and Krick to devise a solution with a composition capable of correcting this condition.

(b) Since in all stages of cortical insufficiency more base (Na) than acid (Cl) is lost through the kidneys, an acidotic condition, as indicated by a fall of the serum pH, may develop (118, 215, 328). The substitution of sodium alone does not prevent a low blood alkali reserve. For this reason one has to supply extra base in form of $NaHCO_3$ or sodium citrate together with NaCl. Animals maintained that way have survived in apparently normal condition for long periods of time.

(c) The changes in the water distribution in cortical insufficiency cannot be considered a primary defect of this condition. They are secondary to the changes in the electrolyte distribution just as the beneficial influence of cortical hormone on the water distribution is exerted via the regulation of the extracellular electrolytes. The volume changes of intra- or extracellular fluids per se are not essential aspects of cortical insufficiency (112). Forced water administration in an Addisonian had no beneficial effect on the hemoconcentration or electrolyte levels (332).

(d) The clinically important cardiovascular disturbances (211) in cortical insufficiency are secondary to other disturbances described above. They include anhydremia, hemoconcentration, decreased blood flow, decreased heart volume, reduced size of the heart, reduction of blood pressure to shock levels, peripheral stagnation, and they terminate in diastolic standstill, often with considerable dilatation.

The fact that insufficiency symptoms can be strikingly similar to shock has been frequently emphasized. Shock, from whatever cause, presents an inadequate circulating blood volume in a vascular system of abnormally large capacity due to peripheral vasodilatation. It results in reduced blood volume, inefficient circulation, inadequate oxygen transport, and tissue anoxia. A sudden increase in capillary permeability has been considered the basic etiological mechanism of the picture.

The syndrome of insufficiency with its low blood pressure, the reduced blood volume, the imminent heart failure, and the immobilized blood water in the tissues and interstitial spaces, invites a comparison of this condition with shock.

A dissimilarity, namely the hypoglycemia in insufficiency and the normal or elevated blood sugar level in shock, can be explained by the medullary activity in shock, and its absence or decrease in insufficiency together with the cortical lack responsible for the actual blood sugar decrease (349).

(e) The apparent similarity between shock and insufficiency and other considerations led to the conception that the cortical hormones might be concerned with the permeability of cell membranes (222). This idea, however, has not found universal recognition (298). A recent report (173) stresses that there is no evidence for the hypothesis that shock as encountered among surgical patients is associated with cortical insufficiency. The same investigation does not lend support for the administration of DCA as a means of preventing surgical shock.

(f) It is obvious that the employment of salt-free diets in insufficiency must lead to a catastrophe if continued long enough. The application of salt restriction tests in Addison's disease or suspected adrenal insufficiency cannot be considered safe enough to be justified. The same can be said about the potassium tolerance test or the low NaCl - high K diet for diagnostic purposes. Deaths have been reported occurring during such diagnostic tests (90, 190). The therapeutic tests, therefore, are much more suitable since they do not entail the dangers and aggravations of the other procedures.

7. Effects of the administration of sodium chloride on the insufficient organism

References: 11, 13, 14, 16, 17, 93, 100, 114, 116, 117,
242, 268, 279, 299, 300

From considerations in previous chapters it is evident that NaCl cannot be a true substitute for the cortical hormones. Nevertheless, it affords to both human and experimental animal at least a partial and temporary amelioration. This palliative effect has not been the least contribution to the increasing success in the treatment of the Addisonian patient.

The administration of NaCl to the insufficient organism
improves and restores general health,
promotes growth and prevents weight loss,
increases the fluid intake and restores the desire for food,
corrects the impaired carbohydrate absorption,
prevents further retention of potassium, favors normal potassium concentration in serum and muscle, and increases the excretion of potassium,
increases protein breakdown and nitrogen excretion,
favors normal renal function and NPN,
favors blood dilution and hydration,
restores the lost Na and Cl and prevents further NaCl wastage; however, a normal serum sodium level cannot be maintained indefinitely,

restores the resistance to toxic doses of drugs almost completely, provided food is given,

improves work capacity and voluntary activity, but not completely; the administration of 1-5% NaCl to insufficient rats which brought about a full restoration of body weight and food intake led to only partial return of activity; the activity level increases with the increased concentration of the salt solutions offered.

No benefit can be observed as to the blood sugar disturbance. The intraperitoneal administration of NaCl is ineffective in severe insufficiency in dogs. This is not surprising because even the saline-treated dog is still exceedingly frail. On the other hand, the salt-treated adrenalectomized rat enjoys fairly good health.

One probably is not wrong in assuming that this good response of the rat to treatment with NaCl has to do with the readiness of this animal to develop accessory cortical bodies or microscopic islands. Thus, the great practical importance of this treatment in the rat is that its palliative action allows enough time for the development of accessories from microscopic cortical cells, or for remnant cortical tissue to hypertrophy, under the stimulating influence of anterior pituitary corticotropin.

IV. METHODS

Aside from animals used for breeding, 212 albino rats of both sexes were used in this study for operative procedures. The strain employed had been inbred for several years but with no particular emphasis on selective breeding, so that the individual size and weight of the animals and the litter-size varied over a considerable range. In order to obtain offspring more closely approaching each other in hereditary similarity, only a few males were used for breeding purposes.

The animals were housed in home-made, all-metal wire cages placed above newspaper-covered sheet-iron trays, an arrangement which permitted frequent and convenient cleaning in a short period of time without frightening the rats. Obvious advantages of this type of cage are its cleanliness and the difficulty for parasites to find corners and gaps to live in. Furthermore, fecal material and food rests could freely fall through the raised wire bottom and could be removed at convenience. A disadvantage is that these cages do not permit a quantitative determination of the animal's food intake since the checkers fall through when gnawed down to a certain size. As this study is not concerned with the quantitative aspects of solid food intake, this cage equipment could be used with advantage.

Pregnant and lactating animals and their litters were

placed in special litter cages similar to the normal stock cages but having a narrower wire bottom in order to prevent loss of, or trauma to, the young. All of these cages contained paper shavings for nest building, a material preferred by the mothers to other materials.

Only animals bred under the care of the author were used for the experiments to be reported. The average litter size was seven (3 - 12). The young animals were weaned and separated according to sex four weeks after birth. The parent animals were given adequate rest periods between pregnancies and matings.

Every cage was equipped with a flat one-pint bottle firmly attached to the cage wall in an inverted and tilted position by means of a wire holder. The containers were standardized to contain 450 cc of fluid. Constricted nipples (glass) prevented leakage as was confirmed by tests in which the cages were shaken. The nipple at the end of the drinking tube was placed in easy reach of the animals so that they could obtain fluid even when prostrate from severe cortical insufficiency.

The fluid intake of the animals after operation was measured by emptying the remaining fluid into a graduated cylinder; the difference from 450 cc gave the intake for a certain period of time. The bottles were refilled up to the engraved 450 cc mark. Measuring, refilling, feeding, cleaning, and weighing were done at approximately the same time of the day.

Previous to being operated upon, all rats were maintained on the same adequate standard diet and were offered tap water ad libitum. The diet consisted of Purina Dog Chow checkers which, according to the makers, contain 0.56% of potassium and 0.5 - 0.67% of sodium. In the latter half of this investigation a new Dog Chow replaced the previously used checkers. According to a statement by the makers, the changes in the sodium contents do not seem to be significant (from 0.67 to 0.5%). Potassium assays of the new Dog Chow were not available at the time of this report. In order to introduce some variety in the diet, all animals received in addition a dog cake once a week (Old Trusty Dog Food). The checkers and cakes require gnawing which meets the natural food habits of the rodents and helps to keep the teeth from becoming too long. Greens, particularly lettuce, but also leaves of cabbage, spinach, beet and carrot were given at least once a week.

Prior to operation, all animals received tap water ad libitum. Care was taken not to offer ice-cold tap water during the winter months but to bring it approximately to room temperature. This was done to avoid gastro-intestinal disturbances in the operated and unoperated rats.

It was attempted to keep the animals under conditions as uniform as possible. This met with some difficulties. The location and arrangement of the animal quarters, the lack of airconditioning, the placing of the radiators, and the

large size of the windows did not permit the strict maintenance of uniform temperature and humidity. These conditions, although not interfering directly with the pursuits of this study, certainly did not exert beneficial effects on the state of health of normal or operated animals. Snuffles and upper respiratory infections were encountered not infrequently and may have influenced pregnancy of normal or survival periods of operated animals. In one instance, an animal to be operated on a very hot and humid midsummer day, was found dead in his cage on the very morning of the day of the planned operation. Though free from external signs of disease and without having exhibited any significant weight change, the autopsy revealed a pulmonary infection with fibrosis, induration, congestion, and suppuration. This being an exception and the only case of its sort, it nevertheless shows that the conditions in our colony were not ideal.

Before the actual start of this work and shortly after an influenza wave in Boston, an outbreak of pneumonia occurred in the colony which at that time, fortunately, did not yet include operated rats. Several old animals and some breeding adults succumbed.

Another difficulty, though temporary in character, arose when cimices (not uncommon in this neighborhood) appeared in our animal quarters and necessitated the use of pyrethrum and similar sprays. Though carefully protected from the direct large size of the windows did not permit the strict maintenance of uniform temperature and humidity. These conditions, although not interfering directly with the pursuits of this study, certainly did not exert beneficial effects on the state of health of normal or operated animals. Snuffles and upper respiratory infections were encountered not infrequently and may have influenced pregnancy of normal or survival periods of operated animals. In one instance, an animal to be operated on a very hot and humid midsummer day, was found dead in his cage on the very morning of the day of the planned operation. Though free from external signs of disease and without having exhibited any significant weight change, the autopsy revealed a pulmonary infection with fibrosis, induration, congestion, and suppuration. This being an exception and the only case of its sort, it nevertheless shows that the conditions in our colony were not ideal.

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Another difficulty, though temporary in character, arose when chinces (not uncommon in this neighborhood) appeared in our animal quarters and necessitated the use of pyrethrum and similar sprays. Though carefully protected from the direct influence of the spraying, a certain number of animals reacted with a slight decrease of fluid and food intake associated with a slight delay of weight gain on the day of spraying and on the following day or two. It is thought, however, that the use of the insecticides did not inflict any more serious ill-effects on the animals.

Despite the described shortcomings, all animals received the optimal attention and care as far as adequate nutriment, housing, cleanliness, and operative procedures were concerned. The success of these painstaking attempts to create as good an environment as possible under the given circumstances, was reflected in the normal or even better growth and development, the general behavior and state of health (apart from occasional upper respiratory infections) of all the animals, and in the survival periods of those operated upon.

All of the 212 experimental animals were subject to the same operative procedures. However, only 182 operated rats were used for the investigation proper; the first 30 animals served as "practice operations". It was felt that it would be necessary to master the anesthesia and operative technique before being able to rely on observations in connection with such operations and to draw any conclusions. Moreover, it was desirable to acquire a thorough knowledge of the symptomatology of experimental adrenal insufficiency and to gain experience in the interpretation of its severity. For these

reasons the first 30 rats (survivors were killed after different periods of time) were not included in any of the figures and tables of this report. The record benefitted from this series of practice operations: anesthesia accidents, hemorrhages, crushed glands, intestinal manipulations, and surgical shock were practically absent from the series of 182 animals.

Since it is well known that immature rats succumb much more readily than adults to adrenalectomy, young adult animals three months of age were chosen for the operation. The average age of the 182 rats was 90.2 days. Their average weight was 200.2 gms. (138 - 266) for the males, and 146.4 gms. (114 - 180) for the females. These figures are slightly higher than those collected for the same strain over a number of years. They also compare favorably with Donaldson's figures (76) for 90 day old albino rats as far as males are concerned. The average weight of his group is 184.8 gms. (103 - 238). Donaldson's average weight for females is 148.0 gms. (95 - 178), and almost identical with the average of our animals.

The routine procedures to be described were worked out in the practice series and strictly followed in all of the 182 animals. They proved to be satisfactory in our hands. A similar technique had been developed in this laboratory and used successfully by Wyman and tum Suden; it was also employed by Grollman (102).

Although rats are known to possess an unusual immunity and resistance to infections, and the absence of infections or complications following adrenalectomy under "soap and water cleanliness" has been reported (157), aseptic technique was employed in this investigation. This did not entail any considerable inconvenience, and it assured optimal results. The standard set of instruments prepared was

1 pair of medium-sized scissors	for extraabdominal work
1 " " " " surg.forceps	
1 " " fine straight "	for adrenalectomy and transplantation
1 " " " curved "	
1 " " small scissors	

needle and thread

Hemostats, retractors, needle holders, etc. were used in some of the practice operations but were soon discarded because they were unnecessary.

The anesthetic used in all operations was ether. The animals were placed in a glass jar containing cotton with a small amount of ether and were removed at the first signs of relaxation. The anesthesia was maintained by means of a gauze mask with ether.

An adequate area of the animal's back was freed from hair by means of curved scissors. The rats were individually numbered by holes punched in their ears. The animal was then placed on the well-lighted and warmed operating table, fastened with four elastics, and its back painted with an iodine-alcohol mixture.

The dorso-lumbar approach for adrenal extirpation was chosen instead of the transperitoneal route used by some workers. The dorsal approach for adrenalectomy is a relatively easy procedure and convenient for the subsequent transplantation.

A small skin incision was made at the lower end of the back. By spreading the blades of the scissors beneath the skin toward the cranial region of the back, subcutaneous connective tissue attachments were loosened. A 6-7 cm longitudinal midline incision was made in the cephalad direction. Tissue bands in the flank regions were loosened by blunt dissection when necessary. Care was taken that no hemorrhage occurred.

The adrenal glands were located and removed through separate openings. The proper and exact placement of the incision is of great importance for the successful and quick execution of the operation. With the adrenal directly beneath the place of entrance, it is unnecessary to widen the abdominal opening, or to search for the gland and thereby run the danger of shock following manipulation of the viscera.

The left adrenal was always located, extirpated and transplanted first. After feeling the left costovertebral angle, the lumbar muscles were penetrated with the fine straight forceps and the wound was widened by spreading the prongs to an opening of approximately 5 mm. Particular care was

taken to pierce just the abdominal wall but not to go deeper. The gland lies directly below or slightly cephalad to the opening and can easily be recognized by its light color and its shape. It moves slightly with the respiratory excursions.

While the abdominal wound was kept open, the second pair of fine forceps was used to grasp gently the periadrenal tissue and by slight pull to bring the entire adrenal tissue complex into full view near the opening, thus preventing it from slipping back into the abdominal cavity. This slight dislocation permitted the application of the curved fine forceps for pinching the adrenal stalk, assuring complete hemostasis due to clot formation in the crushed vessels with rapid coagulation. The adrenal tissue was cut loose with the fine scissors below the curved forceps. The adrenal or part of its tissue should never come out by itself, for this would indicate too strong a tension on the tissue. Such poor technique almost always results in some cortical remnant.

The removal in toto of the intact gland, without any traumatization, together with the entire capsule and all periadrenal tissue and mesenteric attachments, is the essential goal of the operation. In the large animal the periadrenal tissue (areolar, vascular, fat and connective tissue, which may contain aberrant accessory cortical tissue, microscopic

in size and capable of hypertrophy in the absence of the main glands) sometimes exceeds the main gland in bulk whereas it is only a thin veil in small animals. The touching or squeezing of the adrenal must be avoided under all circumstances. The strict observation of this rule led in our series to a very small percentage of cortical remnants.

Special instruments in different sizes according to adrenal size (319) and with spoon-like prongs (23) have been devised by some experimenters in order to facilitate the extirpation of this organ. The use of instruments of this sort appears to be entirely unnecessary and undesirable since they may do more harm than good by crushing the gland or squeezing off tissue fragments. Also, the use of a high-frequency knife to prevent hemorrhage (319), or cauterization with piecemeal removal of the gland (122) cannot possibly lead to more satisfactory results - at least not in the rat. All of these instruments and procedures may introduce an unnecessary trauma while failing to ensure removal of all adrenal tissue.

Special precautions were observed to avoid any manipulating, stretching or squeezing of the omentum and the intestines.

The excised adrenal-periadrenal tissue was halved, the halves being placed on the animal's back, and a small subcutaneous pocket prepared on each side of the animal adjacent to a large subcutaneous vessel. One half of the gland together

with its periadrenal tissue was then placed immediately into the left frontal dorsolateral pocket, the other half plus its periadrenal tissue into the right. The whole transplantation beginning with the completed adrenalectomy of one gland need not take more than five seconds. Because of varying amounts of periadrenal tissue and in order to avoid unnecessary dissection of the adrenal, it was not always possible to get two halves exactly equal in size, a condition which, however, is not of importance. This fast transplantation procedure if done with all precautions and great care yields optimal results. It avoids rough handling and drying of the fragile tissue, and it leads to a minimal interruption of the connection with the nutrient media. In order to facilitate the quick transfer of the adrenal to its new bed, the organ was not weighed or measured because an exact quantitative determination would require the stripping of the gland from the periadrenal structures with possible injury, loss of time, and little if any gain. It will be pointed out in the discussion that size and function of takes are conditioned by the anterior pituitary and are probably independent of the bulk of transplanted material as long as a certain amount of capsule is grafted.

This subcutaneous type of heterotopic autoplastic transplantation was chosen for several reasons. The location of an intramuscular transplant in a living animal frequently

requires examination and dissection of tissues, manipulations favoring shock which occurs only too easily in animals with even a slight degree of adrenal insufficiency. Subcutaneous grafting, on the other hand, promised a much easier accessibility with a view to subsequent exploratory operations with removal of the transplants. Aside from the convenience of subsequent examination and removal, it was also of interest to compare the incidence of takes and the efficiency of functioning intramuscular and subcutaneous autotransplants. The large number of adrenal grafts done over a number of years in this laboratory had been performed intramuscularly. Subcutaenous transplantation must not necessarily entail poorer vascularization since our grafts were placed invariably adjacent to large subcutaneous vessels, thus facilitating the quick establishment of a generous vascular supply as could be seen from autopsy findings at different intervals after operation. Any appreciable movement was eliminated by the formation of adequate pockets at operation. On the whole, it was felt that this subcutaneous type of graft was not less protected than the intramuscular type.

Adrenalectomy and transplantation of the left organ was followed immediately by the same procedure on the right. While the left adrenal in the rat is situated slightly more posterior and central than the right, the latter is found between the last rib and the vertebral column. The bisected right gland was transplanted into two subcutaneous pockets

in the animal's right and left flank close to large subcutaneous vessels. The adrenalectomy is slightly more complicated on the right side than on the left due to the topographical relation of the right organ to the liver and the inferior vena cava. With some experience the extirpation of the right gland is achieved with the same ease as in the case of the left adrenal.

Adrenalectomy is more difficult in older and heavier animals on account of the greater distance between adrenals and abdominal wall and the larger amount of peritoneal fat. In these animals, it is particularly important to place the abdominal wound correctly.

The small size of the abdominal opening made a suture superfluous. The skin incision was closed by a continuous suture with a subsequent iodine-alcohol painting. The time between incision and suture of the skin did not exceed 10 minutes and usually was between 6 and 7 minutes.

The ether mask was removed as soon as the skin suture was started so that the animal became conscious right after being removed from the operating table and placed into a separate cage. Within a fraction of an hour the rats operated upon appeared normal in every respect and were frequently seen eating and drinking with relish within 15 minutes after being removed from the table. They often appeared playful and responded as usual. The only difference from normal

animals was that they appeared somewhat sleepier for some time.

The operated animals were left in separate cages for at least one week for observation. This prevented the chewing of the wound by cage mates. Eventually, - provided they were in excellent health, active, had gained weight, thus suggesting takes and indefinite survival, - several operated animals of the same sex and experimental group were placed together, although never more than four animals in one cage. The slightest sign of insufficiency, a weight loss for any other reason, or behavior different from that of normal rats, led to separation and special observation with frequent weight determinations.

The operated animals were distributed into four experimental groups with litter mates of the operated series equally divided whenever possible. No mating was allowed at any time; all females were virgins. The only exception was rat #177 which became pregnant after being placed in a wrong cage for a short time before operation. This female destroyed her litter of seven within a week after delivery.

All experimental groups received the same care and were kept under the ordinary conditions of the colony with free access to the regular stock diet. Group I received tap water ad libitum as the only source of fluid; group II, 1% NaCl solution; group III, 1% KCl solution; and group IV, 0.5%

KCl solution. This diet was started immediately after operation. The salt solutions were prepared from the pure salts and distilled water. A route of fluid and salt administration other than the oral was not used. Intraperitoneal injections, for instance, repeated over a three month period, would have been a rather harmful procedure. The oral administration was more suitable despite the uncertainty of intestinal absorption.

The surviving animals were, as a rule, observed for approximately three months following operation, some as long as 121 days after transplantation (213 days of age). Detailed records were kept of every animal's complete history, including operations, postoperative course, weight, fluid intake, and autopsy findings. The fluid intake figures of the tables include only unquestionable data; all doubtful values were discarded. Changes in weight proved to be fairly reliable indicators of cortical insufficiency.

All surviving animals of the NaCl group were put on tap water for some time during the postoperative period in order to exclude the possibility that NaCl in the absence of viable cortical tissue might have been responsible for the prolonged survival. Rat # 143 serves as an example: the animal had survived for more than two months in fairly good health, but without appreciable weight gain; she died of acute adrenal insufficiency three days after being put on tap water.

No viable cortical tissue could be found at autopsy.

Surviving animals, unless used for removal operations or functional tests, were sacrificed from 70 to 116 days after adrenalectomy and transplantation. They were killed with ether.

In order to verify the completeness of the adrenal ablations, all animals were autopsied except for a few. The latter were rats that had died and decayed during an exceptionally hot and humid summer period or had been destroyed by cage mates. A particularly careful search for remnant or accessory adrenal tissue was undertaken and all viscera were examined for changes possibly related to cortical insufficiency. The transplants, or whatever remained of them, were carefully removed with the surrounding tissues for fixation in 10% formalin for subsequent histological examination of their cellular and vascular structure. Again, the grafts were not weighed since it was thought that the preservation of the graft and its bed might yield more information than the recording of their weights. A representative number of takes, accessories, remnants, and questionable structures was imbedded in paraffin, sectioned and stained (H.E.).

A series of 24 surviving animals, including both sexes and all experimental groups, underwent exploratory operations 76 to 95 days after the transplantation. Under the same routine of anesthesia and asepsis as described above, the

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Surviving animals, unless used for removal operations or functional tests, were sacrificed from 70 to 116 days after adrenalectomy and transplantation. They were killed with ether.

In order to verify the completeness of the adrenal ablations, all animals were autopsied except for a few. The latter were rats that had died and decayed during an exceptionally hot and humid summer period or had been destroyed by cage mates. A particularly careful search for remnant or accessory adrenal tissue was undertaken and all viscera were examined for changes possibly related to cortical insufficiency. The transplants, or whatever remained of them, were carefully removed with the surrounding tissues for fixation in 10% Formalin for subsequent histological examination of their cellular and vascular structure. Again, the grafts were not weighed since it was thought that the preservation of the graft and its bed might yield more information than the recording of their weights. A representative number of takes, accessories, remnants, and questionable structures was imbedded in paraffin, sectioned and stained (H.E.).

A series of 24 surviving animals, including both sexes and all experimental groups, underwent exploratory operations 75 to 95 days after the transplantation. Under the same routine of anesthesia and asepsis as described above, the sites of transplantation were searched for takes which, when found, were carefully removed with some surrounding tissue for fixation in formalin. This operation served a threefold purpose. In the first place, it was intended to be a check on the function of the grafts. Secondly, it was of great interest to see whether, under certain conditions, heterotopic dormant cortical cells are capable of regeneration. Finally, the incidence of accessory masses and their response to deprivation of the organism of transplanted cortical tissue deserved examination. These animals, operated upon for the second time, were placed in individual cages and received tap water and the normal stock diet ad libitum. All of them were carefully autopsied regardless of whether they had died of insufficiency of were sacrificed after three or four weeks.

A batch of ten surviving animals of group I, II and IV was turned over to Dr. Wyman for a functional (hemodilution) test described elsewhere. The sacrificed animals were autopsied by the author.

V. RESULTS

Group I (Tap water)

A. Male (Table I)

The operation was performed on 25 animals whose average age was 90.0 days and whose average weight was 198.8 gms.

Six rats died of cortical insufficiency within 24 days, the average being 12.8 days. 19 rats survived the observation period of about three months at the end of which time 15 were sacrificed and 4 underwent removal operations. At the autopsy of #156, a cortical remnant was found on one kidney; grafts were neither found at the removal operation nor at autopsy after the sacrifice of this animal. Since the remnant apparently suppressed the growth of the transplanted tissue, the rat was excluded from the following percentage figures. 75.0% of the rats of this series survived with takes; 25.0% succumbed to cortical insufficiency without takes.

With three exceptions, all surviving animals displayed postoperatively a temporary weight loss, ranging from 0.8 to 13.5% of the weight at operation. The average weight gain of the surviving rats was 0.77 gms. per day, ranging from 0.26 to 1.17 gms. The average weight loss of the animals that succumbed was 14.3%, ranging from 7.1 to 25.9%.

The average fluid intake per day of the surviving rats

was 21.6 cc, that of the succumbing animals 16.0 cc.

Three transplants took in six animals, two in seven, and one graft took in 5 rats.

Animal # 3 had two takes in spite of the presence of three cortical remnants.

B. Female (Table I)

The operation was performed on 26 rats whose average age was 89.9 days and whose average weight was 143.6 gms.

13 rats died of cortical insufficiency; the average survival period was 26.2 days. The rats that succumbed include four cases of chronic cortical insufficiency which died between 32 and 51 days after operation. The other animals died within 28 days of the acute and subacute type of insufficiency. 13 animals survived the observation period. Of these #160 has to be excluded since it possessed a remnant which inhibited the regeneration of grafts. Therefore, 48.0% of the rats survived with takes, and 52.0% died of cortical insufficiency. Two of the surviving animals underwent removal operations; eleven were sacrificed.

With one exception, all surviving animals displayed postoperatively a temporary weight loss ranging from 0.7 to 16.2% of the weight at operation. The average weight gain of the surviving rats was 0.35 gms. per day, ranging from 0.11 to 0.72 gms. The weight loss of the rats that succumbed averaged

16.8%, ranging from 5.6 to 23.8%.

The average fluid intake per day of the surviving rats was 17.4 cc, that of the succumbing animals 15.9 cc.

Four transplants took in two animals, three in four, two in six, and one graft took in one animal.

Animal #36 had three takes in spite of the presence of an accessory cortical body.

For several weeks rat #55 suffered from a severe labyrinthitis resulting in continuous rotatory movements of the whole body and nystagmus of the head, with marked exertion. However, this animal held her own and did not loose weight. This resistance can be ascribed to the presence of three good takes. No accessory or remnant cortical tissue could be found at autopsy at the end of the three month observation period.

Group II (1% NaCl)

A. Male (Table I)

The operation was performed on 26 rats whose average age was 90.8 days and whose average weight was 192.1 gms.

Three rats died of acute cortical insufficiency within 22 days, two of them with signs of lung infection. The survival period was 13.6 days, and the mortality was 12.0%. Animal #22 which died of shock due to hemorrhage during the

operation was excluded from these figures. 22 animals, i.e. 88.0%, survived the observation period with takes. This figure includes animal #13 which died at the end of three months of an intercurrent pulmonary infection, after a normal development and complete absence of symptoms of insufficiency. At autopsy a good take was found. 17 of the surviving animals were sacrificed and 4 underwent removal operations.

13 of the surviving animals displayed postoperatively a temporary weight loss, ranging from 0.4 to 9.6% of the weight at operation. The average weight gain of the surviving animals was 0.80 gms. per day, ranging from 0.47 to 1.38 gms. The average postoperative weight loss of the rats that succumbed was 18.8%, ranging from 14.0 to 21.0%.

The average fluid intake per day of the surviving rats was 32.6 cc, that of the succumbing animals 22.8 cc.

Four transplants took in two animals, three in six, two in seven, and one graft took in seven rats.

Good takes were found in two animals in spite of the presence of remnant or accessory cortical tissue.

B. Female (Table I)

The operation was performed on 26 rats whose average age was 90.7 days and whose average weight was 146.5 g~~r~~ms.

Six animals died of cortical insufficiency , two of the acute type within 15 days, and four of the chronic type

within 72 days. There was a 23.1% mortality. 20 animals (76.9%) survived. This figure includes three rats two of which died in the third postoperative month of an intercurrent lung infection and one of an unknown cause. All three animals had good takes. Seven of the surviving rats were subjected to removal operations and ten were sacrificed at the end of the observation period.

Only four of the surviving animals displayed a temporary postoperative weight loss, ranging from 0.6 to 8.3% of the weight at operation. The average weight gain of the surviving rats was 0.41 gms. per day, ranging from 0.22 to 0.68 gms.

The average fluid intake per day of the surviving rats was 31.8 cc, that of the succumbing animals 29.5 cc.

Four transplants took in three animals, three in five, two in six, and one graft took in five rats.

Rat # 4 which survived 107 days postoperatively was then used for a massive intraperitoneal dextrose injection. Subsequently it died, and decay set in early because of the excessive heat of that day, so that consequently the animal could not be autopsied.

Three animals had takes in spite of the presence of remnant or accessory cortical tissue.

Group III (1% KCl)

A. Male (Table I)

The operation was performed on 14 rats whose average age was 89.9 days and whose average weight was 209.2 gms.

Eleven rats (78.6%) died of acute insufficiency within 17 days, the average survival period being 10.6 days. Three animals (21.4%) survived; of these, two were sacrificed and the third was subjected to a removal operation.

All three surviving animals displayed postoperatively a temporary weight loss, ranging from 2.7 to 10.7% of the weight at operation. The average weight gain of the surviving was o.53 gms. per day, ranging from 0.49 to 0.58 gms. The postoperative weight loss of the animals that succumbed averaged 18.5%, ranging from 14.6 to 22.3%.

The average fluid intake per day of the surviving rats was 20.0 cc, that of the succumbing animals 14.0 cc.

Three transplants took in two animals, and two took in the third rat.

B. Female (Table I)

The operation was performed on 13 rats whose average age was 91.2 days and whose average weight was 149.0 gms.

Eight animals (61.6%) died of acute cortical insufficiency within 16 days, the average survival time being 11.0

days. Five rats (38.4%) survived; one of them was subjected to a removal operation, the other four were sacrificed at the end of the observation period.

Two of the surviving animals displayed postoperatively a temporary weight loss of 2.0 and 6.9% of the weight at operation; the lost weight was rapidly regained. The weight gain of all surviving rats averaged 0.43 gms. a day, ranging from 0.11 to 0.58 gms. The postoperative weight loss of the animals that succumbed averaged 16.1%, ranging from 4.2 to 29.9%.

The average fluid intake per day of the surviving rats was 16.3 cc, that of the succumbing animals 15.3 cc.

Four transplants took in one animal, three in two rats, and two in two.

Two of the surviving animals had takes in spite of the presence of remnant or accessory cortical tissue.

Group IV (0.5% KCl)

A. Male (Table I)

The operation was performed on 27 rats whose average age was 89.1 days and whose average weight was 200.7 gms.

13 rats succumbed after an average survival period of 17.1 days. One animal died of chronic cortical insufficiency 34 days after operation, and one of an intercurrent lung

days. Five rats (38.4%) survived; one of them was subjected to a removal operation, the other four were sacrificed at the end of the observation period.

Two of the surviving animals displayed postoperatively a temporary weight loss of 2.0 and 6.9% of the weight at operation; the lost weight was rapidly regained. The weight gain of all surviving rats averaged 0.43 gms. a day, ranging from 0.11 to 0.58 gms. The postoperative weight loss of the animals that succumbed averaged 16.1%, ranging from 4.2 to 29.9%.

The average fluid intake per day of the surviving rats was 16.3 cc, that of the succumbing animals 15.3 cc.

Four transplants took in one animal, three in two rats, and two in two.

Two of the surviving animals had taken in spite of the presence of remnant or accessory cortical tissue.

Group IV (0.3% KCl)

A. Male (Table I)

The operation was performed on 27 rats whose average age was 59.1 days and whose average weight was 200.7 gms.

13 rats succumbed after an average survival period of 17.1 days. One animal died of chronic cortical insufficiency 34 days after operation, and one of an intercurrent lung

infection 44 days after operation, despite two good takes. The latter animal was excluded from the percentage figures. 14 animals survived the observation period at the end of which time 11 were sacrificed and three subjected to removal operations. 46.2% of this series died of insufficiency and 53.8% survived with takes.

With one exception, all of the surviving animals displayed postoperatively a temporary weight loss, ranging from 1.0 to 15.7% of the weight at operation. The average weight gain of the surviving rats was 0.86 gms. per day, ranging from 0.48 to 1.29 gms. The average weight loss of the animals that succumbed was 22.8%, ranging from 11.5 to 29.1%.

The average fluid intake per day of the surviving rats was 23.0 cc, that of the succumbing animals 18.1 cc.

Four transplants took in one animal, three in 2 rats, two in five, and one graft in seven.

Two animals possessed takes in spite of the presence of remnant or accessory cortical tissue.

B. Female (Table I.)

The operation was performed on 25 animals whose average age was 89.9 days and whose average weight was 146.5 gms.

14 rats (56.0%) died of acute and subacute cortical insufficiency within 26 days, the average survival period

being 14.8 days. Eleven rats (44.0%) survived the observation period with good takes. Nine were sacrificed, and two were subjected to removal operations.

Nine of the surviving animals displayed postoperatively a temporary weight loss, ranging from 0.7 to 17.2% of the weight at operation. The average weight gain of the surviving rats was 0.38 gms. per day, ranging from 0.16 to 0.58 gms. The weight loss of the animals that succumbed averaged 20.0%, ranging from 13.2 to 27.0%.

The average fluid intake per day of the surviving rats was 16.9 cc, that of the succumbing animals 14.6 cc.

Four transplants took in one rat, three in five animals, two in four, and one graft took in one animal.

Three animals had takes in spite of the presence of remnant or accessory cortical tissue.

The results of all experimental groups are summarized in table I.

VI. SYMPTOMS OF CORTICAL INSUFFICIENCY AND AUTOPSY FINDINGS IN THE ANIMALS OF THIS STUDY

1. Symptoms of insufficiency

The symptoms of cortical insufficiency encountered in the animals that did not possess functioning cortical tissue at autopsy, were identical with the picture obtained from adrenalectomized non-transplanted rats of the practice series. In the transplanted animals with insufficiency, the syndrome was sometimes delayed, though not significantly.

The animals, though responding, and taking food and fluid immediately after operation appeared sluggish, and sat quietly in the corner of the cage or slept. This stage was gradually overcome by those animals that later showed good transplants. Most of the rats were somewhat sleepy, but otherwise exhibited a normal behavior. They were as playful as formerly; but while normal animals continued to run and jump around the cage after being petted, the rats in the early postoperative stage soon would huddle in a corner of the cage.

The insufficient rats reacted differently. Their voluntary activity decreased from day to day. At first, the animals still responded when food was offered and showed at least some tendency to play or to bite an instrument put through the wire wall. Later, they did not do that anymore. Whenever they were picked up to be examined or weighed, they

attempted to move backwards, a behavior rarely shown to such an extent by normal animals of the colony. This retreat was also attempted on the weighing pan, and even while the rat was being held in the hand. The animals, when held, whined in a typical way, hardly encountered in normal animals. The decrease in voluntary activity together with the timidity displayed was quite typical of the earlier stages of insufficiency and was invariably noted in the rats which later died of insufficiency. Our observations concerning activity are similar to those of other investigators (242). It has been claimed that inactivity is one of the first symptoms to be observed after the onset of insufficiency, and that it is the last of the test functions to become normal after therapy. This observation was amply confirmed by the author's experiments. The animals with good takes, though by far more active and playful than the insufficient rats, often showed a sluggishness and timidity which were not normal. This difference from the normal, though at times hardly noticeable, is indicative of a latent mild chronic insufficiency. This observation was made in every group of experimental animals. The NaCl rats were just as sluggish as the others. As a matter of fact, it often appeared that the surviving KCl rats were more lively than the sodium animals. The decreased voluntary activity of the rats with takes should not be overemphasized because - in the absence of specific tests - it was not easily noted, and observation

of the difference was made possible only by frequent comparison with normal animals of the same age.

The insufficient animals huddled in the corner of the cage, became progressively disinterested in their surroundings, and soon displayed a ruffled and shaggy fur. Sometimes the hair appeared coarser than in the normal animal, and thinning of the hair on the back and near the flanks was observed. In many animals a rusty dirty-brownish coloration appeared, particularly in the region of the head and the nape of the neck. As long as the animals could move, they did so in a poorly coordinated way. They walked slowly and stiffly, and wobbled. In more advanced stages they swayed and fell against the cage walls, finally not being able to right themselves. At that stage, nasal and conjunctival discharges, frequently dark-brown and bloody, could be observed.

The insufficient rats showed a definite and progressive decrease in the consumption of food and fluid. The latter was not so obvious in the earlier stages. Even quite prostrate rats were observed trying to reach the water bottle in order to drink. The best indication of anorexia was their response to the greens offered them. Normal animals invariably began at once to eat the lettuce, or whatever was offered, and consumed everything put before them. The rats in advanced stages of insufficiency did not even touch the greens. Vomiting was observed, but not frequently. The muscular weakness

and excessive prostration were invariably accompanied by a marked asthenia and emaciation, which was best seen near the hips.

Diarrhea was observed in a few animals; however, it did not appear to be an outstanding symptom of insufficiency. Although many authors have claimed that diarrhea is a significant symptom of insufficiency in the rat, Gaunt (91) has expressed doubts as to whether one should place diarrhea on the list of insufficiency symptoms at all. The author of this investigation tends to agree with Gaunt, at least as far as the rat is concerned.

The animals of our colony have frequently been observed to eat paper or at least to try to grab some paper from the sheet below the cage. This was done by insufficient as well as by normal animals of all ages.

The progressive weight loss of the insufficient animals is one of the outstanding and significant symptoms of this condition. It was observed in almost all rats and was the guiding indicator as to whether an animal should be separated from cage mates.

The more advanced stages of insufficiency were always accompanied by panting and labored respiration with the appearance of foam around mouth and nose. The increase of the respiratory rate was usually an indication that the and excessive prostration were invariably accompanied by a marked asthenia and emaciation, which was best seen near the hips.

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The more advanced stages of insufficiency were always accompanied by panting and labored respiration with the appearance of foam around mouth and nose. The increase of the respiratory rate was usually an indication that the terminal stage had been reached and that one could expect the final collapse within 24 hours. Several animals which could be observed in this final stage displayed convulsions.

Aside from this picture of insufficiency observed in our animals, a few other symptoms in the rat, noted by various investigators but not studied in this series, should be mentioned. The general biochemical and metabolic disturbances of this condition are described in detail in another chapter. The increased sodium excretion, potassium retention, hypoglycemia, and blood concentration in the rat are quite similar to findings observed in other animals and in the Addison patient. The electrolyte and blood sugar changes in the rat have been detected as early as 24 hours after adrenalectomy (15). The amount of excreted sodium in advanced insufficiency may actually be decreased due to diminished blood flow through the kidney. The amount of urine and feces is usually decreased. Increased peristalsis has been observed by some workers. Low blood pressure is noted in the rat as it is in other species. A fall in body temperature is largely a terminal sign and can be expected within 12 - 24 hours of death.

2. Autopsy findings

A comparatively large number of pathological changes in animals dying of cortical insufficiency (especially in the rat and the dog) has been described in the literature. In the following, observations of the author will be recorded. Since the surviving animals with takes presented an essentially normal picture after being sacrificed, this chapter will deal rather with the animals dying of insufficiency. No attempt is made to list the findings according to their frequency. The author is not prepared to make a definite statement as to the frequency of pathological changes, since the number of our animals which died of insufficiency is relatively small. A much larger series of adrenalectomized rats would be necessary to define a scale of frequency of autopsy findings. Moreover, this study was not intended to investigate the pathology of cortical insufficiency. In several animals, no particular gross changes, except for the diminution of body fat, could be found which might be interpreted as the cause of death.

Not infrequently, a thinning of the fur and coarser hair could be observed, sometimes associated with a dirty-brownish discoloration, particularly in the nape of the neck.

The almost complete absence of subcutaneous and intra-abdominal fat was a frequent finding and was almost invariably

associated with severe insufficiency. On the other hand, if the surviving animals were killed and had either takes or accessory tissue, an abundant and normal amount of body fat could be observed.

An increase in the amount and size of lymph nodes was invariably found. These lymph nodes, when present in the neighborhood of the kidney, may sometimes be mistaken for cortical accessory bodies. However, they are easily identified when held against light by showing their typical structure which resembles a giant red blood cell.

Slight adhesions between the upper renal poles and the liver or spleen were observed. They are, of course, to be expected and are of no significance.

Pulmonary findings, usually only moderately severe, were frequently recorded. In some cases, however, extensive abscesses were found. Congestion and edema, fibrosis, emphysema, hepatization in various degrees of severity were observed.

Sinus infections and empyema of the middle ear were noted several times and are to be considered an aggravation of an upper respiratory infection.

Little can be said about gross changes of the genital organs. A very marked testicular atrophy was noted in one case (animal # 118). Congestion of the female genital structures was observed several times.

Congestion of various internal organs, particularly of the gastro-intestinal tract , occurred quite frequently. A few times blood was found in the intestinal lumen. (Gastric ulcers, and bile in the stomach due to reversed peristalsis have been observed in dogs dying of insufficiency. Marked pancreatic congestion (280) and enlargement of the thymus have been observed by many workers in various species.) In this series, congestion of the pancreas did not seem to be more outstanding than that of other internal organs.

Engorgement of subdural vessels and sinuses was not infrequently encountered. It can be assumed that this also is a manifestation of the failure of the right heart.

An earlier report from this laboratory (338) lists the most characteristic autopsy findings in 174 rats according to their frequency as follows:

congested intestine, stomach, lungs; diarrhea, bloody eyes, blood in the contents of the ileum, congested kidney, enlarged thymus, congested thymus or hemorrhagic spots, bloody nose.

The author's experiences differed from this report in several instances. For example, hemorrhagic secretion around the eyes and nose of dying animals was found very frequently and can be related to the terminal cardio-vascular upset. On the other hand, signs of diarrhea were encountered far less frequently. As a matter of fact, diarrhea should not be considered under "autopsy findings" since it is definitely a clinical and not a pathological entity.

The diagnosis of "congestion" has been overemphasized and used indiscriminately by many workers. It is felt that one should not attach too much significance to the word "congestion" unless one has good macroscopic or, whenever in doubt, microscopic evidence for an abnormal accumulation of blood in an organ or tissue. The state of the autopsy material also should always be considered in order to exclude postmortem changes from the pathological picture of cortical insufficiency.

The grafts in rats dying of insufficiency usually had a waxy, yellowish or grayish appearance. They often lay loosely in their pockets without any adherence to the surrounding tissues. In very few instances, they were liquefied. In the great majority of cases, they presented the picture of a dry coagulation necrosis. The medullary part very often could be recognized by the difference in color. (Coenen (52), 36 years ago, stated that the macroscopic structure of degenerated adrenal transplants in dogs was recognizable days or even weeks after transplantation.) If the non-viable graft was somewhat adherent to the surrounding tissue, vessels could be traced in the vicinity of the site of transplantation. This vascularization was similar to that found in takes. Sometimes, three to five vessels could be seen coming towards the graft from different directions. It was frequently found in the animals that had died of subacute or chronic insufficiency that the grafts had been absorbed. In few cases, no

traces of the graft could be found. In most cases, a little brownish or yellowish-black pigment was encountered. This pigment was sometimes scattered over an area of about 1 cm in diameter.

Takes were easily recognized by their protrusion from the subcutaneous tissue and by their color. They invariably were darker than the necrotized grafts and usually brownish-red, often with a bluish tinge. They were well imbedded in subcutaneous fatty tissues, provided that the animal was not emaciated, and several distinct vessels ran from all sides towards the new cortical organ. The diameter of the takes ranged approximately from one to seven mm, the majority being 3 to 4 mm in diameter. As a rule, the grafts were round and appeared as elongated or ovoid bodies only a few times. The takes were always well encapsulated. Sometimes they had a dark blue center which exceeded the lighter outer portion in area.

Accessory or remnant bodies were found only in a few instances. Most of them were of pinhead size and had the yellow-brown color of a normal adrenal gland. If they were found on the upper pole of the kidney, they most likely were remnants of the extirpation. If, on the other hand, they were separate from the kidney and located on the cava or other vascular structures not far from the kidney, or near the lower margin of the liver or spleen, or dissected out of the retroperitoneal tissues, they were probably genuine hypertrophied accessory bodies.

VII. DISCUSSION AND CONCLUSIONS

1. Grouping, weight and size of the animals

With the exception of group III (1% KCl), each experimental group comprises 51 or 52 animals, a number which is considered to be sufficient for drawing conclusions.

Group III comprises only 27 animals. The study started out with a 1% KCl concentration. As soon as we learned how toxic this particular solution is, and how little chance there is for rats treated in this manner to survive and for the transplants to be subsequently incorporated, the series was discontinued and replaced by series IV (0.5% KCl). To be sure, this potassium concentration is still very toxic; but as can be seen from our results, it permits a greater number of survivals and takes.

As has been pointed out, the 182 experimental animals, all three months of age, were of an average size and weight which compared very well with or exceeded that of Donaldson's standard values or that of animals of our colony observed and recorded over several years previous to this investigation. With few exceptions, the general health of the animals at the time of the operative procedures appeared to be good.

2. Animals dying of insufficiency due to failure of incorporation of transplants

The lowest mortality of the transplanted groups of rats is exhibited by the NaCl-treated animals of both sexes. This is to be expected since sodium chloride per se, even without the existence of any gross cortical tissue in the body, is at least able to prolong life and facilitate the hypertrophy of preformed accessories. There is in the NaCl series of this study, however, a 12% and 23.1% mortality rate despite the transplantation of cortical tissue. These mortality figures show that claims which have been made concerning the indefinite survival of salt-treated animals have to be viewed very sceptically. As far as an administration of 1% NaCl is concerned, such claims are probably not correct. In the author's experience it does not seem possible to obtain a 100% survival of rats lacking an adequate amount of cortical tissue.

The tap water control groups show mortality figures about twice as high as in the corresponding NaCl groups. The salt content of the dry food alone is apparently not sufficient to provide any significant protection against insufficiency. The call for hormone production by the grafts is an urgent one, and if the transplanted tissue is not incorporated, a comparatively great number of animals succumb, in a rather short time. The need for cortical hormone in the salt-treated

animals, on the other hand, is less urgent; the electrolyte disturbances and fluid shifts are delayed, at least for some time, and accessory tissue, if present, has a chance to hypertrophy and to attain a functional state.

Both sodium chloride and water groups are quite similar as far as the ratio of mortality in the male and female is concerned. About twice as many females succumb to cortical insufficiency regardless of whether or not they receive NaCl. However, the author does not consider this as a specific sex difference. One has to realize that the size and weight of female animals at the age of operation are far less than those of the male. An average difference of 50 gms. was observed. This does not mean, of course, that the female animals are more immature than the males. (Immature rats are known to be less resistant to adrenalectomy.) However, smaller size and weight in the female per se, possibly associated with less resistance, may account for this greater mortality. This does not exclude constitutional factors. However, too much emphasis should not be placed on the sex difference since nobody at present is able to point to a specific cause which might account for the differences in weight, size, and mortality.

The mortality figures of the KCl series are quite different from the water and NaCl groups. The great toxicity of 1% KCl brings about a higher mortality in either sex than observed animals, on the other hand, is less urgent; the electrolyte disturbances and fluid shifts are delayed, at least for some time, and accessory tissue, if present, has a chance to hypertrophy and to attain a functional state.

Both sodium chloride and water groups are quite similar as far as the ratio of mortality in the male and female is concerned. About twice as many females succumb to cortical insufficiency regardless of whether or not they receive NaCl. However, the author does not consider this as a specific sex difference. One has to realize that the size and weight of female animals at the age of operation are far less than those of the male. An average difference of 50 gms. was observed. This does not mean, of course, that the female animals are more immature than the males. (Immature rats are known to be less resistant to adrenalectomy.) However, smaller size and weight in the female per se, possibly associated with less resistance, may account for this greater mortality. This does not exclude constitutional factors. However, too much emphasis should not be placed on the sex difference since nobody at present is able to point to a specific cause which might account for the differences in weight, size and mortality.

The mortality figures of the KCl series are quite different from the water and NaCl groups. The great toxicity of 1% KCl brings about a higher mortality in either sex than observed in any other of our experimental groups. The number of male animals succumbing is surprisingly higher than that of the females. In the 0.5% KCl series, the number of females dying of insufficiency was only 4% higher than that of the corresponding tap water animals, but the mortality of males on 0.5% KCl was 21% higher than that of male tap water rats. Contrary to the 1% KCl group, the 0.5% KCl treated rats also showed what was conspicuous in tap water and NaCl-treated groups, namely a greater number of females succumbing to the experimental insufficiency. The difference between male and female is less marked in the water and 0.5% KCl rats and amounts to about 10% whereas it amounts to about 27% in group I.

It is not clear why the male 1% KCl animals exhibit a greater susceptibility to the toxic action of KCl and why this is not found in the 0.5% KCl rats. It may be that a greater number of 1% KCl animals would have yielded a different figure. Be that as it may, the exact mechanism of potassium intoxication in relation to the two sexes is not known, and the underlying factors causing the observed differences, therefore, cannot be discussed.

It is of interest to notice that the eight cases of chronic cortical insufficiency in the tap water and NaCl groups belong to the female sex. This observation which does not seem to be related to the electrolyte intake points to

the possibility that the female sex hormones might come into play in these young mature animals and achieve, though not necessarily to a very great extent, a delay of severe symptoms. A beneficial effect of progesterone (of similar chemical structure as the cortical substances) in the insufficient organism is known to occur. The above eight female rats with chronic cortical insufficiency seem to confirm the possibility of sex hormone influence.

However, if that is so, would it not be reasonable to expect a greater number of female rats to survive? The facts presented above actually show that more females succumb, at least in groups I, II, and IV. This may lead to the conclusion that a protective mechanism in the female via progesterone is, at least in the state of acute cortical insufficiency, not sufficiently effective in overcoming possible constitutional disadvantages of the female and thus does not help to approach the lower mortality figures of the male sex.

Such a conclusion is not necessarily correct. This can be seen from the survival periods of the tap water animals. The average survival period of the females is definitely longer, even if one does not include the chronically insufficient animals; it is 19.3 days in the female as compared with 12.8 days in the male. Since only two NaCl females died of acute insufficiency, it is not possible to give conclusive figures for this group. But the above difference in the sur-the possibility that the female sex hormones might come into play in these young mature animals and achieve, though not necessarily to a very great extent, a delay of severe symptoms. A beneficial effect of progesterone (or similar chemical structure as the cortical substances) in the insufficient organism is known to occur. The above eight female rats with chronic cortical insufficiency seem to confirm the possibility of sex hormone influence.

However, if that is so, would it not be reasonable to expect a greater number of female rats to survive? The facts presented above actually show that more females succumb, at least in groups I, II, and IV. This may lead to the conclusion that a protective mechanism in the female via progesterone is, at least in the state of acute cortical insufficiency, not sufficiently effective in overcoming possible constitutional disadvantages of the female and thus does not help to approach the lower mortality figures of the male sex.

Such a conclusion is not necessarily correct. This can be seen from the survival periods of the tap water animals. The average survival period of the females is definitely longer, even if one does not include the chronically insufficient animals; it is 19.7 days in the female as compared with 12.8 days in the male. Since only two such females died of acute insufficiency, it is not possible to give conclusive figures for this group. But the above difference in the survival time of untreated acutely insufficient rats in which grafts are not incorporated shows definitely that there is a factor in the female organism which tends to delay the onset of the terminal stages of cortical insufficiency. There is no reason to exclude the possibility that this factor is an ovarian hormone, progesterone or a similar active substance. This factor, however, does not seem to be efficient and potent enough to counteract the constitutional weakness of the female, and its presence, in spite of its potential beneficial effect, does not prevent the observed higher mortality rate in the female rat with failing incorporation of adrenal transplants.

The survival times of all animals of all groups dying of acute and subacute cortical insufficiency averaged 13.78 days. This figure does not include the survival periods of those rats which died of chronic insufficiency, i.e., after 30 postoperative days. Nine such animals were observed (## 32, 33, 37, 47, 54, 72, 93, 113, 143); their survival periods were 32, 42, 40, 34, 34, 49, 51, 59 and 72 days after transplantation. Spontaneously or on withdrawal of the salt solution they displayed the characteristic symptoms of cortical insufficiency.

The average values of the survival period observed in all of the acutely or subacutely insufficient rats do not, in the opinion of the author, vary sufficiently to permit any

significant conclusion. The females of the tap water group lived an average of five and one half days longer than the general average. This higher figure is in part explained by some subacute cases in this series. A possible relation to female sex hormones as a factor in delaying the terminal symptoms has been mentioned above. Otherwise no pertinent sex or other differences appear in the experimental groups. Thus, the conclusion may be drawn that an early failure of the transplant to establish itself in its new bed will result in death after a survival period similar to that of untreated bilaterally adrenalectomized rats. The survival period of the succumbing animals as such does not appear to be much influenced by the different treatment of the four groups. The average survival period of the NaCl animals lies slightly below and that of the 0.5% KCl rats a little above the total average value. Thus, while the potassium administration definitely influences the mortality, it does not exert any profound effect on the time factor in the mechanism of death from insufficiency.

Another conclusion may be drawn from these figures. Since the survival periods of animals dying of insufficiency without having established viable grafts, are almost identical with the survival time of untreated adrenalectomized rats or, at best, only slightly above that figure, it can be said that the cortical hormone content of the transplanted tissues is significant conclusion. The females of the tap water group lived an average of five and one half days longer than the general average. This higher figure is in part explained by some subacute cases in this series. A possible relation to female sex hormones as a factor in delaying the terminal symptoms has been mentioned above. Otherwise no pertinent sex or other differences appear in the experimental groups. Thus, the conclusion may be drawn that an early failure of the transplant to establish itself in its new bed will result in death after a survival period similar to that of untreated bilaterally adrenalectomized rats. The survival period of the succumbing animals as such does not appear to be much influenced by the different treatment of the four groups. The average survival period of the NaCl animals lies slightly below and that of the 0.5% KCl rats a little above the total average value. Thus, while the potassium administration definitely influences the mortality, it does not exert any profound effect on the time factor in the mechanism of death from insufficiency.

Another conclusion may be drawn from these figures. Since the survival periods of animals dying of insufficiency without having established viable grafts, are almost identical with the survival time of untreated adrenalectomized rats or, at best, only slightly above that figure, it can be said that the cortical hormone content of the transplanted tissues is of little if any significance. It apparently is absorbed immediately; and if the animal is to survive, the reactivation of cells or regeneration with subsequent hormone secretion must follow soon. The actual amount of hormone stored in the grafts seems to be negligible.

The average weight loss of the animals of all groups dying of insufficiency without incorporation of the grafts was 18.2% of the preoperative level. The water group on the whole displayed average weight losses smaller, and the 0.5% KCl rats greater, than the average value. The potassium toxicity cannot be made responsible for the greater weight loss in the latter group since the 1% KCl animals did not show any marked deviation from the average. They could be expected to show the toxic effect of potassium in a different weight figure if the potassium as such would influence it. But such apparently is not the case to any significant extent.

Other workers have found an average weight loss of 17% after complete one-stage adrenalectomy in rats (184, 231). This figure compares well with that obtained in our study.

From the comparison with the weight losses of untreated animals it can be concluded that the non-incorporated transplanted tissue does not exert any influence which might manifest itself in a different weight level at death. Together with the above observations it can be stated that the insufficient animal with non-incorporated grafts does not

differ in any significant respect from the untreated adrenalectomized rat.

The chronically insufficient rat with non-incorporated grafts also does not differ significantly from the animal with chronic insufficiency after complete adrenalectomy. That chronically insufficient animals actually can gain weight above the preoperative level was seen in several cases of this study. Such an increase in weight is in part due to the continuous growth processes of the young adult organism. On the other hand, the weight gain might be the result of either the salt treatment (if the animals belong to one of the salt groups) or the possible existence of microscopic accessory tissue in the absence of gross remnants. The animals start to lose weight as soon as the accessory structures are exhausted. From this moment on, the process of succumbing is similar to that of acute insufficiency. As can be seen from the cases with chronic insufficiency in this study, the weight losses are usually smaller than those in acute insufficiency, or the animals may even gain a little weight or hold the preoperative weight level.

The results of this study do not suggest any consistent sex difference as far as weight loss after operation is concerned. Kroc's experiments (183) show marked variations between the two sexes; his figures, however, cannot be considered conclusive in this respect since part of his animals

were castrated and the number employed in some of his series is not satisfactory.

Reviewing all figures of the present investigation, no definite correlation can be detected between the degree of postoperative loss of body weight, and the length of the survival period or the initial preoperative weight level. A similar observation for adrenalectomized female rats has been made by other investigators (184).

The results of our study are not in agreement with an observation of Soji (278) who stated that the weight losses are most marked when the rats died on the sixth to the ninth day after adrenalectomy. Though the time factor has some bearing upon the loss of weight in acute insufficiency, it appears that postoperative weight losses are primarily conditioned by other factors, among which the pituitary stimulation of accessory structures seems to be significant.

3. Animals surviving with incorporated transplants

Various figures for the incidence of successful cortical autotransplants may be found in the literature. Wyman and tum Suden (355) claimed success in 95% of their rats. Jaffe and his group (162-166) reported a series of intramuscular grafts in the rat in which only four out of 67 animals died after transplantation. Jaffe claims that at least 80% of the autografts in the rat regenerate and that in guinea pigs

84% takes could be obtained.

For reasons mentioned in a previous chapter, any values given for the frequency of the incorporation of transplants are open to question. The comparatively low survival figures of the author's series are, at least in part, due to constant objective sifting of the material and classifying of doubtful cases as negative. The author feels that even such figures might turn out to be too high if more precise and reliable functional tests were available. The histologically normal appearance of a graft, its ability to maintain life for some time, and the maintenance of normal activity and work performance of the animal do not suffice to permit a final conclusion that such a take is perfect. Such a conclusion and the solution of many a question of adrenal and transplant behavior will have to be derived from quantitative biochemical data, which, it is to be hoped, will be made available as the knowledge of the cortical secretions advances.

The highest percentage of surviving animals with takes was expected and found in the NaCl groups, the lowest in the 1% KCl series. The sodium groups top the water animals with 21%, while all tap water rats show an approximately 12% higher number of survivors than all 0.5% KCl animals. The latter group still has a mortality rate 19% lower than the 1% KCl rats. Together with the histological results,

these observations permit the following conclusions.

The increased number of survivors in the NaCl-treated group is due to the beneficial effect of NaCl which brings about a prolonged survival period. The delay of the onset of acute insufficiency provides a respite for the grafts. Vascularization, scavenger processes, and other helpful reactions in the direct vicinity of the graft can go on. Cells of the cortical capsule may remain for some time in a latent state before better nutritive conditions permit and initiate regenerative processes. There is no reason to doubt that cortical cells can withstand such a latent period. Later in the discussion it will be emphasized that pre-cortical cells, persisting perhaps for the entire life-time of an organism, can possibly emerge from their hidden existence at any time, provided they are "called" by anterior pituitary secretion. Moreover, Dornfeld's experiments (77, 78) show that the tremendous ultracentrifugal force, though causing cytologic displacements, do not destroy the regenerative capacity of cortical cells. Why, then, should the essentially undisturbed existence of grafted cortical cells in a "friendly" environment lead to an immediate loss of their regenerative ability? There is no reason to make such an assumption. As long as the administration of NaCl prevents the profound disturbances of acute adrenocortical insufficiency, the possibility of regeneration remains. This path of regeneration, however, is not compulsory. The abrupt

deprivation of the graft of its vascular supply, the possible accumulation of metabolic or breakdown products in its surroundings, the deterioration of the medullary part, the peculiar reactions of the surrounding tissue, and many other factors may cause the doom of the transplant. Yet, the chances of overcoming these obstacles are greater in the salt-treated group than in any other. The result is that the majority of transplants regenerates as is expressed in the high figure of surviving animals with successful takes.

This reasoning leads to another conclusion. Taking into account the fact that the sodium chloride administration prolongs life and facilitates regenerative and preregenerative adjustments which would not be possible in an organism under the greatly disturbed state of acute insufficiency, and considering all histological evidence obtained, it can be said that from these experiments one cannot conclude that NaCl has any direct action on the adrenal graft. There is no good reason to assume that the increased sodium intake has any stimulating effect on the cortical cell just as there is no evidence for an effect of this ion via the pituitary. NaCl acts in the transplanted organism as it does in the untreated animal deprived of its cortices, namely, as a palliative device preventing to a certain extent the impending results of cortical deprivation and permitting adjustments which are otherwise impossible.

One might ask: why then is a state such as chronic cortical insufficiency possible? If the life prolonging influence of NaCl facilitates the regenerative capacity, why does not the more or less extensive survival period of chronic insufficiency, whatever its cause may be, finally lead to takes?

It has to be pointed out again that even under optimal conditions for readjustment, adequate nutrition and regeneration, the capsular cell is not compelled to regenerate. It is just not possible to push a living cell into a phase of activity if possibly there are damages which are irreversible or inherent factors in the cell itself which oppose regeneration. The nature of such factors is entirely obscure. The problem would boil down to the question: why does one cell grow and regenerate, and another does not? This is essentially similar to the question: why does a normal cell stop growing and a malignant cell continue? Needless to say, at the present time no sound explanation can be given.

What has been said positively about NaCl can be expressed in the negative for the potassium salts. If NaCl favors, KCl inhibits cellular adjustments towards regeneration. It has to be emphasized that this does not seem to be a specific influence on the cortical cell but a general one, probably acting on the entire organism. The fact that potassium is toxic in the insufficient animal and the reason for the toxicity have been discussed extensively in a

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What has been said positively about NaCl can be expressed in the negative for the potassium salts. If NaCl favors, KCl inhibits cellular adjustments towards regeneration. It has to be emphasized that this does not seem to be a specific influence on the cortical cell but a general one, probably acting on the entire organism. The fact that potassium is toxic in the insufficient animal and the reason for the toxicity have been discussed extensively in a previous chapter. This toxic action - as any toxic action would do to a certain extent - puts a considerable strain on all functional systems of the organism and, therefore, interferes with any attempts e.g. of the vascular and reticuloendothelial system to clear the way for the reestablishment of a graft. Moreover, the precipitation of crises at an early point interrupt all steps of regeneration which might have been initiated. Thus, it is obvious that the more concentrated KCl solution exhibits a more deleterious action than the less concentrated one. If an animal can withstand these toxic influences successfully by having at its disposal enough reserve forces, for instance in form of small amounts of accessories, and if these reserves are not strong enough to completely inhibit the transplant, a graft can then regenerate, as is seen in the minority of cases of the KCl series. The excellent morphologic and functional state of these few successful takes shows that the overcoming of the initial obstacles opens the road to success regardless of what ion is offered. The majority of animals, however, particularly in the 1% KCl series, is overwhelmed by these initial blows due to the toxic action of potassium in the insufficient organism. This explains the low survival and incorporation figures in the 0.5% KCl series and the even lower ones in the 1% KCl groups.

With the exception of group III which comprised only a

small number of surviving animals, more males than females of all the other experimental groups had successful takes. The possible influence of sex factors on survival and regeneration has been discussed earlier in this chapter.

A considerable number of animals which survived with takes exhibited a varying temporary weight loss immediately after transplantation. In all instances this loss was regained in time. The loss of weight ranged from 0.4 to 17.2% of the preoperative weight level. No correlation could be found between the preoperative weight level, the final success of the transplantation, and the weight loss. In all series, a greater number of males than females exhibited this loss of weight. It can be assumed that the immediate lack of cortical material produces this fall which is eventually corrected by the beginning of active hormone production of either graft or accessory structures. The electrolyte administration did not seem to influence this initial weight loss.

Since weight changes permit, to a certain extent, conclusions as to the growth of the animals, it was interesting to calculate the rate of weight gain of all animals which possessed viable grafts and to relate the results to the functional capacity of the transplants.

The average daily weight gain for all male animals of group I, II, and IV was 0.81 gms.. This figure represents a three month period following transplantation at the age

of 90 days. This value is almost identical with Donaldson's figure of 0.80 obtained from a large number of normal albino rats between the age of 90 and 182 days.

The comparison of the author's results with Donaldson's standard values and previous records of our own colony indicates that the male rats of group I, II, and IV with takes gained weight and probably grew at a normal rate. The individual figures for the three groups are very close to the average, a little above in the 0.5% KCl series. It cannot be said with certainty whether the value above normal is a constant feature of the latter group. Such a statement would require a greater number of animals of this group with successful grafts. Nevertheless, it is conceivable that animals which were able to overcome the initial aggravation through potassium intoxication must be excellent specimens as far as strength, vitality, and reserves are concerned, and might therefore have a growth rate superior to that of less well-developed rats. Moreover, the possibility of an increase of corticotropin production (perhaps only temporary) manifesting the increased call for cortical hormone following the administration of potassium, cannot be excluded.

The figure of the weight gain per day of the male 1% KCl group was not included in the above average value since it is felt that the small number of surviving animals (three) of 90 days. This value is almost identical with Donaldson's figure of 0.80 obtained from a large number of normal albino rats between the age of 90 and 152 days.

The comparison of the author's results with Donaldson's standard values and previous records of our own colony indicates that the male rats of group I, II, and IV with takes gained weight and probably grew at a normal rate. The individual figures for the three groups are very close to the average, a little above in the 0.5% KCl series. It cannot be said with certainty whether the value above normal is a constant feature of the latter group. Such a statement would require a greater number of animals of this group with successful grafts. Nevertheless, it is conceivable that animals which were able to overcome the initial aggravation through potassium intoxication must be excellent specimens as far as strength, vitality, and reserves are concerned, and might therefore have a growth rate superior to that of less well-developed rats. Moreover, the possibility of an increase of corticotropin production (perhaps only temporary) manifesting the increased call for cortical hormone following the administration of potassium, cannot be excluded.

The figure of the weight gain per day of the male 1% KCl group was not included in the above average value since it is felt that the small number of surviving animals (three) cannot give a conclusive average value. This particular figure (0.53 gms./day) is much below the average for all other males of this study and of Donaldson's series.

The average value for the daily weight gain over the post-operative period for all female animals of group I, II, and IV is 0.38 gms. This figure is definitely lower than Donaldson's (0.53 gms.) which was obtained from a large number of normal albino rats between the age of 90 and 182 days.

The individual average figures of the female groups I, II, and IV are very close to the 0.38 value. The figure for the females of the 1% KCl series is not included because of the small number of survivors of this group. It is slightly higher (0.43 gms.)

This discrepancy between the author's and Donaldson's values for female rats does not mean, however, that the females of all experimental groups grew at a slower rate than normal. A comparison with figures obtained from normal animals of our stock over a long period of time show that the approximate weight gain for females of our colony equals 0.4 gms. per day for a corresponding period of development. This value compares very well with the one obtained from this study (0.38). Therefore, it can be concluded that the female rats with good takes gain weight and probably grow at a rate normal for our particular strain. It seems that our female stock animals have a decreased

growth rate in adulthood as compared with the standard Wistar females.

It was observed in several animals - irrespective of diet or sex - (example: # 86) that after a good weight gain for several weeks after operation, a stop and subsequent decrease of weight occurred. This suggests the inability of the apparently well-established takes to maintain growth and body weight to the same extent as do normal cortices.

4. The amount of regenerated cortical tissue

Size and number of cortical grafts which appear viable do not necessarily indicate their functional capacity. It has been observed in this study that even poor-looking, small takes might function better than big grafts of the size of a normal gland.

However, it is quite interesting to compare all of the experimental groups for the number of regenerated grafts. This is done not to obtain a criterion of transplant function, but to judge the readiness of the animals of the different groups to incorporate the transplants offered. This comparison may indirectly furnish information whether or not the treatment of the grafted animals has any influence upon the incorporation of transplants.

The average number of grafts found in animals with success-

ful takes of all experimental groups was 2.4 takes per animal.

The average figures of the individual groups show little if any deviation from the above average figure with the possible exception of the male 0.5% KCl rats which had only 1.8 takes per animal. But even this value is not sufficiently different to constitute a significant deviation from the average.

This leads to the conclusion that all experimental groups regenerated, on an average, approximately the same number of grafts. If this is correct it must follow that none of the treatments employed seems to influence specifically the incorporation of transplanted tissue. This appears to be an important confirmation of the author's conclusions of the preceding chapter.

It is interesting to note that without exception the average values for the number of regenerated grafts of all female groups was slightly higher than that of the corresponding male groups.

A Canadian investigator (3) basing his results on two (!) clinical cases claimed that potassium salts accelerate the regeneration of granulation tissue and maintain the vitality of endothelial and fibrous tissue. Sodium salt supposedly have the opposite effect on these structures. The results of our work suggest that such a specific beneficial or deleterious effect of the ions mentioned does not exist in the

case of cortical regeneration.

Summarizing our findings in 106 rats with successful takes, it can be stated that four takes were found in 10 animals, three in 32, two in 38, and one graft in 26 animals.

An earlier publication from this laboratory (347) gave the results obtained from 109 autotransplantations in rats as follows. Four takes were found in 11 rats, three in 14, two in 25, one graft in 23, and one, two or no takes together with one or two accessory bodies in 36 animals.

While the number of animals posessing four takes is almost identical in these two series, it seems that more rats of our investigation incorporated three and two grafts than in the earlier report. It is doubtful whether this difference is of much significance. Although, as has been indicated above, the treatment in the author's study has probably not much to do with the number of the regenerating grafts, the difference in technique of the earlier work and of this report might at least in part account for the slightly different results. Previous transplantation experiments in this laboratory have been carried out intramuscularly whereas the author employed the subcutaneous site.

As to the size of the individual transplants, it was found that they may attain the size of a normal gland or even exceed it. The latter, however, did not happen often. The takes were never larger than 7 mm in diameter. The presence of several takes in the same animal usually resulted in smaller individual grafts.

5. Gross accessory and remnant cortical tissue and its association with incorporated transplants

It has been mentioned that particular care was taken in the operative procedures in order to avoid the fragmentation of the glands to be extirpated and to avoid leaving remnants of periadrenal tissue. This care was rewarded by a comparatively small number of gross remnant and accessory bodies. 18 individual masses were found among all experimental animals, all of them not very far from the original adrenal site. The location of these bodies included the upper pole of the kidney, the renal vessels, the vena cava, the retroperitoneal fat, and the lower and ventral margin of the liver. The bodies were never bigger than 2 - 3 mm in diameter, mostly smaller. The 18 masses include several accessory bodies which were found after the removal of the grafts from their subcutaneous site of transplantation. Since this removal most likely induced the development of these bodies from microscopic islands, the actual number of these masses which would have been found on sacrifice of all animals without preceding removal operation might have been even smaller. 14 of the bodies were present in spite of viable takes, this again including animals from the removal series. Various papers in the literature (337, 339, 357) also report on successful grafts with accessory tissue in the same animal.

These observations suggest a conclusion with an important

bearing on the judgement of the grafts' adequacy. The discussion on the adreno-pituitary relationship and the formation of accessory bodies (see below) stresses that an adequate amount of cortical tissue, be it the intact glands or remnant or accessory tissue, will not tolerate an additional amount of functional cortex. Some of the excess cortical tissue would atrophy via a pituitary depression through excess cortin. If this is correct, - and it seems to be, - it can be taken for granted that at the time of transplantation no gross accessories were present in the body. This conclusion can be drawn for still another reason. Had there been an accessory body with any appreciable functional capacity, a graft never would have been incorporated since the available corticotropin would have been fully engaged with the already established cortical structure. Thus, it can be stated that in all probability the grafts were the only gross cortical structure present immediately after operation. The accessory or remnant cortical tissue must have developed despite the presence of and in addition to the regenerating transplants. That this could happen in 14 cases clearly indicates that the grafts are not of the same functional capacity as the intact cortex. Corticotropin - under normal conditions probably fully occupied with the established cortical tissue - must have been available in sufficient amounts to permit the hypertrophy of microscopic rests or remnant cortical cells.

The author feels justified in stating that despite the apparently normal growth and behavior displayed by the animals of all groups possessing incorporated grafts, the takes often do not entirely equal the functional capacity of normal cortical tissue. Again, the different treatment of the various experimental groups has apparently not influenced this subnormal character of the grafts in any way.

6. The operative removal of incorporated transplants

24 animals from all experimental groups were subjected to an operation with the intent of removing all or part of the grafts which could be found. All of the animals used for this purpose exhibited a normal postoperative development. This suggested that the transplants introduced three months prior to the exploratory and removal procedure had taken.

In a few animals one take was left in place. The rats survived without any change in behavior, weight gain, or appearance. In the absence of accessory tissues this proves the functional adequacy of the one remaining graft.

The removal operation was done from 76 to 95 days after transplantation. Five male and five female animals succumbed to the operation within 29 days, with an average survival period of 13.7 days, a figure which is identical with the average survival of the animals that succumbed to acute or subac. insuff. after the first operation. The ten rats displayed a weight loss from 1.3 to

26.8% of the preremoval level, apparently unrelated to the survival time.

It is of interest to note that the survival time was either short or belonged to the subacute type. This indicates that those animals which died soon after the removal possessed grafts which were the only support of the organism in its need for cortical hormones. The longer survival, on the other hand, suggests that the removed takes must have permitted a certain development of accessory cortical tissue , even if it could not be found in some cases, which finally became exhausted and accounted for the delayed death. This might mean that these grafts did not have the full functional ability of the others or of normal cortex.

The surviving animals were sacrificed from 17 to 30 days after the removal operation. This period might appear to be rather short; but in all cases, with the exception of three to be mentioned separately, there was good reason to assume that the animals would have survived indefinitely, even if they had not reached the preremoval weight level again. All of these animals had takes at autopsy which were left in situ on purpose, or they had regenerated cortical masses and accessory bodies.

In several instances it was found that regenerated masses had developed in the subcutaneous tissue where at the removal procedure no take could be found. This regenerated tissue then

must have developed rather rapidly from minute masses of "pigment" which apparently still contained cells capable of regeneration if called by corticotropic stimulation. This observation which reminds one of the presence of abdominal microscopic cortical islands led the investigator of this study to a concept which will be dealt with in point 8 of this chapter.

In six instances accessory tissue was found at autopsy after the sacrifice. This again suggests that the transplanted tissues might not have been entirely adequate and functionally efficient. This would have resulted in some development of accessory structures which probably was greatly enhanced after the removal of the takes, thus facilitating the survival of the animals.

As to the three exceptions mentioned above: for technical reasons animals ## 152 - 154 had to be sacrificed on the 19th postoperative day. In contrast to the other surviving animals of the removal series, they showed a progressive loss of weight which indicates that these rats eventually would have died of subacute cortical insufficiency. This would have increased the number of females succumbing to the removal operation above that of the males and also would have led to a higher value of the average survival time. At autopsy these animals did not show any accessory or other cortical tissue; this confirmed the previous assumption of

their imminent death.

The surviving animals possessing cortical tissue were regaining an initial weight loss following the removal operation. Some of them were still below the preremoval weight level at the time of being sacrificed; others displayed a weight gain up to 9.3% four weeks after the removal operation.

Most of the animals received tap water for one to two weeks prior to the removal operation, and all of them received tap water following the operative procedure. The measuring of the fluid intake of these animals yielded the following results. The water intake following removal operation was usually smaller than the intake of the salt solutions previous to the removal of the transplants. This difference was most marked in the animals of the NaCl groups. The amount of tap water taken in by the animals which finally succumbed to insufficiency was, in the majority of cases, below that taken in before the operation. Most of the rats which survived the removal operation showed an increased intake of tap water as compared with that of corresponding preoperative periods.

7. Histological observations

Good grafts, listed and counted in the tables as "takes", display a typical morphological structure which is similar in all of them, though certain variations are noticeable.

The successful graft is invariably surrounded by a thin capsule of connective tissue which often sends strands into the interior of the cortical body. These bands are very fine and apparently form a supportive structure. Usually some subcapsular sinuses can be found. Immediately adjacent to the capsule is a small but definite zone packed with typical cortical cells which has the characteristic appearance of the zona glomerulosa of the normal gland. Though the HE stain is not suitable for a cytologic examination of cells as far as their mitotic activity is concerned, mitotic figures may be found in several instances in this layer of the graft.

These glomerulosa cells, as well as other cells found in the viable grafts, show some degree of fine granulation which might indicate a difference from normal cortical cells.

Next to the packed marginal zone comes the broadest zone found in grafts, a usually orderly arranged zone of fascicular cell rows accompanied by blood sinuses. This arrangement is unmistakable in many of the grafts examined. This orderly fashion is not displayed in tangential sections and in some transplants which, though having cortical cells of good appearance, were lacking this fascicular arrangement, but displayed cells similar to the glomerulosa cells only in a less dense manner.

The innermost cortical layer reminds one of the reticularis with a loose, net-like arrangement of cells.

This usually orderly arranged cortical layout in successful grafts is of great interest in view of certain observations made by other workers. Some investigators working with cortical transplants in varies species have stated that they did not succeed in obtaining grafts arranged similarly to the normal gland (148, 242, 265). The opposite can be concluded from our findings. With the technique employed by the author and regardless of the treatment of different groups it is possible to obtain a structural picture very similar to that of the normal gland. This fact suggests that the regenerative processes in our grafts follow the same pattern of growth as they do in the normal gland as has been described in the chapter on the life cycle of the cortical cell. Moreover, the orderly arrangement together with an abundant blood supply (sinuses, capillaries, venules, and arterioles in and near the cortex) from a morphologic point of view alone, suggests the functional capacity of such grafts.

Adjacent to the reticular layer a fibrotic zone usually is found corresponding to the medullary core. Some sections, however, contain only cortical cells without having a center of different tissue. It seems that these represent grafts which were halved not exactly in the middle of the adrenal gland so that one half contained less medulla than the other. The smaller amount of medullary debris apparently was cleared away and the subsequent regeneration resulted in a compact

cortical body similar to an accessory gland.

The center usually contains connective tissue cells with many fibroblasts and often a relatively large amount of a homogeneous blue material, a typical sign of calcification. The latter can be seen even with the naked eye. Frequently the fibrotic core contains a great number of blood sinuses filled with erythrocytes. This vascular center accounts for the dark blue color of some grafts which has been mentioned above. Deposits of hemosiderin can be found frequently in the fibrous tissue and also, but less often, in the cortical parts.

As a rule, the cortical layers enclose the fibrous core as is seen in the serial sections. Sometimes, however, a hilus-like connection between between the tissues surrounding the graft and the fibrous center is established containing for the most part more or less dense connective tissue and vascular elements.

In younger grafts, necrotic masses are found occasionally, surrounded by vascular and connective tissue structures and apparently without eliciting any profound reaction. This reminds one of other instances (e.g. in tuberculosis) where non-viable tissue also is tolerated for a long period of time without producing reactions from the surrounding tissue and without necessarily interfering with processes of growth in adjacent portions of the organ.

The fibrous center frequently shows vacuoles which suggest the existence of fatty tissue. Smaller vacuoles were found in the reticularis too, a feature which agrees with the loose structure of this particular portion of the cortex.

An occasional giant cell and a number of lymphocytes with some leucocytic elements can be found. Such cells are present least frequently in the cortical portions.

If debris is still present in the center of the graft, it seems obvious that coagulation necrosis led to this state. In some cases structural features of cells can still be recognized but the fading-out of the nuclear elements and the granulation of the protoplasm suggest the death of the cells. In other cases homogenous debris indicates the more advanced stage of cellular breakdown.

The abundance of vascular and cellular elements in all layers of all transplants show that rebuilding processes go on actively even weeks and months after transplantation. This does not mean that it takes so long a time to establish the graft fully. It is the author's impression that such an establishment with adequate functional capacity can be achieved much earlier. What it shows primarily is the most intimate incorporation into the vascular supply system of the organism and the marked cellular activity indicating the power of development and regeneration of the transplanted tissue, the cooperation of the surrounding tissues, and thus

the full success of the grafting.

The tissues surrounding the successful grafts display the typical structure of subcutaneous tissue including a good blood supply to the graft, fat, connective tissue, and fibrocytes. Phagocytic cells are encountered and occasionally a very mild inflammatory reaction can be seen. Hemosiderin deposits suggest that previously more abundant vascular reactions had been going on in the region which finally regressed to the state of the tissue found at examination.

8. "Dormant" cortical cells

The observations and findings recorded in previous chapters lead to a conclusion which may explain many features of adrenocortical regeneration, be it in accessory bodies or in transplants.

The well known fact that in states of cortical insufficiency accessory bodies may develop from non-visible predecessors led to the assumption of microscopic cortical or precortical islands in various places of the abdominal cavity as a remainder of early embryonic cell migrations.

Such microscopic rests cannot by nature possibly occur in the dorsal subcutaneous tissues of the rat. If, however, good takes are removed from an animal, and shortly after a new graft has developed from a barely visible or invisible focus of atrophied and pigmented graft cells, the following

conclusion seems to be imperative. Primitive cortical cells (as in the case of accessories) or regressed former cortical cells (as in the case of non-viable transplants) apparently are able under certain conditions to retain a potential regenerative capacity which, initiated by corticotropin, might come into play in cortical deprivation.

Let us call this type of cell a "dormant" precortical cell. Dormant, because such a cell is most likely not functional in that stage; and precortical, because it might develop into a normal cortical cell. It must be similar in character to the indifferent parent cell of the adrenal capsule.

In the case of the graft that has regressed, this dormant type of cell is actually postcortical since it is a remainder of a once functional cortical entity. This, however, does not detract from the term "precortical"; the latter is warranted by the potential ability of such a cell to reestablish itself.

The small cortical cell accumulations sometimes found in the periadrenal tissue point in the same direction. They and obscure peritoneal cells, being the forerunners of accessory bodies, may constitute a system of potential cortical reserves which finally may decide the fate of the organism in case of cortical deprivation induced experimentally or by disease. The species variations in the amount of precortical accessory cells and the ability to develop accessory organs

seem to be dependent upon the extent of this system.

Though it has nothing to do functionally or embryologically with the sympatho-medullary chromaffine system of the body, this dormant precortical tissue constitutes a certain parallel to the chromaffin system as far as reserve mechanisms of the body are concerned.

The exploration of the cytological properties of dormant precortical cells was beyond the scope of this investigation. An exhaustive histo-cytological study with special cytological methods will be necessary to elucidate the finer properties and the histochemical features of these cells.

The assumption of this reserve system not only explains many of the author's observations but permits correlation with the investigations of other workers.

It has been found that a vigorous regeneration can be obtained from a capsule remaining in the organism after a unilateral adrenal enucleation if the other adrenal gland is extirpated eight weeks later (147). This capsule had been inactive during this period of time, and then showed an apparently unimpaired regenerative capacity. Some of Kroc's ear grafts (183) appeared to be poor looking for as long as 42 days after transplantation, and then suddenly they developed in as little as eight days, sometimes simultaneously with a regression of the graft in the opposite ear. The explanation seems to be that an interference with

the viability or full function of one graft leading to its atrophy or destruction initiates the "awakening" of other cortical tissue via the pituitary corticotropin. The assumption of an "awakability" of accessory, remnant, and atrophied cortical tissue is compulsory. The compensatory hypertrophy of cortical tissue similarly presupposes the potential capacity of non-functional or slightly functioning cortical cells to yield a full functional activity if called upon by the anterior pituitary.

Thus, the adrenopituitary relationship together with the recognition that cortical cells or precortical structures may possess inherent regenerative powers gives a reasonable explanation for many of the phenomena observed.

9. Electrolyte and fluid intake

Measurements of fluid intake were done routinely in all experimental groups over the entire period of observation. The intake of animals on salt solutions or water was measured in some cases as long as 106 days before they were sacrificed or underwent removal operations. The results obtained confirm previous work done by other investigators and demonstrate the electrolyte disturbances in the state of insufficiency as has been discussed in another chapter. The author's figures for NaCl and water intake compare well with values published by other investigators for adrenalectomized rats (10, 93, 94, the viability or full function of one gland leading to its atrophy or destruction initiates the "awakening" of other cortical tissue via the pituitary corticotropin. The assumption of an "awakability" of accessory, remnant, and atrophied cortical tissue is compulsory. The compensatory hypertrophy of cortical tissue similarly presupposes the potential capacity of non-functional or slightly functioning cortical cells to yield a full functional activity if called upon by the anterior pituitary.

Thus, the adrenopituitary relationship together with the recognition that cortical cells or precortical structures may possess inherent regenerative powers gives a reasonable explanation for many of the phenomena observed.

9. Electrolyte and fluid intake

Measurements of fluid intake were done routinely in all experimental groups over the entire period of observation. The intake of animals on salt solutions or water was measured in some cases as long as 100 days before they were sacrificed or underwent removal operations. The results obtained confirm previous work done by other investigators and demonstrate the electrolyte disturbances in the state of insufficiency as has been discussed in another chapter. The author's figures for NaCl and water intake compare well with values published by other investigators for adrenalectomized rats (10, 93, 94, 224, 243, 245, 246, 262).

Within certain variations, the amount of water or potassium chloride solutions ingested by animals which finally succumbed to cortical insufficiency, was the same. The groups on sodium chloride ingested more than the other groups. The increase of the NaCl-female over the male is not considered significant; only a few of the NaCl rats died, and the few figures obtained do not give valid averages.

In general, the surviving animals showed no marked differences in the amount of fluid consumed. However, the groups on NaCl ingested far more fluid than any of the others. The fact that the NaCl intake after establishment of takes is still above normal suggests that there is a certain cortical insufficiency which brings about the increased salt appetite.

The greater amount of fluid ingested by the male survivors of all experimental groups of this study is readily understood if one considers the greater weight of these animals.

The increased salt intake in insufficiency is very well known (50, 262). It has been shown that in the salt-maintained adrenalectomized dog the high NaCl intake and output virtually amount to a saline perfusion of the animal's kidneys. The result is a compensation for an otherwise impaired kidney function with a more or less efficient elimination of waste products, nitrogenous substances, and potassium.

In the Addisonian patients, too, salt craving is common.

They display an increased desire for food rich in salt contents and have an aversion against sweets (243, 246). A striking example is the case of a three and one half year old boy with hyperplasia of the androgenic zone and subsequent diminution of the cortical layers (330). An extraordinary craving for salt was observed with an increased fluid intake and aversion against sweets. The boy died while on a normal hospital diet. He must have kept himself alive for two and one half years previous to hospitalization by consuming very large amounts of salt.

Mineral losses or increased mineral needs are closely reflected by increased appetite for minerals. This has been known by farmers for hundreds of years. Many clinical observations (for instance in pregnancy or lactation, or in parathyroid disturbances) and animal experiments have substantiated this old knowledge. The increased appetite forms the basis for Richter's self selection method (244). In extensive investigations Richter was able to show the close relationship between appetite and nutritional needs not only for minerals but also for vitamins, fats, carbohydrates, and proteins. In the case of the adrenalectomized animal he concluded that the increased salt ingestion is probably not consequent to the experienced alleviation of the insufficiency discomfort but more likely to changes in the taste receptors in the oral cavity with a lowering of the salt taste threshold and an enhanced ability of taste dis-

crimination.

The results of the author's study and the results obtained by many other workers definitely suggest that every animal having adrenal transplants should receive sodium chloride (with or without sodium bicarbonate or citrate) at least immediately after operation in order to tide the animal over the most critical period of acute insufficiency and in order to give the graft a chance to establish vascular connections and get rid of the degenerating masses. It is clear from the work done that this is in no way a specific facilitation of the graft but merely a general osmotic and life-prolonging effect.

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SUMMARY

(1) The present status of experimental and therapeutic adrenal transplantation has been critically reviewed and discussed, and the factors influencing the establishment and functioning of incorporated grafts have been analysed.

(2) Adrenocortical function and insufficiency, particularly in relation to electrolyte and water balance and to renal excretion and reabsorption, have been reviewed. The adreno-pituitary relationship and its bearing on cortical atrophy, hypertrophy, and regeneration, as well as on the existence and amount of accessory cortical tissue, have been discussed.

(3) One hundred and eighty-two rats were subjected to complete adrenalectomy and transplantation of their own adrenals. The operated animals were distributed in four groups which received either tap water, 1% NaCl, 1% KCl, or 0.5% KCl solution in addition to the standard solid diet. 24 animals from all experimental groups underwent a second operation during which established transplants were excised. The postoperative course of all animals including their weight changes and fluid intake, and the gross and microscopic autopsy findings were recorded.

(4) The mortality rate of the 182 animals, sex differences in resistance to cortical insufficiency and in amount of regenerated transplants, the survival period of succumbing rats, the incidence, character, and functional capacity of the takes, the observed changes in behavior, weight, and salt and fluid appetite, and the occurrence of accessory cortical tissue have been discussed and the findings correlated with the known properties of adrenal glands and transplants. A hypothesis of "dormant" cortical cells has been suggested.

THE BEHAVIOR OF ADRENOCORTICAL TRANSPLANTS IN THE RAT UNDER THE INFLUENCE OF INGESTED SODIUM AND POTASSIUM SALTS

Abstract of a Dissertation

Submitted in partial fulfilment of the requirements
for the degree of Doctor of Philosophy
BOSTON UNIVERSITY GRADUATE SCHOOL

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1942

An analysis of the factors facilitating or impeding the incorporation and regeneration of adrenal transplants led to the investigation of the possible influence of ingested sodium and potassium salts on adrenal grafts. The important relationship of adrenocortical function to the normal balance of these electrolytes in the organism made this study all the more desirable since as yet no one apparently has studied their possible effect on the incorporation and character of transplanted adrenal tissue.

One hundred and eighty-two albino rats of an inbred strain, reared and maintained under uniform conditions, underwent at 90 days of age a complete bilateral adrenalectomy via the dorsolumbar route under ether anesthesia and with aseptic technique. The excised glands were halved and immediately transplanted to dorsal subcutaneous pockets adjacent to blood vessels. The subcutaneous site was chosen for transplantation because of its accessibility and in order to establish a comparison with intramuscular adrenal transplants previously done in this laboratory.

The operated animals were distributed in four experimental groups which received the same solid standard laboratory diet.

25 males and 26 females, comprising group I, received tap water and served as control animals. 26 males and 26 females, comprising group II, received 1% NaCl solution in place of drinking water. 14 males and 13 females, comprising group III, received 1% KCl solution. The experiment with this group was discontinued because of the high mortality among the animals exposed to this toxic concentration of potassium. 27 males and 25 females, comprising group IV, received 0.5% KCl solution. If the animals did not succumb to cortical insufficiency or die of another cause, they were observed for approximately three months following operation. After this period they were either sacrificed or subjected to an exploratory operation with subsequent removal of established transplants. A series of 24 rats with all indications of incorporated grafts underwent this second operation. The operative procedures, the postoperative course of all the animals including their weight changes and fluid intake, and the gross and histological autopsy findings were recorded.

Mortality rate in the experimental groups or failure of incorporation of grafts:

	I H_2O	II 1% NaCl	III 1% KCl	IV 0.5% KCl
male	25.0%	12.0	78.6	46.2
female	52.0%	23.1	61.6	56.0

The mortality figures for group II show that the administration

of NaCl does not bring about an indefinite survival of animals deprived of their cortical tissue.

The relatively small number of succumbing animals does not permit a definite statement for or against a specific sex difference in the resistance to cortical insufficiency. However, the longer survival period of females of group I and the fact that eight of the nine rats that reached the stage of chronic insufficiency (group I and II) were females may indicate a more favorable situation for the female sex as to degree and duration of cortical insufficiency. This reasoning is not necessarily contradicted by the observation that the mortality rate of all female groups except for those in group III was higher than that of the corresponding male animals, because one has to take into account the considerable differences in weight and size of the male and female animals at the age of operation.

The survival time of rats dying of acute and subacute insufficiency averaged 13.78 days with only slight variations in the different experimental groups. It appears that the failure of the transplant to establish itself leads to death, unless life is prolonged by NaCl, in a period of time similar to that for untreated bilaterally adrenalectomized rats. The group variations do not appear to be sufficiently consistent and significant to warrant any other conclusion. Potassium per se does not seem to shorten the survival

period in these animals to any great extent. The values for group IV are actually above the average survival time for all groups.

Since the survival periods are little different from those of untreated adrenalectomized rats, it can be concluded that the hormone content of the transplanted tissues is of no significance for the prolongation of life.

The postoperative loss of weight in the animals dying of cortical insufficiency averaged 18.2% of the preoperative level. This figure does not differ significantly from that of untreated adrenalectomized rats. No definite sex difference in the loss of weight could be found. No definite correlation between postoperative loss of weight and length of survival or initial preoperative weight level could be made. The NaCl treatment or the existence of accessory cortical tissue brought about a weight gain in some animals but did not prevent the eventual exhaustion of the hormone reserves. The loss of weight in animals dying of chronic insufficiency was usually less than that in the acute cases. Several animals maintained their preoperative weight level and some even made slight gains.

The incidence of successful transplants appears to be low when compared with data given by several other investigators. This disparity in the percentage of takes appears to be due to different criteria for a take, to different

sites of implantation, and to the author's counting of doubtful cases as negative.

The highest number of incorporated transplants was encountered in the NaCl groups. It is suggested that the the life-prolonging influence of sodium promotes survival until adjustments in and around the grafted tissues, such as vascularization and nutrition, which facilitate regeneration can be made. This effect of sodium is not different from the palliative action of this ion in cortical insufficiency. The relatively small number of incorporated transplants in the KCl groups suggests that the toxic potassium, by putting a considerable strain on all functional systems of the organism, interferes with attempts of incorporation and regeneration or kills the animal before cortical restoration is sufficiently extensive to maintain life. The few animals withstanding the intoxication developed excellent takes. It appears that after overcoming the initial obstacles cortical regeneration can proceed irrespective of the ion offered.

With the exception of group III, a greater number of male than female animals incorporated transplants.

Seventy-three rats of all experimental groups showed a temporary weight loss after operation. No correlation could be found between this loss of weight and the preoperative weight level or the degree of regeneration. A greater number

of male animals exhibited this loss of weight than did females.

The male rats of groups I, II, and IV that survived with takes averaged a gain in weight of 0.81 Gm a day over approximately three months. The values for the individual groups vary but little. The corresponding value for the females of groups I, II, and IV was 0.38 Gm a day. These figures represent a rate of weight gain which is normal for the strain employed.

The average number of regenerated transplants in all experimental groups was 2.4 per animal. The deviation in the individual groups was not significant with the possible exception of the females of group IV. It appears that all animals with successful grafts regenerate on the average the same number of takes, irrespective of the electrolyte offered. This again suggests that the electrolytes administered affect the processes of incorporation only indirectly by improving or aggravating the early period of cortical insufficiency following operation. In the female a slightly greater number of cortical bodies were regenerated than in the male groups. This might perhaps be interpreted as a sex difference in the chances for cortical regeneration.

In 14 animals accessory masses of cortical tissue were found together with viable takes. From what is known about the adreno-pituitary relationship and its influence on the existence of accessory bodies it can be said that, were the grafts fully adequate, they would not tolerate the additional

accessory cortical tissue. In view of the fact that takes and accessory bodies were encountered together in 14 instances it seems that, in these and perhaps in many other transplanted rats, the functionally subnormal grafts do not engage the entire corticotropin output of the anterior pituitary. Thus, the excess of this stimulant is available for the development of microscopic accessory cortical bodies. It can be concluded that the incorporated transplants did not attain the functional capacity of the normal cortical organ, a fact obscured by the normal growth and behavior of the animals. The electrolyte treatment did not influence this functional impairment to any significant extent.

The measurement of the fluid intake of the animals of all experimental groups confirmed data published by other workers on the salt and fluid requirements and appetite of normal and insufficient rats.

The histological investigation of the transplanted material revealed a three layer arrangement of cortical cells similar to that of the normal gland. This structure apparently was achieved irrespective of the electrolyte treatment. This finding is in contrast to the statements of several investigators who were unable to observe a normal cellular arrangement in transplanted cortices. It suggests that the regenerative processes follow the same pattern as do the processes in the life cycle of the normal cortical cell.

Observations throughout this study suggest that primitive cortical cells, such as are present before the development of accessory bodies, or regressed cortical cells, such as are present in non-viable rests of transplants, may under certain conditions retain a potential regenerative capacity which allows them to develop when stimulated by corticotropin. These cells are called "dormant" and may constitute a cortical reserve system which, if called upon, produce a state of the organism which though not entirely equal to the normal may maintain the life of the animal.

Adrenal function and insufficiency, particularly with respect to electrolyte and water balance and renal function; cortical atrophy, hypertrophy, regeneration, and their relationship to the anterior pituitary; and the present status of adrenal transplantation (experimental and therapeutic) were critically reviewed and analysed.

series		sex	# of	av.age	av.wt.	#w.t.	%r.t.	#+ins.		%+ins.	av.sur.		%av.wt.	av.fl.i.	#	%	wt.g.	av.fl.i.	av.#T.	4	3	2	1T	RA	TRA	Rem.op	#rem.	#rem.
			rats	at operation ds.	Gm.	wt.l.	wt.l.	ac.	chr.		+ins. ds.	w.o.chr. ds.	l.+ins.	+ins./d cc	sur.w.T.		sur. Gm/d	sur.w.T. cc/d	sur.		in #	rats					sur.	+
H_2O	IA	♂	25	90.0	198.8	16	0.8-13.5	6	-	25.0	12.8	12.8	14.3	16.0	18	75.0	0.77	21.6	2.1	-	6	7	5	2	1	4	3	1
	IB	♀	26	89.9	143.6	12	0.7-16.2	9	4	52.0	26.2	19.3	16.8	15.9	12	48.0	0.35	17.4	2.5	2	4	6	1	2	1	2	1	1
1% NaCl	IIA	♂	26	90.8	192.1	13	0.4-9.6	3	-	12.0	13.6	13.6	18.8	22.8	22	88.0	0.80	32.6	2.2	2	6	7	7	3	2	4	2	2
	IIB	♀	26	90.7	146.5	4	0.6-8.3	2	4	23.1	(39.7)	12.5	-	29.5	20	76.9	0.41	31.8	2.3	3	5	6	5	3	3	7	3	4
1% KCl	IIIA	♂	14	89.9	209.2	3	2.7-10.7	11	-	78.6	10.6	10.6	18.5	14.0	3	21.4	0.53	20.0	2.6	-	2	1	-	1	-	1	-	1
	IIIB	♀	13	91.2	149.0	2	2.0-6.9	8	-	61.6	11.0	11.0	16.1	15.3	5	38.4	0.43	16.3	2.8	1	2	2	-	2	2	1	1	-
0.5%KCl	IVA	♂	27	89.1	200.7	14	1.0-15.7	11	1	46.2	17.1	15.5	22.8	18.1	14	53.8	0.86	23.0	1.8	1	2	5	7	2	2	3	2	1
	IVB	♀	25	89.9	146.5	9	0.7-17.2	14	-	56.0	14.8	14.8	20.0	14.6	11	44.0	0.38	16.9	2.5	1	5	4	1	3	3	2	2	-

av.age at op.	average age of the animals at the time of adrenalectomy and transplantation
av.wt.at op.	average weight of the animals at the time of adrenalectomy and transplantation
# w.t.wt.l.	number of animals which showed a temporary postoperative weight loss
% r.t.wt.l.	percentage range of this temporary weight loss as compared with preoperative weight
#+ ins. ac. chr.	number of rats dying of acute or chronic insufficiency
%+ ins.	percentage of rats dying of insufficiency
av.sur.+ins.ds.	average survival time (days) of animals dying of insufficiency
w.o.chr.ds.	average survival time (days) of animals dying of acute and subacute insufficiency not counting the chronic cases
% av.wt.l.+ins.	percentage of average weight loss of the succumbing animals as compared with preoperative weight
av.fl.i.+ins./d	average fluid intake of rats dying of insufficiency (per day)
# % sur.w.T.	number and percentage of animals surviving with takes
wt.g.sur. Gm/d	weight gain of the survivors in Gm per day
av.f.i.sur.w.T.	average fluid intake of the survivors with takes in cc per day
av.# T.sur.	average number of takes in survivors
4 3 2 1T in #rats	number of rats in which 4, 3, 2, or 1 take was found
RA	number of rats which possessed remnant or accessory cortical tissue
TRA	number of rats which had takes in the presence of remnant or accessory tissue
Rem.op.	number of rats which underwent removal operation
#rem.sur.	number of rats which survived removal operation
#rem.+	number of rats which died following removal operation

Case#	Author	# of pat.	type of graft	survival after transplantation
1,2	Jabulay 1897	2	het.	† 24 hrs.
3	Busch & Wright 1910	1	het.	† 2½ wks.
4	Morris	1	?	?
(5	Hurst et al. 1922+1941	1	homo 2x	† 27 mos.aft.2.op.)
6,7	Conybeare & Millis 1924	2	homo	† immed. & 12 hrs.
8	Bra	1	het.	† 3 ds.
9	Courmont	1	het.	† 24 hrs.
10	Pybus 1924	1	homo	† "a few wks."
11	"	1	homo 2x	surviving, 3 yrs.
12	Currie 1924	1	het. 2x	?
13	Dmitrijew 1925	1	het.	surviving, 6 wks.
14	Rosenow	1	homo	† immed.
15	Halpern & Arkusenko 1927	1	?	surviving, 1 yr.
16	Reinhart & Leschke 1928	1	homo	surviving, 1 yr.
17,18	Bauer & v.Eiselsberg	2	homo	†
19	v.Eiselsberg	1	het.	†
20	Curschmann 1928	1	homo	† 11 wks.
21	d'Abreu 1933	1	homo 2x	† 9 ds.aft.2.op.
22,23,24	Biedl 1934	3	het.	†
25	Desmarest & Monier-Vinard	1	homo 2x	† 2 ds.aft.2.op.
26	Beer & Oppenheimer 1934	1	homo	† 2 wks.
27	" " "	1	homo 2x	surviving
28	Bayer	1?	?	†
29	Hertzen	1?	?	surviving
30	Kanevskij	1?	het.	surviving, 9 mos.
31	Bailey & Keele 1935+1939	1	homo	surviving, 4 yrs.
32	Goldzieher & Barishaw 1937	1	homo	† 9 mos.
33	Stone et al. 1938	1	homo	† 9 mos.
34	Stone et al. 1938	1	homo	surviving
35	Katz & Mainzer 1941	1	homo	surviving, 15 mos.

Table II

Therapeutic adrenal transplantation
in patients with Addison's disease

Appendix A

ADRENOCORTICAL FUNCTION AND INSUFFICIENCY

Reviews: 19, 35, 101, 119, 175, 176, 195, 305, 319, 325.

"In our present state of knowledge it is impossible to designate any specific function of the organism as being primarily related to adrenal cortical activity." (Grollman, 1941, 102).

Despite the vast amount of work which has been done on adrenocortical physiology there is a wide divergence of opinion as to the primary mode of action of cortical hormones, and the extent of their functions.

The variety of diseases for which denervation or even ablation of the adrenal has been recommended shows clearly the confusion and the lack of an adequate basic understanding of adrenal function among scientists and clinicians. At one time or another - and not very long ago - hypertension, hyperthyroidism, gangrene, epilepsy, gastric ulcer, and diabetes (248) were treated by adrenal resection or denervation.* It is clear from the results that these indications were based on completely ill-defined grounds (101, 248). With the exception of adrenal neoplasms our present knowledge of

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* Some of the material used for therapeutic transplantation in humans was obtained from other patients operated under such indications.

this gland does not warrant any surgical intervention whatsoever.

The wide and often indiscriminate use of cortical preparations shows a more empirical than rational approach to the problems involved. Aside from the well justified application in cortical insufficiency, adrenal hormones are used at present for certain cutaneous disturbances, for the treatment of burns and shock, and for the mitigation of ill effects of fever therapy (119). The use of cortical hormones in the preoperative prophylaxis of shock has been suggested, and successes have been reported (58). However, their effectiveness in circulatory failure has yet to be substantiated.

Extracts from the adrenal glands contain a large number of closely related steroids with different specific effects. More than 20 crystalline substances have already been isolated. And yet, the amorphous residue is still 15 times as potent as the most effective synthetic substance, desoxycorticosterone. It might be that these crystalline products are not the original hormones but more stable derivatives.

The following synthetic cortical hormones have been prepared: desoxycorticosterone (acetate or propionate), corticosterone, 11-dehydrocorticosterone, Kendall's compound E. Desoxycorticosterone (DCA) is the most effective among these substances but is considerably inferior to whole extract. It seems that DCA acts chiefly and perhaps only on the salt and

water balance of the organism (195). The synthetic hormones have the great advantage of not producing a refractory state on repeated administration as do cortical extracts.

Since no one known compound can produce all physiological effects of the cortex, it is very unlikely that this organ produces a single substance, the vital hormone. Compound E, at least to a certain extent, has shown some effects on gluconeogenesis as well as on electrolyte distribution, renal function, and muscle efficiency. However, the quantitative differences in these effects are so great that no one seriously claims that this is an all-around cortical hormone.

Hartman and his group (120, 121) have claimed the existence of a separate "sodium factor" in the adrenal secretion the lack of which appears to be responsible for the disturbance of sodium metabolism. This factor seems to act particularly on the kidney or on tissues generally. It is not essential for the maintenance of life.

It cannot be stated at present what the primary causative mechanism of the symptoms of cortical insufficiency is. It is not the province of this study to review and discuss this still unsettled problem. All that can be said is that most of the evidence as to the primary cause of insufficiency centers around the renal disturbance and the defective salt and water metabolism. Kendall (175) considers the regulation of distribution and excretion of inorganic ions as the main

task of the adrenal cortex. However, the cortical function cannot be fully described in terms of Na, Cl, or K metabolism just as hyponatremia or hyperpotassemia alone cannot be regarded as the primary factor in the picture of cortical insufficiency.

The important electrolyte-water-kidney aspect of cortical function has been outlined in section III. This appendix shall serve to describe the more secondary activities and functional systems which are influenced by the cortex under normal circumstances and which fail in the absence or deficiency of its secretion.

It is likely that at least some of the following functional groups are indirectly influenced by way of electrolyte and excretory changes caused by cortical hormones. These interrelations, however, are not understood as yet.

The claims that cortical secretions act on particular organ or functional systems are contradicted by the assertion of some investigators that the adrenal cortex liberates a general tissue hormone (184).

In addition to the relation of the electrolyte-water-kidney complex to the adrenal cortex, the following functions of the organism are affected by cortical secretions and contribute to the symptomatology of cortical insufficiency.

1. Carbohydrate metabolism

The cortex is concerned with the maintenance of normal blood sugar levels. It regulates and maintains glucogenesis from protein destruction and controls carbohydrate oxidation.

This cortical influence is well demonstrated by the disturbances of the carbohydrate metabolism encountered in experimental and clinical cortical insufficiency:

(a) decreased gluconeogenesis, decreased conversion of non-carbohydrates (protein breakdown products) to carbohydrates (85, 86),

(b) increased utilization (oxidation) of the available carbohydrates (86, 181) resulting in exhaustion of glycogen stores of the liver (119),

(c) decreased glycogen content of muscle and impaired lactic acid formation in muscle (325),

(d) decreased glucose appetite (246),

(e) decreased glucose absorption (9),

(f) hypoglycemia (86, 195, 356),

(g) intolerance to oral glucose administration, altered threshold for hypoglycemic symptoms which occur already at a relatively high glucose level (305),

(h) increased sensitivity to insulin (195).

The defective protein-carbohydrate conversion explains, at least in part, the amelioration of pancreatic or phlorhizin diabetes brought about by adrenalectomy.

The intravenous administration of glucose in cortical

insufficiency is beneficial as far as hypoglycemia, gluconeogenesis, and glucose absorption are concerned. However, the temporary relief is followed by a crisis caused by the transportation of potassium into the cells together with the carbohydrate.

DCA or NaCl have no significant effect on the carbohydrate disturbance in the insufficient organism whereas corticosterone, compound E and their derivatives with an oxygen on C_{11} exert a beneficial influence.

2. Resistance to stress

The cortical hormones maintain the natural resistance of the organism to various types of stresses.

The insufficient patient or experimental animal has been shown to respond with an increased susceptibility and sensitivity to

poisons, drugs, bacterial toxins (119), histamine (due to lack of cortex and medulla) (138, 170, 224, 337), potassium (364), exercise, fatigue (137), infection (119), low oxygen pressure (175), water intoxication (87, 295, 301), trauma, hemorrhage (even if trivial) (293, 298), various types of shock and any procedure which throws a strain on the peripheral circulation (292), temperature changes (342, 348), thyroxin (175), insulin (175), theelin, phlorhizin (176).

A decreased formation of antibodies has been observed in cortical insufficiency (232).

Although a high sodium - low potassium diet with an adequate carbohydrate supply can maintain a normal extracellular electrolyte concentration, normal liver glycogen contents and blood glucose in the insufficient organism, it does not enable the treated animal to withstand the stresses mentioned as does the intact organism.

It is a common observation that all symptoms of clinical or experimental insufficiency become more evident under the influence of stress. As a matter of fact, the chronic, latent or temporary forms of cortical insufficiency are revealed only under abnormal conditions of environment and activity.

3. Muscular efficiency

The increased muscular fatigue of cortical insufficiency has been subjected to extensive experimental investigation. Especially Ingle and his group have been using the work performance of the gastrocnemius muscle as an index of functional adequacy of adrenal tissue or replacement therapy (141, 143). Totally adrenalectomized rats showed a quick loss of muscular response, succumbing to the stress, whereas partially adrenalectomized animals exhibited a good initial performance but eventually showed complete fatigue and also succumbed. The low work capacity is observed even after allowing time for regeneration of the remaining cortical tissue.

These experiments show well the wide difference between

the minimal cortin requirement for the mere maintenance of life, and the amount of cortical hormone necessary to meet adequately a stress such as the work performance test.

Although the unilateral removal of an adrenal gland has been considered an insignificant interference, it is of great interest to note in the work performance tests that the animal with only one intact adrenal does not show a normal work capacity, just as it is incapable of maintaining a normal resistance to cold. The removal of only a small portion of the gland seems to decrease the functional capacity of the remaining part greatly, possibly through circulatory damage.

The skeletal muscles of adrenalectomized dogs show an absolute gain in bulk due to the increase of the intracellular phase. The sodium and chloride contents are decreased whereas water and potassium are increased, thus reflecting the extracellular water and electrolyte changes (216).

Muscle efficiency is influenced by corticosterone, compound E, and their derivatives with an oxygen at C_{11}. A high sodium - low potassium diet, however, produces a work capacity which is still inferior to that of animals with intact adrenals. This inferiority of the treated adrenalectomized animals is evident despite their survival, weight gain, and apparently normal health (142).

Similar tests have shown that the administration of

adrenalin chloride or glucose has a significant influence on the work output (158).

4. Body temperature

The importance of cortical hormones for the regulation of body temperature has been concluded from the inability of the insufficient organism to maintain a normal level of temperature.

A reduction of approximately 10% of the basal heat production in bilaterally adrenalectomized rats has been recorded (132). The exposure of the animals to cold was not met by an adequate heat production. The fall of the colonic temperature is largely terminal. Martin and Maresch (209) emphasize that the cortical secretion is not more closely related to temperature regulation than other endocrine products such as the thyroid or pancreatic hormones. It seems then that an influence of the cortex on the maintenance of body temperature is not specific but rather a result of the gland's other actions on the physiological state of the tissues of the body.

5. Interendocrine relations

The all-important pituitary-adrenal relation with its profound influence on the general well-being and development

and its specific bearing on cortical regeneration is dealt with separately in Appendix C.

At this point it may suffice to mention the marked growth disturbances associated with adrenal insufficiency. Lucke (197, 198) succeeded in producing "adrenal dwarfism" with retarded development. He considers pituitary and adrenal dwarfism as identical, the only difference being that in the former the superior organ fails whereas in the latter the defect is located in the executing organ.

Regressive changes in number, size, and structure of pituitary cells with some attempts at compensation have been observed in the experimental animal as well as in Addison patients (182, 207, 238). The administration of 1% NaCl prevented these changes to a large extent. The presence of pituitary changes in non-tuberculous and their absence in tuberculous Addison patients led to the suggestion that simple adrenal atrophy is due to a primary pituitary deficiency (221).

The relationship between cortical secretion and the function of the mammary glands is possibly also governed by the hypophysis. Adrenal insufficiency is characterized by deficient lactation. While an adrenalectomized animal may deliver a normal litter, it is usually not able to carry the young to weaning (308). The only female of our study which became pregnant delivered seven living young which she destroyed within a few days (rat # 177).

The relationship of the adrenal cortex to the gonads is of particular interest in view of the embryology of these glands. Cortical tissue can elaborate androgens and estrogens. Whether this ability justifies the conclusion that the cortex is an accessory sex gland cannot be answered at present. At any rate, the adrenal-gonadal relationship is established beyond doubt and may be considered another manifestation of interendocrine relations mediated by the pituitary.

Loss of libido and of potency, amenorrhea, and atrophy, degeneration, and disorganization of the reproductive system in Addison's disease have been reported. Atrophy of the seminal epithelium of the cat was observed in insufficiency (320). Absence of normal sex activities and suppresion or irregular appearance of the estrous cycle in experimental cortical insufficiency are well known (184, 207, 336). The degree of the ovarian disturbance seems to depend upon the severity of the state of insufficiency. However, even in the completely adrenalectomized animal, pregnancy can be brought about and may result in normal delivery. The unilaterally adrenalectomized animal does not show any disturbance of the estrous cycle. It has been suggested that the diestrus following adrenalectomy is due to the general ill health of the animal rather than to lack of a specific adrenal sex factor (185). This conclusion may not be right if one considers that cortical insufficiency possibly inhibits the gonad-stimulating cells of the anterior pituitary.

The administration of cortin or the treatment with salt restores the estrous rhythm of the insufficient animal (184, 185, 208). The suggestion has been made that the salt treatment possibly restores the electrolytic equilibrium of pituitary and ovarian tissues.

A general impairment of oxidative systems of the organism has been claimed to be a result of insufficiency manifesting itself in a depressed oxygen consumption in liver and kidney tissue (307). An overall metabolic reduction of 25% has been reported for bilaterally adrenalectomized rats (45). The question whether the depressed metabolic rate is a manifestation of the deranged relationship between adrenal cortex and glands which produce hormones with calorigenic action or whether it is a result of circulatory or respiratory failure has to be left open. Extracts, DCA, or NaCl administration maintain a normal metabolic rate in the adrenalectomized animal.

There seems to be an antagonism between adrenal cortex and thymus. While thymectomy is followed by cortical hyperplasia, the bilateral (but not the unilateral) ablation of the adrenal glands results in hyperplasia of the thymus which may be a manifestation of a general lymphoid hyperplasia. In Addison's disease, too, thymus enlargement has been described. Despite frequent reports of thymic hyperplasia in experimental insufficiency, Selye (268) doubts

whether thymus hyperplasia in the strict sense of the word ever occurs after adrenalectomy.

Trauma, low temperature, excitement, and the administration of drugs result in adrenal enlargement and simultaneous thymic involution. The latter does not take place, however, in the untreated or salt-treated adrenalectomized animal.

6. Pigment metabolism

The pigmentary disturbance of cortical insufficiency is the least understood symptom of this condition. This disturbance appears to be peculiar to man. Its appearance in insufficient animals is a controversial matter. The Addisonian almost invariably displays a characteristic pigmentation of the extensor surfaces, scars, the skin of the face, shoulder and palms, and the buccal, rectal, and vaginal mucous membranes. One probably does not go wrong in assuming an enzymatic derangement as the background of this symptom. None of the available hormone preparations has shown any consistent effect on this pigmentation.

7. Capillary tone

A distinct pressor action has been attributed to the cortical hormone which prevents the reduction of the blood

pressure to shock levels (297). Such a control of capillary tone would facilitate the fluid exchange between vascular and extravascular tissues and maintain a normal blood volume. Its absence in cortical insufficiency brings about circulatory failure with ensuing death as a result of capillary atony with dilatation and vascular stagnation. This has been claimed to be the cause of death from insufficiency.

8. Vitamin metabolism

Cortical hormones supposedly retard vitamin B and C deficiency. It has been suggested that the adrenal affords a protection against destruction of the vitamin. Verzár and his school consider cortical insufficiency a secondary avitaminoses produced by disturbed phosphorylation and incorporation of B vitamins into the various enzyme-coenzyme systems. They and others (234) claimed that cortin cannot maintain insufficient animals which are kept on a flavin-free diet. Their concept became dubious, however, when it was shown that a great number of vitamin B compounds and their phosphorylated derivatives do not have any effect on the insufficient organism (49).

Hemorrhagic cortical necrosis in rats together with lesions in other organs have been observed and correlated with the possible lack of an unidentified factor of the B complex (65, 217). A similar necrosis could be prevented by calcium

pantothenate (213).

The adrenal cortex normally contains a relatively large amount of vitamin C. A deficiency of this vitamin together with a disturbance of its excretion has been described in cortical insufficiency (167).

Interference with phosphorylation processes may contribute to the disturbed vitamin metabolism and may also be responsible for the typical slow recovery in the muscle of the Addisonian, and for the deficient intestinal absorption encountered in cortical insufficiency.

9. Intestinal absorption

The impaired intestinal absorption (e.g. of glucose) in cortical insufficiency and the proposed mechanism (defective phosphorylation) have been mentioned above.

It should be pointed out that there is a marked decrease in the absorption of Na, K, and Cl in the insufficient animal even if the animal is maintained in good health and with normal blood electrolyte levels by means of saline treatment (71). This intestinal disturbance, whatever its mechanism may be, might account for some of the nonspecific and long-standing gastro-intestinal symptoms which are commonly encountered in Addison's disease.

10. Miscellany

Fat and protein are not readily broken down in adrenal (or pituitary) insufficiency. However, the catabolic processes do go on in the absence of either adrenal or pituitary tissue.

Psychoneural disturbances in Addison's disease (mental fatigue, insomnia, restlessness, abnormal reactions of the sensory apparatus) suggest an effect of cortical hormones on the nervous system. It is probable, however, that these disturbances are primarily due to the hypoglycemia or the decreased cerebral blood flow.

Claims for cortical influences on healing processes and callus formation have been made. Occasional observations in adrenal insufficiency such as hypercalcemia, increased serum phosphatase, and gynecomastia have been recorded. The mechanism of these and other disturbances is unknown.

11. Chronic cortical insufficiency

Chronic cortical insufficiency has frequently been observed both in patients and experimental animals. The greater diagnostic difficulty in these long-standing cases which often display only vague symptoms explains why probably many patients with a chronic adrenocortical impairment go unrecognized. A recent report (199) describes a syndrome

including exhaustion, anorexia, loss of weight, and low blood pressure, blood sugar, and BMR, which led to the discharge of soldiers from their duties (even after they had been doing only the lightest work) and which was diagnosed as a constitutional cortical (or general endocrine) inferiority. Observations of temporary symptoms of insufficiency after exhaustive diseases, overexertions, and infections (84) suggest the existence of a latent cortical defect which manifests itself only under stress.

In the experimental animal (104, 122, 161, 231, 356) a chronic type of cortical insufficiency could be shown conclusively

after partial adrenal extirpation (the severity of the insufficiency is roughly inversely proportional to the size of the fragment left);

in some cases after bilateral adrenalectomy with eventual exhaustion of accessory cortical tissue;

in the adrenalectomized animal maintained with a minimal amount of cortin or under salt treatment;

after periadrenal vascular ligation with subsequent atrophy of the gland.

The symptoms are cessation of growth, failure of normal reproductive activity, subnormal body temperature, marked diuresis (93), slight asthenia, progressive emaciation (if there is any weight gain it may be due to skeletal growth), low resistance to infections, and blood sugar values in the lower normal range. At autopsy, loss of body fat, gonadal

and thyroid atrophy, and lymphatic and thymic hyperplasia are found. The observations that relatively large amounts of cortin do not relieve these symptoms (whereas pituitary preparations do) or that cortical hormones may fail to bring about the recovery from crises suggest that chronic insufficiency may be the cause or the result of a pituitary deficiency which in the long-standing cases may have led to an irreparable pituitary damage. The observation that the clinical picture of chronic cortical insufficiency is essentially that of pituitary cachexia fits the above conception very well.

It has to be mentioned that it is possible that a moderate insufficiency of a chronic type may cause multiple nutritional deficiencies which in turn may aggravate some of the symptoms of the cortical impairment.

12. The survival period in experimental cortical insufficiency

The reports of survival periods following complete adrenalectomy in experimental animals display a considerable diversity and lack of uniformity. There are a great number of papers dealing particularly with untreated adrenalectomized rats (51, 70, 77, 78, 91-94, 161, 166, 183, 184, 206, 209, 224, 230, 231, 242, 243, 246, 261, 266), and other species (36, 89, 165, 206). The mortality rate actually ranges from 0 to 100%, the majority of reports state between 85 and 100%. The average survival time is usually under three weeks, in

the majority of cases 6 - 14 days after operation. If an animal dies immediately after the adrenalectomy, there is every reason to believe that the cause of death is traumatic shock rather than adrenal insufficiency.

A number of factors exert a definite influence on the survival time after cortical deprivation.

(a) Freedom from disease is essential, since animals which are sick at operation succumb rapidly to the operative trauma.

(b) The shorter the anesthesia the better. Ether is supposedly deleterious (89).

(c) It is obvious that a skilful operative technique and the completeness of adrenal ablation have much to do with the subsequent survival period.

(d) Optimal postoperative care and favorable environmental temperature will facilitate an optimal survival time. Maximal survival has been reported at 30°C (323).

(e) The postoperative diet as to its electrolyte, vitamin, and carbohydrate contents is of great importance for the length of the survival period (290). A commercial diet containing e.g. 1% NaCl and 0.9% K is adequate as far as health, growth, and reproduction of normal rats is concerned, but it does not favor the survival of adrenalectomized animals (138). Additional amounts of salt or a low potassium content definitely prolong the survival period (11, 13, 16, 156) whereas

the administration of potassium reduces the survival time (93). The mortality seems to vary inversely with the sodium intake (243). However, there is an optimal amount of about 650 - 940 mg of NaCl per day for the adult rat (16). An excess of NaCl is injurious and leads to a short survival period similar to that of untreated rats. Rats on 1% NaCl were observed in good health and growth for as long as 176 days postoperatively (14). The growth rate for the surviving animals on various salt diets may approach normal or can be subnormal (94, 182). Though one cannot doubt the life-prolonging property of various sodium-containing salt diets, the obtained survival is not indefinite (6, 7, 77, 102, 103, 206, 323). Vitamin B deficiency has been shown to shorten the survival time of adrenalectomized animals (261).

(f) Age and weight of the animal at the time of the operation seem to exert an influence on the survival period. Rats below 50 gms were shown to survive only one day whereas those above 200 gms survived nine days (89). Short survival times of young rats were observed by several investigators (51, 91) while rats operated at puberty showed an increased survival period with little or no significant relation to the initial body weight (276).

(g) The possible influence of species and strain differences and its relation to a variable frequency of accessory tissue has been dealt with in another chapter. The assumption of a wide distribution of accessory cortical

tissue is not necessary. It usually occurs in the neighborhood of the main glands. Likewise, an assumption of preformed cortical cells is not necessarily compulsory. Possibly a more primitive structure such as celomic epithelium gives rise to accessory bodies (51). Any influence prolonging life would increase the chances for such tissue to regenerate.

(h) The life-prolonging influence of pregnancy, pseudopregnancy, and heat has been shown in many experiments (250, 252, 253, 280, 308). However, pregnancy could not prevent insufficiency and death occurring in rats after removal of transplanted adrenals.(229). Besides natural or synthetic active cortical hormones, progesterone, too, prolongs the life of the insufficient organism (233, 308), and ameliorates the symptoms. Adequate amounts of this hormone can maintain life. A beneficial effect of gonadotropic substances can be explained by the subsequent luteinization. Pituitary implants or extracts (83) prolong life by means of an increased hormone secretion from the corpora lutea. Male hormones are ineffective as to prolongation of life of the insufficient organism.

(i) Seasonal influences on the survival after adrenalectomy have been observed. Consistently longer survival of cats was obtained in winter, whereas the shortest survival periods occurred in summer (36). Striking seasonal differences as to survival time were observed in the groundhog, a hibernating animal, depending upon whether the operation was done in

the dormant or active period (34). In rats, a shorter survival in spring as compared with that during the winter months has been observed (321).

(j) Thyroidectomy and glucose administration have been shown to prolong the survival period, whereas the administration of thyroid substance hastens the fatal outcome (359).

A decreased water elimination in the former and an increased one in the latter case might account for the observed effect (361).

(k) A life-prolonging effect of cardiac glucosides has been described (363).

Appendix B

THE ADRENAL MEDULLA AND ITS FATE IN TRANSPLANTATION

Although this paper deals with the adrenal cortex, some remarks must be made about the medullary portion of the adrenal gland and its relation to the cortical organ.

The medulla is only a part of a widely distributed chromaffine system of ectodermal origin, formed from sympathoblasts and consisting of modified ganglion cells. The adrenal medulla is not essential for the maintenance of life. It is provided with preganglionic cholinergic fibers.

The cortex, on the other hand, is derived from the mesodermal "interrenal" zone in close proximity to the genital ridge and the Wolffian body. The cortical organ is essential for life. It is without innervation (27).

The medulla in the rat is about 8 - 9 times smaller than the cortex. The medullary volume of normal rats was found to be 8% as compared with a cortical volume of 92% (109).

The function of the medulla as part of the sympathicoadrenal system is to serve for rapid adjustment of the organism in "emergencies" and to guard the constancy of the internal environment. Thus, it reinforces the activities of the sympathetic nervous system.

Despite its different embryologic, morphologic, and functional properties and its merely "accidental" association with the cortex, the medulla supports cortical influences on the "steady maintenance of certain bodily conditions" (340). For the understanding of homeostasis it is, therefore, worthwhile emphasizing the potential synergism between the medulla as the organ of the "first line of defense", and the cortex, an organ of the "second line of defense" (348).

The adrenal medulla and transplantation

The vast majority of successful auto- and homoplastic transplantation of the adrenal gland results in regeneration of cortical tissue only.

However, there are several reports which definitely state, and some which produce histological evidence, that medullary cells could be found in adrenal grafts. These medullary cells were present only in small amounts, irregularly arranged, of abnormal structure and doubtful functional significance, but yet sufficiently well defined to be recognized as such.

Instances of preservation of medullary cells in autografts were reported in 22, 41, 80, 145, 174, 189, 337.

Baker and Baillif (22) who found medullary islands in four regenerating autografts which were removed 20 days after operation, were able to trace nerve fibers to these cell groups.

It is of considerable interest that among the reports of successful transplantation there are three instances in which special techniques of transplantation were employed that deserve mention.

(a) Levy and Blalock (189) employed renal-carotid anastomoses. Their experiments have been described elsewhere. The medullary cells found were apparently normal, but no epinephrine could be demonstrated from the venous blood of the transplants. Ingle and Harris (145), too, showed that extracts of transplants containing some medullary cells were physiologically inactive when tested for epinephrine.

(b) Keeley et al. (174) performed two-stage operations which involved at first only partial mobilization of the adrenal and, thus, permitted the preservation of its blood supply. In two of their dogs they found medullary cells which appeared to be normal.

(c) Dunphy and Keeley (80), using a similar three-stage procedure ("sandwiching"), always obtained medullae smaller than normal, but in excellent condition. It seems that the difficulty of establishing an adequate blood supply to the central portion soon enough with routine techniques was overcome by this special method which facilitated a new blood supply before the original one was interrupted. These medullary cells, though appearing viable, did not help to bring about a hyperglycemic response in the animals. The in-

creased sensitivity to insulin indicates that no adrenalin is secreted into the blood stream due to lack of sympathetic impulse transmission. However, biologic assays of the excised grafts (one year or more after transplantation) showed that the established grafts with medullary cells did contain adrenalin, i.e., the grafted medullae were actually viable and capable of producing adrenalin or adrenalin-like substances. The absence of acetylcholine (a humoral sympathetic impulse mediator) seems to explain the failure of the graft to release adrenalin into the circulation.

Medullary cell groups could be found in a number of successful homotransplants (200, 313-315).

Medullary tissue was found to be incorporated in some cases of transplants to the anterior chamber of the eye (313-315). Seven out of 12 transplanted adrenals of newborn rabbits showed medullary cells, and 68% of adrenals of newborn mice transplanted to the kidneys also contained medullary tissue. The functional capacity of these medullae was not demonstrated. Since double adrenalectomy of the hosts did not benefit the survival of medullae from which the cortices were teased away, Turner concludes that the medullary incorporation is apparently not conditioned by the anterior pituitary. He believes that the survival of medullary cells depends upon the accessibility of nutritional fluids and the possibility of a rapid vascularization.

Early investigators (41) describe four rabbits which had takes containing medullary cells that gave a reaction with chrome salts. Two of the animals - one with an autograft, the other with a homotransplant - survived after the removal of the transplants and were found to contain accessory tissue at autopsy.

Which factors, as a rule, make medullary tissue degenerate in transplants?

(1) The failure to obtain medullary regeneration in grafts may be due, at least in part, to the peculiar blood supply of this organ. The cortex receives an abundant supply from the aorta, inferior phrenic and renal arteries. The cortical sinusoids empty into medullary veins. Thus, most of the medullary blood has passed through cortical capillaries, i.e., it probably carries cortical metabolic waste products. The medulla, in addition, receives oxygenated blood from medullary arteries which penetrate the cortex. It is obvious that it is exceedingly difficult to reestablish such a vascular supply through the cortical organ which, despite its better position, hardly gets enough nutritional fluids for itself.

(2) Aside from the difficulty of reestablishing vascular connections which is a more advanced stage in the process of transplant regeneration, the simple and immediate

diffusion of nutritional fluids to the medulla would be very difficult on account of its central position. Diffusing fluids would have to pass the cortex. More than that, metabolic and destruction products of the medulla, and possibly of the inner cortical layers, may accumulate in the interior without being drained, and may actually damage the already anoxic tissue.

(3) The highly differentiated chromaffine tissue per se seems to be even more fragile and susceptible to damage than cortical tissue. This is evident in the readiness of the medulla to respond immediately to damaging influences with degenerative processes.

(4) The medullary cell, embryologically related to nervous tissue, exhibits little or no regenerative capacity, which is characteristic of both these ectodermal structures.

These nutritional and cell factors, possibly together with others, account for the common failure of adrenal grafts to regenerate the medullary portion.

As for the exceptions mentioned, it can be said that the admittedly abnormal and irregular condition of most of the medullary cells found does not justify their being called "takes". The few apparently normal and functioning medullary grafts, however, may possibly present a truly successful result. They should provoke more investigation of this problem which is an interesting companion to the more urgent problem of cortical transplantation.

Appendix C

ADRENAL ACCESSORY TISSUE, REGENERATION, HYPERTROPHY, ATROPHY, AND THE ADRENO-PITUITARY RELATIONSHIP

1. Adrenal accessory tissue

The very close relationship between survival of insufficient rats, and the existence and amount of accessory cortical tissue, furthermore, the properties common to accessory bodies and transplanted tissue, and the assumption that their growth or regeneration are governed by the same mechanism, all of these problems make it desirable to deal with accessory adrenal tissue more in detail.

Many conflicting results in the literature and their misinterpretation seem to be due to the limited and vague knowledge of the frequency, distribution, and species and strain differences of adrenal accessory tissue.

Such tissue may occur in man (98, 218) and has been found in the broad ligament (Marchand's organ), on the spermatic and ovarian veins, on the spermatic cord, in the inguinal canal, in the vicinity of testis and epidydimis, under the capsule of liver and kidney, and near the main adrenal glands. H.M.S.Turner (316) suggested that the prognosis for an insufficient female might be less unfavorable as compared with a male if she lives long enough for the accessory

structures to develop and to take over the cortical functions.

Accessory bodies are rarely encountered in dogs. In over 150 dogs no accessory masses could be found even with microscopic examination (169). Another paper (249) reports that in 120 dogs no accessory tissue was found; the same workers later described a small accessory in a single dog (251).

Accessory cortical tissue is also uncommon in the cat. A frequency of 5-10% has been estimated. One report mentioned 11 animals with accessory tissue out of 167 cats (206). Another investigator (360) described one accessory body in a series of 100 cats; the tissue showed all three cortical zones. A cat with accessory adrenal tissue displaying a typical cortex and medulla was reported in another communication (289).

In the guinea pig, accessory cortex is also rare (165); a frequency of about 4% has been reported.

Three marmots with accessory cortical tissue have been described (34).

While accessory tissue in cattle may attain the size of a walnut, it may still be visible to the naked eye in the cat, rabbit, and dog, but only microscopically noticeable in the rat and mouse (101).

In the rat, mouse, and rabbit, accessory adrenal tissue seems to occur frequently. The finding of adrenal accessory

tissue in 70% has been claimed for the rabbit. One investigator (70) found accessory tissue in every animal of a series examined. Typical cortical and medullary cells were encountered in the accessory tissue of the rabbit.

Adrenal accessory bodies which, according to Grollman (101), were first described by Hartmann in the dog in 1699 and by Morgagni in man in 1740, have been the subject of discussion relative to occurrence, frequency, and localization to a greater extent in the rat than in any other species.

It has frequently been observed that adrenalectomized rats survive and show accessory adrenal tissue at autopsy. Undoubtedly, many of these accessory bodies were not true accessory glands but remnants resulting from incomplete extirpation. It is difficult if not impossible to distinguish between accessory and remnant adrenal tissue. If such a body is found on the upper pole of the kidney, it is likely that this is a hypertrophied remnant, particularly if it is distinctly macroscopic in size and similar to the normal gland in color and attachment. Its presence in other locations suggests the existence of genuine accessory, provided the operator was not so careless as to fracture and crush the gland inside the body and spread fragments all over the abdominal cavity. Grollman (101) claims that accessory tissue occurs relatively infrequently in the rat and mouse, and that with meticulous care at adrenalectomy, the same fatal outcome

can be obtained in the rat as in any other species. Many earlier workers had doubted this. It is easy, particularly for anyone without sufficient experience and practice with adrenalectomy to detach some of the friable cortical tissue which subsequently hypertrophies to form a remnant body. This accounts for many socalled "accessory" masses.

Earlier workers claimed that many accessory glands occurred in the neighborhood of the male sex organs. Biedl (28) supposedly found in 96% of rats macroscopic accessory bodies near the testes. This has never been confirmed (70, 101). Grollman doubts that accessory tissue is scattered throughout the abdominal cavity (101). True accessory bodies usually are found in the vicinity of the main glands and may occur as islands in the medullary part of the gland. If present in the abdominal cavity they show the structural character of Grollman's "androgenic" tissue.

It is conceivable that in early embryonic phases, e.g. at the time of cortical and medullary fusion, single precortical cells or whole cell buds are separated from the main gland and persist in ectopic positions to form true accessory bodies. Such a development may possibly occur at any time of the extrauterine life. Such cell groups may gradually atrophy and disappear. It has been claimed (163) that if searched for carefully, one can find microscopic accessory tissue in at least 50-70% of newborn or very young infants which gradually

disappears with advancing age since it is not needed.

The persistence of some of this tissue and its hypertrophy may account for the greater incidence of accessory bodies in adrenalectomized than in normal animals. Gross accessory glands in the normal rat are found only very infrequently. In but one rat out of 500 investigated a gross accessory body was found (231). None at all in normal and about 3% in adrenalectomized rats (160) were found with numerous microscopic accessory masses in all groups of rats.

Colony and strain differences in the rat have been observed influencing the length of survival after adrenal extirpation (92), and a difference in the amount of cortical accessory tissue was made responsible for the divergences reported. Other workers (51) attributed these differences to inherited distinctions in pituitary activity. Grollman (101) who adrenalectomized all recognized strains could never find an accessory-endowed strain of rats, thus deprecating the claims of numerous investigators.

There is every reason to assume the existence of accessory cortical or cortical-like tissue in all animals which survive complete adrenalectomy indefinitely or beyond the critical period even if no accessory structures can be demonstrated at autopsy. One report (242) describes four animals which survived adrenalectomy and the removal of transplants with no accessories found, being totally inactive. Since the

adrenal cortex is essential for the maintenance of life, it must be concluded that in these animals there is just enough microscopic accessory cortical tissue present to keep them alive. The inactivity is a manifestation of the severe insufficiency. Gaunt (91) revived three rats from unmistakable crises with extract; the animals survived indefinitely after only a few extract treatments. The extract apparently permitted a sufficiently long survival for the development of an adequate amount of accessory tissue. However, the presence of accessory tissue does not guarantee the life of the animal. Death from insufficiency may occur despite the presence of accessories which undergo exhaustion atrophy or are not adequately supplied with blood (323).

It has been claimed that accessory bodies may increase to the size of a normal adrenal gland (161). This has not been confirmed by the Mayo workers (151) who stated that the accessories do have a capacity for growth and are able to prevent cortical insufficiency but never attain the size or functional capacity of normal adrenal glands.

2. Adrenal regeneration, hypertrophy, and atrophy

Regenerative and regressive changes in the adrenal gland have to be considered together since they are mediated by the same mechanism, namely, the action of corticotropin from the anterior pituitary.

Although it has been claimed (46, 47) that the adrenal as a highly differentiated organ is not capable of regeneration, the work presented by the Mayo investigators and by others on the enucleated adrenal (127, 147, 149, 151) not only shows the now unquestioned ability of cortical tissue to regenerate and hypertrophy under certain conditions but also throws light upon general problems of cortical growth and inter-endocrine relations.

It is well known that a large variety of stimuli brings about reversible cortical hyperplasia in the normal animal. Pregnancy (125), ovulation, castration, infection, inanition and starvation (68), administration of thyroxine and various drugs (69), avitaminosis (vitamin B deficiency) (97), experimental tropical conditions of temperature and humidity (48), shock, exercise (109), cold, toxins, - any type of maintained stress will lead to increased adrenal weight. Many of these stimuli will also accelerate cortical regeneration in the enucleated gland. The inhalation of carbon tetrachloride, the administration of thyroxine, and the application of formaldehyde have been used to demonstrate the regenerative capacity in normal, unilaterally adrenalectomized or enucleated animals.

It has been claimed (109) that heavy work will bring about not only cortical but also medullary hypertrophy. On the other hand, it has been shown that demedullated adrenals

do not develop chromaffin tissue (85).

The fact that castration may produce an increase in adrenal weight of mice and rats can possibly be interpreted in the following manner: the removal of the gonadal end-organ (e.g. testes) might make the anterior pituitary gonadotropins, now superfluous, available for other purposes or might modify their actions.

Experiments employing the gastrocnemius work performance test in rats (140) showed an adrenal weight gain as early as 12 hours after the beginning of the test. This did not occur in hypophysectomized or cortin-treated animals. The administration of adrenotropin to the hypophysectomized rat, however, promptly induced cortical hypertrophy.

Other experiments (156) showed that unilaterally adrenalectomized rats on various salt diets did not respond with hypertrophy since there was no significant change in the physiological requirements for cortin. As soon as these animals were subjected to stress (work, thyroxin, or temperature extremes) a marked hyperplasia occurred due to the increased cortin requirement. Thus, the weight response appears to be due to the functional adaptation of the cortical tissue to the increased cortin requirement, an adaptation that is mediated by the anterior pituitary.

Unilateral extirpation or ligation of an adrenal (resulting in complete atrophy and subsequent replacement with

connective tissue) brings about compensatory hypertrophy of the remaining intact organ. This is shown by its increased volume, mitotic activity, and the appearance of cells with two nuclei (212). This compensation occurs early after adrenalectomy. After a while the histological changes become less obvious. The processes of hypertrophy occur most rapidly at a time when insufficiency symptoms would appear in a completely adrenalectomized animal. The enlargement of the glomerulosa and fasciculata apparently take place at the expense of the reticularis (302).

If the unilateral adrenalectomy is done in an hypophysectomized rat, no compensatory hypertrophy is observed; the latter occurs only in the presence of corticotropin (240). The initiation and progress of hypertrophy seems to be entirely due to pituitary stimulation. A diminished amount of circulating cortical hormone - following unilateral adrenalectomy - may increase the production of corticotropin. The cortex-stimulating substance is available for the remaining cortical tissue and facilitates its hypertrophy. The administration of cortical extract prevents the compensatory hypertrophy (202).

It is obvious that the cortical changes are mediated by corticotropin. The administration of pituitary extracts in normal animals leads to cortical hypertrophy as seen by a marked weight increase of the adrenal gland (82). With an

excess of cortin in the body fluids, the corticotropin production seems to be suppressed, whereas increased physiological requirements for cortin lead to an increased corticotropin output. Such is the case in the presence of various stimuli imposing an increased demand for the cortical hormone. The anterior pituitary principle stimulates adrenal regeneration and growth neither of which is possible in its absence. It thus enables the cortex to meet the physiological requirements for hormone.

The absence of the pituitary in normal animals leads to cortical atrophy; no hypertrophy will occur on stress. The atrophic condition can be repaired by the administration of corticotropin.

The amount of adrenal capsule left in the unilaterally adrenalectomized animal and the time allowed for regeneration also determine the extent of this regeneration. This is to be kept in mind. One should not expect miracles from pituitary stimulation. A certain amount of cortical tissue has to be available to ensure regeneration.

It is very important to realize that cortical hypertrophy does not necessarily mean increased hormone production. It may merely be a manifestation of a lengthened survival of postsecretory cells (27). There is, however, little doubt that hyperplastic cortical tissue can produce hormones in excess. If the hypertrophic processes overshoot their goal,

and true neoplasia is present, the effects of faulty or excessive hormone production become manifest: precocious puberty, early maturation, changes in secondary sex characteristics, and possibly reversal in sex.

The administration of cortical hormone or the presence of intact cortical tissue suppresses the regeneration or hypertrophy (if under stress) of the enucleated adrenal gland (155). This inhibition occurs regardless of the age of the experimental animal, just as regeneration after unilateral adrenalectomy seems to take place in young and senile animals. Hypophysectomy completely suppresses cortical regeneration. The cortical atrophy following hypophysectomy can be prevented by anterior pituitary extracts or repaired by hypophysial implants.

As in other endocrine structures (e.g. gonads, thyroid) the administration of large amounts of a hormone leads to regressive changes in the organ producing this active substance. Thus, in normal rats large doses of cortin bring about adrenal atrophy which is restricted to the cortex only. (139, 152, 154). Cortin also depresses the regeneration of transplants and, as mentioned above, of enucleated adrenals. The excess cortin seems to suppress the corticotropic activity of the anterior pituitary. On the other hand, anterior pituitary extracts prevent such a cortin-induced adrenal atrophy in normal rats. It appears that the cortin level in the

organism influences the anterior pituitary secretory activity in both directions as far as corticotropin is concerned.

Selye (269) observed adrenal atrophy in all three cortical zones in the mouse and rat following the administration of DCA. Some degeneration in medullary cells was observed, too. This atrophy differs from that induced by hypophysectomy in that the DCA atrophy is not very severe in the reticularis. Androgens and progesterone also caused cortical involution particularly in the female animal, while estradiol caused cortical hypertrophy especially in the male.

Selye calls the atrophy of the cortex induced by the administration of exogenous hormone "compensatory atrophy" since it appears to be the reverse of compensatory hypertrophy in the mechanism of its production. It seems to be identical with the contralateral cortical in adrenal neoplasm. The cortical atrophy cannot be produced by cortical preparations or other steroid material in the normal or hypophysectomized animal when adequate amounts of adrenotropic substance are administered simultaneously.

It is of considerable interest that substances other than the main products of the cortex, e.g. androgens, also can elicit cortical atrophy and that medullary atrophy can be produced by DCA. Selye calls this a "transferred compensatory atrophy" and believes that it is also mediated by the hypophysis. This atrophy does not seem to last permanently.

What can be done in the experimental animal occurs in the patient with a cortical tumor. The excessive hormone production which may be present in an actively growing neoplasm suppresses the function of the contralateral adrenal and leads to atrophy of that gland (322, citat.in 25). On removal of the tumor, profound shock and fatal outcome is observed in the majority of cases. This obvious insufficiency definitely indicates an appropriate pre- and postoperative therapy with cortical extracts or synthetic compounds.

In chronically underfed rats it has been observed (214) that a relatively greater adrenal weight loss occurs than corresponds to the concomitant loss of body weight. This, too, has been ascribed to a depressed pituitary function.

3. The adreno-pituitary relationship

Reviews: 55, 277, 291

The role of the adrenal cortex in Cushing's syndrome, "diabetes of bearded women", precocious maturity, virilism and hirsutism, feminization, pseudohermaphroditism, reproductive dysfunctions, and abnormal sexual behavior in patients or experimental animals point strongly to inter-endocrine relations and particularly to a pituitary influence. The capability of the adrenal to elaborate male and female sex hormones tempts one to consider this gland as a potentially bisexual accessory sex gland under pituitary control. However,

much caution is indicated until more will be known about both pituitary and cortical physiology, especially about the extensive steroid metabolism of the adrenal cortex.

The socalled adrenogenital symdrome has been recently reviewed by Wintersteiner (335).

Simmonds' syndrome caused by the destruction of the anterior pituitary is characterized by cortical atrophy among various other features. This makes the differential diagnosis between the pituitary disorder and cortical insufficiency extremely difficult as exemplified by a recently reported case from the Massachusetts General Hospital (203).

There is no doubt that the morphologic and functional maintenance of the adrenal cortex depends to a great extent on the anterior pituitary. The hypophysial fraction concerned with adrenal control is corticotropin which, as all pituitary hormones, probably is of protein nature (whereas the adrenal and gonadal active substances are chemically simpler steroids). It is not yet known whether corticotropin has only one specific function or possibly other effects, too. This fraction is distinct from thyrotropin and gonadotropins. The normal anterior pituitary can secrete corticotropin quickly in increased amounts as a response to a variety of nonspecific stimuli. This constitutes an important defense mechanism of the body.

The ablation of the pituitary is rapidly followed by cortical atrophy; the medulla retains for the most part its

normal size and histology. The administration of corticotropin to the hypophysectomized or normal animal leads to a cortical (not medullary) hypertrophy of all cortical layers.

As has been shown in the chapter on cortical hypertrophy, many stimuli and disturbances of homeostasis lead to cortical hypertrophy and hyperplasia. This does not take place in the hypophysectomized organism as the cortex cannot respond without corticotropin. It was emphasized that an excessive amount of endorgan hormone leads to a diminished secretion of end-organ stimulating factor in the pituitary and subsequently to the atrophy of the end-organ. On the other hand, an end-organ hormone deficiency results in an enhanced secretion of the pituitary end-organ stimulating factor with subsequent end-organ hypertrophy. This automatic adjustment of pituitary secretion to the end-organ hormone level in the body fluids and its effect on the functional state of the end-organ itself represents an excellent example of an endocrine equilibrium in the organism. This mutual relationship between pituitary and end-organ is well examplified by the adrenal cortex. Not only does a pituitary secretion influence cortical structure and function, but cortical hormones in turn affect pituitary secretion rates. This endocrine equilibrium is clearly expressed by its disturbances in clinical or experimental pituitary defects (272) resulting in a striking cortical atrophy which can be repaired by hypophysial

substitution therapy. The hypopituitary organism does not respond to unilateral adrenalectomy with compensatory hypertrophy. Hyperpituitarism, on the other hand, results in cortical hyperplasia. Adrenal insufficiency exerts a definite morphologic effect on the pituitary as evidenced e.g. by a change in the basophils. Both pituitary and adrenal insufficiency result in stunting of growth, inactivity, and atrophy of the reproductive, symptoms probably due to a common pituitary defect.

As has been stated in earlier chapters, the pituitary is not necessary for the maintenance of life. Hypophysectomy is not immediately fatal (except in the fowl). Atrophic adrenals of hypophysectomized animals are not entirely without function. The cortices of animals deprived of their pituitary secrete a sufficient amount of life-maintaining cortical hormone, but any stress shows that this secretion is not adequate. This ability of limited function in the absence of the hypophysis fits into Swann's conception (291) that the vital functions of endocrine glands though profoundly influenced by the anterior pituitary are to a certain extent independent of the pituitary control.

A relation between adrenal cortex and pars nervosa has been anticipated (291) but cannot yet be discussed. An antagonistic effect of adrenal and posterior pituitary substances on renal function and salt and water balance have been observed (37).

Swann (291) summarizes the present knowledge of the degree of pituitary control over various cortical functions as follows:

> the pituitary exerts a slight influence on the cortical role in salt and water metabolism, dextrose absorption, anorexia, and inanition; a partial influence on the resistance to stresses (with marked species differences); probably a complete control over sugar, fat, and protein metabolism . Pituitary control of cortical effects on muscle metabolism, experimental hypertension, and reproduction in some species has been suspected but not clearly demonstrated as yet.

The clue for sex differences in cortical size and growth may lie in a different pituitary behavior in the two sexes. Female rats have heavier pituitaries than males (123) and show the following differences from males in cortical structure and function (76, 347, 351, 352, 355): under normal conditions they have a larger cortex, show greater regeneration of cortical grafts, regenerate more accessory tissue, and give more successful non-sibling adrenal homotransplants. Female pituitary implants have been claimed to be better able to cause adrenal hypertrophy. Female adrenals gain more weight after estrogen administration than do male glands. The lack of a change, or a decrease in adrenal weight after ovariectomy and the absence of significant changes in the incidence of successful homotransplants (355) might point to estrogen as stimulating corticotropin function.

The above sex differences might be attributed to a larger larger amount of corticotropin in the female or at least to a greater availability of this fraction in the female organism.

Appendix D

THERAPEUTIC ADRENAL TRANSPLANTATION

It is obvious that the present lack of understanding of the mechanism of cortical function and dysfunction impedes the progress not only of experimental but particularly of therapeutic adrenal transplantation. The ill-fated attempts of therapeutic application clearly prove this point. The lack of a comprehensive review of the literature on therapeutic adrenal grafting makes it advisable to cite and discuss the results obtained so far.

The exaggerated and extravagant claims of quacks and charlatans for the relief afforded in certain disorders by transplantation and the publicity given to their "successes" have certainly not been beneficial to surgical progress. Not very long ago, gonadal transplantation in connection with the "rejuvenation" craze fooled a gullible public and many a physician, too, as to the value of endocrine transplantation. This era seems to have disappeared, fortunately, and the increasing knowledge of tissue transplantation in general, its indications, contraindications, limitations, and dangers have been spread. It has to be emphasized, however, that we stand at the very beginning of endocrine grafting as an attempt to conquer deficiency and disease. Any hopes for a successful application in therapy will remain justified more

emotionally than scientifically until laboratory men and clinicians will be able to provide a sound physiological basis for the problem.

The scattered information about therapeutic transplantation of cortical tissue and the lack of an adequate and complete survey prompted this critical review of the cases of patients with cortical insufficiency who have been subjects to or victims of this form of treatment.

The question may be raised whether a discussion of endocrine transplantation is timely in the face of recent chemotherapeutic advances with relatively inexpensive synthetic active materials.

With all due recognition of the chemotherapeutic successes attained so far, there is no doubt in the author's mind that one goal will remain, now and ever, namely, to restore the organism's genuine power to provide its own secretions. And this goal will continue to be a steady and urgent call for the experimental physiologist and surgeon.

One might possibly consider the use of adrenal transplantation in the therapy of various forms of cortical insufficiency, particularly Addison's disease.

The symptoms of cortical insufficiency can be brought about by a variety of pathological conditions in the adrenal among which tuberculosis ranks high. Other causes are cal-

cification, amyloid degeneration, syphilitic gummata, neoplasms, "simple atrophy" due to anterior pituitary atrophy or ischemic necrosis (Simmonds' disease), and massive bilateral adrenal hemorrhage. The latter condition is called Waterhouse-Friderichsen syndrome, occurs mostly in children, is probably caused by a fulminating septicemia, and is almost invariably fatal within 24 hours after its sudden onset. Thrombotic lesions of the adrenal vascular system have been found in this condition (79).

The symptomatology of cortical insufficiency is described in appendix A.

The seriousness of the disease, the insidious onset of Addisonian crises, the frequently moribund condition of the patient, and his enhanced sensitivity to any kind of stress - be it operative procedures, introduction of a foreign protein, or a multitude of other burdens to the organism - together with difficulties inherent to adrenal transplantation itself , make the reasons for the failures of a surgical treatment apparent.

Aside from specific therapeutic measures required for the underlying disturbance, such as tuberculosis, the present treatment of cortical insufficiency which has led to a conspicuous success comprises:

(1) the administration of specific hormones in form of

a) cortical extract: oral, subcut., i.m., or i.v.

b) synthetic desoxycorticosterone: subcut. pellets, i.m., or sublingual

c) crystalline steroid compounds from the adrenal such as compound E, which, however, are not yet generally available

(2) supplementary therapy: sodium chloride and sodium bicarbonate or citrate, low potassium diet, adequate amount of carbohydrate.

According to Thorn (306) no striking benefits have yet been seen after the administration of anterior pituitary substance, but it is hoped that better results may be obtained as soon as a potent and moderately priced corticotropic material is made available.

A remarkable and true progress in the general and specific management of Addisonian patients has been made during the last 10 - 15 years. While prior to this era the condition was almost invariably followed by death in a short time, recent papers report of survival periods up to 18 years after the onset of the syndrome (254, 255). Even if these cases are only moderately severe ones, they indicate the great advance achieved.

In spite of this success this treatment is still far from being ideal. This can be seen, for instance, from the potential dangers of the administration of DCA. Although this material constitutes a tremendous advance in therapeutics, it is a two-edged weapon and must be used wisely. Too rigid

a rehydration of the Addisonian is undesirable. The powerful salt and water retention due to DCA overdosage might lead to hypertension, edema, and heart failure; fatalities have been reported. If potassium is not available, the result may be an abnormal increase of intercellular fluids with subsequent increased venous pressure. For this reason, the combination of DCA treatment with a low potassium diet or extra sodium is undesirable as has been pointed out by the Mayo workers (309, 327), although high sodium - low potassium treatment is otherwise excellent in cortical insufficiency. But under a DCA management a moderate potassium and a relatively low sodium intake should be maintained.

Transplantation in man, as far as adrenal tissue is concerned, is necessarily homo- or heterografting. A dislocation of a patient's adrenal gland might become necessary at times, e.g. in the case of nephrectomy when adhesions between adrenal and renal capsule necessitate the loosening of these connections and fixation of the adrenal structures in the retroperitoneal tissues. This, however, cannot be called true free autotransplantation. Any application of adrenal autotransplantation for other than experimental purposes in animals does not seem to be intelligent, because if the glands are intact and functioning there is no reason whatsoever to touch them, and when they are diseased there is no hope that this tissue might become functional and healthy again in some

other site of the body. It is now generally agreed that heterotransplants of adrenal tissue with our techniques have no chance of being incorporated into the organism as a functional unit.

Successful adrenal transplants must entail the survival of the undoubted case of Addison's disease beyond the expected period of life together with clinical and laboratory evidences of improvement over a long period of time. It is exceedingly difficult to judge whether an adrenal graft has taken or not. Indeed, at the present time this seems often to be impossible. A short period of improvement in the patient's condition does not justify the conclusion that the transplant is functioning. It is well known that spontaneous remissions and clinical variations of Addison's disease occur; their nature, however, is entirely obscure. Moreover, the additional salt and supportive therapy as practiced today, not to speak of administration of cortical extracts or synthetic preparations, might account for the improvement. Nor does histological examination of transplanted adrenal tissue give absolutely adequate information since an apparently normal histological structure does not guarantee an equally normal function. Another factor introducing uncertainty in the judgment of the possible functioning of a graft in a not quite certain case of insufficiency (with a therefore questionable indication for transplantation) is the other site of the body. It is now generally agreed that heterotransplants of adrenal tissue with our techniques have no chance of being incorporated into the organism as a functional unit.

Successful adrenal transplants must entail the survival of the undoubted case of Addison's disease beyond the expected period of life together with clinical and laboratory evidences of improvement over a long period of time. It is exceedingly difficult to judge whether an adrenal graft has taken or not. Indeed, at the present time this seems often to be impossible. A short period of improvement in the patient's condition does not justify the conclusion that the transplant is functioning. It is well known that spontaneous remissions and clinical variations of Addison's disease occur; their nature, however, is entirely obscure. Moreover, the additional salt and supportive therapy as practiced today, not to speak of administration of cortical extracts or synthetic preparations, might account for the improvement. Nor does histological examination of transplanted adrenal tissue give absolutely adequate information since an apparently normal histological structure does not guarantee an equally normal function. Another factor introducing uncertainty in the judgment of the possible functioning of a graft in a not quite certain case of insufficiency (with a therefore questionable indication for transplantation) is the difficulty of diagnosing the insufficiency in some cases. How can one judge the efficiency of a grafted tissue if one does not even know whether there is any need for the particular secretion and whether this secretion is not produced by the organism's own structures? An unmistakable proof of the diagnosis is the most important factor prior to consideration of transplantation therapy. The diagnostic difficulty in Addison's disease still exists despite recent advances in the biochemical knowledge of this disease. Pluriglandular disorders, pituitary basophilism, adrenogenital syndrome, Simmonds' disease, and other conditions may complicate diagnosis and treatment considerably. The case of Hurst (# 5) is an example of some of the difficulties encountered. It is to be hoped that quantitative biochemical methods - aside from the routine electrolyte and glucose determinations - will be devised which will conclusively answer questions concerning the amount of cortical hormone that is produced, broken down, and excreted and whether or not this amount constitutes a deficiency. If techniques like these could be made available for use in any well equipped laboratory, many a question of adrenal and transplantation physiology could be solved. At present a well-rounded clinical, biochemical, histological, and cytological picture is necessary as a basis for the evaluation and understanding of the processes connected with adrenal transplantation.

Unfortunately, the available data of adrenal transplantation

in man do not fulfil these requirements. The lack of precise and comprehensive information, the inadequacy of diagnosis and technique together with the failure to follow the patients long enough (if they happen to survive), and the general lack of sufficient knowledge of adrenal physiology per se make the following series of cases contribute only little to one of the really pertinent problems of medicine: how to influence diseased endocrine structures.

Case

1,2 Jabulay (159) in 1897 was the first to report the transplantation of animal adrenal tissue into humans, a venturesome undertaking which for many years thereafter evoked the amazement of writers. He attempted this therapeutic grafting after beneficial results were obtained with thyroid transplants in clinical cases. Fresh dog adrenals were implanted under the abdominal skin of two patients with Addison's disease. Both of them died after 24 hours with hyperthermia and prostration.

3 Busch and Wright (42) in 1910 grafted an adrenal from a hog into the testicle of a 35 year old Addisonian man. After some improvement for about two weeks, a sudden exacerbation was followed by death in coma. The authors, nevertheless, assumed from the histological examination that part of the transplant had survived. The medulla became necrotic.

4 Morris (discussing 204) reported a case of adrenal tuberculosis in which he transplanted a normal adrenal. No details were given. "It takes kindly" and "..even though the graft undergoes absorption, we may obtain some control over the tuberculosis ... by increasing the patient's general resistance", were his conclusions.

5 *Hurst* et al. (136) in 1922 reported the case of a 41 year old man with Addison's disease in whom extract treatment did not meet with success. A subcutaneous homotransplant of an adrenal from a male victim of an accident was not followed by improvement; the wound suppurated. Two weeks later a second transplantation was undertaken. This time the adrenal tissue was obtained from an eight month old fetus immediately after its death and grafted into the patient's testicle. After a temporary aggravation of the man's condition, an improvement with rise of blood pressure was noted; the pigmentation, however, did not disappear. The patient eventually was able to do light work but the symptoms gradually reappeared and he died 2 years and 3 months after the operation. The autopsy (135) revealed perfectly healthy adrenals and complete disappearance of the grafted tissue. The diagnosis of "Addison's disease" in this patient had previously been confirmed by many British physicians and accepted as a typical case by the Clinical Section of the Royal Society of Medicine. In accordance with these postmortem findings the diagnosis had to be changed and corrected to liver cirrhosis possibly with Addison's anemia. This case demonstrates very well the extraordinary diagnostic difficulties which one may encounter in patients with marked pigmentation, asthenia, and low blood pressure, all of which signs were present in the described case and led to the diagnosis of "undoubted" typical Addison's disease. *Conybeare and Millis* (57) conclude from this case and other experiences that it is almost impossible to be certain of a diagnosis of Addison's disease without confirmation by autopsy. In the light of more recent work and diagnostic techniques, this seems a little exaggerated; however, it points to diagnostic difficulties which still exist.
6 These authors in 1924 described two cases from Guy's Hospital. A fetal adrenal was transplanted into the testis of a patient; he died within 12 hours after operation. No details were
7 given. Another Addisonian patient received a fetal adrenal

into his kidney with an almost immediate fatal result. An autopsy was not performed.

8 Bra (cited in 53) transplanted the adrenals of a dog into a
child with Addison's disease. The patient died three days
9 after operation. A similar operation was done by Courmont
(cited in 53). Death occurred within 24 hours following considerable
hyperthermia and cardiac collapse.

Pybus (237) who failed to obtain successful pancreatic homotransplants
in two patients with severe diabetes, reported in
1924 adrenal homotransplantation in two Addison patients.
10 A 25 year old woman who received grafts from a male donor
11 died a few weeks after operation. A 40 year old miner received
subcutaneous implants of adrenal glands obtained from
a healthy young man immediately after his death from an accident.
The subsequent improvement enabled the patient to work
again in the mine for some time, but symptoms returned 28
months after operation. Three years after the first transplantation
no trace of the previous grafts could be found
and another half of a gland was transplanted. Some improvement
has been noted and the patient has been working for
three years since then, without enjoying full health, however.

12 Currie (63) reported in 1924 a case of a 50 year old woman with Addison's disease who did not respond to extract treatment. He transplanted a sheep adrenal in the abdominal wall of the patient. A short period of improvement was noted. The subsequent suppuration of the graft necessitated another transplantation of two sheep adrenals which after being sliced were administered by means of syringe into the subcutaneous tissue. Improvement followed. Unfortunately, a follow-up study could not be done since the patient left for another country.

13 Dmitrijew (74) in 1925 applied subcutaneous heterotransplants obtained from dogs to a 26 year old man with Addison's disease and noted definite improvement. The patient had no complaints for six (!) weeks after operation. This investigator also transplanted adrenals to seven patients who suffered from gangrene. In all cases reported the grafts underwent atrophy but the author claims a stimulating effect of the transplanted tissue. Dmitrijew relates gangrene of the foot to adrenal hypofunction. Transplantation of rabbit and dog adrenals into 12 other patients with spontaneous or senile gangrene (cited in 130) supposedly resulted in improvement, local cure in eight and return of the tibialis pulse in three of the patients.

14 Rosenow (cited in 188) transplanted the adrenal gland of an epileptic into a patient with Addison's disease. Death occurred immediately.

15 Halpern and Arkusenko (cited in 95, 171) in 1927 supposedly succeeded in transplanting adrenal tissue to an Addison patient, with subsequent general improvement for one year.

16 Reinhart (239) and Leschke (188) reported in 1928 a case of a 20 year old woman with Addison's disease in a cachectic condition, in whom homotransplantation was done. The adrenal was obtained from a patient who was nephrectomized because of tuberculosis of the kidney. The non-tuberculous adrenal was grafted into an abdominal muscle pocket. In addition, adrenal preparations were administered per os. Progressive improvement followed with fading of the pigmentation, rise of blood pressure, return of menstruation, and gain in weight. Leschke reported that the patient was well and able to work and travel one year after operation. Reinhart expressed doubt as to whether this graft would be able to provide a lasting substitute for the diseased glands. He thought a

partial success may be obtained as long as the transplant secretes and possibly stimulates the patient's own adrenals. He mentioned that it might become necessary to do another homotransplantation in this patient or, if human tissue should not be available, to try a heterograft.

7,18 Bauer and v.Eiselsberg (cited in 64) transplanted adrenal tissue obtained from epileptics into the abdominal wall and into the sternal bone marrow of two patients, both of whom died. In one of them, the autopsy showed a rapid necrosis of the graft. 19 In another case, v.Eiselsberg used a heterotransplant with tissue obtained from a macacus. The transplantation was not successful, and the patient died.

20 Curschmann (64) in 1928 transplanted adrenal tissue obtained from a unilaterally adrenalectomized young and healthy epileptic into the abdominal muscles and subcutaneous fat of a 42 year old Addisonian man. The patient left the hospital improved eleven days after operation. However, later his condition became progressively aggravated and he died in coma 11 weeks after operation. No autopsy was performed. Despite this complete failure the author emphasized that homotransplantation will have to be tried again and again. Perhaps, particularly in the non-tuberculous patient, a temporary substitution can be achieved which will give the diseased adrenals a chance to recover.

21 d'Abreu (2) reported in 1933 the case of a 27 year old woman with far advanced Addison's disease. Extracts had been without beneficial effect; intravenous saline and glucose provided only temporary relief; the blood pressure was very low. Adrenal tissue from a 73 year old woman who had just died of cerebral thrombosis was transplanted in slices into subcutaneous pockets. Improvement with rise of blood pressure was

noted. Ten days later an adrenal of a stillborn premature infant was grafted into the abdominal wall of the patient. He died nine days after the second operation. Autopsy revealed cortical atrophy, but no tuberculosis of his own glands, and suppuration of the grafts.

22,23,24 Biedl (cited in 72) applied heterotransplantation in three patients. All of the three operations were failures.

25 Desmarest and Monier-Vinard (72) in 1934 obtained a small fragment of adrenal tissue from a patient adrenalectomized because of hypertension and inserted it subcutaneously into a 33 year old man with Addison's disease. This man had shown temporary improvement prior to operation after administration of cortical extract. The extract therapy was continued postoperatively. However, no essential improvement could be obtained. Another transplantation was undertaken. The donor was a 47 year old man and was of the same blood group as the recipient. This donor, too, was adrenalectomized for arterial hypertension. The whole gland was placed into the abdominal muscles of the Addisonian. Extract was given as usual. Following hyperthermia and shock death occurred on the second postoperative day. The transplant was completely necrotic.

Beer and Oppenheimer (25) in 1934 attempted adrenal homotransplantation in two Addisonian patients.

26 (a) 24 pieces of cortex (not medulla) obtained from a woman nephrectomized because of renal tuberculosis were transplanted into the rectus muscles of a 41 year old female. The blood groups of donor and recipient were not compatible. The patient died two weeks after the operation despite additional postoperative administration of extract. The postmortem examination revealed extensive caseous tuberculous necrosis of both adrenals. The transplants showed degeneration of most

of the adrenal cells. However, there were viable parts as confirmed by several examiners. The authors conclude that these viable parts had"taken" during the two weeks following transplantation.

27 (b) A 23 year old man who was treated pre- and postoperatively with intravenous extract, saline, high salt diet, and glucose, received 45 cortical pieces (not medulla) into the rectus abdominis muscle. The cortical tissue was obtained from another patient nephrectomized and adrenalectomized because of nephrolithiasis. Eight weeks later another transplantation of 19 cortical fragments into the rectus abdominis was performed. Progressive improvement could be observed though the blood pressure remained low. A low NaCl test diet indicated the still existing adrenocortical insufficiency. The pigmentation around the mouth and the lips did not change. Nevertheless, the patient was in good condition after the administration of cortical extract and NaCl. He was discharged and has lived a fairly normal life since. It is the author's impression that though the grafts were functionally not completely capable of making up for the cortical insufficiency, they seem to have taken and to have helped in bringing about the improvement.

In both cases the cortical transplants were placed into rectus pockets within one hour after removal from the donors.

The questionable and unreliable review of Aouslender (18)
28 (no references; misspelling of names) cites Bayer as having transplanted adrenal tissue into Addisonian patient(s ?)
29 with a fatal outcome. Hertzen supposedly was more successful. His patient(s ?) survived.

30 Kanevskij (cited in 95) grafted rabbit's adrenal tissue into a patient. The transplant suppurated and was discharged but

the patient seemed to be "cured"; pigmentation was lost and not relapse occurred up to nine months after operation.

31 Bailey and Keele (20, 21) reported in 1935 the case of a 44 year old woman with the undoubted diagnosis of Addison's disease and X-ray evidence for some pituitary defect. Salt, whole gland, and extract treatment did not prevent a downhill course. The adrenals of a stillborn fetus were transplanted into each rectus sheath; one of them did not take. A steady improvement was noted. The patient was observed for four years following the operation. Symptoms did not return, and the weight remained constant. A slight pigmentation remained. No pituitary change could be found on X-ray examination after four years. The menopause had occurred during this period. The patient has been working long hours. She appeared to be cured. The authors mention that the patient's menstruation had always been regular. They leave the question open as to whether the available female sex hormones might have contributed to the taking of the transplant.

32 Goldzieher and Barishaw (95) in 1937 grafted adrenal tissue into a 27 year old man with Addison's disease who received saline and extract injections and salt orally for one year and a half before operation, resulting in temporary improvement and relapses. The tissue was secured from a woman who was adrenalectomized because of adrenogenital syndrome. About 30 slices were implanted into rectus or fascia pockets. Adrenal and anterior pituitary extracts were given after operation. A remarkable improvement followed: blood pressure, muscular strength, appetite, and weight increased, the pigmentation faded, and the patient went back to work six months after operation. Three months later he died after relapses associated with pneumonia. The autopsy revealed bronchopneumonia, complete simple atrophy of the patient's adrenals

(cause unknown; no tuberculosis); the transplants showed well preserved, persisting cortical tissue with multinucleated giant cells similar to those found in cortical tumors. The authors take that as evidence for the capability of nuclear division. They consider the grafts as having survived and assume that the transplanted tissue had been functioning during the nine months since it showed no necrosis and little evidence of regression at the time of death. The authors express hope that transplantation as a treatment in Addison's disease under improved conditions might approach a real cure. They think that the fact that it was possible for this man to survive after the operation for considerable time without depending upon increased salt intake or cortical extracts (which previously were indispensable) and to show transplants in a satisfactory state of preservation indicates that human homotransplantation of the adrenal can be achieved with beneficial effects. The authors believe that preoperative treatment with hormone and salt makes the patient a better surgical risk and therefore may improve the results of transplantation. They think that the postoperative extract administration may prevent strain and exhaustion of the graft. They assume that the pituitary extract was helpful in stimulating the transplant.

Without giving any details, <u>Stone</u> et al. (288) in 1938 mentioned
two patients who showed "definite improvement" with homografts
33 which previously were cultured in vitro. One of them died of
pneumonia nine months after operation after having gained
weight and exhibited other signs of improvement. The other
34 patient showed increased blood pressure, his libido had returned,
and he was able to do some work. The authors admittedly
failed to obtain successful thyroid and pancreatic homografts
in man after preparations of the tissues in vitro. They claim,
however, some success in a few cases with parathyroid transplants,
unfortunately without giving case histories or any
details.

Other investigators (72) have stated that immediate transplantation is to be preferred to the passage of the graft through serum, Locke or Ringer solution. They also advocate the fragmentation of tissue to be grafted and the implantation of individual pieces apart from each other.

35 Katz and Mainzer (171) reported in 1941 an "indisputable" case of Addison's disease which they observed for about three years before and 15 months after transplantation. An atypical feature of this case was the absence of pigmentation. The 56 year old woman had received for one year cortin and salt treatment to which she responded well. Adrenal tissue was obtained from a 52 year old man who had just died from cerebral tumor; the tissue was transplanted into the abdominal muscles of the patient. Donor and recipient belonged to the same blood group. Despite the administration of extract and salt a further decline occurred which, however, was followed by improvement on the third day after operation. She left the hospital on the sixth postoperative day feeling absolutely well and with an increased blood pressure. The extract administration was discontinued on the seventh postoperative day. The patient is well compensated as long as she lives under normal conditions. Following exertion or excitement she experiences somnolence and pain in the back. The administration of extract became necessary only once during an influenza attack with lowered blood pressure.

Conybeare (56) doubts the correctness of the diagnosis of Katz and Mainzer. The long-standing symptoms in their patient (10 years), the lack of pigmentation, the not uncommon occurrence of low blood pressure values in even healthy adults, and the lack of biochemical evidence do not support the diagnosis of Addison's disease and certainly do not

justify the term "indisputable". Similarly, Simpson (275) considers the case of the above authors as a rather doubtful one, though the absence of pigmentation does not necessarily mean an incorrect diagnosis. In Simpson's experience the results of adrenal transplantation in Addison's disease have been either negative or at best have afforded temporary improvement.

Turner (316) also questions the diagnosis of Katz and Mainzer. He never saw a patient with Addison's disease without pigmentation.

On the other hand, Spanish workers (318) claim to have observed many cases of non-pigmented atypical cortical insufficiency.

All 35 cases are summarized in table II.

A sober and critical analysis of these 35 cases - a difficult undertaking due to lack of adequate information in several of them and unavailability of the original reports of a few - does not lead to encouraging results.

Cases 4 and 29 offer so little information that they have to be omitted for any serious consideration. Little is gained from case 12 since a follow-up study could not be done. Case 13 has to be discarded because the freedom from symptoms for 6 weeks after transplantation is of no significance.

Case 5 has to be excluded because of the admittedly incorrect diagnosis.

Cases 1-3, 6-10, 14, 17-26 and 28 represent obvious failures. It is, of course, not possible to state exactly whether

and how the transplants per se influenced the more or less serious condition of these patients. One can go so far as to say that the marked septicemic state, the pronounced collapse, and the exceedingly short survival time of some of the patients certainly do not indicate any beneficial effect. This is not surprising in the light of animal experiments - particularly with heterotransplantation - which are discussed elsewhere.

Cases 25, 26 and 32 succumbed despite the postoperative administration of adrenal and pituitary extracts. This shows that transplantation per se constitutes a stress for the diseased organism which could not be mastered. The Addison patient is enormously sensitive towards all interferences and therefore an extremely poor surgical risk so that even insignificant manipulations such as subcutaneous implantation become a most severe disturbance. This is particularly true for the introduction of foreign protein. Trauma, exertion, and psychic influences may accelerate the downhill course of the Addisonian due to the altered reaction to such stimuli. For instance, slight acute nephritis and "poisoning" in two fairly well compensated Addison patients led to coma and death (64).

Cases 32 and 33 also seem to be failures in spite of the longer survival which may be entirely unrelated to the transplantation. It has to be noted that the postoperative

administration of pituitary or cortical extracts or both in case 32 may have something to do with the nine month survival.

Patients 16 and 27 are surviving, but they also received adrenal preparations, saline or glucose postoperatively. These cases as well as patients 11, 27, 30, 32-35 may very well be examples of a more or less mild form of chronic cortical insufficiency or may be interpreted as spontaneous remissions.

The assumption of the investigators of cases 3, 26 and 32 that parts of the grafts survived, is of little significance if one considers the apparent functional inadequacy of these supposedly vital transplants and the subsequent fatal outcome. The implanted cortical tissue was obviously unable to meet emergencies such as infection and intercurrent disease. It was not even capable of providing enough hormones to answer the minimal requirements of the organism. It has been pointed out that an apparently normal histological picture of a graft does not necessarily justify the conclusion that the particular tissue is functioning normally. The multinuclear giant cells in case 32 which were interpreted as indicating nuclear division might well be a regressive sign similar to foreign body giant cell reactions.

Case 30 cannot be considered a success since the suppurating graft was discharged. This "cure" cannot possibly be related to the transplantation. Spontaneous remission, chronic

adrenal insufficiency, or incorrect diagnosis are the only possible interpretations.

This leaves us with the "successful" cases. Case 15 cannot be considered since the history is not available. Report 34 does not offer sufficient data. Patient 11 cannot be called a full success since the first transplant appeared to have been completely absorbed, and, moreover, the man did not enjoy normal health. As to case 16, Reinhart himself doubts that the graft will be capable of providing a lasting substitution. His assumption that a transplant might stimulate the patient's own adrenals cannot be agreed with. It is well known that the opposite, depression of an endocrine gland, is induced by the administration of the particular hormone. Thus, such grafts, having not only little chance to take in the presence of some glandular rests, their hormone content might have a depressing rather than a stimulating effect on the remaining cortical tissue. In case 16 as in 27, the postoperative administration of cortical extract might account for the amelioration of the symptoms. In the latter case, saline and glucose were given in addition to extract, and yet, there was still a cortical insufficiency in existence as revealed by pigmentation and test diet. Case 35 is doing well as long as she is under non-stress conditions. This, too, clearly indicates residual chronic cortical insufficiency. Moreover, the diagnosis in this case has been questioned

by several competent investigators, a fact which tends to belittle a partial success even more.

Case 31 would sound promising if one could be sure of the diagnosis, and the nature of the suspected pituitary disorder, and if more biochemical data were available. However, the remaining slight pigmentation suggests that the "cure" is not a complete one and the best one can do is to await further reports on this case.

The small amount of hormone contained in transplanted cortical tissue does not seem to be of any other significance than to provide a little active material for a transitory improvement which ceases as soon as the hormone is absorbed. It is difficult to conceive that these minimal amounts of hormone could have any appreciable effect in prolonging life. If a graft is to exert the desired effect, it must produce active substances in adequate amounts as soon as possible and must continue to secrete over a long period of time. An amelioration of several weeks or a couple of months cannot be considered a success, and no surgeon should consider it desirable to perform transplantation in order to bring about such a temporary success. It is now possible to produce such a condition much more easily and without taking chances by means of synthetic preparations or extracts or even with mere dietary adjustments. The goal of cortical transplantation should remain the true cure and permanent

correction of a deficiency. The present therapeutic state of affairs is that we are well able to give palliative and substitutional treatment to the patient with cortical insufficiency, but nothing more. The aforementioned series of cases shows quite clearly that, including the few doubtful successes, not a single instance warrants the term "cure".

At present, nothing can be expected from heterotransplantation. The results in the above patients and experimental evidence suggest that it is not reasonable to attempt therapeutic heterografting when even homotransplantation does not lead to the desired results. Future work must concentrate on homotransplantation with young donor tissue. Various of the above authors and others (326) have voiced hopes that - in spite of the obvious therapeutic failures - the encouraging results of experimental grafting might lead to an improved application in the treatment of Addison's disease. Whether tissue culture or other methods of "conditioning" of the tissue to be transplanted will be of value is open to discussion and doubt. Anterior pituitary corticotropic preparations may afford better prospects. Several workers have suggested the use of adrenotropic substances in order to stimulate the viable cortical rests of the patient, and the transplant. Whatever will be done and tried, the greatest scepticism and sober judgement will have to be employed in estimating the merits of any form of therapy.

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AUTOBIOGRAPHY

I, Frank Lothar Plachte, was born on October 26, 1913 at Glogau in Germany, the son of Max and Nathalie Plachte. From 1920 to 1933 I attended the Glogau Oberrealschule and Ludwig - Maximilians University in Munich. From 1933 to 1938 I went to Munich University Medical School where I passed the Preliminary Medical Examinations in 1935 and 1936. During the summer of 1935, 1936, and 1937 I worked as a student intern in the departments of surgery and pathology of a Berlin hospital.

After my arrival in the United States in 1939, I did volunteer work in the Department of Neurosurgery of the Hospital of the University of Pennsylvania. Since September 1939 I have been a student at the Graduate School of Boston University majoring in physiology. In addition, since June 1940 I have been assisting in research in the Department of Pharmacology of Harvard Medical School.