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OXON. 1893.

PRACTICAL EXERCISES IN PHYSIOLOGY.

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PRACTICAL EXERCISES IN PHYSIOLOGY.

1. Electrodes.

Prepare two straight moderately thiek wires, about four ineches long. Taper each to a blunt point at one end. Solder to the opposite end of each about two feet of thin wire. Cut two bits of stronger wire, eaeh half an inch long, and solder each to the unattached end of one of the thin wires. Prepare two three-ineh lengths of glass tubing (which should be thiek walled and of narrow bore). Warm them, and introduee the wires, after covering them with packing wax, so that their points project half an inch. Bind the tubes together for eonvenienee of handling, bare the wires by seraping the wax off the points, and bend the opposite ends so that they diverge from eaeh other.

Another useful form of electrodes is to be made as follows:—Take a bit of gutta-percha sheeting two inches long and one-third of an inch wide; after warming one end, bend it so as to form a trough quarter of an inch deep, with a long handle. Make two holes in each side of the trough, one millimeter apart, and at an equal distanee from the bend. The two pairs of holes must be opposite each other. Perforate the end of the handle in a similar manner. Prepare two lengths of thin covered wire and remove the covering of one end for at least two inches. Pass the bared ends through the holes, so that the wires lie outside the handle, and are exposed in the floor of the trough. Cover all the parts which are exposed externally with fused gutta-percha.

2. The Daniell Cell.

The positive element is a well amalgamated zinc rod immersed in ten per cent. sulphuric acid contained in a porous cell; the negative element is a copper cylinder immersed in solution of sulphate of copper. Put a wire in each binding screw. The end of the wire attached to the zinc (negative wire), is called the cathode; that attached to the copper (positive wire), the anode.

3. The Induction Apparatus.

Arrange cell and coil for single shocks, *i.e.*, join the ends of the battery wires to the two top screws of the du Bois' induction apparatus, in which the primary wire ends, interposing a key by which the current is made and broken at will. Insert the ends of the electrode wires (first form) into the binding screws of the secondary coil. Gradually sliding the secondary towards the primary coil with the points of the electrodes on the tip of the tongue, observe that the break shock is first felt, then the make. Observe the effect of withdrawing the core of the primary coil in these experiments.

Arrange cell and coil for repeated shocks (faradisation), by bringing the battery wires to the two screws *A* and *E*, Fig. 1. The circuit now includes the vibrating hammer or automatic interrupter. On closing the current the hammer is drawn down and causes a break; the current ceasing, the hammer is released, and contact is restored by the spring. You thus obtain a succession of make and break induction currents in alternately opposite directions.

To obtain successive make and break excitations of nearly equal intensity, modify the arrangement as follows:—Connect the two ends of the primary coil (the top binding screws), by an extra derivation or "short circuiting" wire broken by a key. The wires to the battery remaining closed, the effect of closing

the derivation circuit is to diminish,—of opening to increase the current in the primary coil. The diminution and increase

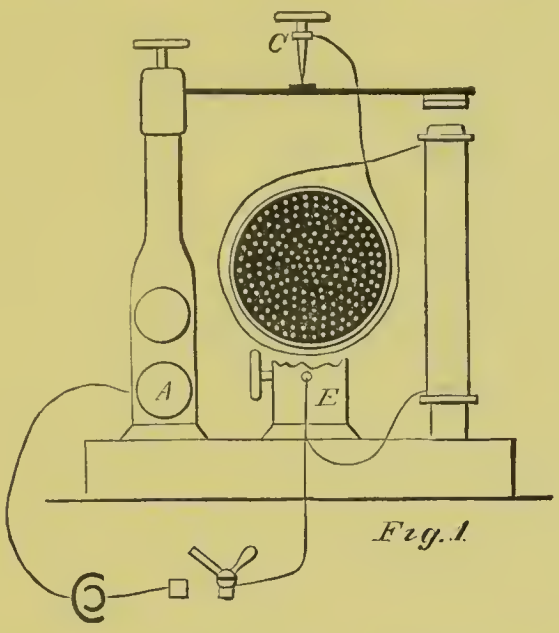


Fig. 1.

give rise to induction currents, of which, the directions are opposed like those produced by make and break. Both of them are *cæt. par.* weaker than the make induction current, and they are sensibly equal to each other in their excitatory effects.

Helmholtz' Modification.—When it is necessary in faradising that the excitatory effects of the make and break shocks should be equal, the apparatus is arranged as in Fig. 2. Connect the battery wires as before. Bridge the interrupter by a wire extending from *B* to *C*. Raise the upper contact screw *C* out of reach, and bring the lower (*F*) within reach of the spring. Here, as in the other case, the current in the primary coil diminishes by derivation, when the descending hammer touches *F*; increases to the same amount when it rises, but is never broken.

4. The du Bois Key.

Before proceeding further, note that a key may be used for throwing a current into or cutting it off from a nerve or other

excitable structure in two ways, viz., (1) in such a way that when it is closed the current is made, when it is opened the

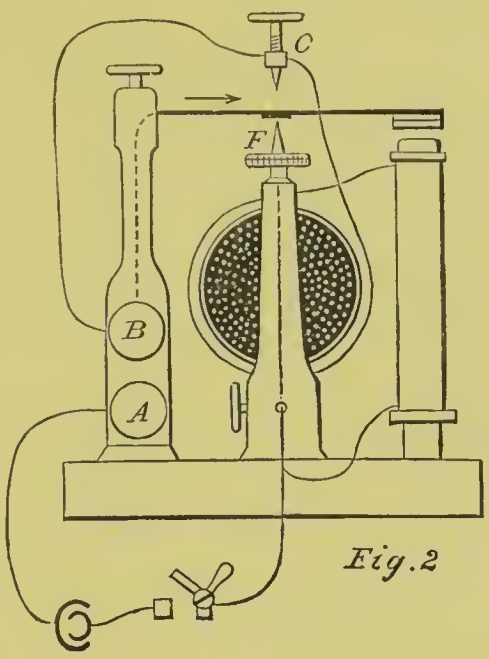


Fig. 2

current is broken; or (2) so that when the key is closed it acts as a bridge, by which so large a proportion of the current is derived, that it in effect vanishes in the part of the circuit beyond the bridge.

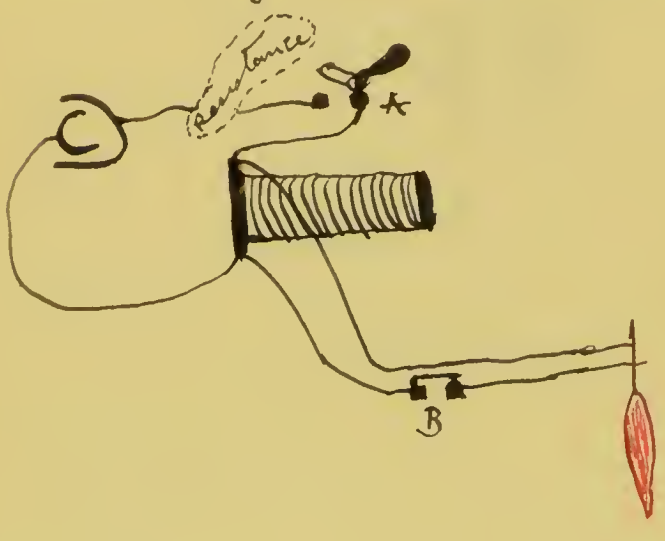
In using induction currents for excitation, always employ the second method.

5. Physiological Proof of the Break Extra Current.

The extra current is the current produced in a coil by the inductive influence of contiguous turns on each other, when a voltaic current through the coil is closed or opened; its direction is *against* that of the battery current at *make*, *with* it at *break*. Establish connections, as in Fig. 3, placing the electrodes on the tongue. Close the key, *A*, so that the current is cut off from the coil. Observe that opening and closing the current by the key, *B*, produces little or no appreciable effect. Now open

For fuller description of the break extra current vide 2nd edition. A simpler way of demonstrating the extra C, is to connect a cell with one terminal of a Coil & then take the other terminal with the other wire from the cell. On breaking a spark is obtained, but not on making.

Another way of demonstrating the extra current. (BBS)



Key B being kept closed, on opening A a twitch is felt on the tongue, or a contr occurs in a prep. This is due to the extra current at breaks, for it is not obtained by opening or shutting B.

the key, *A*, so that the current passes through the coil. Opening the key, *B*, gives rise to a strong effect which is due to the break extra current.

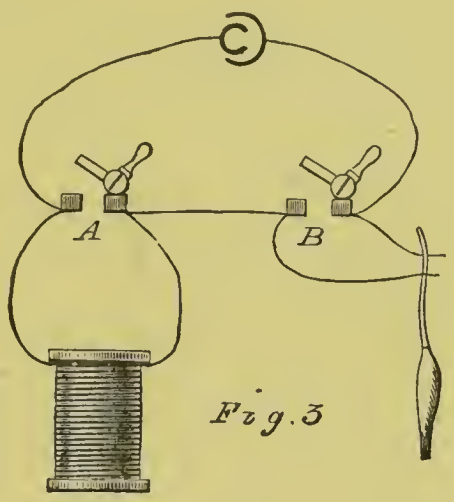


Fig. 3

6. Indirect Electrical Excitation of Muscle.

Pith a frog and prepare a sciatic nerve without dividing it. (See Hdb. p. 343.) In the process the gastrocnemius should not have twitched. Connect the electrodes (second form) with a Daniell cell, interposing a key in the circuit, and apply them to the nerve. Contraction follows make and break of the circuit. It does not continue during the passage of the current.

Repeat Experiment 3, substituting a sciatic nerve prepared as above instead of the tongue. In all cases note the distance of secondary from primary coil at which contraction is first observed.

7. Unipolar Excitation.

Connect one electrode with the secondary coil, and apply it to the nerve. If the preparation is completely insulated, there should be no response to make or break or to faradisation. If the insulation is destroyed by touching the preparation, or otherwise, contraction occurs.

It is to avoid unipolar excitation that, as a rule, the induction circuit is directed to be thrown in and out by using the key as a bridge (See §). Unipolar excitation is more apt to occur with the break shock than with the make, in consequence of the greater intensity of the former. Consequently, it is avoided by using Helmholtz' modification. Prove this by experiment.

8. Comparative Excitability of Nerve and Muscle.

Note the approximation (in centimeters) of the secondary to the primary coil which is required to obtain a response (1) to the make induction shock; (2) to the break; (3) to faradisation, applying the electrodes to the nerve. Compare these effects with those observed when the electrodes are applied directly to the muscle.

9. The Rheochord.

Whenever very weak voltaic currents are required, we use the Rheochord. The simplest and most convenient form is a long wire of German silver, of about 20 Ohms resistance, which, for convenience of space, is wound on glass pegs which are fixed at equal distances in two rows at opposite ends of a well-varnished mahogany board. The wire is thus divided into as many equal lengths as there are pegs. On the board, underneath the wires, is a scale, each division of which is $\frac{1}{100}$ of the whole length of the wire. The wire ends in two blocks, *A* and *B*, each of which has a double binding screw. In use, the battery wires are connected with these two blocks. One of them (*A*), also receives the wire of the second electrode. The other electrode wire is attached to a moveable block, by which contact can be established with the rheochord wire at any distance from *A*. In this arrangement, the current through the nerve, or

other structure to which the electrodes are applied, is *in proportion to the length of wire between the slider and the block.* This relation would not hold good were it not that the resistance of the nerve is always very great as compared with that of the wire.

10. Polarisation of Electrodes.

Place a pair of electrodes under the sciatic nerve, and join them by a key, and satisfy yourself that opening and shutting the key gives no contraction. Connect the two wires of a Daniell with each side of the same key, which, therefore, bridges the current. Allow the current to pass through the nerve for a few seconds by opening the key. Disconnect the wires from the battery. Close the key, the muscle will contract, the electrodes having been polarised by the previous current. Repeatedly close and open the key, and observe that the contractions diminish as polarisation subsides. This gives a reason for using the key as a bridge to cut off the constant current.

11. Galvani's Experiment.

Connect two dissimilar, *e.g.*, zinc and copper, wires; and apply their points to a nerve, or one point to a nerve, the other to any part of the frog; contraction occurs at make, or if the preparation is very excitable, at make and break.

12. The Contraction without Metals.

Prepare a nerve-muscle preparation, choosing a vigorous and lively frog. Allow the nerve to fall on the muscles of the lower limb stripped of its skin. The muscle of the preparation contracts because different parts of the surface with which the nerve is suddenly brought into contact are at different potentials.

13. The Secondary Twitch.

Prepare the sciatic nerve of one limb of a vigorous frog, and prepare a nerve-muscle preparation from the other limb. Strip the skin off the first limb, and lay on the gastrocnemius the nerve of the muscle to be secondarily excited. On stimulating the nerve first prepared both muscles contract. The secondary muscle contracts because its nerve is stimulated by the sudden electrical changes which accompany the contraction of the primary muscle. Finally constrict tightly the secondary nerve by a fine ligature; no contraction of the secondary muscle takes place.

14. The Paradoxical Contraction.

Dissect out one of the two main divisions of the sciatic, and divide it as far as possible from its origin. Galvanic excitation of the central end of the divided branch gives rise to contraction of the muscles supplied by the other branch. The second nerve is stimulated by the electrotonic alteration of the first nerve.

15. Action of Curare on Muscle.

Inject one drop of curare solution (1 per cent.), having stopped the circulation of one limb by tying a silk ligature round the sciatic artery. In a few minutes, test muscle and nerve of both limbs. Both react on the ligatured side; on the other side, muscle reacts, nerve does not.

In another preparation use the ordinary dose of curare, 1 to 2 drops of 0·1 per cent. solution, and wait longer. Inject 1 drop of strychnia solution (·05 per cent.). Pinching or touching curarised limbs, which have lost motility, will excite reflex motion in the protected limb.

For the preceding experiments the frog is killed by destroying the hemispheres: the spinal cord must be left intact.

16. The Graphic Method.

For experiments in which this method is used the requirements are (1) a revolving cylinder tightly covered with paper, and then smoked, of which the rate of rotation is known; (2) suitable means for supporting a muscle, so that it may act upon a lever which presses lightly against the moving surface of the paper.

By noting the time required for a given number of revolutions of the cylinder, and accurately measuring its circumference, the rate of motion of the surface which is to receive the record may be fairly determined. But for more accurate purposes, we must make a simultaneous record of the oscillations of a tuning fork, or of an electro-magnetic time-marker, introduced into a circuit in which a vibrating reed acts as an electro-magnetic interrupter.

When for the purposes of the experiment it is desirable that the horizontal motion of the recording surfaces should be slow, *i.e.*, less than two inches per second, an interrupting clock regulated by a pendulum or a metronome is substituted for the vibrating reed in the circuit of the time-marker. This is so constructed that it makes and breaks the circuit at intervals of one or more seconds.

The Myograph.—A simple and useful myograph is constructed as follows:—An oblong block of wood, on which a cork plate is glued, supports the preparation. At one end of the block is a vertical stem or pillar, of which the height and distance from the preparation can be varied. This pillar is surmounted by two steel points, which face each other at a distance of about three-quarters of an inch. On these the axis of a bell-crank lever rotates, of which the horizontal arm carries weight and is prolonged by a straw, and ends in a writing point. To the vertical arm, the tendon of the muscle of which it is desired

to record the contractions, is attached. A moveable vertical rod for carrying a time-marker is provided with the instrument. The whole is supported by a stand in such a way that it can be either moved up or down or rotated on a vertical axis.

17. Graphic Record of a Single Contraction (Twitch).

First arrange the recording apparatus. For the present purpose the rate of horizontal movement of the recording surface must be about 30 centims. per second. Solder to each of two pins, one of which must be of ordinary size, the other as small as possible, two feet of thin covered wire, the opposite ends of which must be prepared as in 1. Cover the cylinder with glazed paper, and smoke it over a petroleum lamp. Arrange exciting apparatus as in 3, interposing the time-marker in the primary circuit, and a du Bois key in the secondary circuit (See 4). Cut off the hind limbs of a frog just killed by pithing, and sever them from each other. Place one of them on the cork plate, in such a position that the tibia is in a line with the long arm of the lever. Expose the tendon of the gastrocnemius, and after severing and freeing it from surrounding parts, tie to it a bit of strong ligature thread immediately above the sesamoid cartilage. Then expose the lower end of the femur, and having drilled a hole thrust through it the larger pin. Secure the covered wires in the cork fixed to the side of the block for this purpose. This serves to fix the femoral attachment of the gastrocnemius. The fine pin is to be thrust through the tendon close to the ligature. Attach the thread to the short vertical arm of the lever, bring the myograph (which has up to this time been on the table) into position, connect its wires with the du Bois key. Charge the lever so that the unexcited muscle is stretched by a weight of 20 grm. The next step is to ascertain to what distance it is necessary to approximate the secondary coil, in

order to obtain a full response to break induction shocks. Bring the ends of the writing lever and of the time-marker into contact with the smoked surface, and ascertain that the two writing points are in the same vertical line. Set the cylinder in unrestrained motion. Close the key in the primary circuit and keep the primary circuit closed while the du Bois key is opened. On breaking the primary circuit, the preparation is excited and the moment of excitation marked on the smoked surface. Observe the period of "latent stimulation" and the characters of the muscle curve. Inscribe a succession of curves, with varying loads (10, 30, 50, 70, &c., grms.).

After Load—The screw underneath the horizontal arm of the bell-crank lever renders it possible so to arrange the weight that the muscle shall be loaded only during action. In most cases it is expedient to use this method.

18. Influence of Temperature on Muscular Contraction.

The prolongation of the single contraction produced by cold may be observed by placing a few bits of ice in contact with the skin by which the muscle is covered. To study the influence of heat, remove the ice, and fix over the limb a copper wire grating, the prolonged end of which can be heated by a small flame. You find that the contraction is shortened. If the temperature of the muscle rises above 40° C., it may become rigid.

19. Influence of Fatigue.

Experiment 1. Arrange the recording apparatus and myograph as in 16. Introduce into the primary circuit in addition to the time-marker and key, a suitable automatic interrupter, by means of which a break induction current may be led through the preparation at regular intervals. The frequency of the excitations must nearly correspond to that of the rotation of the

cylinder (one rev. in 2″). Record every fifteenth contraction, for which purpose the arm at the base of the stand is to be used.

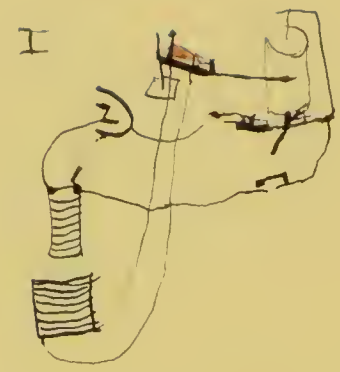
Experiment 2. Arrange the apparatus as in Experiment 1, but dispense with the time-marker. Use a recording surface of which the rate of motion is not more than a millimeter per second. Record each contraction. It is seen that during the observations the rate of diminution of effect is uniform, so that the line connecting the apices of the contraction curves is straight.

20. Extensibility of Muscle.

For experiments on this subject a counterpoised metal writing lever at least two feet long is used. By means of a pulley a weight can be drawn along the lever. This is best effected by attaching to the weight pulley an endless line, which passes round two other pulleys, situated at opposite sides of the axis, and a couple of yards from it. The line must of course be in the plane of oscillation of the lever. One of the pulleys is driven so as to move the weight at a rate of about one centimeter per second.

The pen of the lever is arranged to write on a recording surface, of which the rate of movement should not be more that one millimeter per second.

The gastrocnemius is prepared and fixed firmly above the end of the lever by grasping the femur in a muscle clamp; its tendon is then tied by a strong hemp ligature to the lever as near the writing point as possible. The travelling weight of 500 grm. must be arranged at such a distance from the fulcrum of the lever as just not to bear upon the muscle. Allow the weight to travel along the lever by setting the driving pulley in motion, and inscribe the movement of the lever. Compare muscle with india-rubber.


I


II

Arrange a preparation so that it can be faradised (See 16), and compared the line inscribed with that of fresh muscle.

21. Effect of two Successive Excitations.

For the purpose of observing this it is very advantageous to substitute for the revolving cylinder the "pendulum myograph" or du Bois Reymond's spring myograph. Each of these instruments is provided with two trigger keys, which can be so adjusted that they are opened by the action of the instrument. According to the distance at which they are placed, two circuits in which they are severally introduced, are broken at any desired time after each other. Connect two Daniells severally with two induction coils. Arrange the two trigger keys of the recording instrument, each in one of the two primary circuits. Connect the two secondary coils by one terminal of each; connect the remaining two terminals with a du Bois key, which bridges the electrodes to the muscle, which must be fixed on the myograph block in the same manner as in 17. Make a preliminary experiment so as to ascertain that the contractions produced by the two break excitations are nearly equal. Then proceed to observe the effects of two successful excitations at gradually increased intervals.

This can be done without any special instrument by the following method, in which the curves are inscribed on the revolving cylinder. The axis of the cylinder carries two arms, of which the angular distance can be varied at will. At the end of each arm is a platinum wire, which by striking a stretched copper wire produces at each revolution a closure of the primary circuit of extremely short duration. Introduce the revolving cylinder into the primary circuit by means of the binding screws provided for the purpose. The general arrangement is that described in 17; only one battery and induction coil is required.

22. Composition of Tetanus.

Use a recording surface of which the horizontal movement is five centimeters per second. The arrangement of the circuit and of the preparation must be as in 17 (the muscle being after loaded with 30 grms.), with the exception of the time-marker, which may, however, be advantageously used for determining the rate of horizontal motion of the recording surface. Work the key in the primary circuit so as to produce a succession of closures of extremely short duration. Strike as regularly as possible with gradually increasing frequency. If the muscle is fresh, fusion will be incomplete. Faradise it once or several times and repeat the observation.

Better results can be obtained by using an automatic interrupter, the frequency of action of which can be varied at will, which must of course be brought into the primary circuit. Make several experiments, first with three or four interruptions per second, then with seven or eight, and so on until the spasms which were in the first experiment distinct, become completely fused.

23. Continuous Contraction.

For the purpose of recording the various forms of this curve (complete tetanus), an automatic interrupter must be used, of which the frequency can be increased to at least fifty per second, and which is so constructed as to produce induction currents of sensibly equal strength. The rate of horizontal motion of the recording surface must be about one centimeter per second. Time must be recorded by the electro-magnetic marker. Arrange preparation and apparatus as in 22. Inscribe a curve of ten seconds' duration and twenty excitations per second. At regular intervals of a few minutes, inscribe similar curves, the duration being the same, but the frequency varying from twenty to fifty

per second. Repeat the experiments, varying the after load from 30 to 70 grm., the frequency of excitation remaining constant.

24. Influence of Veratrin on Contraction.

Use a recording surface of which the horizontal movement is five centimeters per second. Arrange apparatus as in 17. Inject about 0·5 c.c. of 0·1 per cent. solution of veratrin into the lymph sac of a brainless frog. After a few minutes, separate the lower limbs from the rest of the preparation, and record the contraction of the gastrocnemius, as directed in 17.

25. Unpolarisable Electrodes.

The form in common use consists of (1) a smooth, amalgamated zinc rod dipping into (2) a saturated solution of zinc sulphate, with which the tissue is electrically continuous by (3) a plug of china clay made into a paste with saline solution ·75 per cent. Threads may be used to connect the plug with the tissue. Such electrodes are of high resistance. (For the method of testing them with reference to their freedom from polarity See 10.) To make amalgamating liquid for electrodes, dissolve with gentle heat 3 c.c. of mercury in a mixture of 50 c.c. nitric acid, and 150 c.c. of hydrochloric acid. Dilute this liquid for use with its own volume of hydrochloric acid, and eight times as much water. In amalgamating the zinc rods care must be taken to use as little liquid as possible.

26. The Moist Chamber.

This is a box made of well varnished mahogany with glass ends and lid. It contains a block of cork soaked in paraffin, on which the nerve-muscle preparation can be extended in such a position that the tendon can be attached to and act upon a myographic bell-crank lever outside. It is also fitted with moveable holders for metallic and for non-polarisable electrodes.

There is room on each side for the wet sponges which are necessary in order to keep the air in the chamber moist.

27. Changes of Excitability of Nerve after Arrest of the Circulation.

For this purpose a moist chamber, two pairs of non-polarisable electrodes and induction apparatus are required. Arrange the apparatus as in 3, interposing between the du Bois key bridging the secondary coil and the two pairs of non-polarisable exciting electrodes, a Pohl's reverser, without cross wires. The muscle and nerve must be prepared as follows:—Strip off the skin, cut across the trunk half way down the back. Remove the viscera and the wall of the visceral cavity. Lay the preparation on the cork plate, ventral surface downwards. Expose the sciatic nerve in the hollow of the knee. Thrust one blade of the scissors between nerve and femur with its back to the nerve and divide the femur about a third of an inch from the knee. Free the nerve from surrounding parts following it to its origin, carefully severing its muscular branches. Cut away everything excepting the remainder of the vertebral column to which the nerve is still attached. Drill a hole through the femur, through which a pin is to be thrust, so as to fix it on the cork of the moist chamber. Then pin the bit of the vertebral column on the cork in such a way that the nerve does not touch it.

Apply one pair of electrodes to the nerve as high as possible, the other close to the knee. Test the excitability of the nerve in both situations, as directed in 8. Leave the preparation in the chamber, without changing the position of the electrodes, and repeat the testing from time to time. In another preparation examine in a similar way the effects of injury or section.

28. Experiments relating to Electrotonus.

The requirements are as follows:—Moist chamber with a

pair of non-polarisable and a pair of platinum wire electrodes, a recording surface with horizontal movement of one centimeter per second, three Daniells, a Pohl's reverser, a rheochord, three keys, and an induction coil provided with an interrupter of 100 per second.

Use two Daniells for the "polarising" current. Connect them through a key with the middle binding screws of the Pohl's reverser, from the binding screws of one side of which two other wires proceed to the end blocks of the rheochord. Connect one end block with one of the non-polarisable electrodes, and the moveable block with the other. Introduce into the primary circuit of the induction apparatus the interrupter and a key. Connect the secondary coil with a du Bois key which bridges the platinum wire (exciting) electrodes. Having prepared a sciatic nerve as in the preceding experiment, ligature and attach the tendon, as directed in 17 and 26; bring the exciting electrodes into contact with the nerve, near its muscular attachment, and apply to it the threads of the non-polarisable electrodes. The lower thread should be close to the exciting electrodes, and the other at least a centimeter above.

Find the minimum distance of coil at which contraction occurs in the absence of the polarising current, and then bring the coil within that minimum. Having arranged the apparatus so as to obtain a myographic tracing, open the key; the muscle is feebly tetanised. After three seconds close the polarising current which must be descending. The muscle enters into strong contraction. Reverse the direction of the polarising current; contraction ceases. Proceed in the same way, alternating the direction of the current at intervals of three seconds. On shutting off the exciting then breaking the polarising current, the muscle may be tetanised (Ritter's tetanus).

In another preparation employ excitation by single shocks to test the after effects of polarisation. The excitability is

	Ascending.		Descending	
Weak	C.		C.	
Moderate	C	C	C	C
Strong		C	C	
	close	open	Close	open

increased after the passage of the continuous current in either direction.

29. The Law of Contraction (Pflüger's Law).

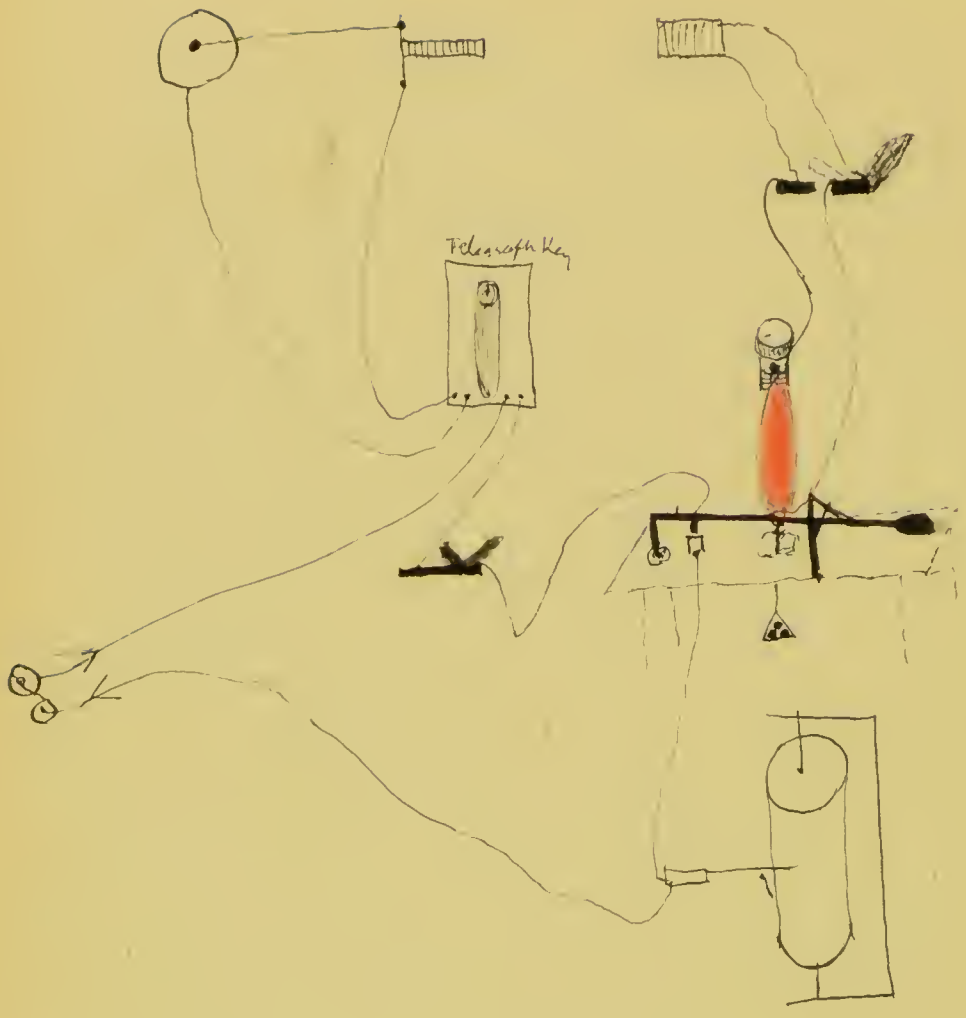
For this purpose the arrangements required are the same as for experiments on electrotonus, with the exception that the induction apparatus, and the battery and electrodes connected with it, are not wanted. The ordinary du Bois key with rubbing contact must not be used. The mercurial key or a key in which contact is made as in the Morse key, must be substituted for it. Having completed the connections, close and open this key at intervals of two seconds, so as to make and break the circuit, gradually increasing the distance of the moveable block of the rheochord from the end block until the muscle begins to contract at make, when the direction of the current is from the muscle. At once shift the slider to the second length. The muscle will probably respond to both make and break. Now substitute for the two Daniells half a dozen Groves or Leclanchés, and dispense with the rheochord. Under these conditions contraction occurs at break only.

In another preparation repeat the observation with the current directed towards the muscle. In the first two cases the result is similar, but with the strong current the contraction usually occurs only at make. Note that the closing contraction is observed with a weaker current when its direction is from the muscle.

30. Ritter's Tetanus.

Conduct the current of two or more Daniells, without rheochord, through a nerve for a short time, the direction of which should be towards the spinal cord. On breaking the current, the muscle enters into tetanus, which can be instantly arrested by again closing the current. It is not abolished by

Helmholtz's Latent period measurement apparatus.



By pressing on the Telegraph Key the muscle is stimulated. At the same time the Time marker circuit is made. Consequence, a mark on the Cylinder. But after a short time the muscle contracts and lifts the lever. In so doing the Time marker circuit is broken. Result there is another mark on the Cylinder. The distance between the two marks is the latent period. It gets longer as the muscle is weighted. Vide tracings.

Measurement of tracings shows the latent period

cutting the nerve midway between the electrodes. Severance of the nerve near the muscle necessarily arrests it. It is sometimes possible to observe the same effect on opening a current directed towards the muscle. If so, it can be abolished by severing the nerves between the electrodes. It is therefore dependent on conditions which have their seat at the anode.

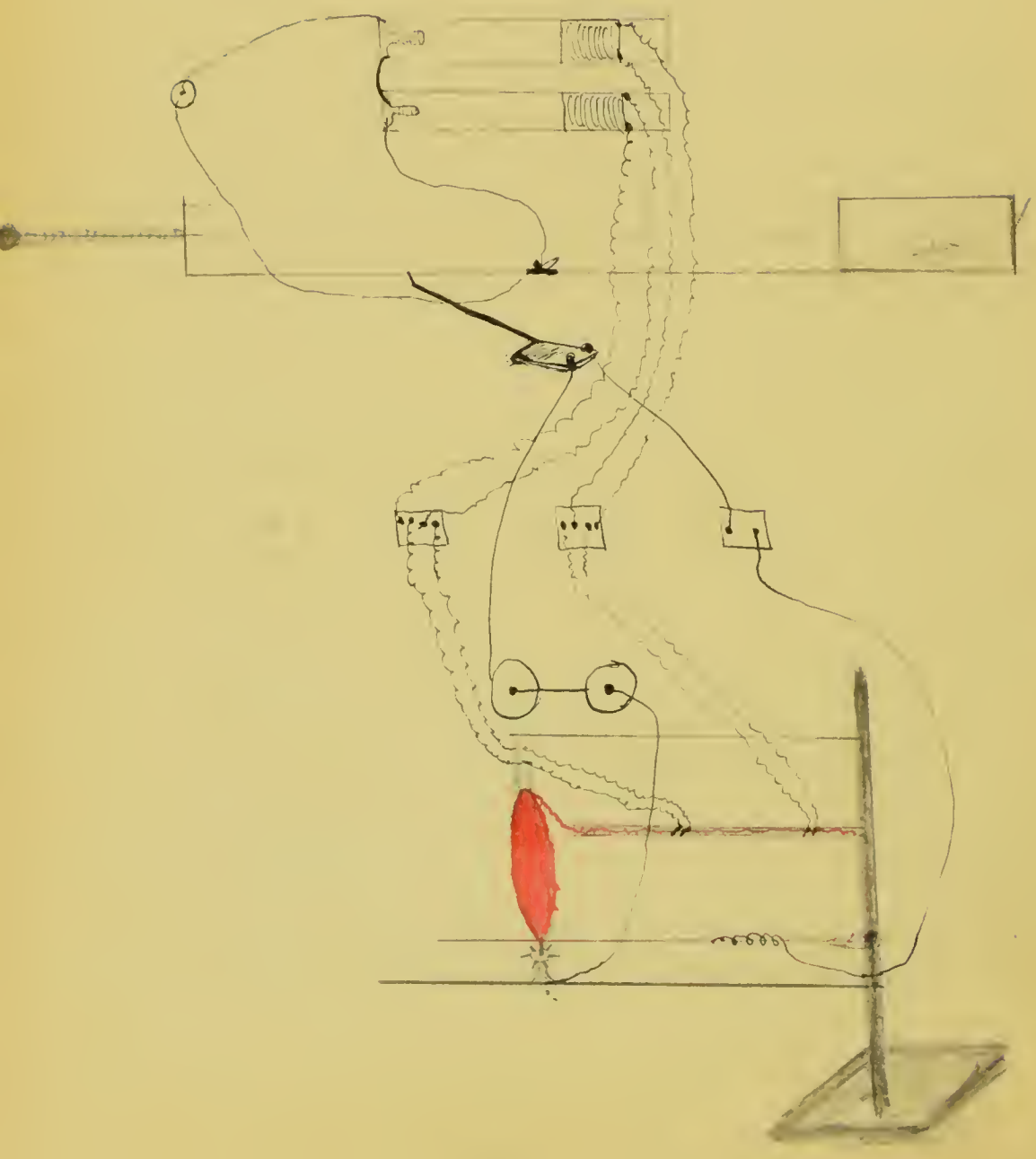
By leading through the nerve a current of which the direction is reversed every three seconds, the tetanic after effect of opening becomes more marked.

31. Measurement of the Period of Latent Stimulation by the Pendulum Myograph. (Helmholtz.)

Preparation of the apparatus. Cover the glass plate smoothly with paper, smoke its surface as before, and fix it to the pendulum. Arrange the "detent" and the "catch" so that the pendulum, when detached from the former, just catches on the latter. Test the instrument by taking tracings with a tuning-fork vibrating 100 times a second, on the smoked paper, when the pendulum is moving at several different velocities (the velocity varying with the positions of the detent and catch). Arrange the induction apparatus for single shocks, including in the primary circuit one of the trigger-keys of the pendulum-apparatus. Prepare the gastrocnemius as in 17. Great care must be taken in fixing the femur immoveably to the cork plate, in attaching the ligature (for which thin wire may be advantageously substituted) to the tendon and lever. See also that no part of the apparatus touches the surface of the glass plate, as the pendulum swings, excepting the writing point, and that the pressure of the point on the plate is slightly greater towards the end than at the beginning of the swing. Bring back the pendulum to its place and see that everything is in order—the trigger-key closed, the key which bridges the secondary circuit open, the lever in its position. On liberating the pendulum, a

Rate of propagation in nerve.

Tiegerstedt.



muscle curve is inscribed on the smoked surface. Withdraw the lever from its writing position, by means of the arm on the lever stand, bring the pendulum back past the key, close the latter, keeping it closed by firm pressure of the finger, allow the pendulum to rest against it, bring the lever into the writing position, and make a mark on the surface, which indicates the moment of excitation. Draw a base line by allowing the pendulum to swing, without exciting the muscle. Take three or four similar curves, raising or depressing the table an equal distance after each observation by the winch. Remove the muscle lever, and take a tracing with a tuning-fork vibrating at least 100 times a second, carefully arranging the style of the fork in the position previously occupied by the writing end of the muscle lever. Remove the paper, varnish and measure the tracings. From the mean result of the measurements, the latent stimulation may be computed. If du Bois' spring apparatus is used, the course of the experiment is the same, but the manipulation somewhat different.

32. Rate of Propagation in Nerve.

For this purpose the same recording apparatus is used as in 31. The arrangement of the exciting apparatus is the same as in 27, two pairs of platinum wire electrodes, of which the supports are fitted to a moist chamber (the dimensions of which are especially adapted to the purpose), taking the place of the non-polarisable electrodes. The muscle nerve preparation is arranged as in the experiments on electrotonus. The nerve must be prepared as long as possible; the distance between the two pairs of electrodes should be about an inch. The part of the nerve between them should lie on a lacquered copper-plate, of which the temperature must be about 5° C. The writing lever must be weighted as little as possible. It is advantageous to check

its upward motion. Make two observations one after the other, exciting first at the spinal, then at the muscular end of the nerve, and inscribe the two records on the same base line. In doing so take care to mark the first before the second is taken. Repeat the observations in reversed order, and so on.

Inscribe the tracing of a tuning-fork below each base line. The interval between the two records corresponds to a time-interval not exceeding $\frac{1}{1000}''$. The pendulum myograph may also be used to measure the rate of propagation in human motor nerve, the observer experimenting on himself. Arrange a Marey's tympanum, so that its lever may write on the glass plate. Connect the tympanum by an elastic tube in which there must be a small aperture for the escape of air, with a pair of toy bellows held between the thumb and finger of the left hand. Arrange the primary circuit as before. Connect one electrode of large area from the secondary coil with any part of the body. Apply a small metal disc covered with wash leather steeped in strong solution of salt to the skin, first at the bend of the elbow, and for a second observation above the clavicle, arranging the lever of the tympanum so that the two curves shall be close together. Then make a series of similar observations in pairs, taking care to allow the pendulum to draw a base line to each curve. Measure on the base line the distance between the curves of each pair, rejecting all records in which the two are not of the same amplitude. The results obtained by this rough method are surprisingly constant. They show that the rate of propagation in man much exceeds that observed in the frog.

33. The Seat of Exhaustion in Nerve and Muscle.

For experiments to demonstrate this, the moist chamber, with exciting and non-polarisable electrodes, two Daniells and induction apparatus with interrupter are required. Arrange

the induction apparatus for faradisation in connection with the exciting electrodes, and connect one Daniell with the non-polarisable electrodes through a key. Prepare both sciatic nerves; cut off the limbs above the knee, leaving the leg and foot intact.

Fix the preparations A and B in the moist chamber, so that the nerves may be together on the same pair of exciting electrodes. Now apply the non-polarisable electrodes to the nerve of B between the point of excitation and the muscle. Lead the current of the Daniell through the nerve. It is preferable that it should be descending. Faradise: the muscle A will become tetanised; B will remain quiescent, the stimulus being blocked by the electrotonic zone. Continue faradisation until the tetanus of A has subsided, then break the polarising current; B will become tetanised. Both nerves have been equally stimulated, and are therefore equally fatigued; the tetanus of B shows that its nerve is still excitable, and that the seat of fatigue is not in the nerve trunk.

Make a nerve muscle preparation, but arrange it in the moist chamber, so that one pair of exciting electrodes is in connection with the nerve, and another with the muscle (as in 17). Connect their wires with the two sides of a switch interposed in the secondary circuit. Faradise the nerve until the tetanus has subsided, then excite the muscle. The apparently exhausted muscle enters into tetanus, hence the seat of fatigue in this case is not in the muscle.

34. Acidification of Muscle.

Test the reaction of a fresh sectional surface of curarised muscle. For this purpose use two strips of glazed litmus paper of different colours. Place them edge to edge on a varnished board, and affix the cut surface over the junction. The reaction

is neutral or feebly alkaline. Test it again half an hour later, it will be found to be acid, notwithstanding that the muscle has been kept in a moist atmosphere, or under mercury.

Repeat the experiment, keeping the muscle at 45° C., observe that it becomes acid much sooner.

Repeat after subjecting the muscle to prolonged tetanus. This is accomplished by faradising the spinal cord in a decapitated frog, after previous division of the sciatic on one side, and comparing the reaction of the cut surface of the muscle which has been tetanised with that of the other.

35. Rhythmical Motions of the Heart of the Frog.

In a curarised preparation of which the hemispheres have been destroyed, expose the sternum, and cut across the episternal cartilage. Then sever the sternum from its connections by a cut on either side, and turn it down over the belly. The heart is seen still covered by the pericardium, which may now be divided. Note the condition of each of its cavities, and the mode of its rhythmical action.

Destroy the spinal cord by pithing, and observe the changes thereby produced in the state of the circulation, and particularly in the mode of action of the heart.

36. Experiments relating to Cardiac Inhibition.

The Inhibitory Centre.—For the purpose of observing the effect of passing a series of induction shocks through the inhibitory centre of the heart, a fine silk ligature is attached to the frænum (the thread-like ligament which stretches from the dorsal aspect of the ventricle towards the lower part of the pericardium). The heart having been prepared as above directed, carefully divide the frænum between the ligature and its fixed point of attachment, then by means of the ligature raise the heart out of

its place and turn it upwards. The inhibitory centre is recognised by the whitish crescent-shaped line, which marks the junction of the wall of the sinus with that of the right auricle. Faradise this spot for a second or less, placing the electrodes on the line, the points of which should not be more than a couple of millimeters from each other. Observe the mode and order in which the cavities of the heart resume their rhythmical action.

Stannius' Experiment.—Using the same or a similar preparation, pass a thick ligature under the bifurcation of the aorta between it and the venæ cavæ superiores. Then seizing the frænum ligatures with the forceps, turn the heart up. Carefully observe the position of the crescentic line, and loop the ends of the ligature so that when it is tightened it may embrace it. On tightening, the heart will stop in diastole.

In the heart so prepared, sever the ligatured parts from the rest of the preparation with sharp scissors. The auricles and ventricles resume their normal rhythmical action.

Cardiac Vagus of the Frog.—Use for the following dissection one of the preparations which has been used for the preceding experiments. Expose the trunk of the vagus nerve as it escapes from the cranium as follows:—Remove the integument so as to bring into view the muscles of the back of the neck on one side, avoiding injury to the cutaneous vessels. Then expose the scapula, and sever with the scissors the cartilaginous from the bony scapula; remove the former, dividing the muscles attached to it, then expose the sterno-mastoid muscle which connects the outer part of the petrous bone and the posterior border of the cartilaginous ring of the membrana tympani with the concave anterior border of the scapula. Introduce a small test-tube into the gullet, and remove the sterno-mastoid so as to expose the slender muscles (petrohyoidei) which run from the petrous bone to the posterior horn of the hyoid bone, embracing

Reflex inhibition

Tap abdomen of recently killed frog. Heart stops for a bit. This is reflex inhibition.

accelerator stellate

Stim ~~sub-maxillary~~ ganglion.

the cavity of the pharynx. Parallel with these muscles, and in close relation with them, are seen the carotid artery and several nerves, of which the two nearest the cranium are the glosso-pharyngeal, and the vagus. It is advantageous to remove the projecting part of the lower jaw.

Having completed this dissection, expose the vagus in a freshly pithed preparation. Prepare the heart as in 35, and fix the preparation in such a position on a cork that the electrodes can be conveniently applied to the nerve, at the same time that the motion of the heart can be observed. Observe the effect of faradising the nerve, beginning with moderately strong currents, and gradually increasing their strength until the heart stops.

Action of Muscarin and Atropin.—In an entire heart, whether removed by severing the vessels or *in situ*, stop rhythmical action by applying to the organ a drop of serum containing a trace of muscarin. Observe the relaxed and motionless condition of the ventricle which, if the circulation is going on, becomes much distended. After a few minutes, apply (in serum) a drop of 0·2 per cent. solution of atropin. Observe the gradual restoration of rhythmical action in the atropinised heart. Observe that faradisation of the inhibitory centre is now without effect.

37. Experiments relating to the Motor Centres of the Heart.

In a freshly pithed preparation, cut off the auricles and the bulb, leaving the ventricle and auriculo-ventricular septum. The heart continues to beat normally, or, if the beats cease, they are renewed by a pinch, by an induction shock, or by bringing a hot wire into the neighbourhood of the cut surface. In this condition it is called a "ventricle preparation." Cut off the whole of the auriculo-ventricular furrow with sharp scissors. The preparation so obtained (the ventricle apex) does not con-

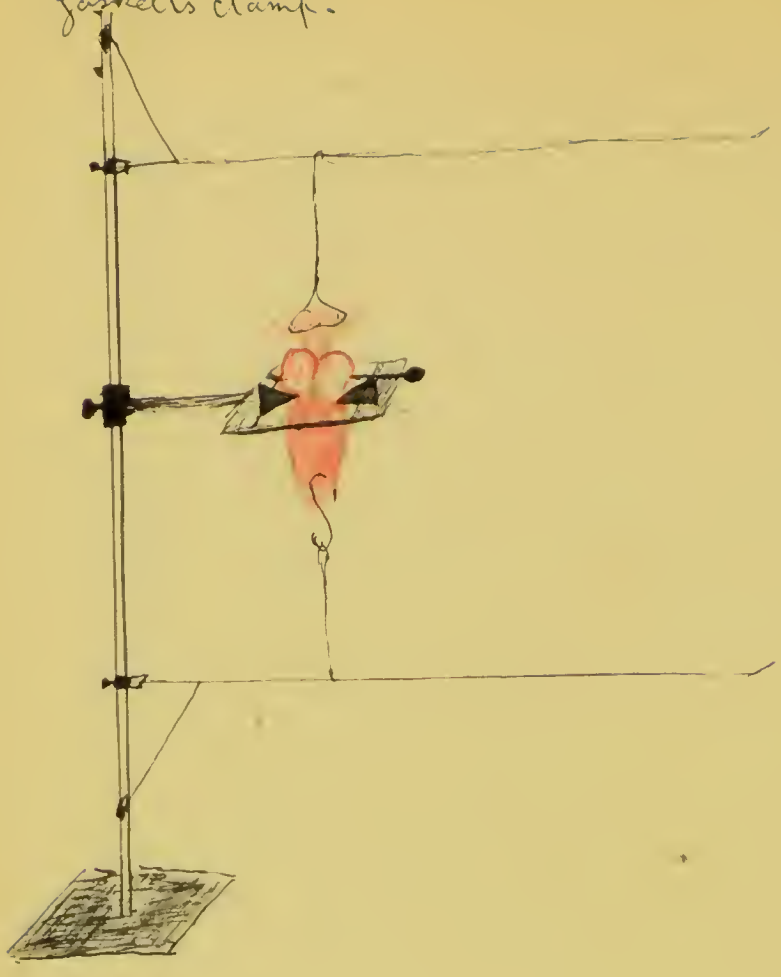
tract spontaneously, but *responds* to a single excitation, whether mechanical or electrical, *by a single contraction*, the duration of which is dependent on the temperature.

38. Study of the Ventricular Contraction by the Graphic Method.

For this purpose a cooling-box, fitted with electrodes and writing lever, a recording surface and an induction apparatus, arranged as in 3, are required. The cooling-box is a cylindrical brass vessel, through which water at any desired temperature can be passed. The heart is supported on its upper surface, which for this purpose is well lacquered. The lever consists of a straw about five inches long, bevelled at one end and bent to form a writing point. The straw carries three pieces of cork, *a*, *b*, *c*; *a* serves as a counterpoise; through *b* a needle passes at right angles to the straw, while by *c* the straw rests on the heart. The needle is the axis of the lever, and works on bearings attached to the box, the height and position of which can be adjusted.

Make a ventricle apex preparation as in 37, and place it in position, resting on the cut surface; an imperfect ring of glass of proper size serves as a support for it. Remove a few drops of blood from the pericardium, and mix it with twice its bulk of saline solution. Allow it to coagulate in a watch-glass, and after removing the clot use the liquid for moistening the preparation. Ascertain that the electrodes are in effectual contact with the preparation, and that the lever works in a vertical plane. The rate of movement of the recording surface should be five centimeters per second. The heart-box is supported by a stand of the kind described in 16, which also carries an electro-magnetic time-marker. As in 17, the writing points of the lever and of the time-marker should be in the same vertical line, and the method as regards the measurement of time is the

Gaskell's clamp.



By clamping the auriculo Ventricular groove it is easy to prove the independent rhythm of the auricle & Ventricle.

NB. Do it gradually & gently. At first the auricular beat is faster, then the Ventricular & then the Ventricular beat stops, the auricles beginning again. On unclamping gradually the Ventricle slowly recovers.

same. The period of latent stimulation is much longer than in voluntary muscle. Its duration is above 0·15″.

With apparatus arranged as above draw a succession of ventricular curves at temperatures varying from 12° to 18° C.; it is found that the duration of the systole is decreased by about 0·1″ for every degree of temperature. In this and the three following experiments, a Stannius' heart may be substituted for the ventricle apex preparation.

39. Summation of Effect in Successive Ventricle Contractions ("Staircase").

The arrangements are the same as for the previous experiment, but in addition the recording cylinder is introduced into the primary circuit, as in 21. One arm only is used, so that the circuit is momentarily closed once in each revolution of the cylinder. When the ventricle is thus excited by single induction shocks following each other at about 10″ intervals, each curve is observed to exceed its predecessor in amplitude, the augments gradually diminishing from the beginning to the end of the series.

40. Period of Diminished Excitability.

The arrangement is as before, but both arms are used as in 21. Beginning with the two contacts synchronous and induction currents just strong enough to be responded to with certainty, gradually increase the time-interval between them. Repeat the experiment using much stronger induction currents.

41. Effect produced by Frequent Excitations (Faradisation).

The ventricle preparation is to be excited by frequent induction shocks at regular intervals (thirty per second), and the

Result of heat & Cold on heart beat.

In 10 seconds periods.

Normal 8 beats.

Cold stim. 8. 7. 7. 6. 6. 6. The curves are larger.

Hot stim. 6. 7. 7. 8. 8½. 9. 9. 9. The curves are smaller

∴ Cold slows frequency & increases force;
and Heat quickens frequency & diminishes force. 3/3/94.

contractions recorded as before, but the rate of motion should not exceed one centimeter per second. Compare the effect observed when strong and weak induction currents respectively are used. In the former case the contractions are "fused," but not in the latter.

42. Influence of Temperature on the Rhythmically Contracting Heart.

Expose the heart as in 35. Raise it out of the pericardium by a ligature attached to the severed frænum, and cut through the vessels. Place it on the heart-box, keeping it moist as directed in 37. The rate of motion of the recording surface should be one centimeter per second. Allow water at 12° C. to pass through the cylindrical box and record the rhythmical contractions of the ventricle. Repeat the experiment, substituting water at 17° and at 22°, and compare the tracings.

43. Experiments relating to Coagulation of the Blood in the Frog.

Blood.—Collect frog's blood by exposing the heart of a brainless frog and cutting across the ventricle. Allow the blood to flow into a test-tube surrounded by ice, and containing not more than 5 c.c. of 0·75 per cent. salt solution. Observe the subsidence of the corpuscles. After a time pipette off the liquid, and observe its coagulation, which may be hastened by stirring with a needle.

Expose the heart of a pithed frog as before. Attach a ligature to the frænum. Pass two ligatures between the bulb and venæ cavæ, and tighten one round the bulb. Divide the frænum beyond the ligature. Raise the ventricle by means of the frænum ligature, and then, taking care that the heart is full of blood, tighten the second ligature round the veins. In doing

this care must be taken not to interfere with the inhibitory region. Cut away the organ by dividing the vessels beyond the ligatures, and hang it up full of blood in a small moist chamber (a wide-mouthed corked bottle). At the end of some hours the heart should be beating, and the blood uncoagulated.

44. Coagulation of Mammalian Blood.

Buffy Coat.—Collect a quart of horse's blood in a wide-mouthed stoppered bottle. Allow it to stand for two days in a cool place. Draw off the serum, and observe the character of the clot (buffy coat). Study the properties of fibrin by placing a small strip of the upper layer (buffy coat) in a watch-glass, and pouring over it some solution of peroxide of hydrogen. Bubbles of oxygen are given off. If some tincture of guaiacum be added, a blue colour is developed. Gluten, potato peelings, and many other substances develop a blue colour under the same conditions.

Lister's Experiment.—In a horse just slaughtered the external jugular is exposed, and ligatured near its termination. A second ligature is passed round its origin, and tightened as soon as the vein is distended. The tied part is then cut out. It can now be removed from the slaughter-house and hung up in a cool place. Two days afterwards pipette off the liquid plasma from which the corpuscles have by that time completely subsided. Examine the plasma microscopically with regard to the formation of fibrin. For the success of this and the subsequent experiments it is essential that they should be done in the winter.

Influence of Neutral Salts upon Coagulation.—Prepare sodium sulphate plasma by receiving in the cold three parts of blood into one part of saturated sodium sulphate solution. Great care should be taken to keep the blood cold and mix the liquids thoroughly immediately.

The mixture must stand in ice. After subsidence pipette off the clear liquid and mix it with three times as much water. Observe the coagulation.

45. Properties of Pericardial Fluid.

Remove the contents of the pericardium of the horse with a clean glass syringe, taking the greatest care to avoid admixture with blood. The liquid should be clear, and of a pale amber colour. If freshly collected it either fails to coagulate, or coagulates very tardily. Whether it coagulates or not it yields a precipitate of globulin when shaken with powdered salt. If hydrocele fluid can be obtained it may be used instead of pericardial.

Mix equal parts of serum and pericardial fluid and allow the mixture to stand in a warm chamber at 35° C. Add a fragment of fresh buffy coat to pericardial fluid in a test-tube, and place it in the warm chamber.

In both experiments coagulation occurs, but in the second it is often delayed.

46. Spectroscopic Examination of the Blood.

Observe the solar spectrum, noting and making a diagram of the positions of the dark lines D, E, *b* and F, in relation to the colours. Compare it with the spectrum of a gas flame, which shows no dark lines.

Observe the spectrum of a flame coloured with sodic chloride, noting the position of the bright yellow line.

Oxy-hæmoglobin.—Introduce defibrinated blood into a test-tube, and observe its opacity when undiluted.

(a.) Dilute by adding five to ten times its bulk of water. Place the test-tube in front of the slit of the spectroscope and direct it to a gas flame. The only light which passes through is that of the red end of the spectrum.

(b.) Add water until the green appears. Note the dark space (**absorption band**) between the red and green.

(c.) Dilute still further until the yellow-green light is distinguishable in the middle of the dark space, dividing the single broad band into two.

(d.) After a further addition of water, note that the band nearest the D line is somewhat more sharply defined than the other. The spectrum is still shortened by the absorption of its violet end.

(e.) On diluting until the solution is almost colourless, two faint bands are still visible.

Map on the diagram the appearances observed.

Reduced Hæmoglobin.—To some blood diluted as in (d), add a drop of solution of ammonic sulphide, and warm gently. The colour becomes purplish. Place the tube in front of the slit as before, and observe the change which has occurred. A single absorption-band, with ill-defined edges, takes the place of the two bands previously observed. Map its position on the diagram. On shaking the blood vigorously the two bands of oxy-hæmoglobin reappear for a short time.

CO-hæmoglobin.—Saturate blood diluted as in (d) by passing a stream of carbonic oxide through it. The blood acquires a cherry-red colour. The two absorption-bands have nearly the same position as those of oxy-hæmoglobin, but no change is produced when the liquid is treated with reducing agents.

Alkaline Hæmatin.—Add to solution of blood, rather stronger than the last, a drop of solution of caustic potash. Warm gently; the colour completely changes. An absorption-band appears to the left of the line D, and much of the blue end of the spectrum is cut off.

Reduced Alkaline Hæmatin.—To the solution obtained above add a drop or two of ammonic sulphide, and warm gently. Observe the change of colour. Dilute if necessary. A strongly

marked band is seen to the right side of the line D, and a second less defined, which nearly coincides with the line E. The band of alkaline hæmatin reappears for a short time after vigorous shaking.

47. Functions of the Reflex Centres of the Spinal Cord of the Frog.

The preparation to be used in the following experiments is obtained by severing the spinal cord immediately behind the medulla oblongata, and introducing by the opening made for this purpose a wooden plug into the cranial cavity, so as to destroy its contents. This having been done, it is placed on a sheet of moist filter-paper, resting on its ventral surface with the hind limbs extended, and covered with a bell-jar. For a time it remains motionless, but eventually assumes a position which differs but little from that of a living frog.

Prepare half a dozen pieces of filter-paper, each an eighth of an inch square, and some strong acetic acid. Turn the preparation over, and after observing that the natural position is not resumed, apply one of the squares, after moistening it with acetic acid and drawing off excess by touching with dry filter-paper, to the inside of the right thigh, and observe the result. Repeat the experiment, holding the right foot. Next, attach the preparation to a suitable holder in such a way that the trunk may be steadily supported and the limbs may hang freely, and apply the squares in succession to different parts of the surface, as *e.g.*, to the skin on either side of the tendon of the gastrocnemius, or to either flank. Observe in each case that the muscular response which results from excitation of the same part of the surface of the body is always the same.

Arrange a second preparation as last described, using a holder so constructed that the limbs may be suspended at any desired

height above the table. Prepare several beakers of water acidulated respectively with 1, 2, 3, 4, and 5 per thousand of sulphuric acid, and place some of each mixture in a saucer. Beginning with the weakest of the acid liquids, bring down the preparation with the rack and pinion, until the tip of the longest toe is immersed. Repeat the experiment at intervals of three minutes with the stronger liquids, in order, carefully washing the foot after each excitation, by dipping it into a beaker of water. Measure the time which intervenes between the beginning of the excitation and the muscular response in each case, with the aid of a metronome.

Proceed as above, substituting a preparation in which, after destruction of the brain, a couple of drops of a 0·1 per cent. solution of sulphate of strychnia have been injected under the skin of the back. Observe that instead of co-ordinate muscular responses, cutaneous excitation produces, under the influence of strychnia, paroxysms of convulsion, in which the body and limbs assume a characteristic attitude.

48. Starch.

Prepare potato starch by grating potatoes into water, stir the mixture thoroughly, and allow it to stand. After partial subsidence pour off the turbid liquid and set it aside. Collect the white deposit of starch, mix it with a fresh quantity of water, and again separate it by subsidence and decantation.

Examine the product microscopically. Each granule exhibits concentric markings, which become more distinct after the partial action of solution of iodine. In the dark field of the polarisation microscope, each shows a dark cross on a bright area. Compare potato starch with that of the cereals (wheat, rice, maize).

Starch is insoluble in cold water; it dissolves imperfectly in hot water; the resulting liquid is opalescent. When a beam

of sunlight passes through it the beam is luminous. When viewed transversely through a Nicol's prism, held between the thumb and forefinger, and rotated, the luminosity appears and disappears alternately. Contrast in this respect a solution of sulphate of quinine, which is fluorescent.

It gives a blue colour with iodine, which vanishes when the liquid is heated, but returns on cooling, if the heating has not been prolonged.

49. Derivatives of Starch.

Dextrin is soluble in water. The solution gives a red-brown colour with iodine, which vanishes on heating, and returns on cooling.

Commercial **Grape-sugar** is a yellowish-brown, crumbly substance, which is readily soluble in water. Its solution is usually slightly coloured, and reduces alkaline solutions of cupric hydrate.

The **Copper test.**—To a trace of 10 per cent. solution of cupric sulphate, add about 5 c.c. of the liquid to be tested; then solution of caustic potash drop by drop until the solution is clear, and heat gradually. If dextrose is present, the blue colour vanishes, and a yellow precipitate appears of cuprous hydrate, or a red precipitate of cuprous oxide.

Conversion of starch into reducing sugar. Boil about 50 c.c. of starch solution in a flask with 4 c.c. of 25 per cent. sulphuric acid for five minutes. The liquid becomes limpid. It contains in addition to dextrose much soluble starch (**Amidulin**).

50. Volumetric Estimation of Sugar by Fehling's Method.

Dissolve 34·639 grm. of pure cupric sulphate in about 200 c.c. of distilled water, also dissolve 173 grm. of pure double tartrate

of potassium and sodium (Rochelle salt) in 500 to 600 c.c. of a 14 per cent. solution of caustic soda (1·12 sp. gr.), mix the two solutions, and dilute to a litre. 10 c.c. of the solution so prepared are equivalent to 0·05 grm. sugar.

Dilute 10 c.c. of the above solution with 40 or 50 c.c. of water, and heat to boiling in a white porcelain basin, or in a flask against a white background. Now run in from a burette the sugar solution (previously diluted so as not to contain more than one half per cent. of sugar) and continue to do so until the blue colour of the copper solution disappears, *i.e.*, until all the copper is reduced. Read the burette; the quantity used contains 0·05 grm. of sugar, whence the quantity and the amount of dilution being known, the total quantity of sugar may be easily calculated.

51. Action of Malt upon Starch (Maltose).

Dry the starch obtained as before directed in a water-oven, nd prepare a half per cent. solution by boiling it in water.

Prepare some "malt extract" by digesting 10 grm. of powdered malt with 50 c.c. of water at 30° C. for three hours, and subsequently straining.

To some half per cent. starch solution add one tenth of its volume of malt extract, and place the mixture in the warm chamber at 40—45° C. If the temperature be much higher than this the reaction is so rapid that the intermediate stages cannot be observed. From time to time test portions of the liquid by mixing a drop with a drop of iodine solution on a testing slab; the blue colour at first produced is soon replaced by a red reaction, which gradually vanishes. The liquid also contains a reducing sugar (copper test), and alcohol precipitates from the liquid "achroodextrin."

Treat 150 c.c. of the half per cent. solution of starch with one-tenth of its volume of malt extract as before, keeping it in

the warm chamber for three hours. Take 55 c.c. of the product and determine the reducing power by Fehling's volumetric method. Add to another 55 c.c. 1 c.c. of strong sulphuric acid, boil for half an hour in a flask; after cooling bring the liquid to its original volume by the addition of water, and estimate the additional reducing power by the same method.

52. Examination of Flour and Bread.

Wash about a dessert-spoonful of sound flour in a muslin bag.

A milky liquid passes through containing much starch, but no sugar.

After washing for some minutes, a sticky and tenacious material remains on the muslin, which can be collected; this, after further washing, forms an elastic mass (**gluten**) which can be drawn out into threads, and on burning, gives off the smell of burnt feathers characteristic of a proteid.

Bread.—Digest with warm water. The extract contains starch and dextrose. The residue consists principally of starch and gluten.

53. Milk.

Milk has (in London) usually an acid reaction, and a specific gravity of from 1025 to 1030.

Determine the specific gravity of a sample of new milk with the hydrometer. Allow it to stand for a day in a narrow cylindrical vessel, syphon off the milk, and determine its specific gravity. By the addition of water it may be reduced to the original specific gravity.

Curdling of Milk.

By Acid.—Warm 50 c.c. of milk in a flask to 40° C.; add a few drops of acetic acid. Swing the flask gently round, and

when the curd has lumped together, pour off the whey, which will be found to contain sugar.—(Copper test.)

By Rennet.—Warm 25 c.c. of milk in a flask to 40° C.; add twenty drops of commercial extract of rennet. Set it aside for ten minutes; a coagulum is formed from which the whey gradually separates.

Repeat, first diluting the milk with at least its own volume of water. No coagulum is formed.

Repeat, but boil the extract of rennet before use. There is no visible change.

The coagulated casein contains much fat (**butter**) which can be extracted by ether. The ether extract, when evaporated on paper, leaves a greasy stain.

Comparison of Acid and Rennet Whey.

Coagulate 25 c.c. of milk with extract of rennet and precipitate the same quantity with acetic acid in the cold. Examine each whey for proteid (by adding Millon's reagent and boiling), and for earthy phosphate (ammonia).

Separation by Salt.—Treat milk with twice its volume of a saturated solution of common salt, with the addition of powdered salt, and shake thoroughly. (This is best effected by a machine; it can, however, be done on a small scale by shaking milk in a test-tube with powdered salt.) The casein and fat rise to the surface of the clear whey, which may be syphoned off. Test the liquid for sugar.

Separation by Filtration.—When milk is filtered under pressure through a porous disk, its casein, being particulate, remains behind. The clear filtrate contains **lactose** (milk-sugar).

54. Experiments on the Coagulation of Milk (Hammarsten).

Prepare a solution of rennet by extracting, with 200 c.c. of glycerine, the mucous membrane of the fourth stomach of the sucking calf.

Precipitate 100 c.c. of milk by adding to it its own volume of 5 per cent. sulphuric acid in the cold. Mix thoroughly, and filter. Observe that a perfectly clear filtrate is thus obtained: the filtration will take some hours. Remove the material from the filter, and if necessary, dry in a water oven, wash repeatedly part of the precipitate with distilled water in a capsule, separating by subsidence and decantation after each washing.

Dissolve a portion of this precipitate in very dilute caustic soda (1 per cent.). Add a drop of glycerine rennet extract, and warm to 40° C. The milky fluid does not coagulate.

Mix thoroughly with about its bulk of calcium carbonate another portion of the washed precipitate. Add some litmus, and cautiously add lime water until the milky liquid is neutral or slightly alkaline. Add a drop of glycerine extract of rennet, warm gently; the characteristic rennet coagulum almost immediately appears, floating in a clear fluid.

After prolonged and careful washing in the manner described above, add to a portion of the precipitate calcium carbonate as before, and mix thoroughly; then litmus and lime-water. Warm, add rennet; if the precipitate has been washed free from phosphates, there will be no change, but on adding a drop of 0·5 per cent. phosphoric acid, the rennet coagulum will appear.

These experiments show that the precipitation of casein by acids in the cold (souring of milk) is a process fundamentally distinct from that of its coagulation by rennet (curdling); moreover that the presence of calcium phosphate is necessary for the efficient curdling action of the rennet ferment.

55. Action of Acids and Alkalis on Albumen.

Prepare the whites of two eggs, disintegrate them by clipping with scissors; dilute the albumen to twenty times its volume with distilled water. Introduce the mixture into a corked flask, which must not be more than half full, and shake briskly. Strain through muslin and filter, and if necessary carefully neutralise. The liquid so prepared is faintly opalescent; it contains a proteid body, *albumin*, which diffuses through animal membrane with great difficulty. Such a liquid, containing 5 per cent. of albumen, is to be used in the following experiments. It coagulates on heating at about 70° C. if neutral.

To some of the liquid add a few drops of 0·1 per cent. solution of caustic potash, and warm gently for two or three minutes. Boil. The liquid will no longer coagulate, the albumin having been transformed into the alkaline modification (**alkali-albumin**).

Cool some of the liquid. Colour it with litmus solution, and add carefully very dilute acid. A precipitate falls at the point of neutralisation, which is soluble in any excess of acid.

Treat another portion with a few drops of 0·1 per cent. sulphuric acid. Warm very gently for not less than ten minutes. On boiling no coagulation occurs, the albumin having passed into its acid modification (**acid-albumin**).

Cool some of the liquid. Colour with litmus, and add carefully very dilute solution of potash. A precipitate occurs on the point of neutralisation, which is soluble in any excess.

Take two equal quantities of alkali-albumin solution. To one add two or three drops of 10 per cent. solution of sodic phosphate. Colour both with litmus, and neutralise with weak acid. The portion without sodic phosphate is precipitated. The other portion is not precipitated until enough acid has been added to convert the sodic phosphate present into acid sodic phosphate.

Prepare Lieberkühn's jelly by adding strong potash solution drop by drop to a little undiluted white of egg in a capsule, stirring vigorously.

56. Characters of Proteid Solutions.

Tests for proteid bodies in solution.

To some 5 per cent. albumin solution add strong nitric acid. The precipitate obtained turns yellow on boiling. Cool the liquid, and add strong ammonia. The precipitate assumes an orange tint (**Xanthoprotein reaction**).

To another portion add **Millon's reagent**. (Mercury is dissolved in its own weight of strong nitric acid. The solution so obtained is diluted with twice its volume of water. The decanted clear liquid is Millon's reagent.) A precipitate is formed, which turns dull red on boiling.

To a third portion add solution of potassic ferrocyanide, and a drop of acetic acid. A white precipitate appears.

Introduce a fourth portion of the liquid into a test-tube containing a trace of 10 per cent. solution of cupric sulphate. On adding solution of potash, a violet colour is obtained.

Indiffusibility of Proteids.—Suspend a parchment paper tube containing diluted blood, in a beaker of distilled water, so that the two open ends are above the surface. The colouring matter and proteids do not pass through the membrane. The soluble salts pass through readily, and their presence in the water can be recognised by the usual tests.

57. Determination of Temperature of Coagulation of Proteid Solutions.

"A glass beaker containing water is placed within a second larger beaker also containing water, the two being separated by a ring or cork. Into the water contained in the inner beaker

there is immersed a test-tube, in which is fixed an accurately graduated thermometer, provided with a long narrow bulb. The solution of the proteid of which the temperature of coagulation is to be determined is placed in the test-tube, the quantity being just sufficient to cover the thermometer bulb. The whole apparatus is then gradually heated. With the arrangement described the rise in temperature takes place very slowly and equally throughout. Care being taken to have as good illumination as possible (the best plan being to place the apparatus between the operator and a well lighted window) the experimenter notes the temperature at which the liquid first shows signs of opalescence; he afterwards notes again the temperature at which a distinct separation of flocculent matter occurs."—Gamgee's *Physiol. Chem.*, iii.

Take three portions, of 5 c.c. each, of the 5 per cent. albumin solution in three test-tubes, and colour them with litmus. Dilute the 0·1 per cent. acid about five times, and add a drop of it to one of the portions; to another add a drop of 0·1 per cent. potash solution similarly diluted. Heat all three tubes gradually, and note the temperature at which each coagulates.

58. Comparison of Serum Albumin with Egg Albumin.

Dilute serum of blood with distilled water, so that the mixture shall have as nearly as possible the same specific gravity as a solution of albumen (55). Carefully neutralise with weak acid, until the mixture has become slightly cloudy.

Compare the properties of the two forms of albumin, according to the following table:—

Serum albumin is precipitated by strong hydrochloric acid. The precipitate is readily soluble in excess.

The precipitate, by boiling serum albumin, is soluble in strong nitric acid.

Serum albumin solution is not coagulated by ether.

Egg albumin is easily precipitated by strong hydrochloric acid. The precipitate is *not* readily soluble in excess.

The precipitate, by boiling egg albumin, is soluble with difficulty in strong nitric acid.

Egg albumin solution is coagulated by ether (if the solution be not alkaline).

For the ether test dilute 5 c.c. of each liquid with two or three times its bulk of 0·1 per cent. sulphuric acid. Add ether, and shake briskly.

59. Properties of Serum-Globulin.

Neutralise 5 c.c. of serum with a few drops of 0·1 per cent. sulphuric acid. Dilute with about 75 c.c. of water, and allow the precipitate to settle. The precipitate is insoluble in water, but soluble in excess of acid.

To some serum in a test-tube add solid magnesium sulphate in large excess, and shake briskly for some time. A dense precipitate is obtained (serum-globulin), which on the addition of water redissolves. This body is an example of a class of proteids which are distinguished by being insoluble in water, soluble in weak solutions of neutral salts, but insoluble in saturated ones.

Precipitate a fresh portion of serum as above; filter. Add to the filtrate powdered sodium sulphate in excess, a further precipitate is obtained. The liquid still gives the proteid reactions. (Test by Millon's reagent.)

Panum's Method of Separating Serum-Globulin.—Dilute serum with fifteen times its bulk of water, and add five drops of 20 per cent. acetic acid to every 160 c.c. of the mixture. The precipitate was formerly called "serum-casein."

Alex. Schmidt's Method.—Dilute serum with twenty volumes of water, and pass a stream of CO_2 through the mixture.

Collect the precipitate by decantation, and observe that it is insoluble in ordinary water which contains CO_2; in boiled water it dissolves with great difficulty.

An additional precipitate may be obtained from the decanted liquid by treating it with a trace of acetic acid, the "serum-caseïn" mentioned above.

Repeat the CO_2 method without dilution. No precipitate is formed.

Construct a dialysing cell by closing the ends of a length of dialysing tube with india-rubber corks, one of which is perforated for two glass tubes. Of the two tubes the longer must reach to the lower cork and be bevelled at its end; the other must be cut off short. Through this arrangement send a current of water from the tap. Fill a narrow cylindrical vessel, not much larger than the tube, with serum, and plunge the latter into it. Allow the water to flow for twelve hours, and observe the precipitation of the globulin.

60. Diastatic Properties of Saliva.

Prepare some starch solution as directed in 48, and ascertain that it contains no dextrose.

Add some saliva, and place the tube containing the mixture in a water-bath at 35° C. Observe the changes, as directed in 51.

61. Peptic Digestion.

Introduce some fibrin into a test-tube, and just cover it with 0·2 per cent. solution of HCl. Allow it to stand for forty-five minutes in a water-bath at from 35° to 38° C. At the end of this time the fibrin is swollen and transparent, but has not dissolved.

Repeat, using instead of hydrochloric acid, water to which a

drop of glycerine extract of gastric mucous membrane has been added. The fibrin remains unaltered.

Repeat, adding a drop of the same extract to the acid liquid. The fibrin dissolves gradually.

Colour with litmus the liquid thus obtained. Neutralise carefully with 0·1 per cent. solution of caustic potash. The acid albumin formed during the first stage of digestion is precipitated.

Gastric Digestion.—Digest a portion of mucous membrane scraped off the stomach, with 0·2 per cent. hydrochloric acid at 38° C. Observe its rapid disappearance.

Prepare peptone solution from commercial gastric peptone: neutralise, precipitate other proteïds by boiling with ferric acetate, and filter.

(Ferric acetate is prepared by precipitating a solution of 40 grm. of iron alum with 63 grm. of lead acetate, allowing it to stand overnight, and syphoning off the clear red liquid.)

The peptone solution yields no precipitate either by boiling or by neutralisation, but is precipitated by alcohol.

To a trace of sulphate of copper in a test-tube, add some peptone solution. On addition of potash a red colour is produced (other proteids give a violet colour).

Peptone, although more diffusible than other proteids, does not diffuse through parchment paper.

62. Pancreatic Digestion.

Glycerine extract of pancreas is prepared by triturating the gland with sand, and adding for every grm. of pancreas 1 c.c. of 1 per cent. acetic acid and 10 c.c. of glycerine.

Introduce 5 c.c. of 1 per cent. solution of sodium carbonate, to which a couple of drops of glycerine extract of pancreas have been added, into each of two test-tubes. Boil one of them and allow it to cool. Add some boiled fibrin to each, and place

them both in the water-bath at 35° C. Compare the changes produced in the tube which has not been boiled with those observed in peptic digestion.

A finely divided ox pancreas is allowed to digest at 40° C. for twenty-four to thirty-six hours in a litre of 1 per cent. solution of sodium carbonate, to which the white of one egg must be added every ten hours. Strain and filter the product.

Boil some of this liquid after acidulating slightly. Albumin is coagulated. Colour another portion with litmus, and neutralise carefully; alkali-albumin is precipitated. To another portion of the liquid add Millon's reagent; filter. On boiling the filtrate the presence of tyrosin is indicated by a red colour.

Leucin and Tyrosin.—The liquid product of pancreatic digestion from which the proteids have been separated is reduced to a small bulk by evaporation, treated with excess of alcohol to precipitate the peptone, and filtered. After further concentration, the liquid if left to itself is found to contain leucin in spheroidal crystalline clumps, and tyrosin in stellate groups of long slender needles.

Examine the liquid microscopically, using the polarisation microscope if possible.

63. Bile.

Observe colour and reaction of ox bile. It is usually brown. Neutralise and boil in a test-tube. Bile does not contain albumin.

Mucin.—Add to bile diluted with an equal volume of water, excess of alcohol. Mucin is precipitated; filter, wash the precipitate with dilute spirit, dissolve in lime water. From its alkaline solution, mucin is reprecipitated by acetic acid.

Prepare a solution of acid albumin by digesting albumin in water containing 0·2 per cent. of hydrochloric acid. On the

addition of a drop of bile, the mixture curdles *en masse*. If a large quantity of bile be added, little or no precipitate may be formed, the liquid being rendered alkaline.

Boil bile with three times its bulk of strong hydrochloric acid for ten minutes. The bile is decomposed into bile-resin (cholic acid with colouring matter) and glycin and taurin, the two last-mentioned substances remaining in solution.

Pettenkofer's Test for Cholic Acid.—Spread a drop of bile in a thin film on a white porcelain capsule. Mix with a drop of strong solution of cane-sugar. Add concentrated sulphuric acid drop by drop, and, if necessary, warm. A deep purplish-red colour appears.

Alcoholic Solution of Bilin.—To prepare this evaporate the bile to a thick syrup, and extract with strong alcohol, decolorise the alcoholic extract with animal charcoal, filter, and test the solution for bilin by Pettenkofer's reaction.

Gmelin's Test for the Colouring Matter.—Spread a drop of bile in a thin film on a white porcelain capsule. Allow a drop of strong nitric acid to fall into the middle of the film and observe the effect. The drop becomes surrounded by rings of green, blue, red, and yellow, in the order in which they have been named. Consequently, the green which is first formed is eventually farthest from the drop of acid. If, instead of allowing the liquid to remain undisturbed, the acid be mixed with the bile, the liquid passes through the same tints, in the same order.

Warm a little nitric acid in a test-tube. Incline the tube and pour bile down the side, so as to form a layer over the acid. The colours appear as in 7, at the line of contact of the two liquids.

Cholesterin.—Allow a few drops of ethereal extract of gall-stones to crystallise in a watch-glass. Examine the crystals microscopically. Allow a drop of iodine and then a drop of

sulphuric acid to come into contact with the crystals, and observe the play of blue and red colour. (Iodine and sulphuric acid test.)

Cholesterin is also soluble in chloroform, and the addition of strong sulphuric acid to its solution produces a red colour.

64. Glycogen.

Feed a rabbit on carrots, and, five or six hours afterwards, kill it by opening the carotids. Open the chest and abdomen quickly. Insert cannulæ into the vena portæ and vena cava inferior respectively, and allow a gentle stream of water to flow through the liver until it becomes uniformly pale and the wash-water is free from sugar. Now cut it out quickly, mince it, and throw the pieces at once into boiling water acidulated with acetic acid. Filter the liquid hot, and test with iodine solution. The liquid assumes a red colour identical with that yielded by dextrine in the cold.

To separate the glycogen from this solution evaporate to a small bulk, and add a large excess of alcohol. The glycogen is precipitated as a flocculent powder.

Glycogen solution may be also obtained by taking the livers of two or three oysters, mincing finely, and throwing the pieces into boiling water acidulated as before.

Add to a small quantity of glycogen solution, prepared as above, a few drops of saliva. Place the mixture in the warm chamber for five minutes, and test for sugar.

65. Preliminary Examination of Urine.

Observe reaction and colour. Determine the specific gravity either by weighing or with the urinometer, observing the effect of temperature.

Compare fresh with stale urine as regards appearance, smell, and reaction.

Sulphates.—Add baric chloride after acidifying with hydrochloric acid. A white precipitate of baric sulphate is formed.

Chlorides.—Add argentic nitrate after acidifying with nitric acid. A white curdy precipitate of argentic chloride is produced.

Phosphates.—Add ammonia; the basic phosphates are precipitated. Add ammonic molybdate to urine which has been mixed with half its volume of nitric acid. Boil. A yellow crystalline precipitate falls.

Urea Nitrate.—To urine evaporated to one third, add a drop of nitric acid in a watch-glass. Glistening scales of urea nitrate are abundantly formed in the liquid.

66. Preparation of Urea from Urine.

Evaporate the urine to a small bulk. Add strong nitric acid (pure and free from other oxides of nitrogen) in excess, taking care to keep the mixture cool during the addition of the acid. Pour off the excess of fluid from the crystals of urea nitrate which are formed, strain through muslin, and press between filter-paper. Add to the dry product barium carbonate in large excess, mix thoroughly with sufficient methylated spirit to form a paste. Dry on a water-bath and extract with alcohol; filter, evaporate the filtrate in a watch-glass on the water-bath, and set aside to crystallise. The product may be further purified by animal charcoal and re-crystallisation.

Another method is the following:—Take 20 c.c. of urine. Add "baryta mixture," (two volumes of barium hydrate solution, and one volume barium nitrate solution, both saturated in the cold), until no further precipitate is produced; filter, evaporate to a thick syrup on the water-bath, and extract with alcohol. Pour off the alcoholic extract, filter; again evaporate

to dryness on the water-bath, and take up with water. Place a drop of the watery solution on each of the two slides, add to one strong nitric acid; allow both to crystallise, and examine under the microscope.

Biuret Reaction.—Heat urea to 150—160° C. in a dry test-tube; ammonia is given off. Dissolve the product in a little warm water and pour it on to the surface of a solution of cupric sulphate in caustic potash; a characteristic violet tint is produced at the junction of the two liquids.

67. Preparation of Uric Acid Crystals.

To 100 c.c. of urine add 5 c.c. of strong hydrochloric acid. Allow the liquid to stand for forty-eight hours. Dark red crystals of uric acid separate from the liquid.

Boil serpent's excrement with a 10 per cent. solution of caustic soda or ammonia. Dilute, allow the liquid to stand, and decant the clear fluid. Pour it into a large quantity of water to which 10 per cent. by volume of hydrochloric acid has been added. Allow it to stand twenty-four hours, wash and preserve the crystals which are deposited.

Examine the crystals microscopically.

Treat uric acid or a urate in a porcelain dish with dilute nitric acid, and evaporate to dryness; after cooling allow a drop of strong potash to run over the residue. A deep purple colour is produced.

Preparation of Hippuric Acid.—Take 200 c.c. of fresh cow's urine and concentrate it on the water-bath to 110 c.c., add hydrochloric acid and set aside till the next day. Take the brown crystalline mass which forms, wash with cold water, press between folds of filtering paper, dissolve in as little boiling water as possible, add a little pure animal charcoal and filter, concentrate the filtrate and set aside to crystallise.

Hippuric Acid.—Heat some dry crystals in a test-tube, observe that a red oil forms, and that ammonia and benzoic acid are given off. The former is recognised by fuming with hydrochloric acid, the latter by its aromatic smell.

Preparation of Creatinin.—Precipitate 200 c.c. of urine with milk of lime. Filter and evaporate to a syrup. Extract with large excess of alcohol and filter. Add to the filtrate two drops of a perfectly neutral solution of zinc chloride. Set the liquid aside in a dark, cool place for two or three days. Creatinin zinc chloride crystallises in rosettes, in vertical lines on the sides of the vessel.

Urochrome.—Precipitate about 50 c.c. of urine with lead acetate and a drop of ammonia. Filter. The filtrate should be colourless. Scrape the precipitate from the filter-paper into a capsule. Mix with a few drops of strong sulphuric acid and add to the pasty mass a little alcohol. Filter. The yellow filtrate, on boiling with excess of strong sulphuric acid, turns black. Pour the acid liquid into a large quantity of water. After some hours the uromelanine separates in flocks which are characterised by their extreme solubility in ammonia. It can be precipitated from its solution in ammonia by sulphuric acid.

Indigo.—To 500 c.c. of urine add 250 c.c. of pure hydrochloric acid. Allow the liquid to stand twenty-four hours. A coppery scum floats on the surface. Filter. Treat the filter first with ammonia to extract the uromelanine, secondly with cold alcohol, which acquires thereby a red colour. On boiling the residue in alcohol, a blue solution is obtained, which exhibits the absorption spectrum of indigo-blue.

68. Quantitative Determination of Urea.

Urea (CON_2H_4) when decomposed by suitable oxidising agents, yields CO_2H_2O and N. The most convenient reagent for effecting this decomposition is an alkaline solution of sodic

hypobromite. The CO_2 is absorbed by caustic soda. The nitrogen which is disengaged is collected and measured in a suitable apparatus. Every 37·3 c.c. of nitrogen, at ordinary pressure and temperature, corresponds to 0·1 grm. of urea. As the hypobromite solution will not keep, it is freshly prepared by adding 5 c.c. of bromine to 45 c.c. of a forty per cent. solution of caustic soda.

Dupré's apparatus is used. Introduce 25 c.c. of hypobromite into the flask *c*. Measure off 5 c.c. of urine into a small bottle; and placing this carefully in the flask, replace the caoutchouc stopper. Open the screw clip *d*, and lower the measuring tube *a* until the surface of the water is at the zero point of the graduation. Close the screw clip and raise the measuring tube. If the apparatus be tight, mix the urine gradually with hypobromite solution by inclining the flask. Finally, tilt the flask so as to rinse out the small bottle with the solution, and shake well for a few seconds. Immerse the flask in a vessel containing water at the same temperature as that in the jar. At the same time lower the measuring tube. After two or three minutes, raise the measuring tube again until the surfaces of the liquids inside and out coincide. Read off on the measuring tube the point of coincidence.

In Dupré's apparatus the graduations do not represent c.c. of nitrogen but percentage of urea. In this method only 92 per cent. of the nitrogen of the urea is given off. Uric acid yields less than half, the other nitrogenous constituents of the urine, variable proportions of their nitrogen. Hippuric acid is not decomposed. In the graduation of Dupré's apparatus this error is taken into account.

A more accurate method is to employ a measuring tube graduated to fifths of cubic centimeters. After cooling, the volume of nitrogen resulting from 5 c.c. of urine is read off; the temperature and pressure being carefully noted. From

these data the percentage of urea can be calculated, allowance being made for the error described above, which is sufficiently constant.

Liebig's Method.—A solution of pure mercuric nitrate having been prepared, of such a strength that 20 c.c. of it are required for the precipitation of 10 c.c. of a 2 per cent. solution of urea,—of which, therefore, 10 c.c. correspond to 0·1 grm. of urea;—add 20 c.c. of the baryta mixture to 40 c.c. of urine, and filter. Measure 15 c.c. of the filtrate, which represents 10 c.c. of urine. Place it in a beaker, and run in the mercury solution (each c.c. of which corresponds to 0·01 grm. of urea), until on mixing a drop of the mixture with a drop of a saturated solution of sodium carbonate on a white tile, a pale lemon colour appears. Now read the burette, and calculate as follows:—If 10 c.c. of urine contained 0·2 grm. of urea, it would require 20 c.c. of the mercury solution, *i.e.*, 1 c.c. for each grm. per litre. If the daily quantity of urine were 1500 c.c. this would give a daily discharge of 30 grms. of urea.

This method approaches accuracy only when the quantity of urea present is about 2 per cent. The chlorine in the urine must also be estimated, and the quantity of urea indicated reduced by the subtraction of 1 grm. of urea for every 1·3 grms. of sodium chloride found.

The chlorides are estimated by diluting 10 c.c. of urine with five times its volume of distilled water, making it strongly acid with nitric acid; raising to the boiling point and precipitating with silver nitrate. It should be rapidly filtered, and the precipitate of silver chloride washed, dried, and weighed.

69. Quantitative Determination of Phosphoric Acid.

When a solution of uranic nitrate or acetate is added in successive quantities to a hot solution containing phosphates, previously acidified with acetic acid, the whole of the uranium is

precipitated as uranic phosphate, so long as any phosphate remains in solution. As soon as an excess of uranic salt is present, it can be detected by potassic ferrocyanide, which gives a brown colour with uranic salts.

The standard uranic nitrate solution contains 35·5 grms. in a litre. One c.c. corresponds to 0·005 grm. P_2O_5.

To 50 c.c. of urine add 5 c.c. of a solution containing 100 grms. of sodic acetate in 900 c.c. of water, to which 100 c.c. of glacial acetic acid have been added. Heat the 55 c.c. to 80° C. Add the uranic nitrate solution, until a drop of the mixture placed on a white porcelain slab gives a distinct brown colour, with a drop of potassic ferrocyanide. Note the quantity of solution used, and calculate the percentage of P_2O_5 in the urine.

70. Urinary Deposits.

Deposit in Acid Urine.—The so-called lateritious deposit which forms on cooling in clear healthy urine, of high specific gravity, and acid reaction, consists chiefly of *urate of sodium*, coloured by the urinary pigments. This deposit may be obtained from any acid urine by slightly concentrating and allowing it to stand.

Calcic oxalate is also deposited in acid urine, but only under certain conditions. It may be readily recognised by the microscope. To observe its characters add to warm urine a few drops of solution of calcic chloride and not more ammonium oxalate than is sufficient to produce a very slight precipitate. Allow the urine to stand over night. Take a portion of the precipitate and observe under a moderately high power the small octahedral crystals. After the ingestion of any considerable quantity of rhubarb, calcic oxalate is always deposited.

Deposits in Alkaline Urine.—Add to ordinary urine a few drops of urine which is already ammoniacal, and allow it to stand for a few days in a warm place.

The deposit is whitish and consists of amorphous basic phosphates of calcium and magnesium, identical with the amorphous precipitate which is thrown down when urine is neutralised—along with which are seen large prismatic crystals of phosphate of ammonium and magnesium (triple phosphate) and with a high power minute organisms. On the surface of the urine is a scum which contains the same crystalline and organised forms.

Add to urine ammonium chloride and traces of sodium phosphate and magnesium sulphate. Urine thus treated yields on addition of ammonia a precipitate which on standing becomes crystalline. Compare the crystals with those obtained from ammoniacal urine.

Acidulate urine with acetic acid, then add calcium chloride and sodium phosphate in relatively small quantities. A crystalline precipitate of neutral calcic phosphate is formed.

The crystals are arranged in characteristic stellate groups.

71. Preparation of Creatin.

Prepared from aqueous extract of muscle, or best, from Liebig's extract. Dilute the latter with fifty times its volume of water and precipitate it with lead acetate, excess being avoided. Filter, separate the lead by sulphuretted hydrogen and again filter. Evaporate to small bulk and set aside for a week to crystallise. Pour off the mother liquor, and add three or four volumes of alcohol, specific gravity 0·982, filter, wash with alcohol, dissolve all the crystals obtained in boiling water, again filter and set aside to crystallise. Creatin crystallises in transparent, colourless, rhombic prisms, for which those of common salt may be easily mistaken. They are distinguished from them by appearing illuminated in the dark field of the polarisation microscope.

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