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# QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY

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# QUARTERLY JOURNAL OF EXPERIMENTAL PHYSIOLOGY

ON THE TIME TAKEN IN TRANSMISSION OF REFLEX IMPULSES IN THE SPINAL CORD OF THE FROG. By FLORENCE BUCHANAN. (From the University Museum, Oxford.)

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## I. INTRODUCTION.

THE estimate which seems to be generally accepted<sup>1</sup> of the reduced reflex time in the lower part of the frog's spinal cord in a reflex contraction of the simplest kind is that which was formed by Wundt in 1876,<sup>2</sup> after making experiments in which the mechanical responses of a gastrocnemius

<sup>1</sup> See article on "Nerve Cell" in Schäfer's Text Book of Physiology, 1900, vol. ii., p. 609.

<sup>2</sup> Wundt, *Unters. z. Mechanik d. Nerven u. Nervencentren*, Abth. II.: "Ueber d. Reflexvorgang u. d. Wesen der centralen Innervation," Stuttgart, 1876.

were recorded, when, on the one hand, its sciatic nerve, and, on the other, one or two of the posterior roots of this same nerve, were stimulated by a single break induction shock.

The question of the duration of this reflex time, and of the larger one dependent on it, of the time taken to pass a synapse or neurone-junction,<sup>1</sup> is one of such importance, not only to the physiologist but to the psychologist,<sup>2</sup> that one ought to be very sure that the answer to it has been supplied by methods which would be likely to give it correctly.

Wundt's records were made on a pendulum myograph, the two curves being on the same abscissa, and the difference of time taken, after the moment of stimulation, for the muscle to begin to lift the lever in the two cases, was measured. The difficulty in determining latencies with exactitude by such a method is well known, but the chief objection to which Wundt's experiments seem to me to be open is that he intentionally chose, for comparison with the reflex contraction, a contraction to stimulation of motor nerve of equal amount, and therefore a submaximal one, his reason for so doing being, that he found variations of direct latency with varying strength of stimulus to be more marked than the differences between the two kinds of latency.<sup>3</sup> With the method he used of recording the mechanical response, the increase of latency observed when the stimulus to the motor nerve was submaximal instead of maximal was probably<sup>4</sup> due to a smaller number of fibres being excited; for those excited, having the resistance of the whole of the rest of the muscle to overcome, would fail to move the lever as soon as when all, or a greater number of fibres are in action. It does not necessarily follow, and it seems to me improbable (see p. 27), that the smaller contraction evoked when the sensory root is stimulated owes its smallness to the same cause, so long as no stronger contraction can be obtained by strengthening the stimulus to the root. A further objection to Wundt's method is that, as he himself had shown in an earlier work,<sup>5</sup> the latency may vary a good deal with the particular piece of one and the same nerve stimulated, and in his later experiments the stimulus was applied to spots of different excitability (*loc. cit.*, p. 45).

Wundt's experiments showed that the so-called "total latency," i.e. the time which elapses between the excitation of the posterior root and the beginning of the contraction of the whole muscle, varied in different preparations<sup>6</sup> between 0.025 and 0.050 second. From this time he deducted what he found in each case to be the time which elapsed before the whole muscle began to contract, when the motor nerve was excited

<sup>1</sup> Schäfer (*loc. cit.*).

<sup>2</sup> See, e.g., M<sup>4</sup>Dougall, *Brain*, xvi., pp. 588-9, 1901.

<sup>3</sup> Wundt, *loc. cit.*, p. 16.

<sup>4</sup> Judging from some experiments made in the Oxford Physiological Laboratory, of which an account is given in the *Journal of Physiology*, vol. xxviii., 1902, p. 412.

<sup>5</sup> Wundt, *Unters.*, etc., *Abth. I.*: Erlangen, 1871, pp. 192, 193.

<sup>6</sup> He says, "with the strength of the stimulus," but none of the experiments of which he has published the details seem to me to bear out this statement.

by a submaximal stimulus of such strength as to give a curve of the same height as the reflex contraction, and he thus obtained the result that the delay in the spinal cord, together with the time taken in traversing part of the dorsal roots, the ventral roots, and a small part of the sciatic nerve<sup>1</sup> (a time which, he says, was too small to be measurable) varied between 0.008 and 0.015 second. It is Wundt's lowest estimate of this same-limb reflex time to which hitherto most importance has been attached. As this seemed to be hardly justifiable, I have applied another method which I regard as less open to objection (one which I had been using for other purposes for several years in conjunction with Sir John Burdon-Sanderson, by whom it was first introduced for measuring time relations in physiological processes), to the purpose of determining the time-value in question, and others in more complex reflexes.

As indicator I have used, not the mechanical, but the electrical response of a muscle, recording on a photographic plate, moving at a known and equable rate, the movement imparted to the meniscus of the capillary electrometer the moment an electrical change occurs at a spot connected with this instrument. The fact that the electrical response in the organ, as well as its manifestation in the recording instrument, occurs without any delay as soon as the recording spot is reached, and that it is not necessary for the whole muscle to be implicated before a record can be obtained, obviates what seems to me to be the principal objection to Wundt's experiments. The electrical response has the further advantage over the mechanical response, for the measurement of brief time intervals between different events occurring in a muscle, that the effect not only begins without delay, but, when in existence, outlasts the stimulus (which, either directly or indirectly, produces it) by a so much shorter time. A second effect, therefore, occurring in the muscle only a few thousandths of a second after a first, would have quite a distinct manifestation when recorded by such an instrument as the capillary electrometer, whereas in the record of the contraction of the muscle two such effects would be merged into one. This being so, there should be no difficulty in recording on the same photographic plate, and measuring the time interval between, the two electrical effects produced at one and the same spot of the muscle in response to simultaneous excitation of efferent and afferent nerve respectively; nor is there any such difficulty, provided that the cord is sufficiently sensitive for an effectual response to be obtained from it at all when the stimulus applied to the afferent nerve is single and instantaneous, as for the purpose in hand it must be.

To eliminate any effect which the physiological or the physical condition

<sup>1</sup> The frogs he used were large, being sometimes as much as 21 cm. long, so that 3 cm. more of nerve may have been traversed in the case of the reflex response than was traversed when the (upper part of the) sciatic nerve was stimulated. If one may infer, since it is not otherwise stated, that he used the same species of frog and under the same conditions of temperature as were used for the experiments in the first part of his treatise, the species was *R. viridis*, and the temperature between 15° and 17½° C.

of a particular piece of nerve excited might have on the time of arrival of the response in the muscle, and to ensure that the two stimuli were applied simultaneously, the simplest method to adopt seemed to be that of exciting a mixed nerve at one and the same spot, by one and the same stimulus, so as to obtain in succession in the muscle, first the direct and then the reflex effect.

To be able to rely upon this method it was essential to know whether or not there is any difference in the rate of propagation of an impulse, either through nerve, end-organ, or muscle, according to the strength of the stimulus producing it; for the strength of the stimulus immediately producing the reflex effect could hardly be so strong in a normal cord as that which has to be artificially applied to the mixed nerve in order to produce a reflex effect at all. With this object I made some preliminary experiments on excised nerve-muscle preparations, and found that, with a big resistance in the secondary circuit (as was employed in all the experiments referred to in this paper, unless the contrary is expressly stated), there is no difference—at least none that could be measured on plates travelling at the rate of about 85 cm. a second—in the time taken by an impulse just strong enough to produce an effect at all, and that taken by one strong enough to produce a “maximal” effect, when each traverses in turn the same portion of a particular nerve and muscle—provided, however, that the preparation was a sensitive one. In less sensitive preparations a slight difference was occasionally manifested, but one hardly amounting in any of my preparations to as much as a thousandth of a second.<sup>1</sup> As no electrical response to an excitation produced reflexly by the application of a single break induction shock to afferent nerve can be obtained at all, except in very sensitive preparations, there seemed therefore to be no need to use for comparison with such response one produced by the application to the efferent nerve of a stimulus of smaller strength, as Wundt had considered necessary.

In the same and other experiments made with excised preparations, I found, however, somewhat to my surprise, seeing that the exciting current was of such brief duration, that the transmission time is appreciably and very definitely affected by the direction of the induction current applied to the nerve when this is at all strong, i.e. when it is nearly strong enough, or just strong enough, to be felt on the tongue. A strength of excitation so great as this was seldom used in my experiments. When used, the extra delay, which sometimes even amounted to nearly two-thousandths of a second, and always occurred in passing the spot to which the exciting needle connected (indirectly) with the zinc of the battery was

<sup>1</sup> The difference, such as it is, when present, is probably one in end-organ delay, since Engelmann (*A. f. d. ges. Physiol.*, lxxi., p. 574, 1897) has shown with the curarised sartorius, excited by maximal and submaximal stimuli, that there is no difference in the rate of propagation of the mechanical response in muscle, and Gotch (*J. Physiol.*, xxviii., p. 402) has shown that there is none in the rate of propagation of the electrical response in nerve.

applied, was so easily detected and measured in the direct response of the muscle, that allowance for it could be made in estimating the time which elapsed in the cord.

## II. THE SAME-LIMB REFLEX TIME IN THE NORMAL CORD.

It is essential for measuring the transmission-time in the cord that the stimulus should be instantaneous and single. To overcome the well-known difficulty in producing a reflex effect by a stimulus of this kind without the aid of drugs, I have had recourse, in part, to a method recommended by Biedermann,<sup>1</sup> which consists in keeping the decerebrated frog at a low temperature (2°–6° C.) for from one to five days before making the experiment. (Animals caught in warm weather were kept in the cold for some days or weeks before being decerebrated.)

The species used was *Rana temporaria*. For the most part the specimens were small, the body length being about 6 cm. They were decerebrated by section through the optic thalami in the way recommended by Goltz,<sup>2</sup> the great advantage of which is that it is easily accomplished, with little shock, and little, if any, loss of blood or disturbance of circulation.

After ligaturing the iliac artery on one side, the sciatic nerve was freed, so that a pair of needle electrodes could be placed on it without coming in contact with any other part of the preparation. The corresponding gastrocnemius muscle was then prepared, and, the whole preparation being then placed in a moist chamber, its tendon end and a spot on its dorsal surface were connected by non-polarisable electrodes with the Hg and H<sub>2</sub>SO<sub>4</sub> respectively of a capillary electrometer, which was clamped on to the stage of a horizontally-placed projecting microscope. The vertical slit upon which the column of mercury was projected, and the cylindrical lens about 10 cm. behind it, were fixed in the front wall of a long dark-box. Inside this box a trolley for carrying the photographic plate was arranged to run at equable rates, special care being taken that in so doing it should produce no vibrations. The distance of the plate was such as, with the objective used, to magnify the image about 300 times. As the trolley passed the slit, it broke a platinum contact which had been completing a circuit containing a single Daniell cell and the primary, core-less, coil of a Kronecker inductorium. The induction current that it thus produced in the secondary circuit was used for exciting the sciatic nerve, the fine (steel) needles which served as exciting electrodes being placed, 2–3 mm. apart, on the nerve. There was always, unless the contrary is expressly stated, a large resistance in the secondary circuit.<sup>3</sup> The length of the nerve, the position of each needle with regard to it, the length of the muscle, and the distances of the two tied-on, leading-off electrodes from its ends, were all carefully measured and noted. While the plate was passing behind

<sup>1</sup> Biedermann, A. f. d. ges. Physiol., lxxx., 1900, p. 408.

<sup>2</sup> Goltz, Beiträge zur Lehre der Nervencentren des Frosches, Berlin, 1869.

<sup>3</sup> I used one of 60,000 ohms.

the slit, a wheel-like cardboard disc (called by Dr Garten of Leipzig, who first introduced it for this particular purpose, an "episkotister") was rotating in front of it at such a speed that the light was obscured by a spoke between 700 and 850 times a second. The exact rate at which the spokes were passing the slit at the time it was taken could always be determined with accuracy on the developed photograph, either by means of the record on the plate of an electromagnetic signal set into vibration by a 100 fork, or by the vibrations of a rod in connection with the break-key inside the dark-box, the primary function of which was to mark on the plate the moment at which the nerve was excited. The value of 0.1 second was determined (with the aid of the 100 fork record) in terms of the vibrations of this rod as they appear on the photographs on several occasions, and was found to be quite constant (so long, of course, as no alteration was made in the length of the vibrating rod). Whenever the key for any reason had to be interfered with, a fresh estimation of this value was again made. The number of spokes obscuring the light in 0.1 second, as determined by the lines on the plate, was counted, and the value of the interval between two such lines, in time, was calculated for every photograph.

Two methods of distinguishing a reflex response in a record suggested themselves, and, accordingly, when the preparation was ready, the experiment was continued in one of two ways. In the one case, the strength of induction shock to the nerve required for a "maximal" contraction of the muscle was first determined. Then, the preliminary experiments having shown that the maximal electrical effect is produced by the same strength of stimulus as the maximal mechanical effect, the electrical response to a stimulus of this strength, or to one but little stronger than it, was recorded. By means of the graduated scale with which the inductorium was provided, the secondary coil was next so far pushed up that the induction current might be three or four times as strong, and another record was taken. Responses to just maximal and to supra-maximal stimuli were then recorded alternately three or four times, sometimes also those to still stronger stimuli, in which case one record at least was taken with the direction of the current reversed.

Two consecutive records obtained in one experiment of this kind are reproduced in fig. 1. (A) represents the response to a stimulus just over the "maximal," (B) that to one three times as strong. The direct response of the muscle to stimulation of the efferent part of the nerve began under the proximal electrode, in each case four-thousandths of a second ( $4\sigma$ ) after the excitation of the nerve. In the second record there is seen in addition a much smaller effect, the position of which shows that the muscle was again electrically active under the proximal electrode  $23\sigma$  later than when it became so the first time,  $27\sigma$  therefore after the excitation. In the particular experiment to which these records refer it was only in the two first responses to the supra-maximal stimulus that this



second effect occurred, but in each it occurred at the same time after the first. It did not show itself in any of the responses to the just maximal stimulus. That the second effect, which (in records taken without the aid of a drug to increase the excitability of the cord) was seldom larger than in this experiment, is the reflex effect is perhaps more convincingly shown by the records obtained when the second method of experiment was employed.

This consisted in first recording the response when the nerve was excited by a stimulus five or ten times as strong as would probably be required to produce a maximal response to excitation of the motor nerve. Then, after obtaining not more than four records of this response, one of which was taken with the direction of the induction current reversed, the

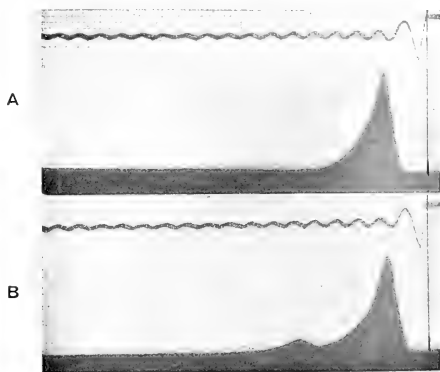


FIG. 1.—First and second electrical responses of the gastrocnemius of a normal preparation, obtained when the intact sciatic nerve of the same side was excited:

A, by a stimulus which was little more than just maximal (1000 units). [Time lines 760 per second.] B, by a stimulus of three times the strength. [Time lines 730 per second.]

sciatic nerve was divided between the exciting electrodes and the cord, and the response obtained when the peripheral end was excited by the same supra-maximal stimulus applied to the same spot on it was recorded two or three times. The small second effect which was sometimes there in all the records taken with the nerve intact was never seen after its severance from the central nervous system. The disadvantage of this method is that one cannot alternate the two things which have to be compared as one can with the other method; and since the reflex effect disappears long before the direct effect, seldom being obtainable in a normal preparation in response to more than a very few excitations of the mixed nerve, it is important in using it to excite as few times as will suffice to make absolutely sure that the effect, if present, is by no possibility accidental, and to ascertain the time of its occurrence, before dividing the nerve.

Controlled, however, by experiments made according to the first method, there is no room for doubt as to what in the records represents the reflex response of the muscle. Moreover, it gives evidence as to the reflex response which is not obtainable by the first method alone. For a supra-maximal stimulus is apt to produce in the record of the electrical response of the gastrocnemius not one, but two, effects which are not there when the stimulus is just maximal. The second such effect, instead of being much smaller than the direct one and occurring some two-hundredths of a second after it, is usually about equal to it in amount (as indicated by the steepness of the rise in the curve), and occurs some six- to ten-thousandths of a second after it. Two records of responses to supra-maximal stimuli in which this

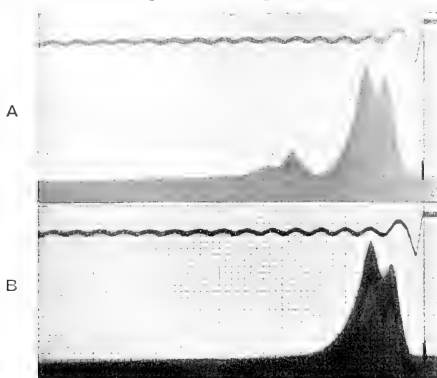


FIG. 2.—Fourth and seventh electrical responses of the gastrocnemius of a normal preparation (Exp. 14) obtained:

- A, when the intact sciatic nerve of the same side was excited by a supra-maximal stimulus. [Time lines 770 per second.]  
 B, when the peripheral end of the same nerve, after dividing it, was excited at the same place by a stimulus of the same strength. [Time lines 780 per second.]

effect is seen are reproduced in fig. 2. The upper curve represents the fourth of four very similar responses obtained from a gastrocnemius when its intact sciatic nerve was excited; the lower one the third of three very similar responses obtained from the same muscle excited at the same place and in the same way, but after the division of the nerve above the place of excitation. In both, and indeed in all the records obtained with this muscle (except the very first, which was that of a response to a weaker stimulus), the second effect, resembling the first direct effect, is seen. It indicates, therefore, that, whether the nerve was in physiological connection with the cord or not, the contact between the muscle and the proximal electrode became again negative to the distal, and by about the same amount as when the impulse first reached that spot along the motor nerve, and, moreover, that it did so after an interval of  $6\sigma$ . In all the records

taken before the severance (including the very first), but in none taken after it, there is seen a much smaller, and again double, after-effect. This indicates that when the nerve was intact, the muscle, as sampled by the two spots connected with the electrometer, began to undergo the same sort of disturbance in its electrical equilibrium as was the case previously in response to the excitation of the motor nerve, but one smaller in amount, and that this occurred 16 to 19.5 $\sigma$  after the first. In the particular response to which the photograph here reproduced refers, it began 19 $\sigma$  later. Its complete absence in the records taken after the severance of the nerve from the cord makes the central origin of the stimulus which immediately provoked it almost a certainty.

What the effect, which I propose to call the second peripheral effect, signifies, does not here directly concern us. I believe it to be in some way dependent on the arrangement of the fibres in the gastrocnemius muscle, and perhaps on the special spot to which the proximal electrode happened to have been applied, since, with this muscle, which I had seldom used before for electrical purposes, I have now come across it frequently in response to strong stimuli, whereas I do not recall ever having seen it in the thousands of records I have taken of the electrical response of sartorius muscles to single break induction shocks, nor has it been present in any of the few excised nerved preparations of sartorius muscles of these cooled frogs which I have tried. I do not, however, wish to express a definite opinion on this point, as I have not really investigated the matter, and the occurrence of a succession of electrical changes resembling one another in response to a single instantaneous stimulus in the sartorius muscles of frogs suffering from drought<sup>1</sup> suggests another interpretation. Whatever may give rise to it is quite independent of the central nervous system, and that is all we need know for our present purpose. The strength of the stimulus required to produce it is sometimes less and sometimes more than that required to produce a reflex effect.

I have thought it superfluous for the chief object I had in view, to deduce from the capillary electrometer records the curves indicating the actual differences of potential prevailing between the two spots of the muscle led off from during each response. To anyone accustomed to interpreting such records it is easy to find, without such deduction, the place which indicates the coming into existence of a difference of potential of one particular sign, and thus to ascertain the time at which it occurs; also, if need be, to see where, and consequently when, its maximum is reached, when it ceases to exist, or is reversed. To those who are not fully conversant with the reading of such records, it may be of some assistance to state here briefly that every rise on the photographic curve indicates a movement of the meniscus which inscribes it towards the orifice of the capillary—an adostial movement—and that such a movement, with the

<sup>1</sup> See Durig, Arch. f. d. ges. Physiol., xxvii., p. 457, 1903.

particular arrangement adopted of connecting the muscle with the electrometer, always denotes that the contact between the proximal electrode and the muscle was during that time (galvanometrically) negative to the other contact. When the mercury is moving most quickly, when the rise in the record is steepest, this negativity is greatest. When the mercury is moving most slowly (as indicated, for instance, by a summit on the curve), this negativity is least, and the difference of potential is either just about to cease, or is being reversed. A descending curve, according to its steepness, i.e. an abostial movement of the mercury, according to its quickness, denotes either that the distal contact is now negative to the proximal, or that there is still no difference of potential between them and that the meniscus is returning in its own time to its original position. It means the former before it means the latter when the contacts are made with two parts of the muscle, each of which in turn becomes electrically, then mechanically, active.

If the electrical disturbance produced by the excitation of the motor part of the nerve is over, under both electrodes as it usually is, before that produced by the excitation of the sensory part has begun to manifest itself under the proximal electrode, the beginning of this second electrical event is marked on the curve either by a fresh rise (as in figs. 1, B, and 2, A) or by a checking of the course of the descending curve.

Having learned to identify the reflex effect in the records, there is no difficulty in ascertaining the time which elapsed between the moment at which the primary direct response reached the first recording spot on the muscle, and that at which the reflex effect reached the same spot; i.e. the time taken for the impulse to travel a known length of nerve to the cord, and, after passing through the cord, to travel the same length of nerve back again.

The shortest time interval between the arrivals of the two effects at the proximal electrode in those preparations which were not under the influence of any drug was  $14\sigma$ ; but it was only in one response (the first) in one preparation [Exp. 15] that I obtained a value so low,<sup>1</sup> it being about  $1\sigma$  longer in the three other responses recorded with the same preparation. In a record obtained in one other experiment [Exp. 31], the interval appeared to be equally short, but this was an experiment in which the comparison of the records taken with the exciting current in opposite directions showed that there was a block at the kathode which it took the impulse  $1\sigma$  to overcome in going straight to the muscle, so that probably

<sup>1</sup> The measurements on which the time values in all my experiments depend were made for each record not only by myself but independently by another person who knew nothing about their significance or the conditions under which the experiments were made. (I have been enabled to obtain this, and other, valuable assistance in the measurement of records, by the kindness of Dr Osler.) On each occasion in which our final results differed by more than  $0.5\sigma$ , and especially if there were any relative difference in the values we each obtained in any one experiment, the whole process was gone over again by the one or the other of us. The number of thousandths of a second in the time values given in the text or in the tables may therefore be relied upon, but the fractions of a thousandth, when given, make no profession of being absolutely accurate.

the real difference of time in this one response, the only one taken with the current descending, was nearer  $15\sigma$  than  $14\sigma$ . In the other responses obtained from this preparation (by means of ascending break induction shocks to the nerve), the interval between the two arrivals appeared to be about  $16\sigma$  in two and about  $19\sigma$  in the third. It was probably slightly, but not so much as  $1\sigma$ , shorter (see p. 14).

In most of the preparations the interval was between  $16\sigma$  and  $21\sigma$ , but in three it was  $23\sigma$ , and in three others it was longer still. Five, out of twenty-three, of the preparations gave records which showed no sign of any reflex effect; and in the records obtained with two others, the reflex effect, though represented in some, was too small for the place at which the curve began to alter its course to be determined with accuracy. Each cord which yielded more than one measurable reflex effect gave it in about the same time in all the responses. In seven preparations from which records of the reflex response were obtained more than once, the recurrence of the same interval (even under different conditions as to strength of stimulus) was very striking. This may be judged of by the measurements obtained from the records in one experiment (the longest of the seven), the conditions obtaining, and the results obtained in which are given here in tabular form.

EXP. 58. Dec. 17, 1906. Room temp.  $12^{\circ}$  C.

Induction current to nerve.		Length, in millimetres, of			Time, in thousandths of a second ( $\sigma$ ).			
Strength. <sup>1</sup>	Direction (descending or ascending).	muscle from entrance of nerve to <i>p</i> . <sup>2</sup>	nerve from <i>Cv</i> electrode to muscle.	nerve from <i>Cv</i> electrode to cord. <sup>3</sup>	taken by impulse to reach <i>p</i> directly (measured).	interval between arrivals of direct and of reflex effects at <i>p</i> (measured).	to be deducted for transmission in nerve <sup>3</sup> (assumed).	Probable delay in cord.
10,000	<i>d</i>	7	14	40	4.4	19.8	2.6	17.2
12,000	<i>d</i>	7	14	40	4.4	19.8	2.6	17.2
10,000	<i>d</i>	7	14	40	4.4	20	2.6	17.4
3,000	<i>d</i>	7	14	40	4.4	20	2.6	17.4
10,000	<i>d</i>	7	14	40	4.4	20	2.6	17.4
3,000	<i>d</i>	7	14	40	4.4	20	2.6	17.4
10,000	<i>d</i>	7	14	40	4.4	19.5	2.6	17.1
10,000	<i>a</i>	7	12	42	4.4	19.8	2.7	17.1

<sup>1</sup> The strength of the current in all the tables is given in units, read off from the graduated scale of the Kronecker inductorium, 1 Daniell being in primary circuit, 60,000 ohms in secondary. With such an arrangement the break induction current could not usually be felt on the tongue with strengths under 12,000.

<sup>2</sup> *p* stands for the proximal leading-off electrode.

<sup>3</sup> The length of nerve from the principal exciting electrode to the cord was not in every case directly measured, although the length of the sciatic nerve itself in the thigh was always measured. I ascertained that the ratio of sciatic plexus length to nerve length in a certain number of preparations was as 1 to 0.73, and have in the majority of cases calculated the length from this. A few millimetres of nerve more or less would make so

[The first record was taken with the right gastrocnemius to excitation of right sciatic. The rest were taken with the left gastrocnemius to excitation of left sciatic. No reflex response was obtained when the strength of the current was 1000 units.]

In three other preparations the variation in the interval was slightly greater, but hardly exceeded  $1\sigma$ . There were, however, three experiments (in two of which four reflex responses, in the third nine, were recorded) in which the shortest and the longest interval on the different occasions varied by as much as  $4\sigma$ . What the conditions were in the longest of these three experiments will be seen from the following table. It will be referred to again later (p. 17).

EXP. 37. Nov. 6, 1906. Room temp.  $11^{\circ}$  C.

Induction current to nerve.		Length, in millimetres, of			Time, in thousandths of a second ( $\sigma$ ),			
Strength.	Direction.	muscle from entrance of nerve to <i>p</i> .	nerve from <i>Cu</i> electrode to muscle.	nerve from <i>Cv</i> electrode to cord.	taken by impulse to reach <i>p</i> directly (measured).	interval between arrivals of direct and of reflex effects at <i>p</i> (measured).	to be deducted for transmission in nerve (assumed).	Probable delay in cord.
5,000	<i>d</i>	17	19	32	4	18.5	2.1	16.4
10,000	<i>d</i>	17	19	32	4	17.8	2.1	15.7
10,000	<i>d</i>	17	19	32	4	18.4	2.1	16.3
5,000	<i>d</i>	17	19	32	4	18.9	2.1	16.8
3,000	<i>d</i>	17	19	32	4	20.2	2.1	18.1
10,000	<i>d</i>	17	19	32	4	22	2.1	19.9
10,000	<i>a</i>	17	15	28	4	20	1.9	18.1
10,000	<i>d</i>	17	19	32	4	21	2.1	19.1
10,000	<i>a</i>	17	15	28	4	22	1.9	20.1

The length of nerve traversed from the place of excitation, through the plexus, to the cord and back, was in most of my preparations about 60 mm. Only occasionally, when large animals were used, was it 70 or even 80 mm. Many different observers have measured the rate of propagation of an impulse along fresh frog's nerve and found it to be about 30 metres per second. It is also known that there is no delay in the dorsal ganglia.<sup>1</sup> To ascertain the time which elapsed in the cord itself during each response we must therefore deduct as a rule about  $2\sigma$ , but occasionally as much as  $2.6\sigma$ ,

little difference to the time to be deducted for transmission in nerve as compared with the time taken in the central nervous system, that this method seemed to me to be exact enough for the present purpose. The relative length of nerve traversed in different responses of the same preparation has, however, been in so far taken into account that usually more has been deducted for transmission when the current was ascending than when it was descending (see also p. 14). The sense in which I have used the words "ascending" and "descending" will be obvious. It is not strictly correct in reference to the muscle when the sensory fibres are being considered, and not the motor only.

<sup>1</sup> Moore and Reynolds, J. Physiol., xxxiii., 1898, Suppl.

from the measured time interval between the arrival of the direct and that of the reflex effect at the first recording spot of the muscle.

Only when the current applied to the nerve to excite it was so strong, or the part of the nerve to which it was applied of such a nature, that a temporary obstruction was produced under the kathode when the current was broken, must a longer time be deducted for transmission in nerve when the kathode was between the anode and the cord, and a shorter one (from the measured interval between the two arrival-times in the particular record) when the kathode was between the anode and the muscle. Although there is no difficulty in detecting a delay-producing moment of this kind, and in measuring the time taken to overcome the obstruction at the kathode by the impulse going straight to the muscle, there is a slight uncertainty about the exact amount to be deducted for delay caused by such obstruction to the impulse starting in the opposite direction. The reason for this uncertainty may be best explained by introducing here the measurements of records taken alternately with (relatively) strong ascending and descending induction currents (in a strychnine preparation) with a view to elucidating the matter.

EXP. 48. Nov. 27, 1906. Room temp. 16° C. One minim 0.01 per cent. liq. strych. injected one hour before preparing nerve and muscle.

Induction current to nerve.		Length, in millimetres, of			Time, in thousandths of a second ( $\sigma$ ),			
Strength.	Direction.	muscle from entrance of nerve to <i>p</i> .	nerve from <i>Cu</i> electrode to muscle.	nerve from <i>Cu</i> electrode to cord.	taken by impulse to reach <i>p</i> directly (measured).	interval between arrivals of direct and of reflex effects at <i>p</i> (measured).	to be deducted for transmission in nerve (assumed).	Probably delay in cord.
10,000	<i>d</i>	11	16	37	5	14	{ +0.8 - 2.5	12.3
14,000	<i>d</i>	11	16	37	5	13.7	{ +0.8 - 2.5	12
14,000	<i>a</i>	11	12	41	3.7	15.6	{ - 0.9 - 2.7	12
14,000	<i>d</i>	11	16	37	5	13.7	{ +0.8 - 2.5	12
14,000	<i>a</i>	11	12	41	3.7	15.6	{ - 0.9 - 2.7	12
14,000	<i>d</i>	11	16	37	5	13.1	{ +0.8 - 2.5	11.4
14,000	<i>a</i>	11	12	41	3.7	15	{ - 0.9 - 2.7	11.4
14,000	<i>d</i>	11	16	37	5	12.5	{ +0.8 - 2.5	10.8
10,000	<i>d</i>	11	16	37	5	12.5	{ +0.8 - 2.5	10.8

Allowing 0.1 $\sigma$  for the extra 4 mm. of nerve traversed when the current

was descending, it took the impulse  $1.2\sigma$  to overcome the obstruction under the Zn electrode on its way to the muscle. Hence, although the reflexly-produced impulse actually arrived at the muscle  $13.7\sigma$  later, the difference between the two times of arrival would have been  $14.9\sigma$ , had it not been for the delay to the direct impulse, unless, as is not at all improbable, the obstruction was still there (in an attenuated form, i.e. causing a shorter delay) when the reflexly-produced impulse reached the same spot later, in which case it would have been somewhat shorter. That it did so persist is one alternative suggested by the preciseness with which the measured time taken for the reflex effect to reach the muscles recurred alternately with the ascending and descending current in the first four responses. Instead of reaching the muscle  $1.2\sigma$  later, when the current was ascending, as one might have expected, it reached it only  $0.6\sigma$  later than when it was descending (in  $19.3\sigma$  in the one case,  $18.7\sigma$  in the other). Allowing  $0.1\sigma$  for the extra 4 mm. of nerve which were traversed by it in the former case, and granting that the obstruction persisted, the whole time taken to reach the muscle was therefore not more than  $0.5\sigma$  longer when the impulse met with the block at the start (current ascending) than when it met with it first on its return from the cord some  $2.8\sigma$  later (current descending). Assuming, as one can hardly help doing, that the delay at the outset at one spot when the current is going in one direction is the same as it is at another spot 4 mm. away, when the current is going in the opposite direction, it looks either as though the reflex effect were delayed  $0.7\sigma$  on its way to the muscle when the current was descending, and not delayed at all on its way to the muscle when the current was ascending, in which case the cord delay would be  $14.9\sigma - 0.7\sigma - 2.5\sigma = 11.7\sigma$ ; or as though the impulse produced by the ascending break induction shock was accelerated when it had to pass a second time, but in the reverse direction, the spot at which it had before been delayed, in which case the cord delay would be  $14.9\sigma - 2.5\sigma = 12.4\sigma$ , and the supposed accelerating factor would be such as to make the impulse traverse the spot  $0.7\sigma$  more quickly than it would otherwise have done. Not knowing which of these alternatives, or what other alternative, best expresses what actually occurred, I have in such experiments (showing cathodic obstruction) given a value to the cord delay intermediate between the two which it seems to me that it might have (see above). The error so introduced is hardly greater than another error which could not be avoided, that, namely, which arises from the assumption made that the rate of transmission along nerve where there is no block, is the same for all nerves and at all temperatures, i.e. all at which my experiments have been made ( $11^\circ\text{C.}$ – $18^\circ\text{C.}$ ). But neither solving the doubt nor removing either error would alter the absolute value of the cord delay by more than a fraction of  $\sigma$ , and would not alter the relative value in successive responses in any one preparation at all; at any rate not in such of them as were taken with the induction current in one and the same direction.



On deducting from the measured time the time which was, according to what has just been said, assumed to have been spent in traversing, in each response in each experiment, the known length of nerve, we find that the time spent in the cord itself in the same-limb reflex, the time taken, as it seems to me, for the impulse to pass from the branched ultimate endings of the several afferent fibres concerned, each across a synapse, to their respective motor cells, may be, though it only once was so in my experiments, as short as  $12\sigma$  in the normal frog's spinal cord, but that it is more frequently something between  $14\sigma$  and  $21\sigma$ . If one may use an analogy, which is possibly something more than an analogy, this is the time taken for the endings of the afferent fibre or fibres, when sufficiently charged, to discharge themselves across the gap to the oppositely charged, or uncharged motor cell.

With regard to the influence of strength of stimulus on such time of discharge, if such it be, it is not easy to make a definite statement, mainly on account of the difficulty in getting the reflex effect (without the use of drugs) sufficiently often in one preparation.<sup>1</sup> In two experiments only, the details of which have already been given in tabular form, did the reflex effects, in response to different strengths of stimulus, give a definite result. Exp. 58 shows that stimuli three or four times as strong as one which cannot be far from just producing the reflex effect, may produce it in almost exactly the same time. In Exp. 37 the reflex times were more varied, but did not consistently vary inversely with the strength of the induction current. It is, however, possible and even probable that there is for each preparation some strength of stimulus, not quite weak enough to be wholly ineffectual, with which the reflex time is longer, although the fact that it is not until the experiment is over and the photographs developed that one knows exactly what has happened creates a difficulty in deciding this point experimentally.

The strength of the reflex effect, as indicated by the steepness of the rise in the muscle record, is almost always less, and very considerably less, than that produced directly by the stimulation of the motor part of the nerve. Only with one undrugged preparation have I obtained records which indicate that the effect of the central stimulus was not much less than that of the artificial stimulus to the motor nerve. In this experiment all the four records of the response, when the intact nerve was stimulated, showed this. In the first response (to an artificial stimulus of 5000 units) the reflex effect was strongest; its strength may be judged by comparing the steepness of the rise in the curves representing the reflex and the direct response in fig. 3. In the second record it was nearly but not quite as strong; in the third and fourth it was decidedly weaker (but about the same for each); the artificial stimulus used to obtain the last three records was twice as strong as that used to obtain the first.

<sup>1</sup> This is a difficulty which I have now overcome by using wholly flexor muscles (see footnote, p. 30) to indicate cord delay, and by keeping the preparation at a low temperature. The results fully confirm those obtained from the two experiments referred to in the text. [November 1907.]

It should here be noted that the strength of the direct effect was appreciably altered in not a few of my experiments by the direction of the induction current the break of which was used to excite the motor nerve, even when the strength of the current was supra-maximal. The steepness was in such cases more frequently less with the induction current descending; but if several responses were recorded with the current in either of the two directions alone, the direct effect was apt to be smaller in a record afterwards taken with the direction reversed. The experiment just referred to affords an instance of this: the first three responses were to the break of ascending currents, the fourth to that of a descending current. While in the second and third the direct effect was about as strong as in the first, in the fourth it was very little stronger than the reflex effect on the same occasion. The direct effect obtained with the descending current did not necessarily, when less in amount, begin to manifest itself later, although sometimes, as in this instance, it did so. Such a

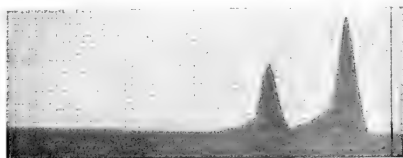


FIG. 3.—First electrical response of the gastrocnemius of a normal preparation obtained when the intact sciatic nerve of the same side was excited by a supra-maximal stimulus. [Time lines 730 per second.]

difference in the strength of the effect (when it occurs) seems to me to indicate some temporary impairment of the particular spot of nerve under the anode, preventing perhaps the participation of the whole number of fibres (in some cases the condition being brought about by this having previously been a kathode), for in some of the preliminary experiments in which the nerve was excited at two different parts of its length in turn, the difference of the effect according to the difference of direction of current might be marked when the one part and not when the other was used. I have, however, made no serious attempt to understand the phenomenon, because it seemed to me to have little, if any, bearing on the matter which is now concerning us. Moreover, the direct effect may get weaker in the course of an experiment even without reversing the direction of the current (see fig. 12 (E), p. 56). In none of my experiments (with the possible exception of No. 49; see note to it on p. 42) was the strength of the reflex effect altered by altering the direction of the induction current applied to (the afferent part of) the nerve either in the normal or in the strychnised cord.

The probable cord delay in the one normal preparation from which

comparatively strong reflex effects were obtained was not shorter than in other normal preparations, nor in this preparation was it shorter the stronger the effect. It was respectively, taking the four responses in order, after allowing for transmission time in nerve (including that taken to overcome the block under the kathode, which was in this preparation  $1\sigma$ ):  $14.3\sigma$ ,  $13.9\sigma$ ,  $16.2\sigma$ ,  $13\sigma$ . The strength of the central stimulus, as estimated by the strength of the effect it produces, is not therefore a function of the time taken by the impulse to affect the motor cell.

There is the same difficulty in making experiments with regard to the influence of temperature on the time occupied in the normal cord, as with strength of stimulus. On every occasion when, after an experiment has begun, the gastrocnemius being used as indicator, I have either cooled or warmed the back of a normal preparation, the reflex effect, if present before, was no longer to be seen in records taken after such treatment. In a few of the experiments I tried to make use of Biedermann's experience that a reflex contraction could be obtained with greater certainty in a cooled spinal frog if, after making the preparation, it was given a long rest with a bag of ice applied to the spine. In eight preparations, among which was the one which gave the lowest time value for the cord delay, there had been for about half an hour, on the back of the frog, a bag (an india-rubber finger-stall) containing ice. This was not the case in the other experiments with normal cords, and it was in three of these that the measured time interval between the two arrivals was longer than  $21\sigma$ , i.e. that the probable cord delay was over  $19\sigma$ . The reflex time in the experiments in which ice was used was not therefore universally longer than in the experiments in which it was not. I do not of course think that this proves that cold has no influence on the delay in the cord, but I have not yet been able to make an experiment that would either prove it or disprove it satisfactorily.<sup>1</sup> In the strychnised cord, which is capable of giving a reflex effect a large number of times in succession, the delay is most certainly increased by cold; but whether we should be justified in applying, without reserve, to a normal preparation what we know to be true of a drugged one is not, in my opinion, to be answered straightway in the affirmative.

The results obtained from the records taken in Exp. 37 (see p. 12) make it appear that fatigue may have some influence on the time of the cord delay. While in the first four responses this did not exceed (when reduced)  $17\sigma$ , in all of the last five it was over  $18\sigma$ , and became finally as long as  $20\sigma$ . None of the other preparations gave evidence of this, but with the one exception of Exp. 58 they all gave (or were allowed to give) too few records of reflex effects to have been able to show it. This is

<sup>1</sup> I have now [Nov. 1907] made two experiments, using the biceps femoris as indicator, which show that the probable cord delay may become some  $10\sigma$  longer in a normal cord by a reduction of the temperature of the whole preparation from  $12^{\circ}\text{C}$ . to  $6^{\circ}\text{C}$ ., and may again become shorter when the temperature is raised once more to  $12^{\circ}\text{C}$ ., though not regaining its original value.

one of the many matters with regard to which further experiments are needed.<sup>1</sup>

### III. THE SAME-LIMB REFLEX-TIME IN A CORD THE EXCITABILITY OF WHICH HAS BEEN RAISED BY STRYCHNINE.

Most of the frogs used for these experiments were treated in the same way as those used for experiments without drugs: that is to say, they were, after being decerebrated, kept in the cold for one or more days before making an experiment. A preparation then made in the same way as has already been described for "normal" animals, but the injection into the dorsal lymph sac of a very minute dose (0.005–0.02 mgr.) of strychnine some hours, or even days, before, or that of a somewhat stronger dose (0.03–0.06 mgr.) immediately before,<sup>2</sup> never failed to produce the reflex electrical response in the gastrocnemius when the mixed nerve was stimulated by a single break induction shock. This response, instead of being usually weaker than that to direct excitation of the efferent part of the nerve, was, as a rule, of nearly equal strength with it, and sometimes even stronger (rise on curve steeper). It was, however, not always its strongest at the beginning. It sometimes lasted no longer than the direct response, but very soon after the administration it began to lengthen, becoming from two to four times as long as the direct response. It was not until the influence of the drug had become so great that it showed itself in the movements of, or in the attitude assumed by, the brainless animals, that the reflex electrical response began to assume the serial character so often described, and capillary electrometer records of which I have published elsewhere.<sup>3</sup> In none of the photographic records reproduced in the present paper does more than the first period of a response to a stimulus of central origin appear, even when more were present. The undulations in the contour which may be seen in most of the records reproduced in figs. 4 to 12 are such as I have shown elsewhere<sup>4</sup> to be of purely muscular origin, although the photographic records which really prove it have not yet been published. When the effect of the strychnine begins to wear off the number of periods of central (proximate and, I think, also ultimate; see p. 29, footnote) origin (recurring five to ten times a second) is reduced, until finally there is again only one such period.

Most of the experiments made for the purpose which is now concerning us were made on preparations in the early stages, when the action of the drug was incipient, or in the late stages, when its action was vestigial.

<sup>1</sup> They have now been made [Nov. 1907].

<sup>2</sup> I cannot state the dose in milligrammes per body weight, because I did not weigh each frog. It seemed to me that little would be gained by doing so, seeing that the effectiveness of the drug varies so much, and in a way that has not yet been sufficiently studied, with the temperature of the frog and other conditions.

<sup>3</sup> Buchanan, *Journ. Physiol.*, xxvii., 1901, Plates VIII. and IX.

<sup>4</sup> Burdon-Sanderson and Buchanan, *Journ. Physiol.*, xxviii., 1902; *Proc. Physiol. Soc.*, p. xxix. Garten (Abh. k. Sächsischen Ges. Wiss., xxvi., p. 333, 1901) has also given experimental evidence of the fact.

In most, therefore, the response with the nerve intact, whether electrical or mechanical, was not much longer than when, after dividing it, its peripheral end was stimulated. A few, however, were made when the preparation was in the attitude characteristic of strychnine poisoning, or only just beginning to recover from it.

The conditions obtaining, and the results yielded in the several responses in nineteen typical experiments, have been tabulated in the same way as were those for normal cords. Certain of the conditions, and the results obtained in thirteen of them, will be found on the left-hand side (first six columns) of the tables beginning on p. 35. In almost every case in which the animal was only lightly drugged, the mechanical, as well as the electrical response was recorded, in order that its strength and duration might be compared with those of the twitch (*einfache Zuckung*) which was recorded at the end of the experiment.

It was only in three experiments that the cord delay was throughout of longer duration than appears to be characteristic of good normal cords under similar conditions. The majority of the experiments do not therefore confirm the conclusion which Wundt came to from his observations on the mechanical reflex response of the strychnised animal, that, namely, the effect of strychnine on the cord is to lengthen the delay. The three preparations in which the cord delay, after deduction for transmission time in nerve in the same way as before, was found to be throughout  $24\sigma$  or more, were the only ones in which not only were the arms flexed, but the legs were rigidly extended when the preparation was made. The reflex electrical responses in all three were serial. Verworn<sup>1</sup> has shown that in acute stages of strychnine poisoning not only the central nervous system but the heart also is affected by the drug. I have therefore examined the heart at the end of these and other experiments. In one of the three [Exp. 50], made on a specimen which had been in the attitude characteristic of strychnine poisoning for about two hours, and in which the buccal respiratory movements had already ceased, the heart was hardly beating at all, the blood was very blue, and the preparation seemed to be nearly asphyxiated; both the direct and the reflex electrical effects were very feeble (rise of curve very gradual); the probable cord delay was between  $25\sigma$  and  $30\sigma$  in four of the responses; it was as much as  $59\sigma$  in the first one, and  $41\sigma$  in the second. Of the other two preparations, the one had been in the attitude characteristic of strychnine poisoning for half an hour only, while the other had been in it for several hours. In both, the buccal respiratory movements were laboured. The heart was beating feebly. The reflex effect was not as strong as the direct effect; and in most, but not all, of the responses, it did not become maximal until some 6-10 $\sigma$  after its commencement; sometimes, in the second of the two experiments (in which the stimuli to the nerve were kept close to the threshold value in strength) [Exp. 55], not until  $20\sigma$  after. Fig. 10, A (see p. 52)

<sup>1</sup> Verworn, *Archiv f. (Anat. u.) Physiol.*, 1900, p. 385.

represents the third of the five responses to excitation of the intact nerve of the same side, recorded with the first preparation [Exp. 52]. It was the only one of the five in which the reflex effect attained its maximum early. Fig. 11, A and C (see p. 54) represent the first and eighth, respectively, of the sixteen responses to excitation of the nerve of the same side, recorded with the second preparation, and both show, as indeed did all but two out of the sixteen, the late development of the maximal reflex effect. It will be seen (pp. 43 and 45) that in the one of these two preparations the probable cord delay varied between about  $26\sigma$  and  $30\sigma$ ; in the other between  $24\sigma$  and  $33\sigma$ .

In the only two other experiments made upon animals with their arms flexed, recovery had so far progressed that the legs were no longer stiff and extended, buccal respiration appeared to be normal, and the heart was beating very fairly. In neither of these were the reflex electrical responses serial, and the cord delay was not longer than is frequently the case in normal frogs. Fig. 7, A (see p. 50) represents the first of six responses from one of these preparations [Exp. 40]. In this and in the second response, but not in the others, the direct effect was double; the reflex effect in this response and in some of the others was less strong; it did not, in this one, reach its maximum at once, although it did so in those in which it was stronger. Fig. 12, A, C, and E (p. 56), represent the first, second, and seventh of the eight responses recorded (by one of the two gastrocnemius muscles used) with the other preparation in which the arms were flexed [Exp. 56 R], when the sciatic nerve of the same side was excited. The reflex effects, as will be seen, were in the first two very nearly if not quite as strong as the direct effects; they attained their maximal strength at once and in all the responses. In four of the later responses they were even stronger than the direct effects owing to the latter having become weaker. One of these is shown in fig. 12, E.

In experiments made upon animals in which the drug was either just beginning to take effect or had nearly lost its effect, the heart was, as a rule, beating quite normally. Measurements of the records taken in these remaining experiments have led me to the conclusion that if strychnine has any direct influence at all on the length of the delay in the cord in the case of the same-limb reflex, it diminishes rather than increases it. Thus, whereas without strychnine only one preparation (out of eighteen) gave a value so low as  $14\sigma$  for the time taken by the impulse to travel from the particular spot on the nerve stimulated, to, and through, the cord and back again, as many as (six out of nineteen) strychnine preparations [Nos. 4, 8, 14, 16, 42, 48] gave as low a value as this, and four of these each gave it more than once or gave a still lower value (shorter by from  $1\sigma$  to  $2\sigma$ ) in one or more of the responses.

In three experiments (one of which only [Exp. 14] is included in the six just referred to) records were taken first of the electrical responses of the one gastrocnemius when its nerve was stimulated, before the administration

of strychnine; then of those of the second gastrocnemius when the second sciatic nerve was stimulated after the administration of strychnine in sufficient quantity, and for a long enough time to make the contraction in response to a single break induction shock to the mixed nerve something stronger than a twitch. Exp. 14, which has already been referred to (p. 8), and of which one of the records obtained when the cord was normal has been reproduced in fig. 2, A, is perhaps the most valuable of these, for the reason that the cord was only excited four times, and gave an excellent response each time, before the administration of the drug, and that as many as nine equally good reflex responses were recorded with the other muscle after its administration. In seven of these it occurred in almost exactly the same time after the direct response, and in all seven the interval was from  $1\sigma$  to  $1\frac{1}{2}\sigma$  less than in any of the responses obtained from the same cord without strychnine. In the other two responses it was shorter still, but in these two (both of which, as it happened, were to stronger stimuli after weaker ones) the effect, when it began, instead of being strong, was very weak, and it only became maximal, in the one case (fourth response)  $2\sigma$ , and in the other (seventh response)  $4\sigma$ , later.

The following table gives the data for both parts of the experiment:—

EXP. 14. Oct. 1, 1906. Room temp.  $18^{\circ}$  C. Records taken first with the right gastrocnemius to excitation of the right sciatic. Cord normal.

Induction current to nerve.		Length, in millimetres, of			Time, in thousandths of a second ( $\sigma$ ),			
Strength.	Direction.	muscle from entrance of nerve to <i>p</i> .	nerve from <i>Cu</i> electrode to muscle.	nerve from <i>Cu</i> electrode to cord.	taken by impulse to reach <i>p</i> directly (measured).	interval between arrivals of direct and of reflex effects at <i>p</i> (measured).	to be deducted for transmission in nerve (assumed).	Probable delay in cord.
5,000	<i>d</i>	7	14	30	2.6	19.5	2	17.5
10,000	<i>d</i>	7	14	30	2.6	15.6	2	13.6
10,000	<i>a</i>	7	17	27	2.6	18	1.8	16.2
10,000	<i>d</i>	7	14	30	2.6	19	2	17

Records then taken with the left gastrocnemius to excitation of the left sciatic, prepared a quarter of an-hour after the injection of  $\frac{1}{4}$  minim 0.1 per cent. liq. stryeh. into the dorsal lymph sac.

10,000	<i>d</i>	8	9	35	2.6	14.2	2.3	11.9
5,000	<i>d</i>	8	9	35	2.1	14.4	2.3	12.1
5,000	<i>d</i>	8	9	35	2.4	14.1	2.3	11.8
10,000	<i>d</i>	8	9	35	2.2	13.4	2.3	11.1
5,000	<i>d</i>	8	9	35	2.2	14.1	2.3	11.8
5,000	<i>d</i>	8	9	35	2.1	14.5	2.3	12.2
10,000	<i>d</i>	8	9	35	2	11.4	2.3	9.1
5,000	<i>d</i>	8	9	35	2.2	14.1	2.3	11.8
10,000	<i>a</i>	8	6	38	2	14	2.4	11.6

It will be noticed that the probable cord delay was not only longer, but more variable before the administration of the drug. The record of the second response of the second muscle is given in fig. 4, for comparison with the fourth of the first muscle reproduced in fig. 2, A. The comparison brings out very forcibly the first effect of strychnine on the electrical reflex response of muscle, namely—the response (to a weaker ultimate stimulus) has become as strong (curve as steep) as that to the direct excitation of the motor nerve; it has also become somewhat longer than it was when produced by the same cord when normal. I believe it to be also characteristic that it occurs somewhat earlier. It will be seen that the direct effect was again double.

With regard to the two other experiments of this kind: they were both made with preparations, in the normal cord of which the delay was long. In the one [Exp. 18] the records taken with the second muscle showed that

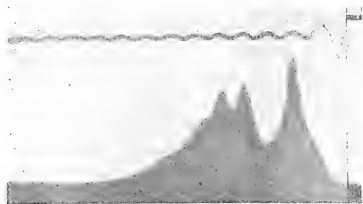


FIG. 4.—Second electrical response of the other gastrocnemius of the same preparation as was used for taking the records to which fig. 2 refers, obtained when the intact sciatic nerve belonging to it was excited (Exp. 14 L). After the first muscle had been used,  $\frac{1}{4}$  minim 0.1 per cent. liquor strychninæ had been injected into the dorsal lymph sac. [Time lines 795 per second.]

the probable cord delay, which did not vary much, and was only just under  $24\sigma$  in the five reflex responses which were obtained with it before administering the drug, was less constant in those taken with the second muscle after so doing. It was in two responses longer than before; in the next, of about the same duration; and in the last, shorter than before. In none of the responses was the reflex effect any stronger, or longer, than before. In the other experiment [No. 45], after five responses of the one muscle to excitation of its nerve, three of which only showed a reflex effect, had been recorded with the cord normal, two records were taken of the responses of the same muscle excited in the same way a quarter of an hour after the administration of strychnine, and before preparing the second muscle and its nerve. These both gave a reflex response no stronger than before, and still much weaker than the direct effect, but in a very much shorter time (probable cord delay in both of them  $12\sigma$  instead of the  $23\sigma$  which it had been in all three responses when



the cord was normal). The records then taken with the muscle of the opposite side when its nerve was stimulated (the first and the fourth of which are reproduced in fig. 8, A and C, p. 51) show that the central stimulus was then, a quarter of an hour later, producing as big an effect in the muscle as the peripheral one, and also that the interval between the arrivals of the two effects was shorter in all the responses, except the very last, than it had been in the records taken before the administration of the drug, the probable cord delay being  $20\sigma$  or  $19\sigma$  instead of the  $23\sigma$ .

Three other experiments [Nos. 16, 42, and 48 (the part of it referred to on p. 13)], all made very soon after injecting the strychnine and when its action was incipient, further lend some support to the view that strychnine, so long as it is affecting the spinal cord alone, tends to somewhat shorten the delay in it in the same-limb reflex, in so far as they all show that the reflex time gets shorter as the time the drug has had to take effect becomes longer.

In four experiments [Nos. 8 (both sides in turn), 38, 46, and 54], all made very soon after the administration of an extremely weak dose, one would not have known from the records that any strychnine had been administered at all: that is to say, the reflex effect was no stronger nor longer than usually obtained from a normal cord. The probable cord delay varied about  $14\sigma$  in the first two, about  $16\sigma$  in the third, and about  $21\sigma$  in the fourth. There was one record obtained with the second muscle in Exp. 8, in which the reflex response was slightly but decidedly stronger than in the rest, and in this one it occurred  $2\sigma$  sooner than in any of the others. Moreover, the delay was  $1\sigma$  longer in the two responses which showed a reflex effect with the first muscle in Exp. 8, than it was in any of the four taken with the second.

Taking all these facts into consideration, I think the conclusion is warranted that strychnine, when it is producing no other effect than one on the excitability of the cord, does not greatly alter the length of time which elapses there in such a simple reflex as the one with which we have hitherto been dealing, but that it does tend to reduce this time slightly, i.e. by one- or two-thousandths of a second. Only when the heart also has been affected by the drug is the time lengthened, owing to insufficiency in the supply of oxygen to the cord or to some other consequence of defective circulation, the impulses arising along the afferent nerve fibres taking then a much longer time to be transmitted to their respective motor cells or else the motor cells taking a longer time to react to their several impulses, some perhaps taking longer than others.

It was particularly when using weak stimuli to excite the dorsal roots, stimuli too weak to have produced any effect at all had the cord been normal, that Wundt found the reflex mechanical latency in strychnine preparations so very much prolonged, it becoming sometimes as much as six or even ten times as long as in normal preparations. The variation of latency with strength of stimulus appears indeed from his curves to become

very marked under the influence of strychnine. It is therefore at first sight somewhat surprising to find that the measurements of my records, examples of which are given in the protocols on pp. 35 to 48, give no more evidence of cord delay varying with strength of stimulus than do those obtained with normal cords. Thus, while in six experiments [16, 40, first part of 42 (p. 28), 46 (which, however, was one of those in which the records give so little evidence of the presence of strychnine), 52, and 53] made with the strength of the induction current kept constant throughout, the cord delays varied in successive responses by as much as from  $2\sigma$  to  $4\sigma$ , they varied hardly at all in five other experiments [14 L, end part of 42, 47, 49 R, and 56 R] made with the strength of the stimulus varying a good deal, even though, as in the three last of these, the strengths used to obtain some of the responses cannot have been much greater than just sufficed to produce a reflex effect at all. It is true that in 56 L the one response recorded with the weakest stimulus had the longest cord delay ( $1.2\sigma$  longer than the rest), and it is also true that in Exp. 14 L (see p. 21) a strong stimulus did on one occasion evoke a response in a time that was  $2\sigma$  shorter than it was on any other occasion; but these are isolated records, and in Exp. 14 L a stimulus equally strong was applied on three other occasions without the delay being shorter than it was with weaker stimuli.

My records, therefore, furnish no evidence of an inverse relationship between cord delay and strength of stimulus, even in preparations in which the effect of strychnine on the cord is well marked, so long as the strength has a certain value which is not far above the threshold value. The strength may be doubled, trebled, or even increased to four or five times its value without altering the cord delay (see footnote to p. 54). But although there can be no doubt as to what is the case with stimuli above a certain strength in relative value, we find ourselves on far less certain ground when we try to discover experimentally what happens when the strength of the stimulus is almost at its threshold value. For here we are met with difficulties as great as those met with in making the same investigation in the normal cord, although they are of another kind. While strychnine cords have undoubtedly so far the advantage over normal ones, for the investigation of the influence, if any, exerted by this and other physical conditions, that any one of them will give as a rule a comparatively large number of responses, they have this disadvantage for the investigation of the particular question as to the influence of strength of stimulus, that the threshold value, i.e. the strength of the stimulus just sufficient to produce an effect, is apt to change during the experiment. This was the case most strikingly in Exp. 55, in which stimuli at first too weak to produce any effect at all became capable of doing so in the course of the experiment. The cord delay, as the table shows, varied throughout a good deal in the different responses, but did not do so in any definite regular relation to the strength of stimulus evoking them, near as this must have been at times to the threshold value.

In spite of this fact the records do, however, it seems to me, afford evidence that there is a region near the threshold where strength of stimulus and synapse delay vary inversely, and that they at the same time suggest an explanation for any discrepancy there may appear to be between such observations as those of Wundt (which have been confirmed in mammals by Sherrington, H. Franek, and others) and mine. I have already mentioned (p. 19) that in so many of the responses in this preparation (as well as in certain others) the reflex electrical effect does not become maximal until some time after its first appearance; and if we grant, as we shall presently find reason for doing, that only a single set of synapses is concerned in the reflex we are here considering, this fact, to my mind, can only mean that there was great want of co-ordinate action amongst the motor cells, due to variations obtaining either in the times taken to pass individual synapses, or in the promptitude with which individual motor cells were reacting. Whichever of these two factors determining what I call synapse delay it may be, the few motor cells which first come into play would control only a certain number of the fibres under the recording spots, not a sufficient number for the whole central stimulus to produce an electrical effect there even equal in amount to the small one which may generally be obtained from a normal cord (to a stronger peripheral stimulus), and not a sufficient number, I think we may add, to overcome the resistance of the rest of the muscle, and cause the whole to contract. Only when the other synapses too have been crossed and the other motor cells have reacted, are all the fibres excited together (the prolongation of the discharge so characteristic of the action of strychnine having brought it about that the cells belonging to the few synapses which were passed first were still discharging at the time the others began to be affected later), and then only does the electrical response attain its maximum; and only when a sufficient number of muscle fibres are brought into play to overcome the resistance of the rest of the muscle would the mechanical response begin.

Since the strength of the stimulus was the same for all, this great variability in the delay at the individual synapses of the same set can only have been due, it seems to me, to great variability in the thresholds, i. e. in the resistances, of the separate synapses. For a few of them the stimulus possesses that strength which by analogy with what is known for muscle and nerve we may call "maximal"; for the greater number it possesses submaximal values. We know that in this preparation the thresholds for those synapses or cells which were most readily affected were changing after the experiment had begun. It is no great assumption to make that others at the end of the experiment had the same threshold which those had had at the beginning, and that these others at the beginning had thresholds lower than those had then. In seeking for the cause of this variability we are struck by the fact that the phenomenon in our records which indicates it (the long delay after the response has begun before it

produces its maximal electrical effect) is only at all commonly present in the responses of those preparations in which not only the cord but the circulation was affected by the drug, and in which (perhaps, to some extent, consequently<sup>1</sup>) all the muscles were in spasm, and in which cord delay (to all strengths of stimulus) was longer than in normal, and still more so than in lightly strychnised, preparations. What strychnine acting on the cord alone effects, appears to be counteracted in certain respects by what strychnine acting on the circulation, and hence indirectly on the cord, effects. We have seen that this is the case as far as cord delay is concerned in the reflex we have so far been considering. It will be still more striking in the reflex we shall next have to consider, and for this second more complex reflex we shall see this conflict between the direct and indirect effect of strychnine on the cord showing itself perhaps in another way also. The cord of the decerebrate frog used in Exp. 55 had been for some hours under the predominating influence of the indirect action of strychnine. By the time the preparation was made, that effect showed signs of wearing off, and it would appear that the more specific action of the drug upon the cord—the lowering of the threshold at any rate—was beginning to re-assert itself, but it had only as yet done so for some of the synapses concerned in the response of this particular muscle, whatever it may have done for others, or possibly the same, synapses concerned in the responses of other (e.g. antagonistic) muscles.<sup>2</sup> The fact that the temperature of the room was rising while this experiment was being made probably contributed to bringing about variability of threshold, since there can be no doubt that strychnine is much more effective at higher temperatures than at lower.

A record such as the one reproduced in fig. 11, C, seems to me, therefore, to show that synapses with higher resistances, i.e. those which required a stronger afferent stimulus to be forced at all, took longer (up to as much as  $20\sigma$  longer) to be passed by impulses hardly above their threshold values in strength than did those with lower resistances for which an afferent stimulus of the same strength would have a higher value; and that thus variations produced in the thresholds of the structures excited have given us information that we could not succeed in obtaining by altering the strength of the exciting stimulus. The question, however, as to whether we may apply this information to a more normal, even though strychnised, animal, remains. It may be that it is only in an animal with circulation

<sup>1</sup> I would here refer to Bethe's observations, in which "Erstickungskrämpfe" (p. 248), "welche sich von Strychnintetanus nicht unterscheiden lassen" (p. 247), are obtained in frogs under conditions which he regards as signifying increased consumption, and therefore requirement, of oxygen (Rosenthal's Festschrift, 1906).

<sup>2</sup> It may well be that this want of co-ordinate action of the synapses concerned in the contraction of one muscle, which seems so often to prevail when strychnine spasms are at their height (I have other examples of the phenomenon indicating it in records taken years ago), is correlated with the opening of paths to other muscles which are normally closed by the high resistance of the synapses or the parts of the synapses (if we regard as a whole synapse, the boundary, or gap, between all the afferent fibre-terminations, and the one motor cell they adjoin) concerned in their contraction, and normally obtaining when the resistance in the first synapses, or parts of synapses, was low.

impaired that synapse time is eventually prolonged by insufficient strength of stimulus, or it may be that the impairment has only emphasised something which does actually hold true for a normal, or more normal, animal, but on a smaller scale, the difficulty in discovering it being in such greater restriction of the threshold region.

I would suggest therefore that in all responses in any of my preparations the records of which showed that the maximal effect was not developed until late, i.e. that the total central stimulus was not exerting its full force at the beginning, there was want of accord in the time taken to pass the several synapses, the greatest effect not being produced until all that can be passed have been passed, and all the muscle fibres belonging to them affected; and that this want of accord is brought about not by the direct action of strychnine on the cord, but by its indirect action.

The fact that, as a rule, when strychnine is affecting the cord only, the effect at the recording spots after the administration of the drug is as great, and becomes so in as short a time, as in the case of the direct response, and that it is sometimes even greater than it (some of the responses in Exps. 45, 47, 49 L, and 56 R), together with the fact that no increase in the strength of the stimulus to the normal cord will make the reflex effect larger than it otherwise is, seems to me, on the other hand, to indicate that the smallness of the reflex response which is usual in a normal preparation is not due to a smaller number of fibres being excited than by the direct stimulus, but rather to the effect produced in each being smaller. In this connection it should be mentioned that the reflex effect, as it is seen in records taken with the normal cord, takes very nearly, if not exactly, the same time to become maximal as the direct effect.

The influence of altering the temperature of the back of the preparation, and consequently of the cord, is well marked after the administration of strychnine. This is shown by the experiment of which the data are given on the following page.

Exp. 42. Nov. 15, 1906. Room temp. 15° C. One minim 0.01 per cent. liq. strychn. injected one hour before making the preparation.

Induction current to nerve.		Length, in millimetres, of			Time, in thousandths of a second ( $\sigma$ ),			
Strength.	Direction.	muscle from entrance of nerve to <i>p</i> .	nerve from <i>Cu</i> electrode to muscle.	nerve from <i>Cu</i> electrode to cord.	taken by impulse to reach <i>p</i> directly (measured).	interval between arrivals of direct and of reflex effects at <i>p</i> (measured).	to be deducted for transmission in nerve (assumed).	Probable delay in cord.
10,000	<i>d</i>	10	14	35	4.2	13.2	2.3	10.9
10,000	<i>d</i>	10	14	35	4.2	14.4	2.3	12.1
5,000	<i>d</i>	10	14	35	4	no reflex effect		
10,000	<i>d</i>	10	14	35	4.2	14.4	2.3	12.1
10,000	<i>d</i>	10	14	35	4.2	13.2	2.3	10.9
10,000	<i>d</i>	10	14	35	4.2	12.1	2.3	9.8

Bag of ice then put on the back of preparation for five minutes.

10,000	<i>d</i>	10	14	35	4.2	23	2.3	21.7
10,000	<i>d</i>	10	14	35	4.2	24	2.3	22.7
10,000	<i>d</i>	10	14	35	4.2	20.7	2.3	18.4
5,000	<i>d</i>	10	14	35	4.2	22.2	2.3	19.9
10,000	<i>d</i>	10	14	35	4.2	21	2.3	18.7

Ice-bag removed. A second minute dose of strychnine given at the same time (in the hope of securing responses to the excitation of the other sciatic also), which fact may (though I do not think it does) detract from the value of the results obtained from the records taken five minutes later.

10,000	<i>d</i>	10	14	35	4	13.1	2.3	10.8
10,000	<i>d</i>	10	14	35	4	14.3	2.3	12
5,000	<i>d</i>	10	14	35	4	14.3	2.3	12
4,000	<i>d</i>	10	14	35	4	14.4	2.3	12.1
5,000	<i>d</i>	10	14	35	4	13.4	2.3	11.1
10,000	<i>d</i>	10	14	35	4	12	2.3	9.7

It is also shown in Exp. 48, of which the data, when the whole preparation was at room temperature, have already appeared in tabular form on p. 13, and of which most of the data, after the back had been cooled, appear on the left-hand side in the table p. 41.

A number of experiments I made some years ago for another purpose, and in which the reflex effect was produced by excitation of the skin, not of nerve, show the same sort of thing. To these I shall have to refer in another connection on another occasion. What is very striking in the two recent experiments is that in the one case [Exp. 42] the cord delay is almost exactly doubled by cold, in the other [Exp. 48] almost exactly trebled. But whether any significance should be attached to these facts the evidence is not as yet great enough to decide. The further question suggests itself as to whether the influence of cold is a direct one on the cells (or the

synapses) of the cord itself, or whether it is some indirect one, as, for instance, on the oxygen supply to the cells.

The fact that strychnine acts on cord delay in the opposite direction to that in which fatigue would act, creates a difficulty in studying the influence of this factor in strychnine preparations. Although Exps. 40 and 45, and the comparison of the two opposite same-limb reflex times in Exp. 56, show that the times got longer as the experiment went on, both Exps. 45 and 55 show that it was not shortened by rest; so that I do not think we should be justified in straightway attributing the increase of length in the three experiments just mentioned to fatigue in the sense in which this word is commonly employed. We shall see that this seems to be otherwise in the case of the more complex reflex which we are going to consider immediately.

A way of investigating the effect of fatigue on cord delay, or rather, as I should prefer to call it, on synapse delay, which seems to me likely to be fruitful, is that of recording long serial responses obtained from well-strychnised preparations, and measuring the time intervals between successive periods. For whatever is the ultimate source of the impulses which give rise to the second and following periods,<sup>1</sup> they must each in turn cross the synapse forced in the first instance by the impulse transmitted through the afferent nerve. It is well known that the periods may get longer towards the end of such a response, if this is a long one; but what determines the duration of the response requisite for them to do so has not yet been sufficiently studied.

#### IV. THE SAME-LIMB REFLEX TIME IN A CORD THE EXCITABILITY OF WHICH HAS BEEN RAISED BY PHENOL.

I have only as yet made two experiments with this drug.<sup>2</sup> As, however, both show that the cord delay in the reflex we are studying was of the same order as in the normal cord or in the strychnine cord, I think they are worth referring to. Ten minims of a 0.1 per cent. solution, containing therefore about 0.6 mgr., were injected subcutaneously two or three hours before making the experiment. In the one preparation a reflex effect was

<sup>1</sup> I still hold that this is to be sought in the central organ itself, and not in the peripheral organs, as Baglioni (*loc. cit.*, and *Z. f. allg. Physiol.*, ii. p. 556, 1903) believes. Besides the evidence against Baglioni's view brought forward by Sir J. Burdon-Sanderson and myself (*loc. cit.* and *Physiol. Centralbl.*, 1902), there is the further objection that the time between two successive periods is often not long enough (it may be as short as 0.05 second) for an impulse not only to have got to the muscle and back to the cord, then back again to the muscle, but for it to have made the muscle contract to such extent the first time as to excite the sensory organs in it (or its tendon) and start the impulse back to the cord. Moreover, by Baglioni's method of treatment of the cord it is difficult to believe that impairment of circulation would not have made cord delays longer than usual. I reserve, however, the discussion of the question for a later paper. As the text shows, it is to something in the cord, other than the motor cell, that I would attribute the periodicity.

<sup>2</sup> Since this paper was written I have made several, but as they all confirm what is said here (and on pp. 31 and 61), and as this paper is already too long, I must leave what they have further to tell for a future communication.

seen from the records to have arrived at the first recording spot of the muscle  $20\cdot2\sigma$ ,  $20\cdot5\sigma$ ,  $20\cdot7\sigma$ ,  $20\cdot1\sigma$ ,  $19\cdot1\sigma$ , and  $24\cdot4\sigma$  after the direct effect, in the successive responses to stimuli applied to the sciatic nerve of the same side, of respective strengths 10,000, 5000, 3000, 5000, 3000, 10,000. A record of one of these (the fourth) is reproduced in fig. 13, A (see p. 62). Above (fig. 13, B) may be seen the record of the response of the same muscle obtained later, when the peripheral end of the sciatic nerve was excited after severing it from the cord. All the records taken with the nerve intact very closely resembled one another.

In the other preparation the reflex responses occurred  $15\sigma$ ,  $15\cdot6\sigma$ ,  $15\sigma$ , and  $25\sigma$  after the direct response, the strengths of the stimuli being respectively 10,000, 3000, 10,000, 10,000 units. The direct and reflex responses very closely resembled one another, and were about equal in strength; both were weak in the last response, strong in the others.

#### V. THE CROSSED-REFLEX TIME.

I have not yet succeeded in getting from a normal cord a true crossed-reflex effect in response to a single induction shock, i.e. a reflex effect in one gastrocnemius<sup>1</sup> when the sciatic nerve of the opposite side was stimulated, although I have tried to get it often in well-cooled decerebrate frogs. Even from the one normal cord, which gave strong same-side reflex responses (fig. 3), no crossed-reflex effect, either mechanical or electrical, could be evoked by such stimulus. I have, however, not infrequently obtained, when very strong induction shocks have been used to excite the sciatic nerve (so strong as to be easily felt on the tongue), responses, mechanical and electrical, in the gastrocnemius of the opposite side, which I might have mistaken for reflex responses, had I not already acquired information about these when the nerve of the same side is excited. Had I only been able to record the mechanical response and to observe the electrical one, it would have been very difficult to know whether they were or were not reflex. The developed photographs, however, showed that the record of the electrical response in these cases was almost precisely identical with the direct effect obtained in response to stimulation of the same-side sciatic, and that it occurred but 1 to  $1\cdot5\sigma$  later than this direct response, and consequently long before any reflex response to excitation of the nerve

<sup>1</sup> Nor in the semitendinosus, nor the biceps femoris (see, however, footnote to p. 59), both of which muscles I have now used a good deal for my experiments (in the summer and autumn of 1907), Professor Sherrington having pointed out to me that muscles which are wholly flexor would be more likely to respond to reflex excitation than the gastrocnemius. The records of electrical responses taken with these two muscles afford confirmatory evidence of all the conclusions come to from experiments made with the gastrocnemius mentioned in this paper. As one would expect, they show that the response is purely reflex (and not preceded by any direct effect) when the sciatic nerve of the same side as the recording thigh muscle is excited near the knee, although it sometimes happens that the conditions become, or can be made, favourable for the appearance in the electrical response of the counterpart of what in a mechanical response is known as "paradoxical contraction," and this then precedes the true reflex effect.



of the same side. In more than one excitable preparation, such a response was obtained by merely removing the large resistance in the secondary circuit of the current used to excite the nerve of the opposite side, or by reducing it to one of not more than 10,000 ohms, if the secondary coil were right up or nearly so. The difference of time in such response, according as the nerve of the same side or that of the opposite side was stimulated, is about that which would be taken for an impulse to traverse between 30 to 45 extra mm. of nerve, and the only suggestion I can make as to what happened when such abnormally strong exciting currents were used is that these escaped to some nerve fibres in connection with the motor cells, or nerve, of the opposite side. The same effect can be produced not only by using abnormally strong induction currents, but also by making something in or about the ventral part of the cord, according to Baglioni<sup>1</sup> the motor cells, abnormally excitable, by the injection of phenol into the circulation. In the two experiments I have as yet made with such phenol preparations, records taken of responses to stimuli not quite but very nearly strong enough to be felt on the tongue, applied to the sciatic nerve of the opposite side, all showed, and in both preparations, besides the true crossed-reflex effect (which I shall have to refer to immediately), an effect occurring but  $1\sigma$  later than the direct effect as obtained by excitation of the same-side sciatic. This had every appearance of being a direct effect itself, and I cannot conceive that it was anything else, in spite of the fact that it was produced by stimulation of the nerve of the opposite side. When the exciting current was weakened, the true crossed-reflex effect appeared as before, and was equally strong, but it appeared alone. I have not, as yet, further investigated the phenomenon, because it has seemed to me that all the evidence obtained by the use of stimuli more nearly approaching, though still exceeding in strength, the stimuli which must occur in nature, goes to show that no physiological interest would be furthered by so doing. That some morphological or even pathological interest would be served thereby seems to me, on the other hand, to be not at all improbable, but it is not with such interests that we are, for the moment, concerned.

I think there can be no doubt that what Rosenthal<sup>2</sup> described as a crossed-reflex contraction to be obtained only with very strong exciting currents, and occurring at the same time as, or sometimes even before, what he considers to be the same-side reflex (whether or not it was so), was nothing but such a direct effect due to the abnormal strength of the current. There is nothing in Wundt's treatise to show that he ever obtained a crossed-reflex contraction without the use of strychnine. The consideration of the conclusion that he came to that the crossed-reflex time was only  $4\sigma$  longer than that of the same-side reflex ("uncrossed," as we may now, if we

<sup>1</sup> Baglioni, A. f. (Anat. u.), *Physiol.*, 1900, Supplement.

<sup>2</sup> Rosenthal, *Abh. Berliner Akad.*, 1873, p. 104. The short mechanical latencies observed by François Franck in what he considers to be the crossed reflex in the guinea-pig, when very strong electrical stimuli were used, would fall, I believe, into the same category.

like, call it), may therefore be postponed until the results obtained from my own experiments on strychnine frogs have been stated. To these we may now turn.

Even in preparations in which the excitability of the cord has been raised by strychnine sufficiently to make certain of the presence of the same-limb reflex effect in the records of the electrical responses, it is by no means certain that a reflex effect will be also obtainable when the nerve of the opposite side is stimulated by a single break induction shock. There is, however, every likelihood of obtaining a response to such a stimulus when the strychnine has been allowed a certain time (varying with temperature, season, dose, etc.,) to act, and there is hardly any doubt about obtaining it in a preparation which has been injected several hours before and is either still showing in its attitude all the external symptoms of strychnine poisoning, or has begun to recover.

The crossed-reflex response has been recorded in sixteen preparations, many of which have already been referred to in connection with the same-side reflex. In all, the sciatic nerve of the opposite side was prepared, usually before beginning the experiment, in exactly the same way as its fellow, and a second pair of needle electrodes was applied to it. A Pohl reverser without crossed wires in the secondary circuit made it easy to excite the two nerves alternately.

The measurements obtained from the records of the several responses, and the probable cord delay estimated therefrom in each, are given for thirteen of the experiments on the right-hand side in the tables beginning on p. 35, which are so arranged that the time, measured and estimated, in any crossed-reflex response may be readily compared with that, measured and estimated in the same way, in the same-side reflex response, recorded immediately before or after it, and given on the left-hand side.

In order to estimate the cord delay when the nerve of the opposite side was stimulated, I have subtracted from the whole measured time, firstly, the time known, from the measurement of the same-limb reflex record, to be taken by an impulse to reach the recording spot on the muscle directly (i.e. through the motor part of the nerve) from the near anode, and, secondly, the time which would have been taken to traverse the measured length of nerve from the far anode to the near one, on the assumption that it travels at the rate of 30 metres per second. The first of these time values would be the same as when the same-limb reflex was recorded, provided that there had been no obstruction at the kathode to be overcome at the time this was taken, in which case allowance would have to be made for the fact. The second of these time values would also be very nearly the same in the two kinds of responses, the distance up one nerve and down the other as far as the near anode being, if the needles were on corresponding parts, just about the same as the distance from the near anode to the cord and back, and the time taken to traverse it only being different if very strong ascending currents were being used. In the last column of the table, I have given

the extra delay in the case of the crossed reflex as it appears to have been in each response. It is estimated by the subtraction of the cord delay which is shown to have probably occurred in the case of the immediately preceding or immediately following same-limb reflex response, from the probable cord delay in the case of the crossed reflex. I have indicated, by bracketing the lines representing them together, which same-limb reflex response I have employed for the purpose in each case.

Two of the experiments in which also crossed-reflex responses were obtained, but which do not appear in the tables, may be first referred to. The one [Exp. 38] was one of the four already mentioned (p. 23) as showing no sign of the effect of strychnine in the records, electrical or mechanical, of the same-side reflex responses. It was one in which an extremely minute dose (0.012 mgr.) had been injected only a quarter of an hour before records began to be taken. After two had been taken with the sciatic nerve of the same side as the recording muscle excited, which subsequently showed on measurement that the probable cord delay had been in the two responses respectively  $13\sigma$  and  $14.5\sigma$ , a third record was taken with the opposite sciatic nerve excited. A reflex response extremely weak, but lasting about twice as long as when obtained by the excitation of the other nerve, was recorded; but measurement showed that it did not occur until  $83\sigma$  after excitation, the cord delay being therefore  $63.4\sigma$  ( $83\sigma - 3.5\sigma - 1.6\sigma - 14.5\sigma$ ) longer than in the simpler reflex. The crossed-reflex response could not be obtained a second time, although three more records, taken when the first nerve was excited, showed that the same-side reflex response was still being obtained unaltered, and with an unaltered probable cord delay, this being respectively in the three:  $14.5\sigma$ ,  $14\sigma$ , and  $13.8\sigma$ . From none of the other three preparations, in which, though strychnine had been given, there was no evidence of the fact in the records of the same-side reflex response, could a crossed-reflex response be obtained at all.

The second experiment, which may be described at once [Exp. 33], was one made on a small frog into which five times as much strychnine had been injected as in the one just mentioned, after the muscle and nerves had been prepared and the response without strychnine recorded. Almost immediately after the injection one good record was obtained of the same-side reflex and one of the crossed-reflex response. These are reproduced in fig. 5. The upper curve (A) represents the response when the nerve of the same side was excited. It shows first the response to direct excitation of the motor nerve, which was in this preparation again double, then that to reflex excitation ( $24.7\sigma$  later). The lower curve (B) is the response of the same muscle when the sciatic of the opposite side was excited. The impulse took rather more than twice as long to reach the muscle ( $54.5\sigma$ ), but when it began to affect it, it affected it much in the same way. There was an unavoidable delay of 10 to 15 minutes before the experiment could be continued. It was then seen that both reflex electrical responses had become serial. Their records showed that both had become

very feeble, but that the crossed-reflex effect manifested itself  $20\sigma$  earlier than before. The probable cord delay in the case of the same-side reflex was still  $22.7\sigma$  ( $24.7\sigma - 2.0\sigma$ ); in the case of the crossed reflex, it had been at first  $47.9\sigma$  ( $54.5\sigma - 4.8\sigma - 1.8\sigma$ ), and was now about  $28\sigma$ , the extra delay in its case having been at first  $25.2\sigma$ , then, after the dose (which was a good deal stronger than was usually employed for a frog of such small size)



FIG. 5.—Electrical responses of the gastrocnemius of a small frog which, after being prepared and immediately before records began to be taken, had been injected with 1 minim 0.1 per cent. liquor strychniæ (Exp. 33).

A, first response obtained when the intact sciatic nerve of the same side was excited. [Time lines 710 per second.] B, First response obtained when the sciatic nerve of the opposite side was excited. [Time lines 700 per second.]

had so taken effect as to produce one of the best known symptoms of the action of strychnine, becoming about  $5\sigma$ .

Before discussing the change which took place in the course of this particular experiment, it will be well to consider what happened in other preparations which were made either a longer time after the injection of a very minute dose of the drug, or still early, but with a more moderate dose than in the second of the two experiments just referred to, and which yielded crossed-reflex responses more frequently than only once or twice. It is to thirteen of these that the following tables refer.

EXP. 4. (L. Gastroc.). Sept. 17, 1906. Room temp. 16° C. Large frog injected with  $\frac{1}{2}$  minim 0.1 per cent. liq. strych. two hours before; spasms now general when any part of skin touched. The right gastrocnemius had already given a few weak same-side reflex responses, which were recorded, but no crossed response could be obtained from it, nor could this be obtained at first with the second muscle.

Induction current to nerve.		Time, in thousandths of a second, in									
Strength.	Direction.	Same-limb reflex.					Crossed reflex.			Extra delay in the case of the crossed reflex.	
		Measured time taken by impulse to reach <i>p</i> directly.	Measured time interval between arrivals of direct and reflex effects.	Assumed time to be deducted for transmission in nerve.	Probable cord delay.	Measured time taken by impulse to reach <i>p</i> .	Time to be deducted for transmission from <i>Cu</i> electrode on opposite side to <i>p</i> (measured).	Time to be deducted for transmission in nerve from one <i>Cu</i> electrode to the other (assumed).	Probable cord delay.		
{	12,000	<i>a</i>	...	...	...	...	30	3.7	2.6	23.7	13.9
	10,000	<i>a</i>	...	...	...	...	25	3.7	2.6	18.7	8.9
	10,000	<i>d</i>	3.7	12.5	2.7	9.8	...	...	...	...	...
	10,000	<i>a</i>	...	...	...	...	27	3.7	2.6	20.7	10.9
	10,000	<i>d</i>	3.7	12.5	2.7	9.8	...	...	...	...	...

All the responses, direct and reflex, were weak; the reflex ones were prolonged, but not serial.

EXP. 16. (R. Gastroc.). Oct. 4, 1906. Room temp. 16° C. One minim 0.1 per cent. liq. strych. injected after preparation of the nerve and muscle and after taking three records with the cord normal (none of which showed a reflex effect) of responses to a fairly strong stimuli. Nor did two records show it, taken respectively five and ten minutes after the injection. Five minutes later still it was present.

Induction current to nerve.		Time, in thousandths of a second, in									
Strength.	Direction.	Same-limb reflex.					Crossed reflex.			Extra delay in the case of the crossed reflex.	
		Measured time taken by impulse to reach <i>p</i> directly.	Measured time interval between arrivals of direct and reflex effects.	Assumed deduction for transmission of nerve.	Probable cord delay.	Measured time taken by impulse to reach <i>p</i> .	Time to be deducted for transmission from <i>Cu</i> electrode on opposite side to <i>p</i> (measured).	Time to be deducted for transmission in nerve from one <i>Cu</i> electrode to the other (assumed).	Probable cord delay.		
{	10,000	<i>d</i>	3.7	17.5	1.9	15.6	...	...	...	...	...
	10,000	<i>d</i>	3.7	18.6	1.9	16.7	...	...	...	...	...
	10,000	<i>d</i>	3.7	17.5	1.9	15.6	...	...	...	...	...
	10,000	<i>d</i>	...	...	...	...	36.3	3.7	1.8	30.8	15.2
	10,000	<i>d</i>	3.7	15	1.9	13.1	...	...	...	...	...
	10,000	<i>d</i>	...	...	...	...	3.4	3.7	1.8	28.5	15.4
	10,000	<i>d</i>	3.7	13.7	1.9	11.8	...	...	...	...	...

All the electrical effects were weak. The reflex effect in the last three responses was two to three times as long as the direct effect. In the first two it was of about the same duration. The contractions were all feeble.

EXP. 18. (L. Gastroc.) Oct. 8, 1906. Room temp. 16° C. Responses of the right gastrocnemius to excitation of the right sciatic had first been recorded with the cord normal. They showed a reflex effect when the stimuli was 5000 units in strength; none when it was 1000 units. After preparing the left gastrocnemius and recording two responses to excitation of the left sciatic, neither of which (though the strengths were 5000 and 10,000 units) showed a reflex effect, 1 minim 0.1 per cent. liq. strychn. was subcutaneously injected. Records taken  $\frac{1}{4}$  hour later.

Induction current to nerve.		Time, in thousandths of a second, in								Extra delay in the case of the crossed reflex.
		Same-limb reflex.				Crossed reflex.				
Strength.	Direction.	Measured time taken by impulse to reach <i>p</i> directly.	Measured time interval between arrivals of direct and reflex effects.	Assumed deduction for transmission of nerve.	Probable cord delay.	Measured time taken by impulse to reach <i>p</i> .	Time to be deducted for transmission from <i>Cu</i> electrode on opposite side to <i>p</i> (measured).	Time to be deducted for transmission in nerve from one <i>Cu</i> electrode to the other (assumed).	Probable cord delay.	
5,000	<i>d</i>	3.7	28	2	26	...	...	...	...	...
1,000	<i>d</i>	3.7	no reflex effect		...	...	...	...	...	...
10,000	<i>d</i>	3.7	28	2	26	...	...	...	...	...
5,000	<i>d</i>	...	...	...	...	no effect		...	...	...
10,000	<i>d</i>	...	...	...	...	43	3.7	1.8	37.5	15.5
10,000	<i>d</i>	3.7	24	2	22	...	...	...	...	...
10,000	<i>d</i>	...	...	...	...	41	3.7	1.8	35.5	15
5,000	<i>d</i>	3.7	22.5	2	20.5	...	...	...	...	...
10,000	<i>d</i>	...	...	...	...	38	3.7	1.8	32.5	12

All the effects (direct and reflex) were weak.

EXP. 40. (R. Gastroc.) Nov. 13, 1906. Room temp. 12° C. One minim 0.04 per cent. liq. strychn. injected the day before making the experiment. Body still somewhat stiff; limbs no longer so. Muscles responding reflexly to slightest touch of skin.

Induction current to nerve.		Time, in thousandths of a second, in								Extra delay in the case of the crossed reflex.
		Same-limb reflex.				Crossed reflex.				
Strength.	Direction.	Measured time taken by impulse to reach <i>p</i> directly.	Measured time interval between arrivals of direct and reflex effects.	Assumed time to be deducted for transmission in nerve.	Probable cord delay.	Measured time taken by impulse to reach <i>p</i> .	Time to be deducted for transmission from <i>Cu</i> electrode on opposite side to <i>p</i> (measured).	Time to be deducted for transmission in nerve from one <i>Cu</i> electrode to the other (assumed).	Probable cord delay.	
5,000	<i>d</i>	5	22	2.3	19.7	...	...	...	...	...
5,000	<i>d</i>	...	...	...	...	48	5	2.4	40.6	20.9
5,000	<i>d</i>	5	20.5	2.3	18.2	...	...	...	...	...
5,000	<i>d</i>	...	...	...	...	46	5	2.4	38.6	20.4
5,000	<i>d</i>	5	20.5	2.3	18.2	...	...	...	...	...
5,000	<i>d</i>	...	...	...	...	55	5	2.4	47.6	21.9
5,000	<i>d</i>	5	28	2.3	25.7	...	...	...	...	...
5,000	<i>d</i>	...	...	...	...	51	5	2.4	43.6	18.1
5,000	<i>d</i>	5	27.8	2.3	25.5	...	...	...	...	...
5,000	<i>d</i>	...	...	...	...	44	5	2.4	36.6	11.1

Reflex effects less strong than the direct effect, except in the 2nd and 3rd same-side and in the 2nd crossed-reflex response. For records of first two responses see fig. 7, p. 50. The direct effect was only double the first two times it was recorded.

EXP. 42. (R. Gastroc.) Nov. 15, 1906. Room temp. 15° C. One minim 0.01 per cent. liq. strychn. injected just before making the preparation. The same-side reflex had been already recorded several times with the cord at different temperatures (see p. 28). No crossed-reflex could be obtained until a second dose (of 2 minims 0.01 per cent. liq. strychn.) had been injected, and then at first it could not be obtained with any strength under 14,000 units.

Induction current to nerve.		Time, in thousandths of a second, in								Extra delay in the case of the crossed reflex.
Strength.	Direction.	Same-limb reflex.				Crossed reflex.				
		Measured time taken by impulse to reach <i>p</i> directly.	Measured time interval between arrivals of direct and reflex effects.	Assumed time to be deducted for transmission in nerve.	Probable cord delay.	Measured time taken by impulse to reach <i>p</i> .	Time to be deducted for transmission from <i>Cu</i> electrode on opposite side to <i>p</i> (measured).	Time to be deducted for transmission in nerve from one <i>Cu</i> electrode to the other (assumed).	Probable cord delay.	
14,000	<i>d</i>	...	...	...	...	32.1	4	2.3	25.8	15
10,000	<i>d</i>	4	13.1	2.3	10.8	...	...	...	...	...
14,000	<i>d</i>	...	...	...	...	29.8	4	2.3	23.5+	11.5
10,000	<i>d</i>	4	14.3	2.3	12	...	...	...	...	...
5,000	<i>d</i>	4	14.3	2.3	12	...	...	...	...	...
12,000	<i>d</i>	...	...	...	...	28.8	4	2.3	22.5+	10.5
4,000	<i>d</i>	4	14.4	2.3	12.1	...	...	...	...	...
11,000	<i>d</i>	...	...	...	...	30	4	2.3	23.7+	11.6
5,000	<i>d</i>	4	13.4	2.3	11.1	...	...	...	...	...
10,000	<i>d</i>	...	...	...	...	28	4	2.3	21.7+	10.6
10,000	<i>d</i>	4	12	2.3	9.7	...	...	...	...	(or 12)
10,000	<i>d</i>	...	...	...	...	28	4	2.3	21.7	12
10,000	<i>d</i>	4	12	2.3	9.7	...	...	...	...	...

The crossed-reflex effects were in the first two responses very weak; they then became stronger, though not always attaining their maximal strength until late.<sup>1</sup> Their duration was somewhat shorter than that of the uncrossed-reflex effects, but, with the exception of the first two, was longer than the direct effect.

<sup>1</sup> The + when it occurs in the tables signifies that the moment at which the effect became maximal was deferred.



EXP. 45. (L. Gastroc.). Nov. 20, 1906. Room temp. 12° C. Five minims 0.005 per cent. liq. strychn. injected  $\frac{1}{2}$  hour before. Records had been taken of the electrical responses of the right gastrocnemius both before and after the injection. No cross-reflex response had been obtainable with that muscle.

Induction current to nerve.		Time, in thousandths of a second, in								
Strength.	Direction.	Same-limb reflex.				Crossed reflex.				Extra delay in the case of the crossed reflex.
		Measured time taken by impulse to reach <i>p</i> directly.	Measured time interval between arrivals of direct and reflex effects.	Assumed time to be deducted for transmission in nerve.	Probable cord delay.	Measured time taken by impulse to reach <i>p</i> .	Time to be deducted for transmission from <i>Cu</i> electrode on opposite side to <i>p</i> (measured).	Time to be deducted for transmission in nerve from one <i>Cu</i> electrode to the other (assumed).	Probable cord delay.	
{ 12,000	<i>d</i>	...	...	...	...	39.3	5	2.5	31.8	11.8
{ 12,000	<i>d</i>	5	22.5	2.5	20	...	...	...	...	...
{ 10,000	<i>d</i>	...	...	...	...	39.3	5	2.5	31.8	12.9
{ 10,000	<i>d</i>	5.2	21.4	2.5	18.9	...	...	...	...	...
{ 10,000	<i>d</i>	...	...	...	...	39.3	5	2.5	31.8	12.3
{ 10,000	<i>d</i>	5.5	22	2.5	19.5	...	...	...	...	...
{ 10,000	<i>d</i>	...	...	...	...	36.9	5	2.5	29.4	10.5
{ 10,000	<i>d</i>	5.5	21.4	2.5	18.9	...	...	...	...	...
{ 10,000	<i>d</i>	...	...	...	...	36.9	5	2.5	29.4	10.5
Ten minutes later:—										
10,000	<i>d</i>	5.3	27.5	2.5	25	...	...	...	...	...
10,000	<i>d</i>	...	...	...	...	no effect	...	...	...	...

The same-side reflex effects were of about the same strength and hardly longer than the direct effect. The cross-reflex effects were weak throughout. For records of the 1st and 2nd responses see fig. 8 (B and A); of the 7th and 8th, fig. 8 (D and C), p. 51.

EXP. 47. (R. Gastroc.). Nov. 23, 1906. Room temp. 16° C.; moist chamber kept at 14° C. Two minims 0.01 per cent. liq. strychn. injected  $\frac{1}{4}$  hour before preparing the muscle and nerve. No crossed effect could be obtained at first with a stimulus under 14,000 units in strength.

Induction current to nerve.		Time, in thousandths of a second, in								
		Same-limb reflex.				Crossed reflex.				Extra delay in the case of the crossed reflex.
Strength.	Direction.	Measured time taken by impulse to reach <i>p</i> directly.	Measured time interval between arrivals of direct and reflex effects.	Assumed time to be deducted for transmission in nerve.	Probable cord delay.	Measured time taken by impulse to reach <i>p</i> .	Time to be deducted for transmission from <i>Cu</i> electrode on opposite side to <i>p</i> (measured).	Time to be deducted for transmission in nerve from one <i>Cu</i> electrode to the other (assumed).	Probable cord delay.	
{ 10,000	<i>d</i>	4.2	19.8	2.5	17.3	...	...	...	...	...
{ 14,000	<i>d</i>	...	...	...	...	35.1	4.2	2.3	28.6	11.3
{ 10,000	<i>d</i>	4.2	19.8	2.5	17.3	...	...	...	...	...
{ 14,000	<i>d</i>	...	...	...	...	35	4.2	2.3	28.5	11.4
{ 10,000	<i>d</i>	4.8	18.3	+0.6	16.4	...	...	...	...	...
{ 10,000	<i>d</i>	...	...	-2.5	...	33.2	4.2	2.3	26.7	10.3
{ 5,000	<i>d</i>	4.2	19.5	2.5	17	...	...	...	...	...
{ 9,000	<i>d</i>	...	...	...	...	31.2	4.2	2.3	24.7	7.7
{ 5,000	<i>d</i>	4.5	19.5	2.5	17	...	...	...	...	...
{ 8,000	<i>d</i>	...	...	...	...	29.5	4.2	2.3	23	6
{ 4,000	<i>d</i>	4.6	20	2.5	17.5	...	...	...	...	...
{ 7,000	<i>d</i>	...	...	...	...	30.2	4.6	2.3	23.3+	5.8
{ 3,000	<i>d</i>	4.6	19.5	2.5	17	...	...	...	...	...
{ 6,000	<i>d</i>	...	...	...	...	31.5	4.6	2.3	24.6	7.6

The records show that the same-side reflex response was, to begin with, somewhat weaker, but of about the same duration as the direct response; but that it soon became as strong, and four times, then six or seven times, as long. The direct response was reduced in strength by the weakening of the stimulus to 4000 and 3000, while the reflex response was not; so that this became finally not only longer, but stronger than the direct response.

The crossed-reflex effect in the 1st response was very feeble. In the two next there was apparently a second stimulus of central origin, affecting the muscle 47 $\sigma$  and 37 $\sigma$  respectively after the 1st. There was this again in the last response but one (the 6th), whereas the only same-side reflex responses which showed it were the 4th and 6th. In the 3rd, 4th, 5th, and 6th responses the crossed-reflex effect was quite as strong as that of the same-side reflex, although in the 6th it did not attain its maximum until some 11 $\sigma$  after it had begun to be effectual. Its duration was shortest, being about twice that of a direct effect, in the 4th.

For records of the 5th and 10th responses (of the whole series) see fig. 9. p. 52.

EXP. 48. (R. Gastroc). Nov. 27, 1906. Room temp. 16° C. (see p. 13). No reflex responses could be obtained to excitation of the nerve of the opposite side, even with strength of current 14,000, while the responses to excitation of the nerve of the same side of which the time measurements are given on p. 13, were showing quite strong reflex effects. It was even then very small, but two records of it were taken.

Induction current to nerve.		Time, in thousandths of a second, in								
Strength.	Direction.	Same-limb reflex.				Crossed reflex.				Extra delay in the case of the crossed reflex.
		Measured time taken by impulse to reach <i>p</i> directly.	Measured time interval between arrivals of direct and reflex effects.	Assumed time to be deducted for transmission in nerve.	Probable cord delay.	Measured time taken by impulse to reach <i>p</i> .	Time to be deducted for transmission from <i>Cu</i> electrode on opposite side to <i>p</i> (measured).	Time to be deducted for transmission in nerve from one <i>Cu</i> electrode to the other (assumed).	Probable cord delay.	
14,000	<i>d</i>	5	12.5	1.7	10.8	...	...	...	...	...
10,000	<i>d</i>	5	12.5	1.7	10.8	...	...	...	...	...
14,000	<i>d</i>	...	...	...	...	35	3.7	2.6	29.7	18.9
14,000	<i>d</i>	...	...	...	...	31.5	3.7	2.6	25.2	14.4
Bag of ice then put on the back of the preparation for $\frac{1}{4}$ hour.										
{ 14,000	<i>d</i>	...	...	...	...	72.8	3.7	2.6	66.5+	34
{ 10,000	<i>d</i>	5	33.3	1.7	31.6	...	...	...	...	...
{ 10,000	<i>d</i>	...	...	...	...	72.8	3.7	2.6	66.5	33.7
{ 8,000	<i>d</i>	5	34.5	1.7	32.8	...	...	...	...	...
{ 10,000	<i>d</i>	...	...	...	...	73.4	4	2.6	66.8	35
{ 10,000	<i>d</i>	5.5	33.3	1.5	...	...	...	...	...	...
{ 10,000	<i>d</i>	...	...	...	31.8+	74.1	4	2.6	67.5	32.8
{ 10,000	<i>d</i>	6	36	1.3	34.7	...	...	...	...	...
{ 10,000	<i>a</i>	4.5	37.5	4	33.5	...	...	...	...	...
{ 14,000	<i>a</i>	...	...	...	...	79.4	4	2.6	72.8+	39.3 or 40.8
{ 14,000	<i>a</i>	4.5	36	4	32	...	...	...	...	

The two kinds of reflexes closely resembled one another in their effects after the cord had been cooled. None of the effects were strong, and in the 1st and 5th crossed-reflex responses and in the 3rd uncrossed one, the maximal strength was not attained at once.

EXP. 49. (R. Gastroc.) Nov. 30, 1906. Room temp. 15° C. One and a half minims 0.01 per cent. liq. strychn. injected the day before. Recovery almost complete.

Induction current to nerve.		Time, in thousandths of a second, in							Extra delay in the case of the crossed reflex.	
Strength.	Direction.	Same-limb reflex.				Crossed reflex.				
		Measured time taken by impulse to reach <i>p</i> directly.	Measured time interval between arrivals of direct and reflex effects.	Assumed time to be deducted for transmission in nerve.	Probable cord delay.	Measured time taken by impulse to reach <i>p</i> .	Time to be deducted for transmission from <i>Cu</i> electrode on opposite side to <i>p</i> (measured).	Time to be deducted for transmission in nerve from one <i>Cu</i> electrode to the other (assumed).	Probable cord delay.	
{ 7,000	<i>a</i>	5.4	19.4	2	17.4	...	...	...	...	...
{ 7,000	<i>a</i>	...	...	...	...	36.3	5	2	29.3	11.9
{ 5,000	<i>a</i>	5.1	19.4	2	17.4	...	...	...	...	...
{ 5,000	<i>a</i>	...	...	...	...	35	5	2	28	10.6
{ 5,000	<i>d</i>	5	19.1	2	17.1	...	...	...	...	...
{ 5,000	<i>d</i>	...	...	...	...	33.3	5	2	26.3	9.2
{ 5,000	<i>d</i>	5	19.5	2	17.5	...	...	...	...	...
{ 5,000	<i>d</i>	...	...	...	...	33.2	5	2	26.2	8.7
{ 3,000	<i>d</i>	5	19.5	2	17.5	...	...	...	...	...
{ 2,000	<i>d</i>	no effect, either direct or reflex				...	...	...	...	...
{ 5,000	<i>d</i>	...	...	...	...	32.9	5	2	25.9	8.7
{ 3,000	<i>d</i>	5	19.2	2	17.2	...	...	...	...	...
{ 5,000	<i>a</i>	5	18.3	2	16.3	...	...	...	...	...
{ 5,000	<i>a</i>	...	...	...	...	33	5	2	26	9.7

None of the reflex effects were quite as strong as the direct. The crossed-reflex effect was, however, throughout, strong; the uncrossed became weaker the 5th and 6th times it was recorded. All the reflex effects had a duration which was about double that of the direct effect.

EXP. 52. (R. Gastroc.). Dec. 4, 1906. Room temp. 16° C. Two minims 0.02 per cent. liq. strychn. injected three hours before. Arms had been flexed and legs extended for  $\frac{1}{2}$  hour. Heart still beating, though slowly.

Induction current to nerve.		Time, in thousandths of a second, in								
Strength.	Direction.	Same-limb reflex.				Crossed reflex.				Extra delay in the case of the crossed reflex.
		Measured time taken by impulse to reach <i>p</i> directly.	Measured time interval between arrivals of direct and reflex effects.	Assumed time to be deducted for transmission in nerve.	Probable cord delay.	Measured time taken by impulse to reach <i>p</i> .	Time to be deducted for transmission from <i>Cu</i> electrode on opposite side to <i>p</i> (measured).	Time to be deducted for transmission in nerve from one <i>Cu</i> electrode to the other (assumed).	Probable cord delay.	
{ 5,000	<i>a</i>	3.6	32.3	1.8	30.5+	...	...	...	...	...
{ 5,000	<i>a</i>	...	...	...	...	55.5	3.6	1.9	50	19.5
{ 5,000	<i>a</i>	3.6	30.5	1.8	28.7+	...	...	...	...	...
{ 5,000	<i>a</i>	...	...	...	...	53.8	3.6	1.9	48.3	19.6
{ 5,000	<i>a</i>	3.6	27.9	1.8	26.1	...	...	...	...	...
{ 5,000	<i>a</i>	...	...	...	...	51.5	3.6	1.9	46	19.9
{ 5,000	<i>a</i>	3.6	29.2	1.8	27.4+	...	...	...	...	...
{ 5,000	<i>a</i>	...	...	...	...	51	3.6	1.9	45.5	18.1+
{ 5,000	<i>d</i>	...	...	...	...	52.9	3.6	1.8	47.5	20.2
{ 5,000	<i>d</i>	3.6	29	1.7	27.3+	...	...	...	...	...

All the reflex electrical responses serial. Maximum strength of crossed-reflex effect sometimes greater than the same-side effect. Maximum not so great in either as in the direct effect. For records of the 5th and 6th responses see fig. 10 (A and B) on p. 52.

EXP. 53. Large Frog (R. Gastroc.). Dec. 6, 1906. Room temp. 14° C.  
Two minims 0.02 per cent. liq. strychn. injected the day before. Recovery almost complete.

Induction current to nerve.		Time, in thousandths of a second, in								Extra delay in the case of the crossed reflex.
		Same-limb reflex.				Crossed reflex.				
Strength.	Direction.	Measured time taken by impulse to reach <i>p</i> directly.	Measured time interval between arrivals of direct and reflex effects.	Assumed time to be deducted for transmission in nerve.	Probable cord delay.	Measured time taken by impulse to reach <i>p</i> .	Time to be deducted for transmission from <i>Cu</i> electrode on opposite side to <i>p</i> . (measured).	Time to be deducted for transmission in nerve from one <i>Cu</i> electrode to the other (assumed).	Probable cord delay.	
{ 5,000	<i>d</i>	4.8	20.4	2.3	18.1	...	...	...	...	...
{ 5,000	<i>d</i>	...	...	...	...	43.3	4.8	2.2	36.3	18.2
{ 10,000	<i>d</i>	...	...	...	...	45.2	4.8	2.2	38.2	19.8
{ 10,000	<i>d</i>	4.8	20.7	2.3	18.4	...	...	...	...	...
{ 10,000	<i>d</i>	...	...	...	...	43	4.8	2.2	36	17.6
{ 10,000	<i>d</i>	...	...	...	...	39	4.8	2.2	32	15.3
{ 10,000	<i>d</i>	4.8	19.	2.3	16.7	...	...	...	...	...
{ 10,000	<i>d</i>	...	...	...	...	43	4.8	2.2	36	19.3
{ 10,000	<i>d</i>	...	...	...	...	40.6	4.8	2.2	33.6	17.4
{ 10,000	<i>d</i>	4.8	18.5	2.3	16.2	...	...	...	...	...
{ 10,000	<i>d</i>	...	...	...	...	40.4	4.8	2.2	33.4	17.3
{ 10,000	<i>d</i>	4.8	18.4	2.3	16.1	...	...	...	...	...
{ 10,000	<i>d</i>	...	...	...	...	35.7	4.8	2.2	28.7	13.6
{ 10,000	<i>d</i>	4.9	17.4	2.3	15.1	...	...	...	...	...

(The big resistance had been removed from the secondary circuit when the last two responses were obtained.)

The same-side reflex effects very nearly as strong as, and hardly longer than, the direct effects, which were double throughout. Crossed-reflex effects extremely weak throughout, and weak out of all comparison with the uncrossed-reflex effects. For records of the last two responses (before section of the nerve), i.e. the 13th and 14th, see fig. 6 (B and A) on p. 49.

EXP. 55. (R. Gastroc.). Dec. 11, 1906. Room temp. rising from 10° C. to 14° C. One minim 0.02 per cent. liq. strychn. injected four days before, and 2 more minims the day before making the experiment. When this was begun, the preparation was in the attitude characteristic of strychnine poisoning. Heart beating but feebly at the end of the experiment. No response of any kind, direct or reflex, could be obtained to begin with, with stimuli weaker than 2000 units.

Induction current to nerve.		Time, in thousandths of a second, in								Extra delay in the case of the crossed reflex.
Strength.	Direc-tion.	Same-limb reflex.				Crossed reflex.				
		Measured time taken by im-pulse to reach p directly.	Measured time inter-val between arrivals of direct and reflex effects.	Assumed time to be deducted for trans-mission in nerve.	Pro-bable cord delay.	Measured time taken by im-pulse to reach p.	Time to be deducted for trans-mission from Cu electrode on opposite side to p (measured).	Time to be deducted for trans-mission in nerve from one Cu electrode to the other (assumed).	Pro-bable cord delay.	
{ 5,000	a	5	28	2	26+	...	...	...	...	...
{ 5,000	a	...	...	...	...	55	5	2	48	22
{ 2,000	a	5	30	2	28+	...	...	...	...	...
{ 2,000	a	...	...	...	...	57	5	2	50	22
{ 1,000	a	no effect, direct or reflex				...	...	...	...	...
{ 2,000	a	5	26	2	24	...	...	...	...	...
{ 2,000	a	...	...	...	...	59	5	2	52	28 or
{ 2,000*	a	5.2	29	2	27+	...	...	...	...	25
{ 2,000*	a	...	...	...	...	58	5	2	51	24
Five minutes' rest now given		...	...	...	...	...	...	...	...	...
{ 2,000	a	5	31	2	29+	...	...	...	...	...
{ 2,000	a	...	...	...	...	64	5	2	57+	28
{ 2,000*	a	5	30	2	28+	...	...	...	...	...
{ 2,000*	a	...	...	...	...	68	5	2	61+	33

Reflex responses could now be obtained with induction currents of 1000 units, but not with any that were weaker. Ten minutes' rest was given before continuing the experiment.

{ 1,000	a	...	...	...	...	61	5	2	54	21
{ 1,000	a	5	35	2	33	...	...	...	...	...
{ 1,000	a	...	...	...	...	68	5	2	61+	29
{ 1,000	a	5	34	2	32+	...	...	...	...	...
Five minutes' rest now given		...	...	...	...	...	...	...	...	...
{ 1,000	a	...	...	...	...	59	5	2	52	21
{ 1,000	a	5	33	2	31+	...	...	...	...	...
{ 1,000*	a	...	...	...	...	69	5	2	62+	31
{ 1,000*	a	5	28	2	26+	...	...	...	...	or 36
{ 1,000	a	...	...	...	...	no effect				
{ 5,000	a	...	...	...	...	58	5	2	51+	22
{ 5,000	a	4.8	31	2	29+	...	...	...	...	...
{ 5,000*	a	...	...	...	...	56	5	2	49+	22
{ 5,000*	a	5.2	29	2	27+	...	...	...	...	...
{ 5,000	a	5	28	2	26+	...	...	...	...	...

The \*, when it appears in the first column, denotes that the big resistance had been removed from the secondary circuit when the particular response was evoked.

EXP. 55.—*continued.*

Induction current to nerve.		Time, in thousandths of a second, in								Extra delay in the case of the crossed reflex.
		Same-limb reflex.				Crossed reflex.				
		Strength.	Direction.	Measured time taken by impulse to reach <i>p</i> directly.	Measured time interval between arrivals of direct and reflex effects.	Assumed time to be deducted for transmission in nerve.	Probable cord delay.	Measured time taken by impulse to reach <i>p</i> .	Time to be deducted for transmission from <i>Cu</i> electrode on opposite side to <i>p</i> (measured).	
500	<i>a</i>	...	...	...	...	56	5	2	49	18
500	<i>a</i>	5	33	2	31†	...	...	...	...	...
500	<i>a</i>	...	...	...	...	69	5	2	62††	31
500*	<i>a</i>	...	...	...	...	56	5	2	49†	22
500*	<i>a</i>	5	29	2	27†	...	...	...	...	...
5,000	<i>a</i>	...	...	...	...	55	5	2	48†	24
5,000	<i>a</i>	5	26	2	24†	...	...	...	...	...

Reflex responses could now be obtained to excitation of the sciatic of the opposite side with induction currents of 500 units; soon afterwards it could be also obtained when that of the same side was so excited. Ten minutes' rest was given before continuing the experiment.

The reflex effects were serial throughout, the separate large rises of the mercury being distinct enough from one another to be counted by the eye in each response. There were sometimes as many as fifteen periods, the number being usually greater in the crossed reflex than in the uncrossed. As the first period was far from being over on a plate which took 0.15 second to pass the slit, they were probably recurring at a rate of about five a second. The records of the responses giving the cord delays marked † showed that the reflex effect was not as strong at the beginning as it subsequently became. All the records so marked had the character seen in the reproduction of the first of these (fig. 11, A, p. 54). The records of the responses giving the cord delays marked †† showed that the moment at which the effect became strongest was yet longer deferred, and that the strength of the effect was very small indeed at the beginning. The records so marked had the character shown in fig. 11, C and D. The crossed-reflex response was more readily produced than the same-side reflex by the weaker stimulus, i.e. one of 1000 units, and, later, one of 500 units was capable of producing a reflex effect when applied to the left sciatic, before it was capable of producing it when applied to the right sciatic. The number of periods in the electrical reflex responses was no fewer with the weaker stimulus. There was the usual two minutes' rest given between recording successive responses, except when otherwise stated.



EXP. 56. Dec. 13, 1906. Room temp. 11° C.-13° C. Two and a half minims 0.02 per cent. liq. strychn. injected two days before. Arms still flexed, but legs no longer rigidly extended.

## RIGHT GASTROCNEMIUS. (EXP. 56 R.)

Induction current to nerve.		Time, in thousandths of a second, in								
Strength.	Direction.	Same-limb reflex.				Crossed reflex.				Extra delay in the case of the crossed reflex.
		Measured time taken by impulse to reach <i>p</i> directly.	Measured time interval between arrivals of direct and reflex effects.	Assumed time to be deducted for transmission in nerve.	Probable cord delay.	Measured time taken by impulse to reach <i>p</i> .	Time to be deducted for transmission from <i>Cu</i> electrode on opposite side to <i>p</i> (measured).	Time to be deducted for transmission in nerve from one <i>Cu</i> electrode to the other (assumed).	Probable cord delay.	
3,000	<i>a</i>	5.3	19.3	2.2	17.1	...	...	...	...	...
3,000	<i>a</i>	...	...	...	...	38.5	4.8	2.2	31.5	14.4
3,000	<i>d</i>	4.8	20.1	2.1	18	...	...	...	...	...
3,000	<i>d</i>	...	...	...	...	38.5	4.8	2.1	31.6	13.6
3,000	<i>d</i>	4.8	20.1	2.1	18	...	...	...	...	...
3,000	<i>d</i>	...	...	...	...	39.2	4.8	2.1	32.3	14.3
2,000	<i>d</i>	4.8	20.1	2.1	18	...	...	...	...	...
2,000	<i>d</i>	...	...	...	...	38.8	4.8	2.1	31.9	13.9
1,000	<i>d</i>	4.8	20.5	2.1	18.4	...	...	...	...	...
1,000	<i>d</i>	...	...	...	...	38.7	4.8	2.1	31.8	13.4
3,000	<i>d</i>	4.8	19.7	2.1	17.6	...	...	...	...	...
3,000	<i>d</i>	...	...	...	...	38.5	4.8	2.1	31.6	14
9,000	<i>d</i>	5.2	19.3	2.1	17.2	...	...	...	...	...
9,000	<i>d</i>	...	...	...	...	37.3	4.8	2.1	30.4	13.2

Interval, during which mechanical responses were recorded.

3,000	<i>d</i>	5.3	21	2.1	18.9	...	...	...	...	...
3,000	<i>d</i>	...	...	...	...	39.1	4.8	2.1	32.2	13.3
500	<i>d</i>	...	...	...	...	66.1	4.8	2.1	59.2	(?)40.3

Both reflex effects lasting about four times as long as direct effect in all, and at first about equal to it in strength. For records of the first four and of the 13th responses, see fig. 12, A, B, C, D, E, on p. 56.

## LEFT GASTROCNEMIUS. (EXP. 56 L.)

Induction current to nerve.		Time, in thousandths of a second, in							Extra delay in the case of the crossed reflex.	
Strength.	Direction.	Same-limb reflex.				Crossed reflex.				
		Measured time taken by impulse to reach <i>p</i> directly.	Measured time interval between arrivals of direct and reflex effects.	Assumed time to be deducted for transmission in nerve.	Probable cord delay.	Measured time taken by impulse to reach <i>p</i> .	Time to be deducted for transmission in nerve from one <i>Cu</i> electrode to the other (assumed).	Time to be deducted for transmission from <i>Cu</i> electrode on opposite side to <i>p</i> (measured).		Probable cord delay.
3,000	<i>d</i>	5.3	23	2.1	20.9	...	...	...	...	...
3,000	<i>d</i>	...	...	...	...	41.5	5.3	1.9	34.3	13.4
2,000	<i>d</i>	5.3	23	2.1	20.9	...	...	...	...	...
2,000	<i>d</i>	...	...	...	...	42.5	5.3	1.9	35.3	14.4
3,000	<i>d</i>	5.3	23	2.1	20.9	...	...	...	...	...
3,000	<i>d</i>	...	...	...	...	43.5	5.3	1.9	36.3	15.4
1,000	<i>d</i>	5.3	24.2	2.1	22.1	...	...	...	...	...
1,000	<i>d</i>	...	...	...	...	47.8	5.3	1.9	40.6	18.5
3,000	<i>d</i>	5.3	23.4	2.1	21.3	...	...	...	...	...
3,000	<i>d</i>	...	...	...	...	43	5.3	1.9	35.8	14.5

Both the direct and reflex effects were less strong (curves less steep) in these records than in those taken with the other muscle, but they were about equal to one another. Reflex effects somewhat longer than when the muscle of the other side was recording. Contractions also not quite as strong, but of somewhat longer duration.

The left sciatic nerve in this preparation was more excitable than the right, for both the crossed reflex and the uncrossed reflex could be obtained with it in response to a stimulus of 500 units' strength. The uncrossed reflex with this strength was recorded mechanically, but not electrically; it was quite as strong as with a current of 1000 or more units. No reflex response of either kind could be obtained when the right sciatic was excited by a stimulus of 500 units' strength. Unfortunately, I did not, after cutting the left sciatic, try whether the muscle still responded when so weak a stimulus as one of 500 units was applied to the peripheral end—so that, although I did ascertain the contrary in the case of the right sciatic, it cannot be stated with certainty whether it was the nerve or cord of which the excitability varied.

In four of these experiments [Nos. 4, 16, 42, 48] the crossed-reflex effect was unobtainable when the experiment was begun, even when, as in the two last, fairly strong reflex effects were being obtained in response to excitation of the sciatic nerve of the same side as the recording muscle. It was not until after respectively nine, three, ten, and nine same-limb reflex responses had been recorded (in all four cases at least a quarter of an hour later) that any response at all could be obtained when a single break induction current, even one strong enough to be felt on the tongue, was applied to the sciatic nerve of the opposite side. In Exps. 42 and 48, it was not until after a second very minute dose had been administered, and even then not for some ten minutes later, that a crossed-reflex effect could

be obtained. In all four experiments the crossed-reflex effect never became strong, though in the first two the same-side reflex effect was no stronger. In all these preparations, in which the first appearance, as it were, of a crossed-reflex effect in response to a single instantaneous stimulus was observed, the extra cord delay in the case of the crossed reflex was very nearly the same as, sometimes somewhat longer than, the whole cord delay in the case of the corresponding same-side reflex. In two out of the four [Exps. 4, 42] it was longer the first time the effect appeared than it was in any of the subsequent responses, and this notwithstanding the fact that in all of them stronger stimuli were used (and had been obliged to be used) to get the effect at all at the beginning.

In one other experiment [No. 53], made on a preparation which had, so far as external appearance went, completely recovered from the effects of a



FIG. 6.—Electrical responses of the gastrocnemius of a large frog which had been injected the day before with 2 minims 0.02 per cent. liquor strychniae, and had apparently quite recovered (Exp. 53).

A, sixth response obtained when the intact sciatic nerve of the same side was excited. [Time lines 810 per second.]  
B, eighth response obtained when the sciatic nerve of the opposite side was excited. [Time lines 815 per second.]

dose of strychnine so small (in proportion to the size of the frog) that these had never been marked, the extra reflex delay in the one case was about equal to the whole delay in the other. Although it could be obtained the first time the sciatic of the opposite side was excited, the crossed-reflex effect was extremely feeble, and in this case it remained so throughout the experiment. It was strongest the eighth and last time it was recorded; but fig. 6, B, shows how weak it was even then. The same-side reflex responses in this experiment, on the other hand, the record of the sixth of which (taken immediately after that of the eighth crossed reflex) is reproduced, had been throughout about as strong as the direct effect (which was again double in all the records taken of it). There was a corresponding difference in the strength of the mechanical responses.

The cord delay in the crossed reflex was about double what it was in the simpler reflex in two other preparations [Nos. 40 and 55], both of which were well strychnised, and in both of which the reflex response could be produced as readily, i.e. at the start, and to a stimulus of the same strength

as the simpler one. Records of the first response to excitation of each nerve in turn in the two preparations are reproduced in figs. 7 and 11 respectively. The muscle used for recording in Exp. 40 was again one of those in which the direct effect to excitation of the motor part of the nerve was double; the same-limb reflex time was longer in the last two responses than in the others, and the whole crossed-reflex time also became longer in the third and fourth responses, so that the extra reflex time at first remained unchanged; it finally in the last response became shorter. Exp. 55 will be referred to again (p. 53).

In all the other experiments the extra reflex delay was shorter throughout than the same-side reflex time in the same preparation, although it was as a rule no shorter than this time frequently is. In three of these [Nos. 18, 45, and 47] the action of the drug was incipient. In Exp. 18 the

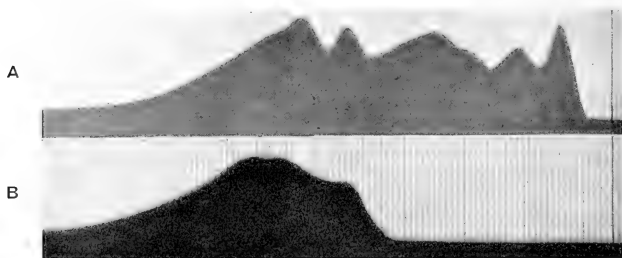


FIG. 7.—Electrical responses of the gastrocnemius of a frog which had been injected the day before with 1 minim 0·04 per cent. liquor strychniae. The preparation was abnormally excitable, but the limbs were no longer at all stiff (Exp. 40).

A, first response obtained when the intact sciatic nerve of the same side was excited. The direct effect was double. [Time lines 745 per second.] B, first response obtained when the sciatic nerve of the opposite side was excited. [Time lines 745 per second.]

same-side reflex delay was exceptionally long; the effects were weak, and resembled one another in both kinds of reflexes. In Exp. 45 the crossed-reflex effect was throughout weak, and at first a good deal weaker than the same-side reflex effect; but as this became weaker each time it was recorded, they were in the end not unlike one another in strength. Fig. 8 allows of the comparison of the two kinds of reflex responses at the beginning and the end of the experiment. In Exp. 47 the crossed-reflex effect was very weak the first time it was recorded, but became in subsequent responses of nearly the same strength as the same-side reflex effect; it varied, however, somewhat in strength as well as in duration in the different responses. Records of the third same-side and of the fifth crossed-reflex responses are reproduced in fig. 9. This was the only preparation, besides No. 33, referred to on p. 33, which gave an extra reflex delay decidedly shorter than the same-side reflex time, as we have now learnt to know it, in strychnine preparations. To begin with, it was about  $11\sigma$ , but became in the fifth and

sixth responses as short as  $6\sigma$ . The records of these two responses show—but it is much more evident in that of the sixth than in that of the fifth (the one reproduced)—that the central stimulus was not exerting its full strength at the start. Three of the crossed-reflex responses, and two of those of the same side, show, as those of no other preparation in so early a stage of strychnine poisoning do, that a second stimulus of central origin was affecting the muscle some  $50-40\sigma$  later than the first began to affect it. This indicates that the drug was taking effect rapidly. In two of the responses in another preparation [Exp. 49] the extra delay became as short as  $9\sigma$ ; but this is hardly so short as to be called exceptional as

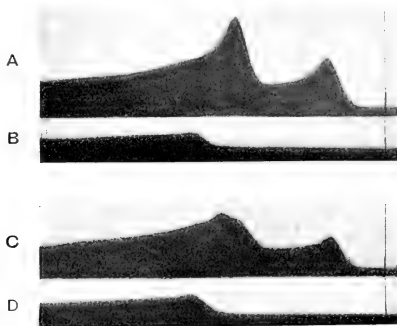


FIG. 8.—Electrical responses of the second gastrocnemius of a frog which, after records had been taken with the other gastrocnemius, before the injection of strychnine, was then injected with 5 minims 0.005 per cent. liquor strychninæ (Exp. 45).

A and C, first and fourth responses obtained when the intact sciatic nerve of the same side was excited. B and D, first and fifth responses obtained when the sciatic nerve of the opposite side was excited. [Time lines 840 per second in all four.]

compared with the whole delay in the same-side reflex, which was once in one strychnine preparation (Exp. 14, see p. 21) equally short.

The extra cord delay in all three preparations [Nos. 50, 52, 55], which were in the attitude characteristic of strychnine poisoning when the records were taken, and in which the heart as well as the cord was affected by the drug, was long, although it was only in Exp. 55 that it was as long as the whole same-side reflex time. With all these preparations the crossed-reflex effect was stronger at the beginning in some of the responses than the same-side reflex effect. This may be seen, for instance, by comparison of the records of the two kinds of response in Exp. 52, reproduced in fig. 10. In Exp. 50, which is not introduced into the table, the extra delays in responses recorded alternately with the same-side responses referred to on p. 19, were, in order,  $35\sigma$ ,  $20\sigma$ ,  $19\sigma$ ,  $14\sigma$ .

The extra cord delay in the case of the crossed-reflex response may, as will have been noticed, vary a good deal in preparations in which the cord delay in the case of the same-side reflex response is keeping fairly

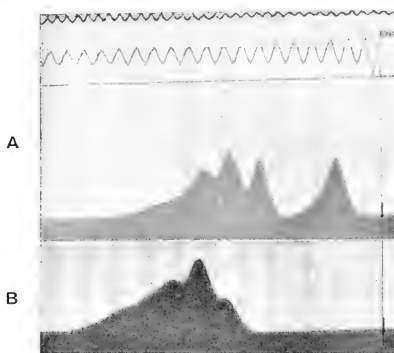


FIG. 9.—Electrical responses of the gastrocnemius of a frog which had been injected  $\frac{1}{2}$  hour before being prepared with 2 minims 0.01 per cent. liquor strychniæ (Exp. 47).

A, third response obtained when the intact sciatic nerve of the same side was excited. [Time lines 820 per second.] (A time marker giving 400 d. v. per second was being used this day, and its record is shown at the top of the photograph.) B, fifth response when the sciatic nerve of the opposite side was excited. [Time lines 820 per second.]

constant. This is especially well illustrated by Exps. 47 and 49. On the other hand, the extra cord delay may remain very fairly constant in preparations in which the same-side reflex delay is getting either longer [Exp. 40] or shorter [Exps. 16, 42, and 52].

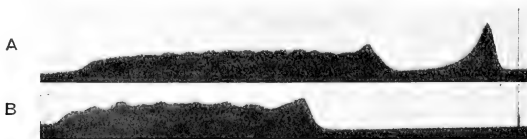


FIG. 10.—Electrical responses of the gastrocnemius of a small frog which had been injected  $2\frac{1}{2}$  hours before being prepared with 2 minims 0.02 per cent. liquor strychniæ, and which has been in the attitude characteristic of strychnine poisoning for half an hour (Exp. 52).

A, third response obtained when the intact sciatic nerve of the same side was excited. [Time lines 830 per second.] B, third response obtained when the sciatic nerve of the opposite side was excited. [Time lines 830 per second.]

The extra delay does not vary with the strength of the effect produced in the muscle, i.e. with that which represents the strength of the physiological stimulus coming from the cord. Thus, both in Exp.

45 (fig. 8) and Exp. 53 (fig. 6) the crossed-reflex effect is very weak throughout the experiment; yet in the one [Exp. 53] the extra delay is approximately equal to the whole same-side reflex delay, whereas in the other [Exp. 45] it is a good deal shorter. Again, in Exp. 55, throughout which there was considerable variation in the length of the delay before the muscle began to respond, and in both kinds of reflexes, the ultimate strength of the effect remained about the same (see fig. 11). On the other hand, when the crossed-reflex effect is variable in strength during an experiment [Exp. 47], the extra delay, although it also may vary, does not vary with the strength of the effect.

Nor does the extra delay in the case of the crossed-reflex response vary, any more than the same-side reflex time does, inversely with the strength of the artificial stimulus evoking it: that is to say, it does not do so when this is once fully above the threshold value, whatever it may do when it is very close to it. Thus, in Exp. 47, in which the stimulus was being gradually weakened, the extra delay was getting shorter, not longer; and in Exp. 56 R, the extra delay remained very fairly constant, notwithstanding a good deal of variation in the strength of the stimulus, only in the last response, one to an extremely weak stimulus, apparently becoming considerably longer. (As, however, no response could be obtained with this muscle when the sciatic of its own side was excited by an equally weak stimulus, we do not really know how much should be deducted to find the extra delay in the case of the crossed reflex; moreover, the effect in this last response was so weak that, for a reason which will appear immediately, I think its significance for giving information about cord delay at all is doubtful.) The same preparation possibly gave direct indication of lengthening of extra cord delay with the weakening of the artificial stimulus when the left gastrocnemius was recording; for, as with the whole delay in the same-limb reflex, only to a more marked degree, the extra delay was in this case longest in the one response recorded with weakest stimulus, but here at any rate this must have been very near its threshold value (see note to experiment).

To obtain more information regarding the influence of stimuli very close to threshold strength we must again, as with the same-limb reflex, have recourse to observing what happens in preparations in which there is reason to believe that the threshold resistance of the individual synapses is variable, when these are attacked by the impulses, presumably of equal strength, produced by a single stimulus. So far as Exp. 55 is concerned, all that I have said on p. 25 with regard to the same-limb reflex delay would apply to the crossed-reflex delay, and it is perhaps significant that a long extra delay in attaining the maximal effect was more frequently observed when the stimulus was applied to the nerve of the crossed side than when it was to that of the same side. Thus there were three other records closely resembling fig. 11, D, and none other resembling fig. 11, C. The evasiveness of the threshold under the influence of strychnine is well seen in most of the preparations in which the drug was only beginning to

take effect, and in which the crossed-reflex response was coming into existence under our eyes, as it were. Thus, in Exps. 42, 47, and 48, the first time it could be obtained it was only to a much stronger stimulus than such as afterwards sufficed to produce it. In two of these [Nos. 42 and 48] the extra cord delay was longer in the first response recorded than it was subsequently, the stimulus, although stronger, being nearer to the threshold value than weaker ones were later. This seems to me to be the best piece of evidence we have at present that strength of stimulus, when near its

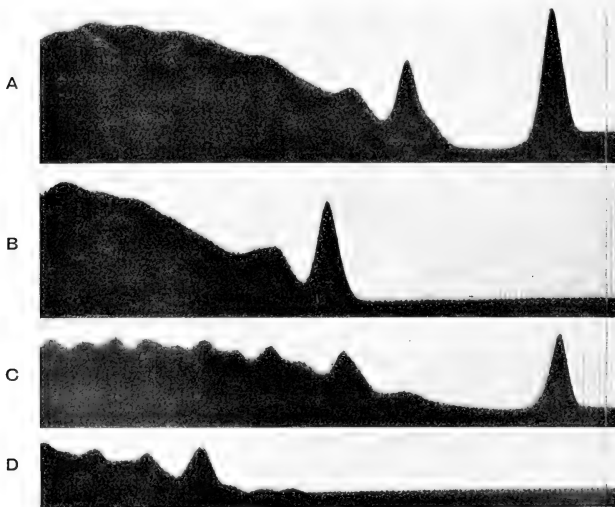


FIG. 11.—Electrical responses of the gastrocnemius of a frog which had been injected the day before with 2 minims 0.02 per cent. liquor strychniae, and which was in the attitude characteristic of strychnine poisoning when it was prepared (Exp. 55).

A and C, first and eighth responses respectively obtained when the intact sciatic nerve of the same side was excited. [Time lines 840 and 770 per cent. respectively.] B and D, first and tenth responses respectively obtained when the sciatic nerve of the opposite side was excited. [Time lines 830 and 820 per second respectively.]

threshold value, is likely, in the normal animal, to affect synapse time, since the cords in these two experiments were in less abnormal condition than was that used in Exp. 55.<sup>1</sup>

The effect of altering the temperature of the cord on the extra cord

<sup>1</sup> Since this paper was first prepared for the press I have made a good many more experiments in which the strength of the stimulus was altered. The results amply confirm the conclusion already come to, namely, that, increasing the strength of the stimulus when once its value is above a certain amount does not shorten the time taken to pass individual synapses, although it may increase the number crossed abreast. They will be dealt with in a future communication, together with the results obtained in more complex reflexes in which cord delay, and possibly the number of synapses crossed in series, appears to be less constant in any one preparation than it is in the two which are here considered.



delay can only be studied in one of the experiments [No. 48], since it was only in this one (of the two which had the temperature altered) that a crossed-reflex could be recorded, before, as well as after, the cooling. As may be seen from the table, not only was the same-side reflex delay lengthened by cooling in this experiment, but the difference between the two cord delays was likewise lengthened, showing that the temperature of the cord affects the extra cord delay in the same way as it affects the same-limb reflex time.<sup>1</sup>

We have, therefore, in the case of the crossed reflex, a loss of time additional to that obtaining in the same-limb reflex, which is of about the same order, and which varies, or does not vary (as the case may be), in the same way with external physical conditions; but which, when these are constant, may vary independently of this same-limb reflex cord delay.

These facts suggest very strongly that there is interposed in the conductive path, in the case of the crossed reflex, some element of the same nature as the one which is alone interposed in the path of the same-limb reflex. In other words, one can hardly refrain from inferring that, whereas in the same-limb reflex a single synapse has, in the case of each fibre, to be passed; in the crossed-limb reflex two such synapses have to be passed in turn by every individual fibre concerned.

The one set of synapses in the case of the crossed reflex would be the same, or would belong to the same set, as those which are alone concerned in the simpler reflex. They may, therefore, be called the primary synapses. The principal cells concerned in them would be presumably the motor cells of the ventral cornua. The second set involved in the case of the crossed reflex may be called secondary synapses. They would lie on the opposite side of the cord, and in view of the most interesting experiments of Baglioni with regard to the action of strychnine on the cord, the locating of them somewhere in the dorsal part of the cord suggests itself.

Whether the primary synapses involved are individually the same, and necessarily so, in the two kinds of reflexes, is another question to which my records suggest an answer. Certain experiments show in the records a very striking difference in the muscle response in the two kinds of reflexes. The difference in Exp. 53 (in which all the six same-side responses very closely resembled the one of them reproduced in fig. 6, A, and all the eight crossed-reflex responses the one of them reproduced in fig. 6, B) was chiefly one in strength, and might either mean that each primary synapse cell was giving a weaker discharge when the impulses arrived by way of the secondary synapses, or that a smaller number of them were brought into play. But in other experiments, e.g. Nos. 55 and 56 R (in both of which especially weak exciting stimuli were being employed), the two kinds of reflex responses were equally strong, and yet exhibited characteristic differences. To illustrate this, I have had reproduced in fig. 12 the records of four

<sup>1</sup> I have now made several experiments which confirm this [Nov. 1907].

successive responses of the same spots of the right gastrocnemius, obtained by the alternate stimulation of the right and the left sciatic in Exp. 56. No one can fail to see (and it is not only in these four records that the phenomenon is to be seen) that the undulations on the curves which represent the crossed-reflex response have a character of their own, different

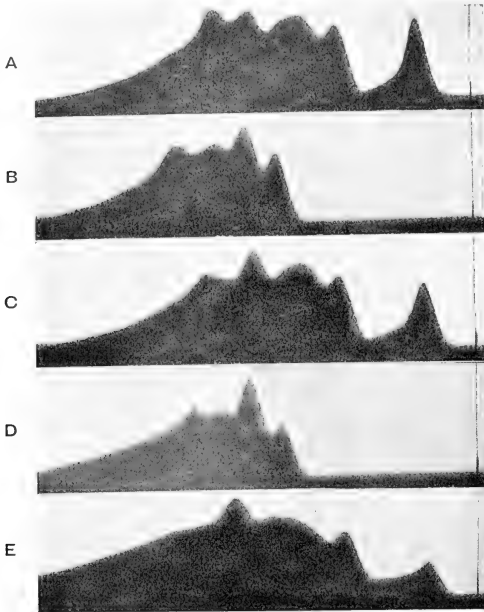


FIG. 12.—Electrical responses of the gastrocnemius of a frog which had been injected with  $2\frac{1}{2}$  minims 0.02 per cent. liquor strychniae two days before, and which had been in the attitude characteristic of strychnine poisoning for some time, but in which the limbs were no longer more than somewhat stiff (Exp. 56 R).

A, C, and E, first, second, and seventh responses respectively obtained when the intact sciatic nerve of the same side was excited. [Time lines 835, 830, and 827 per second respectively.] B and D, first and second responses obtained respectively when the sciatic nerve of the opposite side was excited. [Time lines 830 per second in both cases.]

from that of those on the curves representing the same-limb reflex response. I have elsewhere shown (*loc. cit.*), and I have still better evidence than any that has yet been published (namely, the records themselves) of the fact that these undulations represent something for which the muscle alone is responsible. The difference between the records, therefore, suggests that not all the same muscle fibres, and consequently not all the same efferent nerve

fibres, and not all the same primary synapse cells were in play in the two kinds of reflex response. In Exp. 55 all the records of the same-limb reflex responses referred to with a † in the table had at the beginning the character exhibited in the first of them (the one reproduced in fig. 11, A), while the crossed-reflex responses more usually began in the way shown in fig. 11, B.

At first sight the suggestion that some at any rate of the primary synapses concerned may be different in the two kinds of reflexes may seem to be incompatible with a suggestion I made before (p. 27) with regard to the same-limb reflex response in the normal cord. It is, however, quite conceivable that all the primary synapses and their cells, controlling the particular muscle, may be brought into play when either sciatic alone is stimulated by a strong induction shock, and yet that some come into play more readily when the afferent fibres of the one nerve are excited, others when those of the other are excited, and that such differentiation takes place, though it may not always be manifest, when the exciting currents are weak (even when strong enough to excite all the afferent fibres of each nerve), or when certain synapses have failed to become, or to remain, sufficiently responsive. The records obtained in Exp. 55, showing, as they do (see fig. 11), that neither reflex effect was ever so strong as the direct, would lend support to such a view. The records obtained in Exp. 56 might, however, at first sight seem to make it untenable, in so far as they show that the reflex effects were, to begin with (fig. 12), about as strong as the direct effect. But such a fact does not necessarily always imply that all the muscle fibres have been excited by the stimulus coming from the cord when this has had its excitability increased by strychnine. The increased discharge which this drug enables the motor cells to give may quite well so increase the strength of the effect in the muscle fibres as to obscure a small reduction in the number of fibres which are in play, as compared with the number brought into play in the direct response. That this is sometimes actually the case is shown, I think, by records obtained in a few of my experiments (in 56 R amongst others) of same-limb reflexes in which the direct effect was less strong than the reflex effect. Occasionally it was so when the experiment began, as in the one [Exp. 45] to which fig. 8, A, refers; the curve derived from this record would certainly show that the difference of potential when it first came into existence in each response was somewhat greater in the case of the reflex than in that of the direct response. Usually, as was the case in Exp. 56 R, the reflex effect only became the stronger by the direct effect becoming weaker (compare, in fig. 12, E with A or C). Since the one muscle effect remained strong, the weakening of the other is likely to have been due to some difference in the part producing the excitation, i.e. in this instance in the motor part of the nerve, and the difference seems to me to be most probably one in the number of fibres excited, some of them having become in some way temporarily or even per-

manently impaired.<sup>1</sup> Thus, at the end, in such an experiment as 56 R, the whole reflex discharge was producing a stronger electrical effect in the muscle than the artificial stimulus to the nerve could produce, whether or not more fibres were in play, and in neither case (probably) all being in play. As Exp. 45 went on, the reflex effect as well as the direct effect got weaker, as may be seen by comparing curves C and A in fig. 8, the effect of the strychnine, if I am right in my interpretation, not being great enough in this preparation to overcompensate for a reduction in the number of efferent nerve fibres, and consequently of muscle fibres, which were in play.<sup>2</sup>

The comparison of the crossed-reflex responses, among themselves even, in Exp. 55 suggests a further differentiation of synapses belonging to one and the same set; only it appears to me more probable that the set differentiated in this case was that of the secondary synapses, and that the agent which differentiated them was fatigue. A glance at the table on p. 45 shows how much the extra delay in the case of the crossed reflex was reduced by an extra few minutes' rest each time this was allowed. The examination of the records brings out certain peculiarities in the four responses referred to by the ††, one of which is reproduced (fig. 11, D), which are not presented by the other crossed-reflex responses. The fact that in all of these the total cord delay was almost the same, and 10–13σ longer than it was in most of the other crossed responses, suggests a further inference, the discussion of which, I will, for the present, postpone.

Results such as these, and others obtained in the few experiments which have hitherto been made with stimuli not far from the threshold value in strength, seem to me to indicate that it will be more fruitful for the further investigation of processes occurring within the central nervous system to continue to use weak stimuli rather than strong ones. At the same time it must not be forgotten that the experiment in which stimuli nearest to the threshold value were used [Exp. 55] was one of those in which the circulation was not quite normal, and that some of the peculiarities in the responses may be due to the effect which defective circulation has been shown to have in lengthening cord delay, this being probably greater (see p. 26) on some of the synapses than on others.

We have seen that one of the first actions of strychnine on the cord delay, in the case of the same-limb reflex (on the time taken, if we may so consider it, to pass the primary synapse), is to somewhat shorten it.

<sup>1</sup> In those cases in which the direct effect was weakened by a change in the direction of the current (see p. 16), the reflex effect again usually retained its full strength.

<sup>2</sup> Another interpretation of the regularly recurring difference occasionally met with between the two kinds of reflex effects in the muscle would have suggested itself if the fibres of the gastrocnemius were supplied, as are those of the sartorius and certain other muscles of the frog, by more than one efferent nerve fibre; but Sandemann (A. f. (Anat. u.) *Physiol.*, 1885, p. 246) has shown that this is not the case, or rather he has shown that each fibre of the frog's gastrocnemius is provided with but one motor nerve end-organ, which makes it improbable that it should be the case.

This action appears to be far stronger on the factor which determines the extra delay in the case of the crossed reflex, i.e. on the time taken to pass the secondary synapse. This time may apparently be very considerably shortened by it. In two preparations, as has been seen, in both of which the drug was taking effect very quickly, it was reduced in the course of the experiment to  $5\sigma$  and  $6\sigma$  respectively. According to Wundt, it may sometimes be reduced to as little as  $4\sigma$ . Are we to infer from this, and from the fact that the only time a crossed-reflex response was obtained in a preparation which was hardly influenced at all by the drug [Exp. 38], the delay was as much as  $63\sigma$  longer than in the corresponding same-limb reflex response, that the time taken in passing the secondary synapse is in the normal cord a good deal longer than it usually is in the strychnised cord? I think not, although, if ever I succeed in obtaining and recording the crossed reflex in response to a single instantaneous stimulus in a normal preparation, I should not be at all surprised to find that the delay in the manifestation of the effect in the muscle was still longer.<sup>1</sup> With regard to its long duration in normal or very nearly normal preparations, I would suggest that the same sort of explanation may apply as applies to the postponement of the moment at which the effect becomes maximal in the case of some of the responses to just efficient stimuli in fully strychnised preparations. If we compare, for example, the maximal strength of the reflex effect attained in the responses to which fig. 11, C and D, refer, with what it was when it started in these responses, and then compare the maximal strength of the reflex effect in a normal cord as it usually is, and as it was in Exp. 38 (steepness of rise about the same as in the record reproduced in fig. 1, B), with something less than it, in the same proportion (or in the proportion which the crossed-reflex effect sometimes bears to the uncrossed in strychnine preparations, e.g. fig. 6), it is evident that this something, if it existed, would be imperceptible with the recording instrument and arrangements which were being used. Whatever is the explanation of the postponement of the moment at which the effect reaches its maximum in the one case, seems to me likely to be the explanation of the late appearance of any effect at all in the other case.

The postponement in the strychnine preparations, since it may appear in the response of the uncrossed as well as in those of the crossed reflex, is likely to be due, in part and sometimes entirely, to some factor in the set of primary synapses preventing the appearance of the full effect at once. I have already suggested (p. 27) that this factor is the want of accord in the moment of discharge of the separate cells, brought about by impair-

<sup>1</sup> Since this paper was sent in I have at last succeeded in obtaining in two much cooled but undrugged preparations a true crossed-reflex response in the biceps femoris. Although the records of it were extremely minute, the moment at which it began could be determined. In one preparation the effect appeared after an interval which was twice as long as in the same-limb reflex; in the other, after an interval which was, both relatively and absolutely, considerably longer. The cord delay in the same-limb reflex was very long (about  $50\sigma$ ) in each preparation. [Nov. 1907.]

ment of circulation, and variations in the threshold resistances of individual synapses, some of them being forced and not others, or others not until later.

The late appearance of a crossed-reflex effect, if it appears at all, in the normal preparation, seems to me, on the other hand, to be much more probably due to some factor in the set of secondary synapses preventing its earlier appearance, since, while the same-limb reflex response is comparatively easy to evoke in the normal cord (it was obtained in about 70 per cent. of my cooled preparations), the crossed-reflex response is rarely, if ever, evokable from it by a single instantaneous stimulus to the afferent nerve fibres, and even in the strychnine cord the crossed-reflex response is so often not to be evoked when that of the same side is showing in its record the effects of the drug. It is clear that in such case something else has to happen before the extra excitability thus shown to have been brought about in the primary synapses or their cells, can be taken advantage of by the impulses which may come to them from the opposite side of the cord. If these arrive at all at the primary synapses in the normal, or in the lightly strychninised cord, they behave like impulses produced by artificial stimuli too weak to be effectual, applied to the same-side afferent nerve fibres. It is only when the collective discharge from the cells of the secondary synapses has reached, and passed, its threshold value with regard to the primary synapses and their cells, that it is able to produce an effect in the muscle supplied by these. Yet we have seen that strychnine may, when it has acted for a longer time (insufficient, however, to make the electrical effect serial or the mechanical one a spasm), have a considerable influence on that factor in the secondary synapse which determines the delay in it, and it is most unlikely that what it influences so strongly, it influences less soon than what it influences less strongly, namely, the delay-regulating factor in the primary synapse. Just for this reason, and for the one that other factors also, such as fatigue, seem to have greater influence on secondary than on primary synapse time, I would suggest that it is the individual variability of the time taken in passing the secondary synapses (the variability in their threshold resistances perhaps) in the normal cord which, by preventing the discharges from being synchronous, prevents them from affecting the primary synapse cells and hence the muscle. When, either by making the excitability of the secondary synapse cells more alike, or by making the resistances of the secondary synapses more equal, the separate discharges become not only stronger, but more synchronous, they are able to effect what even discharges of the same strength could not do, one by one, with broken step (if one may use the expression). To make effectively to work together things the temporary capacities of which vary, would take a longer time for the drug to accomplish than when it has to deal with things the capacities (Fähigkeiten) of which vary but little, as in the case of the primary synapses. The fact that the first time a crossed-reflex effect appears after failures to obtain it, in any particular

preparation, it is almost always very weak, even when it subsequently becomes strong, lends support to this view. If it is correct, it implies that the probable cord delay (or extra cord delay, as the case may be) given for each response in my tables represents the time taken to pass the primary (or secondary) synapse by a sufficient number of individual impulses at the same moment, to produce an effect on the muscle. They therefore give the shortest time taken by a sufficient number of them to pass simultaneously. When the effect is its strongest, as it usually is, almost as soon as it begins, the probable cord delay given is the time taken by the large majority, if not all, of the impulses arriving through their several afferent channels to pass the synapse in that response. If any have passed it more quickly, they were too few in number to produce an effect in the muscle. Those that passed their synapses more slowly would only produce an additional effect if a sufficient number of them do it in the same time, and this apparently was only sometimes the case.

That strychnine actually does make the time taken to pass the individual secondary synapses get shorter, and does not only serve to make them work more synchronously, seems to me to be attested by the measurements of records in several individual experiments. If we may express it in terms of what I have just ventured to set forth, these show that the time taken by the greater number, the modal time, to borrow Professor Karl Pearson's word,<sup>1</sup> gets shorter, not longer, as the drug becomes more effective, and it would be most unlikely that those synapses which can be passed in shortest time would be the last to be affected by the drug. I hold, therefore, that the probable extra cord delays obtained from records taken with strychnine preparations just so much affected by the drug as to give a decided and definite crossed-reflex response at all, are likely to be those which would be obtained from each of the cords when normal, could we from such a cord evoke a measurable response; and that the extra cord delays obtained from records taken at, or very soon after, what we may call the critical moment in the action of the drug, represent each the modal time (possibly also the average time of the whole set) taken by the secondary synapses in any particular normal cord. To know this modal time, the time taken to pass the greater number of synapses, and to know the variations from the mode in any particular set of synapses, seems to me far more important than to know the shortest time in which a synapse may be passed.

Another reason for the conclusion that the results obtained with strychnine cords may be applied to normal cords is that, if the excitability of the cord is raised by other drugs, the delay in traversing the cord, when the nerve of the opposite side to the recording muscle is stimulated, is longer than the delay when the nerve of the same side is stimulated, by an amount of the same order as when strychnine was employed. This was so in the two experiments I have so far made with phenol preparations.<sup>2</sup>

<sup>1</sup> Phil. Trans., 186, Series A, 1895, p. 345.

<sup>2</sup> See footnote to p. 29.

A record of one of the crossed-reflex responses taken with the first of these is reproduced in fig. 13, C. The cord delay was  $12.5\sigma$  longer than it was in the same-limb reflex response recorded immediately afterwards (with the same strength of the artificial stimulus), and reproduced in fig. 13, A. It was of about the same duration in two other responses, one to a stimulus somewhat stronger, and the other to one a good deal weaker than was employed when the record reproduced was taken. But there is something

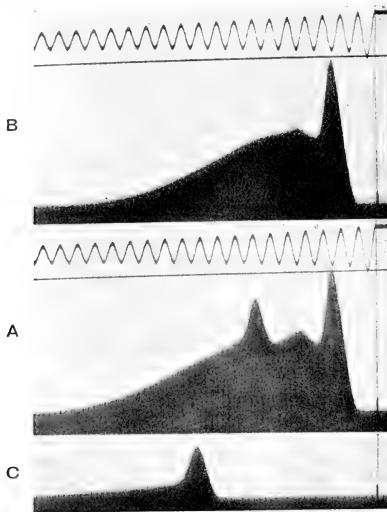


FIG. 13.—Electrical responses of the gastrocnemius of a large frog which had been injected  $2\frac{1}{2}$  hours before being prepared with 10 minims 0.1 per cent. phenol.

A, fourth response obtained when the intact sciatic nerve of the same side was excited. [Time lines 750 per second.] B, response to excitation of the peripheral end of the same nerve after it had been divided. Record taken at the end of the experiment. [Time lines 835 per second.] C, third response obtained when the sciatic nerve of the opposite side was excited. [Time lines 750 per second.]

peculiar about the extra delay when the crossed sciatic is excited by strong stimuli (10,000 units) in phenol frogs (besides that which has already been mentioned on p. 31), the discussion of which I must defer.

My experiments should, and I hope will, when they are carried further, throw light upon the results obtained from the most ingenious and interesting experiments of Baglioni made for the purpose of ascertaining the action of strychnine and phenol on nerve cells in Vertebrates and Molluscs. I do not think, however, that they will confirm his



conclusions that one kind of cell behaves in a different way from another to the same drug, the one having its excitability increased greatly, the other remaining wholly unaffected. They rather suggest, so far as they have gone at present, that strychnine can increase the charge made by both the sets of cells; but that in the one case and not in the other it has first to bring them into array, and that the difference lies not so much in the cells themselves as in the way in which collateral or other fibres are distributed to the one or the other. With regard to these matters, it seems to me that the electrical responses of muscle, when recorded photographically by such an instrument as the capillary electrometer, are capable of giving information about the physiological stimuli which evoke them which can never be obtained from records of the mechanical response alone.

It will give me pleasure to supply any physiologist with copies of photographs relating to any particular experiment in which he may be interested. A great deal of the value of some of the experiments lies in the resemblances (or differences, as the case may be) between the records of successive responses, and it is only samples of them, and those only of comparatively few experiments, that could be reproduced within the limits of this paper.

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All the experiments described in this communication were made with apparatus which belonged to Sir John Burdon-Sanderson, and on which he had spent a great deal of labour, time, and money in trying to make as complete as possible. I had already been using it in its different stages for some years, and any work that has yet been done or will be done with it may in a sense be regarded as a continuation of his work. That this should be continued, and that what was already done in his lifetime should be made use of to the fullest extent possible for suggesting new lines of research and for the acquirement of new knowledge, is, I feel sure, the tribute to his memory which he himself would most desire.

The recording part of the apparatus was made under the direction of Mr H. S. Souttar, by whom it was to a considerable extent designed. He has also supplied me with the very quick capillary electrometers with which all the records have been obtained, and I am greatly indebted to him for so doing. I have also to thank Professor Sherrington for valuable criticism and for the interest he has taken in the work.

The working expenses have been defrayed out of a grant from the Government Grant Committee of the Royal Society.

## SUMMARY.

By means of photographic records taken with the capillary electrometer, it has been shown that:—

1. The delay in the normal cord of the frog, in the same-limb reflex, as determined by the interval of time between the two electrical responses of a particular spot of a muscle (the gastrocnemius) when the efferent and afferent fibres of the mixed nerve supplying it (the sciatic) were simultaneously excited by a single break induction shock, varies in different preparations between 0.012 and 0.022 second. It is very rarely as short as 0.012 second, and only occasionally longer than 0.022 second. These numbers refer to cord delay alone, time for transmission along the known length of nerve having been deducted on the assumption that an impulse travels at the rate of 30 metres a second along fresh frog's nerve.

2. When the cord and the cord alone has been acted upon by a very weak dose of strychnine, this delay is somewhat diminished. It then varies in different preparations between 0.009 and 0.020 second at room temperatures, though it is rarely as short as 0.009 second. It is seldom longer than 0.020 second, except when the circulation as well as the cord has been affected by the drug. In such cases the cord delay may become as long as 0.033 second, and the central stimulus may fail to exert its full strength when it first begins to affect the muscle.

3. Alteration in the strength of the artificial stimulus applied to the nerve does not alter the delay in either the normal or the strychnised cord. This statement is certainly true for stimuli above a certain strength (varying with the sensitiveness of the preparation), but probably does not apply to stimuli the strength of which is only just above the threshold value. The difficulties in the way of determining the threshold value for any one preparation have so far prevented direct satisfactory investigation of the effects of just adequate stimuli.<sup>1</sup>

4. Cooling the cord by applying ice to the back of the preparation greatly increases the delay in the strychnised cord. Cold seems also to increase to some extent the delay in the normal cord.

5. Repeated stimulation (fatigue) may lengthen the delay in the normal cord.

6. The direct action of strychnine on the cord consists principally, so far as the same-limb reflex is concerned, in making the discharge stronger, and eventually longer, in response to a weaker stimulus. So far as the crossed reflex is concerned, it also brings about something which before prevented an effectual response from being obtained in the muscle to a stimulus so brief as a single induction shock, applied to the sciatic nerve of the opposite limb.

<sup>1</sup> A little direct evidence has, however, been obtained since this paper was written that cord delay, even in the same-limb reflex, is longer when the strength of the stimulus is only just adequate to produce a reflex response in the muscle.

7. In strychnine preparations, from which an effectual response can only just be obtained when the nerve of the opposite limb is stimulated, the cord delay is always roughly about double what it is when the nerve of the same limb is stimulated.

8. Whereas the delay in the case of the same-limb reflex is only somewhat shortened by the action of strychnine on the cord, the extra delay in the case of the crossed reflex may be very considerably shortened by the continued action of the drug, so long as its action is confined to the cord. It may be reduced to half, or even to a fifth of what it was, in the course of an experiment in which the drug was taking effect rapidly. It seems, however, never to be reduced to less than 0.004 second, and seldom becomes so short as this. It may vary in any one preparation independently of the whole delay in the simpler reflex, but if the circulation has been affected by the drug it also is long.

9. The extra delay in the crossed reflex is no more affected by the strength of the stimulus applied to the nerve than is the whole delay in the same-limb reflex. It is affected, and in the same way, by changes in the temperature of the cord.

10. The cord delays in the uncrossed and in the crossed reflex are of the same order when the excitability of the cord has been raised by phenol instead of by strychnine, and bear the same sort of relation to one another, provided that in the case of the crossed reflex the strength of the artificial stimulus is not very greatly in excess of (not more than five times) the strength just necessary to produce it.

It has been inferred:—

11. That in the same-limb reflex there is normally a single synapse interposed in the conductive path of each individual fibre concerned, and that the time taken to pass it in the normal animal probably lies between 0.010 and 0.020 second.

12. That in the crossed reflex investigated there are normally two synapses interposed in the conductive path of each individual fibre concerned, and that the time taken to pass the additional ("secondary") one is about the same as that taken to pass the primary one in a normal animal, but that it is subject to greater variation in each individual cord, and that there is, determining it, something which is more susceptible to such agents as drugs and fatigue.

An abstract of this communication, under a somewhat different title, appeared in the Proceedings of the Royal Society, B., vol. lxxix., 1907, p. 503.

## EXPLANATION OF FIGURES.

On each positive used, an exactly vertical line was ruled from the spot in the record of the signal which represents the moment of excitation to the zero line of the curve. Subsequently (for the sake of economising space) the signal-record itself was cut off in all the positives, with the exception of those used for figs. 1, 2, 4, 9 A, and 13 A and B. The photographs have not been "touched" in any other way, except that in some the shaded part has been darkened for purposes of photographic reproduction. They are to be read from right to left, and the ruled vertical line is consequently to be seen on the right-hand side of each. In the photographs themselves the vertical lines mark the time, and their number per second is stated below for each. The horizontal lines, when present, mark in millimetres the height to which the projected image of the meniscus rose.

SOME COMPARISONS BETWEEN REFLEX INHIBITION AND REFLEX EXCITATION. By C. S. SHERRINGTON. (From the Physiology Laboratory, University of Liverpool.)

I. GRADING OF INTENSITY OF REFLEX.

OPINION regarding relation between strength of the stimulus exciting a reflex action and the intensity of the resulting reflex is undergoing change. It was thought that something like the "all or nothing" rule observable for the relation between stimulus and response of the vertebrate myocardium held good for spinal reflex arcs. The statement still often is that within but a narrow range does variation of intensity of external stimulus affect the intensity of the spinal response. Internal condition of the reflex arc does certainly enormously influence the intensity of the arc's reaction. But recently instances have been forthcoming to show that grading of reflex effect follows closely the grading of the external stimulus,<sup>1</sup> and in some cases through a wide range of intensity of stimulus.<sup>2</sup>

There has to be remembered, in dealing with this question, the property of temporal summation so transcendently displayed by reflex arcs.<sup>3</sup> A series of weak stimuli may by summation become more potent than a stimulus of much greater physical intensity, but single or of relatively few or infrequent repetitions. Mere duration of the stimulus comes, therefore, to be equivalent to intensity. A simple way of eliminating this source of confusion is to employ as external stimulus an agent of variable intensity, but of duration practically infinitely brief; its period then becomes negligible. A single induction shock may be regarded as furnishing such a stimulus. The single induction shock has, however, figured very rarely as the stimulus for evoking a reflex reaction. There has existed a belief that a single induction shock must, in order to excite a reflex reaction, be employed in very high strength. Indeed, authorities have questioned whether a single induction shock can excite a reflex at all. Some reflexes are, it is true, extremely difficult to evoke by a single induction shock; thus, the "scratch-reflex" of the spinal dog I was on no occasion able to elicit by a

<sup>1</sup> Merzbacher, L., *Pflüger's Arch.*, lxxxi, 1900. Langelaan, J. W., *Arch. f. Physiol.*, Suppl. Bd., 1903. Sherrington, C. S., *Proc. Physiol. Soc.*, March 1904; *Journ. of Physiol.*, xxxi, p. xvii.; *Journ. of Physiol.*, xxxiv, p. i.; *Integrat. Action of the Nervous System*, p. 70, 1906. Pari, G. A., *Zeitschr. f. allgem. Physiol.*, iv, 1904; *Arch. italiennes de biolog.*, 42; *Atti. Instit. Veneto*, 65, 1906. Baglioni, S., *Analyse d. Reflex-funktion.*, 1907.

<sup>2</sup> Sherrington, C. S., *op. cit.*

<sup>3</sup> Stirling, W., *Ludwig's Arbeiten*, 1874, p. 245.

single shock. But in various other mammalian reflexes this is not the case, and the "flexion-reflex" of the limb is elicitable by a single induction shock, either make or break, and of such slight intensity as to be imperceptible to the tongue.<sup>1</sup>

With the single induction shock as stimulus, therefore, and with the "flexion-reflex" as reaction, observations can be made on the relation between intensity of stimulus and intensity of reflex response. For obtaining comparable break shocks in the following observations, the opening of the primary circuit has been operated by a pendulum. To obviate changes in resistance, a box of 100,000 ohms has been employed in the secondary circuit. The "flexion-reflex" can be readily excited by a break shock, whether applied to an afferent nerve of the limb or to some point of skin in the "receptive field" of the reflex. For the following observations it seemed preferable to apply the stimulus directly to the afferent nerve rather than to the skin. It is true that, as Baglioni<sup>2</sup> has pointed out for the frog, application to the nerve is probably not so favourable as application to the skin for the obtaining of the full amount of grading of the reflex. On the other hand, however, by applying the external stimulus to the afferent nerve direct, the unknown factors entering into the reaction are somewhat reduced, a desideratum where so many variables are perforce included. The afferent nerve to which the stimulus was applied remained the same for all the experiments; it was the musculo-cutaneous branch of the peroneal taken about 4 centimetres below the knee. The electrodes were silver pins placed on each side of the nerve 5 mm. distant along its length. The direction of the current in all the observations was the same.

The "intensity" of the reflex reaction has several forms of expression.<sup>3</sup> The reflex reaction as it increases in intensity tends to involve an increasing number of muscles. It also involves with greater intensity the several muscles individually.<sup>4</sup> The greater reflex movement of the limb which distinguishes a stronger reflex effect from a weaker is probably usually due to both these factors.<sup>5</sup> In the following observations the increase of intensity of reflex reaction has been examined as it occurs in the individual muscle. The question of irradiation of the reflex discharge over a wider or narrower field of musculature has not here been entered on. In regard to any such grading of intensity of action as might be found in the individual muscle, the inquiry had in view its comparison in the excitatory and inhibitory sides of the reflex respectively. The "flexion-reflex"<sup>6</sup> is a reflex of simultaneous double sign ( $\pm$  reflex); flexors of hip, knee, and ankle, and

<sup>1</sup> Sherrington, C. S., Proc. Roy. Soc., vol. lxxvi. B, p. 270, 1905.

<sup>2</sup> Baglioni, S., *ibid.*

<sup>3</sup> Sherrington, C. S., *Integrat. Action etc.*

<sup>4</sup> Sherrington, C. S., *Journ. of Physiol.*, loc. cit.; Langendorff, O., Nägel's Handbuch d. *Physiol.*, iv. i., footnote, p. 240, 1904.

<sup>5</sup> Sherrington, C. S., *Integrat. Action etc.*

<sup>6</sup> Sherrington, C. S., Proc. Roy. Soc., loc. cit.; *Integrat. Action etc.*, p. 83; *Ergebn. d. Physiol.*, 1905, p. 834.

abductors and internal rotators of hip express the excitatory (+) side of the reflex, by contracting; extensors of hip, knee, and ankle, and adductors and external rotators of hip express the inhibitory (-) side of the reflex by relaxing. To serve as samples of these opposed groups reacting under the reciprocal reflex innervation, two muscles were chosen, which act on one and the same joint, but oppositely. These were semitendinosus as flexor of knee, vasto-crureus as extensor of knee. The reflex preparation was made as in previously reported observations,<sup>1</sup> deep chloroform narcosis being employed until after the destruction of the brain.

The reflexes obtained from the isolated semitendinosus show clear grading of intensity of contraction following grading of intensity of the break shock, figs. 1 and 2.<sup>2</sup> The grading, even under experimental conditions, is sufficiently delicate and covers a sufficient range of gradation of stimulus to indicate that under natural conditions strength of stimulus must influence minutely and widely the extent and force of the actual

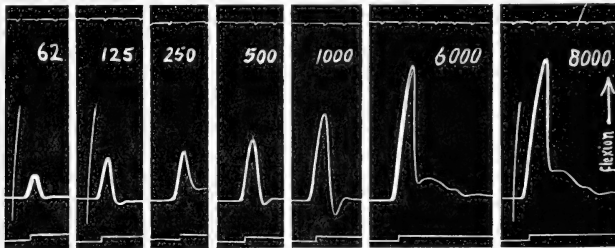


FIG. 1.

movement in the flexion-reflex of the limb. This result is welcome, because it conforms with what would be expected from the standpoint of teleology. In the examples figured (figs. 1 and 2), the sequence of change of intensity of the external stimulus has been from weaker to stronger. The grading of the reflex response has, however, been equally obvious when the direction of the sequence has been reversed. A feature noticeable in the myograms is that not only does the amplitude of the contraction increase with increased strength of stimulus, but also the duration of the contraction increases. There is marked persistence of contraction in the stronger reflexes, recalling the "after-discharge"<sup>3</sup> of strong reflexes excited by longer stimuli. An advantage in using the single induction shock as a stimulus is that fatigue tends to occur hardly at all. The interval between the successive reflexes in the examples shown was one minute, but even with much shorter intervals no evidence of fatigue was obvious. Care has to be

<sup>1</sup> Proc. Roy. Soc., loc. cit.

<sup>2</sup> All the figures read from left to right; in all the time record is marked in fifths of seconds.

<sup>3</sup> Sherrington, C. S., Journ. of Physiol., loc. cit.; Integrat. Action etc.

taken, however, lest the interval be so short as to allow facilitation (Bahnung) of a reflex by its immediate predecessor. This I have seen

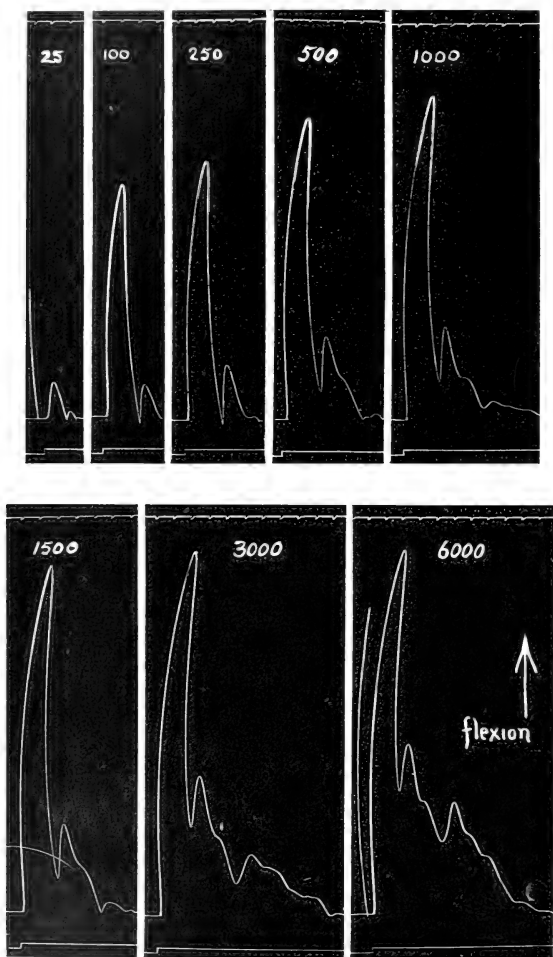


FIG. 2.

occur even across an interval of 15 seconds (fig. 3). Bahnung is particularly likely to occur when a number of reflexes are excited in fairly



quick succession from a preparation that has previously lain quiescent for some length of time. The staircase phenomenon seen in skeletal and cardiac muscle may then make its appearance in the reflexes (fig. 3).<sup>1</sup>

Turning to the inhibitory side of the reflex, the reactions exhibit there a grading not less delicate and extensive than those of the excitatory side. With increase in the strength of the stimulus (break shock), the amplitude of the reflex relaxation increases (fig. 4). Also the speed of the relaxation becomes greater (fig. 4). Increase in speed of progress of the relaxation is particularly evident when, instead of a single momentary stimulus, the stimulus used is faradic. Fig. 5, A B C, illustrates this. For each of the three reflexes in the figure the stimulus consisted of a series of double shocks obtained by an interruptor vibrating 40 per second in the primary circuit. In the reflex of fig. 5, A, the secondary coil stood at 40 units on the Kronecker scale; in reflex B at 100 units; in reflex C at 500 units. The

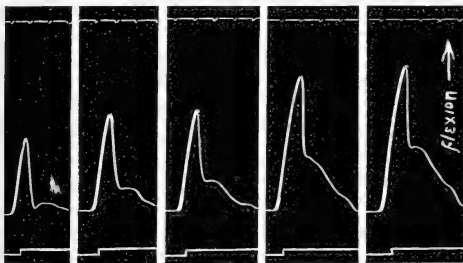


FIG. 3.

reflex relaxation is not only greater in B than in A, but its progress is more rapid; and in C than in B. This greater speed of progress of the relaxation under stronger stimuli finds a close counterpart in the greater speed of augmentation of contraction in the excitatory reflex under stronger stimuli.<sup>2</sup> It is a point of likeness between the inhibitory and the excitatory sides of this "flexion-reflex." The relaxation in reflex C (fig. 7) is followed, after a latency longer than that of the initial reflex inhibition, by the contraction termed the "rebound-contraction,"<sup>3</sup> ascribable to "successive spinal induction."<sup>4</sup> That this occurs in C and not in B or A is in accord with the description of the phenomenon given elsewhere,<sup>5</sup> and with the explanation there offered.

When the inhibitory relaxation is weak it is, in my experience, not rarely accompanied by tremor. This is seen in fig. 5, A and B; also in the

<sup>1</sup> Cf. Stirling, W. (in the frog), *op. cit.*

<sup>2</sup> Sherrington, C. S., *Proc. Roy. Soc.*, lxxvi. B, pp. 272, 274.

<sup>3</sup> Sherrington, C. S., *Proc. Roy. Soc.*, vol. lxxvi. B, p. 160, 1905; *ibid.*, lxxvii. B, p. 478, 1906; *ibid.*, lxxix. B, p. 347, 1907; *ibid.*, lxxx. *Integrat. Action etc.*, p. 206.

<sup>4</sup> *Ibid.*

<sup>5</sup> *Ibid.*

weaker reflexes in fig. 4. The tremor is sometimes more marked than in those examples, as in fig. 6. In the reflex of fig. 6 the stimulus was

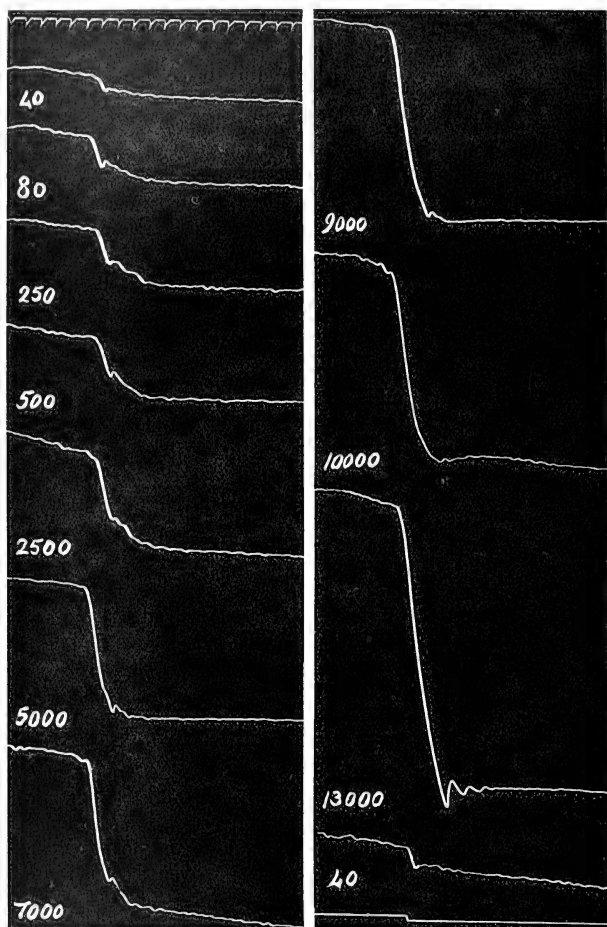


FIG. 4.

faradic and of little more than threshold value; the inhibitory relaxation is correspondingly weak, and when it occurs the muscle exhibits tremor, and this outlasts considerably the period of application of the inhibitory

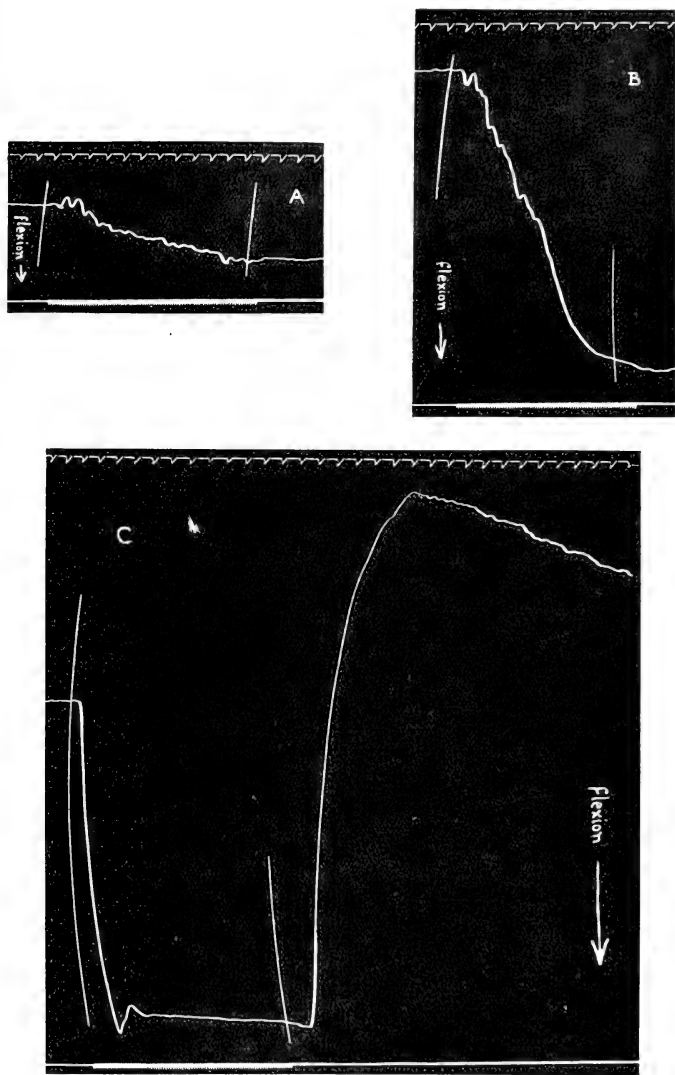


FIG. 5.

stimulus. Weak reflex relaxations come thus to have a hesitant character, very obvious even to simple inspection. This hesitant character constitutes a point of likeness between weak inhibitory and weak excitatory reflexes; examples of the latter have been furnished elsewhere.<sup>1</sup>

The myogram of the inhibitory reflex differs from that of an excitatory reflex in the former's showing little or no recovery by the muscle (under the conditions of experiment) of the length it had prior to initiation of the reflex. Subsequent to the inhibition-reflex the muscle continues to remain of the new length it assumed under the relaxation due to the reflex<sup>2</sup> (fig. 4). After an excitatory reflex the muscle fairly quickly resumes the length it had before its reflex contraction.

The amplitude of the reflex relaxation caused by a single induction shock, although showing regular increase, within certain limits, with in-

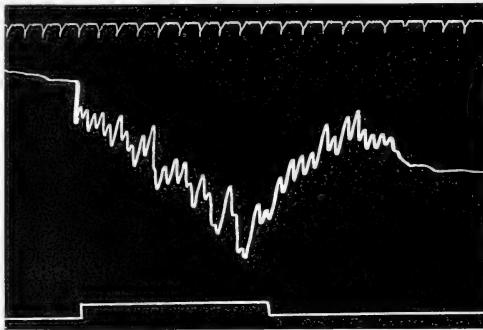


FIG. 6.

crease in the strength of the momentary stimulus, does not in my experience reach, even under the strongest of such momentary stimuli, the extent it attains when, instead of a single induction shock, a short series is delivered, as in faradic stimulation (fig. 7, A and B). The extent of the reflex relaxation which a faradic stimulus, even far below maximal, can produce exceeds the relaxation which a maximal single induction shock produces. This constitutes a further point of resemblance between reflex inhibition and reflex excitation. The most ample and powerful of the reflex contractions elicited by the single break shocks (figs. 1 and 2) fall in amplitude and strength much below reflex contractions easily elicitable from the same preparation by faradic stimuli, even brief and quite submaximal. Fig. 7 exhibits in A the reflex relaxation of vasto-crureus evoked by a single break shock of 12,000 units of the Kronecker scale. Fig. 7, B, is the reflex

<sup>1</sup> Sherrington, C. S., Proc. Roy. Soc., vol. lxxvii. B, p. 494; Integrat. Action etc., p. 70.

<sup>2</sup> Sherrington, C. S., Proc. Roy. Soc., vol. lxxvi. B, p. 273.

relaxation from a short series of double shocks at 6000 units delivered at 30 per second. The reaction B was observed one minute after the reaction A, no change having been made in the position of the electrodes, or in the reflex preparation during the interval. From the difference between the two reflexes it is evident that summation is in marked degree a physiological property of the reflex arc in its inhibitory as well as in its excitatory reactions.

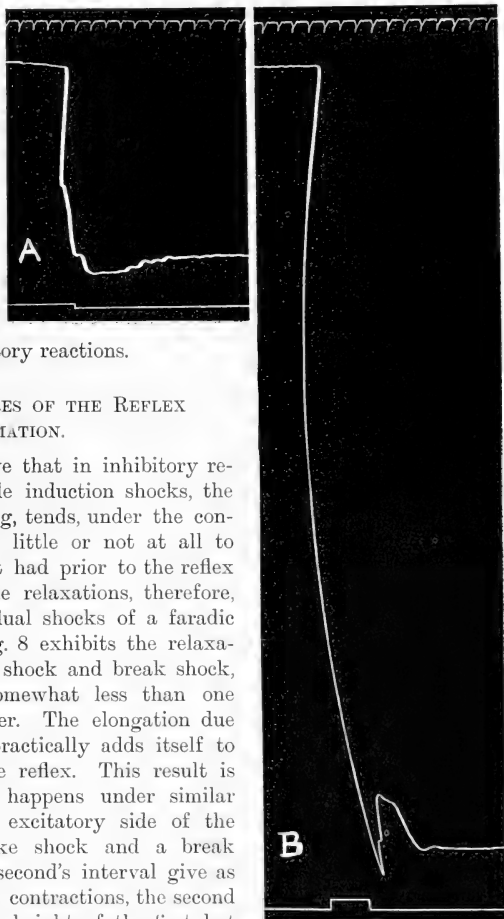


Fig. 7.

## II. SOME FEATURES OF THE REFLEX SUMMATION.

It was shown above that in inhibitory reflexes excited by single induction shocks, the muscle, after elongating, tends, under the conditions of experiment, little or not at all to return to the length it had prior to the reflex which relaxed it. The relaxations, therefore, evoked by the individual shocks of a faradic series easily sum. Fig. 8 exhibits the relaxation caused by a make shock and break shock, the latter ensuing somewhat less than one second after the former. The elongation due to the break reflex practically adds itself to that due to the make reflex. This result is not like that which happens under similar circumstances in the excitatory side of the reflex. There a make shock and a break shock following at a second's interval give as result two short reflex contractions, the second not superposed on the height of the first, but starting practically from the same base line as its precursor. The reflex contractions are not superposed unless the time interval between the two single stimuli be less than some  $150\sigma$ .<sup>1</sup> With this the inhibitory reflexes stand in apparent

<sup>1</sup> Sherrington, C. S., *Integrat. Action etc.*, p. 44.

contrast. Superposition of the mechanical results of single stimuli evoking the inhibitory reflex occurs readily when the time interval between the stimuli extends to  $4000\sigma$ – $5000\sigma$ ; in some records the interval is very much longer still.

Stimuli which, taken singly, produce no perceptible or scarcely perceptible relaxations, produce on repetition relaxations of large extent by summation. Fig. 9 exemplifies this. On the signal line each descent marks a break of the primary circuit. Fig. 9, A, gives the effect of sixteen feeble break shocks, each of 20 units, on the Kronecker scale, delivered in the course of 4.4 seconds. The total relaxation is considerable. Fig. 9, B, shows the degree of correspondence between the rate of succession of the single weak stimuli and the incidence of the separate reflex relaxations.

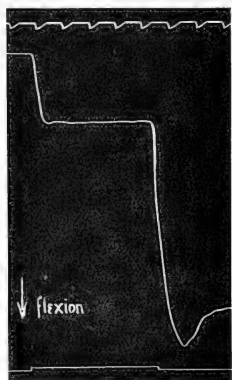


FIG. 8.

The relaxation produced by a single occurrence of the weak stimulus (break shock of 15 units of the Kronecker scale) is seen at the top left-hand corner. Then follow twenty-two similar break shocks of slow and irregular repetition. The effect of each of these is evident on the graphic record. Next ensues an interval of nearly one second without stimulus, and no further relaxation occurs during that time. Then follow twenty-four more of the break shocks at more irregular intervals, and these again sum as to the mechanical effect of their reflexes on the muscle. The later members of the series of stimuli succeed each other rather more rapidly than do the earlier, and their combined effect is seen to be greater. The greater effect of the summation when the individual stimuli follow each other more rapidly is seen better in fig. 10.

Here sixty feeble break shocks (intensity 10 units of the Kronecker scale) were delivered. The first twenty-five of these were delivered at the average rate of 4.5 per second, and produced relatively little relaxation; the last thirty-five shocks of the series were delivered at an average rate of sixteen per second, and they produce much more relaxation—more than four times as much. The more quickly delivered shocks were, owing to the quicker rotation of the interruption key, given by more sudden opening of the primary circuit, and were individually therefore somewhat more intense; and this applies also to the reflex of fig. 9. But the much greater reflex effect of the more frequent than of the less frequent series suggests that with the former their central reactions, and not merely their mechanical elongations of the muscle, summed. With summations such as that shown in fig. 8, an interpretation which obviously can be put upon the result is, that the effect of each stimulus may be divisible into two successive parts. The first part appears to be

a depression of the motoneurone's discharging activity; the second appears as the supervention of a state in which, although there is no further depression, there is no restitution, or only a very slow one, of the moto-

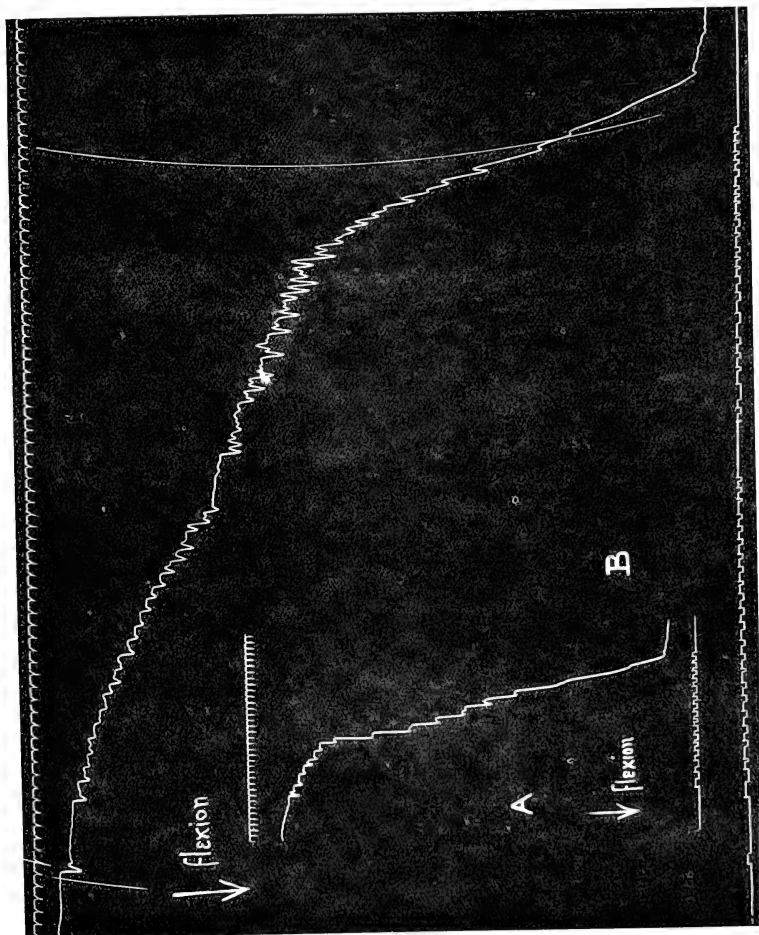


FIG. 9.

neurone's previous discharge. In the first part or period of the effect central inhibition is active; but nothing appears to indicate that it is active in the second. In such summations as that of fig. 8, the summation can be explained without supposing that the process of central inhibition due to

the second stimulus overlaps in time with that occasioned by the first stimulus. In such summations as that, for instance, of fig. 10, it seems probable that the greater reflex effect of the more rapidly following stimuli

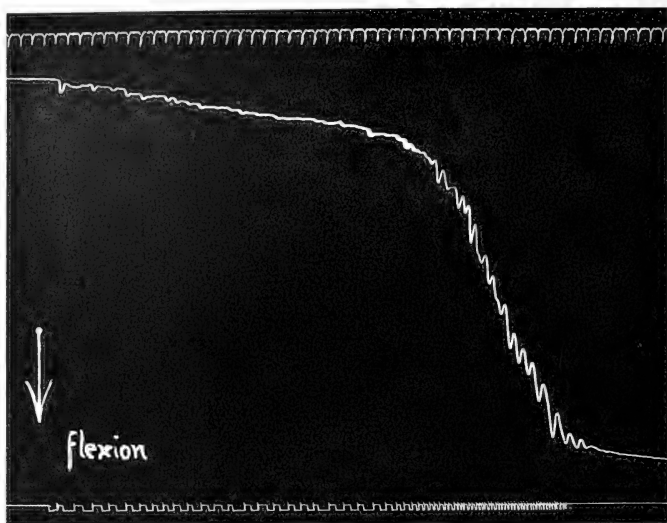


FIG. 10.

is due to summation and overlapping in time of the periods of inhibitions occasioned centrally by the individual stimuli.

It is evident that the reflex arc is delicately responsive to the time relations of its external stimuli in the inhibitory aspect of its function, as well as in the excitatory.



THE FREEZING OF FROG'S NERVE, WITH SPECIAL REFERENCE  
TO ITS FATIGABILITY. By JOHN TAIT. (From the Laboratory  
of Physiology, Edinburgh University.)

DURING the course of an investigation into the influence of low temperature on the conductivity of frog's nerve (1), (2), I often had experiments interrupted by the freezing of the nerve. I used the gastrocnemius nerve-muscle preparation, taking the muscle response as an index of the state of the nerve, of which the middle portion was cooled, while the extreme central and peripheral ends, as well as the muscle, were more or less protected by insulation from cold radiation. When freezing occurred the muscle would suddenly begin to twitch, or then pass at once into a condition of prolonged tetanic contraction, followed after a minute or so by irregular convulsive movements. These continued for two or three minutes, the contractions becoming feebler and feebler until they finally ceased. The nerve then refused to conduct. In other cases again, one or two little twitches of the muscle, followed by absence of conductivity, was all that indicated the onset of freezing. In every case after such irregular muscular movements the nerve was found on examination to be hard and stiff, and had a white, opaque appearance as if covered with hoar-frost.

At first it seemed that the frozen nerve was killed, for no recovery occurred after thawing. Further observation showed that the death of the nerve in such instances was due to mechanical injury during the frozen condition, for, out of curiosity, I had been in the habit of touching the frozen nerve with a pencil to test its rigidity. On ceasing to interfere with it in this way I found that subsequent thawing restored the conductivity. Since then I discovered that Boruttau had previously made similar observations (3).

The conductivity of the frozen nerve does not, as a rule, come back immediately on thawing. Some time usually elapses before conductivity is restored. In cases of doubt as to the actual vitality of the nerve, I always found that after a rest of an hour or two in Ringer's solution the conductivity did return. We may consequently take it that a frozen nerve, while very susceptible to mechanical injury, is not necessarily killed by freezing.

Such a result is not at all surprising when we consider that poikilothermic animals in general may be frozen hard without sacrifice of life. The experiments of Raoul Pictet (4), to whom most of our know-

ledge of this subject is due, showed that fishes which had been cooled down in a block of ice to  $-15^{\circ}$  C. still remained alive after careful warming, although others similarly treated could be pounded like ice into powder. After a temperature of  $-20^{\circ}$  C. the fishes were found to be dead. Frogs, again, withstood a temperature of  $-28^{\circ}$  C.

The exact temperature at which freezing of frog's nerve occurs I have been unable to determine. Boycott (5), who also records some experiments on freezing of nerve, was inclined to place the freezing-point somewhere about  $-7^{\circ}$  C. With my apparatus, which probably gave a more exact determination than his, I found that freezing occurs at a temperature somewhat higher than this, viz. between  $-3^{\circ}$  and  $-5.5^{\circ}$  C.

To study more exactly the changes produced in nerve by means of freezing, it seemed better to freeze a minute length than to freeze a longish portion. By means of a piece of glass-tubing with an hour-glass constriction in the middle, on to which the nerve suspended between two pairs of electrodes could be lowered, it was possible to freeze a very short length of the nerve by running cold alcohol through the tube, and to subsequently thaw it again by running in warmer fluid. The outside diameter of the constricted part of the tube was 2 mm. The alcohol was cooled by means of an ice and salt cooling mixture, consequently the temperature of the frozen part of the nerve might vary from the freezing-point right down to within a few degrees of  $-22^{\circ}$  C. The vessels containing the supply of cold fluid and of warm fluid respectively were connected with the glass tube by means of a three-way stop-cock. A small screw clamp connected to a piece of rubber-tubing at the further end made it possible to regulate the rate of flow and thus to roughly graduate the temperature. The two pairs of stimulating electrodes on which the nerve was suspended were connected by means of a Pohl commutator, from which the cross wires were removed, to a standard Kronecker induction coil, in the primary circuit of which was placed a small accumulator charged to  $4\frac{1}{2}$  volts. By means of these electrodes the nerve could be stimulated either centrally or peripherally to the cooled portion.

#### I. CHANGES DURING FREEZING.

It is difficult to determine the exact cause of the muscular twitchings that accompany freezing of the nerve. Prolonged observation showed that by the time the muscle twitchings began the whole of the small portion of nerve lying over the cold tube was already opaque in appearance. This might indicate that the nerve is frozen right through before twitchings occur; but, on the other hand, the white appearance may be due to deposition of hoar-frost from the atmosphere before the salt solution inside the nerve has reached its freezing-point. The generally accepted idea is that the twitchings and subsequent loss of conductivity in the nerve are due to mechanical compression owing to the expansion of the water-substance in the nerve during freezing, and it has been shown (6) that mechanical

compression applied from the exterior to a normal nerve may temporarily abolish conductivity. On physical grounds it is improbable that the outside coating of ice nips the nerve. A hollow cylinder of water undergoing freezing would expand and not contract.

Observations made by botanists on the freezing of plant tissues (7) show that crystals of ice become deposited first of all between the cells, and only at a later period, when the temperature is still further lowered, inside the actual cells. Although there are no observations published, so far as I am aware, on the histological appearances inside frozen nerve, we cannot imagine that the solution of salts inside the nerve becomes suddenly solid throughout. Once freezing starts the water is probably extracted from the surrounding solution by degrees, thus raising the concentration of the salts in the non-frozen portions. To any definite fixed temperature below the freezing-point there would probably correspond a definite concentration of the solution in the still fluid portions, while only at a very low temperature would the nerve be solid throughout. The twitchings would, from this point of view, correspond rather to the twitchings that occur in a muscle when its nerve is dried.

At one time it seemed as if some light might be thrown on the freezing process by a study of the muscle twitchings which ensue upon freezing of the nerve, for at first I imagined I could detect a correspondence in the behaviour during freezing of two preparations from the same frog. Further investigation, however, showed that such a correspondence, even when all precautions are taken to make the conditions similar, is much less constant than I at first thought.

Another method of investigation which occurred to me was to subject some frogs before dissection to continuous evaporation. Durig (8) found that frogs may lose in two to three days, by evaporation, from 20 to 30 per cent. of their weight: in one case he succeeded in diminishing the weight of a frog by 39 per cent. without killing it. In the process of drying a great concentration occurs in the body fluids, the concentration being least marked in the brain and heart of the animals. On the assumption that the nerves might participate in this drying-up process, I took a number of frogs and, after weighing them, placed them in an open wire cage outside the laboratory window. The outside temperature was but a few degrees above zero, and yet in eighteen to forty hours they had lost from 12·8 per cent. to 23 per cent. of their body weight. Preparations were examined in various conditions of dryness, care being taken not to moisten the nerve with saline solution before freezing. Except that one case, viz. the one which was least dried, showed a more markedly tetanic and longer-lasting response than usual, the muscle twitchings were not different in character from those in ordinary cases. Apparently little help can be got from this method of investigating the question.

The character of the muscle response during freezing of the nerve depends to a large extent on the method of freezing. When one freezes

a minute length of nerve, using the thin glass tube previously described, the muscular response tends to be convulsive and not tetanic, while the period of time during which the twitchings continue is not long. When, on the other hand, 2 or 3 cm. of the nerve is frozen in a cold chamber, the cooling being effected by radiation, the muscle tends at first to go into a prolonged tetanus, while the succeeding convulsive movements may last for many minutes. Whether this difference is due to more rapid freezing in the case where the cooling is by conduction, or whether it depends simply on the shorter length of nerve frozen, I have not as yet determined.

On looking over my charts, I find that freezing of thick nerves from large-sized frogs is, as a rule, accompanied by more twitching of the muscle than is the case when preparations from smaller frogs are used. Whether this is a general rule or merely accidental I cannot say.

One thing, however, became apparent during the investigation of the muscular twitchings from freezing of the nerve, viz. that under certain conditions freezing may occur without any twitching of the attached muscle. Previous experiments on the cooling of nerve (9) had shown that conductivity may disappear at temperatures lying entirely above the freezing-point, while in other cases again no such disappearance of the conductivity occurs even though a considerable length of the nerve be cooled right down to any temperature short of the freezing-point. It was a natural idea that, in those cases in which freezing was unaccompanied by muscular twitching, the temperature of such disappearance of conductivity was high; for then, before the molecular disturbance due to freezing occurred, the nerve for some considerable distance around might be quite incapable of conducting as the result simply of cooling. One or two experiments sufficed to show that this is the correct explanation of the phenomenon.

Nerves in which conductivity disappears at a high temperature may undergo freezing without any excitation being transmitted from the site of the freezing to outlying parts. Other nerves, in which the temperature of disappearance of conductivity lies low, are thrown into a condition of excitation throughout their whole extent when freezing occurs.

Seeing that absence of conductivity in a cooled nerve may arise in two ways, (1) in certain cases as the result of cooling to a temperature still above the freezing-point, (2) as the result of freezing, it is well to clearly distinguish the two conditions. Botanists use the term "cold rigor" to indicate the condition in which function ceases in a plant as the result of cooling when the temperature still remains above the freezing-point. Without committing ourselves to any theory as to the process by which conductivity becomes abolished in a nerve which has not been cooled to the freezing-point, we shall, for convenience, use the term "cold rigor" to denote the condition in question.

One phenomenon I got to look on as indicative of the near approach of freezing in a cooled nerve. If one gradually lowers the temperature,

testing the conductivity at every stage, a sudden marked improvement in conduction occurs just before freezing sets in. Before we discuss this question, however, a word or two is necessary as to the method adopted to test the conductivity.

The work of Wedensky (9) and of Fröhlich (10) on anæsthetised nerve has shown the value of rapid rhythmical stimulation at varying intensities for detecting slight changes in conductivity at a time when, by ordinarily employed methods, such changes are not discoverable. By the method of applying single maximal shocks to the nerve, depreciation of conductivity is indicated only by a falling off in the height of the muscle twitch following stimulation of the nerve. Apart from this change, conductivity seems to remain unaltered almost up to the point at which it suddenly disappears for good. By the method of rhythmical stimulation, on the other hand, as Fröhlich showed, one is able not only to determine roughly the degree to which the amplitude of the excitation is cut down, but also to show changes in the refractory period.

With progressive anæsthesia the refractory period of the nerve becomes longer and longer; consequently, when one stimulates at such a rate that the interval between the individual excitations is less than the refractory period, or about equal to the refractory period, the muscle response becomes abnormal. It may be simply an initial twitch of the same height as that evoked by one maximal excitation; it may be a tetanus which attains its maximal height at the very start, to fall off very rapidly thereafter, and finally to drop to the base line again, thus showing that the conductivity of the nerve has temporarily ceased. Indeed, Fröhlich proved that this latter form of tetanus was an expression of fatigue on the part of the nerve, and a tetanus of this special form he names a "fatigue tetanus."

Such effects are more readily obtained with strong than with weak stimulation, for the duration of the refractory period seems to vary with the intensity of the corresponding stimulation. As previously mentioned, they are to be detected at a time when the conductivity, as tested by the method of isolated maximal break shocks, is apparently present in undiminished degree. Consequently, in testing the conductivity of cooled or of frozen nerve, I availed myself of this method. The rate of rhythmical stimulation used was such as could be obtained by means of the springs supplied with the Kronecker coil, and varied with occasion from 30 to 256 stimulations per second.

Fig. 1 is a record of an experiment in which a portion of nerve was gradually cooled from  $0^{\circ}\text{C.}$  to  $-3.5^{\circ}\text{C.}$ , the state of the conductivity being meantime tested every few seconds by short rhythmical stimulation of constant intensity (30 Kronecker units) and rate (144 per second). In this case the cooling was not effected by means of the thin glass tube, but about 7 mm. of the nerve was cooled in a cold chamber. The first six stimulations produce a kind of tetanic response of the muscle, each successive response, apart from the preliminary uprise, being less marked than the

preceding. These are typical "fatigue tetani." As cooling proceeds there comes a time when only single maximal twitches are got; these gradually

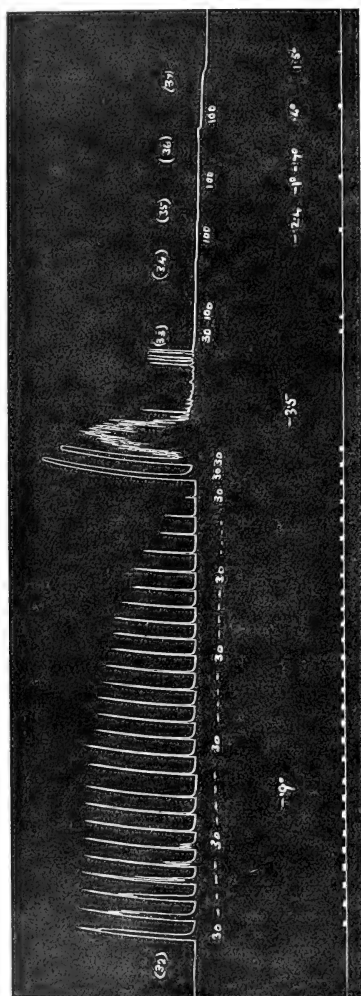


FIG. 1.—The figure shows the condition of the conductivity of the nerve as it is gradually cooled from about  $0^{\circ}\text{C}$ . to  $-35^{\circ}\text{C}$ . (at which point freezing occurs), and then warmed again. Short series of rhythmic excitations (14 per second) at an intensity first of 30 and later of 100 Kroecker units were applied to the nerve at intervals, as shown in the lower line. Observe (1) the gradual abolition of the conductivity as cooling occurs; (2) the sudden improvement in conductivity just before the onset of freezing (this is indicated by the fact that the muscle response is a more or less elevated tetanus and not simply a feeble initial twitch); (3) the absence of conductivity immediately after thawing.

decline in height until finally, when the conductivity is just on the point of going away, the muscle response takes on the form of a complete tetanus

The next stimulation also evokes tetanus, but of a less height. Immediately thereafter the muscle goes into freezing tetanus. When next tested, conductivity is seen to be abolished. This is a case in which, by an accident, the temperature of cold rigor corresponding to the definite length of nerve cooled and the freezing-point of the nerve coincide, and the immediate improvement in conductivity just before freezing sets in is well shown. Similar tracings, none of them, however, so strikingly illustrative as this one, might be repeated indefinitely.

Once or twice it was noted that mere stimulation of the nerve, when the temperature was very low, sufficed to send the muscle at once into a tetanic contraction as if over-cooling had occurred and the molecular commotion due to stimulation had suddenly induced freezing: but such cases are rare.

During the process of freezing the conductivity is not necessarily at once abolished. After the muscle has begun to twitch convulsively, rhythmical stimulation of the central end of the nerve may produce a smooth and elevated tetanus which stands up above the irregular twitches immediately preceding it, and corresponds exactly in duration to the period of stimulation on the cessation of which the irregular twitching begins again. If the freezing be interrupted during this stage, the conductivity is not necessarily abolished. Further, even after the muscle twitchings due to the occurrence of freezing have entirely ceased, the property of conductivity may still be retained by the frozen portion. The muscle may react by single twitches to isolated shocks, and to rapid rhythmical stimulation applied centrally; but these twitches quickly fall off in height and the frozen portion soon ceases to conduct. Hitherto I have not observed tetanus of the muscle to follow upon rapid rhythmical stimulation under these conditions.

To sum up:—When freezing takes place the nerve first becomes white externally. About the same time an improvement in conductivity suddenly occurs: excitatory processes are not so much cut down in amplitude on passing through the cold area and the refractory period becomes shorter.

Immediately thereafter, in consequence of the molecular disturbance due to freezing, the whole nerve is, as a rule, thrown into excitation. In cases, however, where the temperature of cold rigor is normally high, the disturbance arising at the site of freezing is not propagated outwards because of the existence of cold rigor in immediately adjoining parts of the nerve.

When the nerve is in this state of more or less tumultuous agitation due to the occurrence of local freezing, it may retain for some time the property of conducting rhythmical stimuli of external origin throughout its whole length, including the actual site of freezing. Conductivity, finally, becomes abolished if the nerve be sufficiently cooled, though often enough it does

not disappear until some little time after the convulsive twitchings of the muscle accompanying freezing of the nerve have ceased. These facts indicate that the absence of conductivity induced by freezing does not come on abruptly, but gradually and progressively.

During the frozen condition the nerve, while very susceptible to mechanical injury, is not necessarily dead.

## II. CHANGES ON SUBSEQUENT THAWING.

The process of thawing of the nerve, unlike the process of freezing, is not accompanied by any muscular twitchings. In the case of a nerve which has been frozen until all convulsive twitchings of the muscle have stopped, the only external sign of subsequent thawing is the disappearance of the hoar-frost round the frozen part of the nerve. If the thawing take place while the muscle is still contracting vigorously as a result of the freezing process, the muscle contractions cease almost abruptly, and the lever, after one or two diminished excursions, drops back to the base line, to remain there till external stimulation of the nerve be applied. The melting of the ice in the nerve, and the presumable re-dilution of its fluid contents, consequently sets up no marked excitation of the structure.

Nor is the vitality of the nerve in any way prejudiced by the rapidity with which thawing occurs. Pictet (4) found that, when the living animal is frozen, the thawing process must be gradual if life is to continue. Although I have thawed nerves at very different rates, in no case have I seen the slightest ultimate harm ensue from rapid thawing.

As was said before, the conductivity of the nerve does not necessarily return at once on thawing. The time taken for recovery varies considerably in different experiments. According to the time taken and the mode of recovery, we might distinguish three different types of cases:—A. In some cases recovery is rapid, what is apparently full conduction being restored within the space of a minute after thawing. B. In other cases (the majority in my experiments) recovery is delayed for a period varying from a few minutes to an hour or even longer. C. In a few cases a partial recovery of conduction occurs after a minute or two, this is succeeded again by absence of conductivity, and finally, after a more or less prolonged period, full recovery occurs.

As to the conditions under which these various eventualities occur, I have no information to offer. In any one experiment there are a number of variable factors, e.g. the original state of the nerve itself as regards nutrition, thickness, temperature at which cold rigor occurs, etc.; the length of nerve frozen; the rate at which freezing occurs; the ultimate temperature to which the frozen nerve is lowered; the rate of thawing and of subsequent rise of temperature; the degree to which the temperature is ultimately raised; and finally, the extent to which the nerve has been



stimulated before freezing, during the frozen condition and after thawing. For all we know, any one of these factors may influence the result, and although I have carried out experiments to determine the possible influence of various of them, I have as yet reached no positive conclusion.

It must not be assumed that in every case recovery of the nerve occurs after freezing. In a certain small number of cases in my experiments the nerve did not recover even after a prolonged rest. In each of these cases, however, there was reason to believe that the nerve before being frozen was not in a normally healthy condition, so that we may look on death of the nerve from freezing as quite an unusual phenomenon.

No matter whether the return of conductivity after thawing be rapid or delayed, it is always possible to detect stages in the re-establishment of full conduction. As recovery occurs the nerve first of all regains the power of transmitting strong excitations; only at a later period is it able to conduct weak excitations, and in a case where recovery takes place slowly the gradual improvement of the nerve in the transmission of successively weaker and weaker excitations is easily followed. Such observations may be made by using single-break shocks of different intensity to excite the nerve.

When one uses, on the other hand, series of rhythmical stimuli of varying rate so as to examine the duration of the refractory period, one finds that at first single initial twitches are got even with low rates of stimulation, at a later stage tetanus; only after some time does tetanus occur on rapid stimulation, but eventually even with the most rapid rates at one's command the muscle is always thrown into tetanus. This points to a gradual and progressive shortening of the refractory period throughout the stage of recovery.

As regards the duration of the refractory period corresponding to stimuli of different intensities, the result seems to fall out in two different ways according to circumstances. As a rule it is found that the refractory period corresponding to a strong excitation is shorter than that corresponding to a weak one. This is indicated by the fact that rhythmical stimulation of high intensity tends to produce full tetanus of the muscle at a time when stimulation at the same rate but of lower intensity causes only an initial twitch. In a minority of cases an opposite effect is got, viz. full tetani occur on weak stimulation, while initial twitches follow on strong stimulation, pointing to the fact that in these cases the refractory period is shorter for weak stimulations than for strong. The conditions determining which of these two effects should follow I have been unable to discover.

The fact that, under the special conditions of freezing followed by thawing, the refractory period of nerve may apparently be shorter the more intense the preceding excitation, is worthy of special attention. Fröhlich, who first systematically investigated changes in the refractory period of nerve by means of the method of rapid rhythmical stimulation (10), was inclined to conclude, from the coexistence in anaesthetised nerve of long

refractory period with strong excitations, that such coexistence is a general phenomenon. As I have found exceptions to this rule, not only in nerve that has been frozen and thawed again, but also in nerve which is recovering from simple cold rigor, we are not at liberty to generalise and say that a strong excitation in nerve is always accompanied by a longer refractory period than a weak excitation. The exceptional behaviour in this regard of nerve which has been frozen and then thawed would indicate that the internal state of such nerve is not quite comparable with that of anæsthetised nerve.

If at any stage during the recovery of a nerve which has been frozen and warmed again to room temperature, the nerve be once more frozen at the same spot as before, freezing occurs this time without any twitching of the muscle. The reason of this is clear when, instead of actually freezing the recovering nerve, we simply lower its temperature a few degrees below that of the room.<sup>1</sup> Cold rigor then occurs at a temperature many degrees above zero, although the nerve, previous to being frozen, may have retained its conductivity at all temperatures down to the freezing-point. Evidently, therefore, a nerve which has been frozen, and subsequently thawed, is, for a time at least, very susceptible to the influence of low temperature.

This condition of the nerve, whereby it loses its conductivity so readily with depression of temperature after having been once frozen and warmed again to room temperature, is not limited to the first stages of recovery after freezing. Long after the conductivity, as tested by rhythmical stimulation at the fastest rate possible with the Kronecker apparatus, may have to all appearance completely returned, the nerve still tends to go into cold rigor at moderately high temperatures, though as time goes on this tendency progressively diminishes. By testing the nerve in this way we see that changes are produced by freezing which persist for a long time after thawing.

In showing such susceptibility to the depressing action of low temperature, thawed nerve does not stand alone. A similar condition is found in nerve which, without being frozen, has been cooled to some temperature sufficiently low to abolish its conductivity, and has then, during the stage of recovery which ensues upon warming, been fatigued by rhythmical stimulation.<sup>2</sup> Furthermore, under certain nutritive conditions nerve may normally, i.e. without any special experimental treatment, show a similar extreme susceptibility to the action of slightly lowered temperature. The temperature relations in regard to the conductivity of nerve under different conditions have as yet been very imperfectly worked out.

As was previously mentioned, the time for recovery of conductivity after

<sup>1</sup> By arranging that the freezing apparatus should fit on to the apparatus which I used for simple cooling of nerve, it was possible, after freezing and then thawing a minute portion of the nerve, to subsequently cool this portion to any desired temperature without any shifting of electrodes.

<sup>2</sup> For details as to the method of treating the nerve, see paper previously referred to on Fatigue of Medullated Nerve by the Method of Cooling (2).

freezing may be long. If the nerve be frozen twice at the same spot, the time for recovery of conductivity after the second freezing is still further prolonged, though full recovery of conduction, as far as it can be tested with the most rapid rate of stimulation possible with the Kronecker apparatus, does ultimately occur. Hitherto I have carried out no experiments to test the effect of repeated freezings and thawings carried out on the same nerve. Botanists have shown that plants subjected to repeated freezings show gradual diminution in vitality with each freezing and ultimately die. Arguing from the result of two freezings on nerve, it is at least possible that freezing never occurs in excised nerve without some slight irreparable alteration in its structure.

To sum up at the present stage:—

Nerve which has been frozen and thawed again is not, for some time at least, in normal condition. After thawing, slow changes begin to occur which ultimately lead to the more or less complete restoration of its function. The time for such restoration may vary greatly in different cases. As recovery proceeds the nerve first of all becomes capable of transmitting strong excitations; only at a later period is it able to transmit weak excitations, and this improvement in functioning power takes place gradually. Meantime, the refractory period becomes progressively shorter and shorter. At any given stage the refractory period corresponding to strong excitations may apparently be shorter than that corresponding to weak; in other cases again the reverse holds true, and the refractory period for strong excitations is longer than that for weak.

After a frozen nerve has been warmed to room temperature and its conductivity is beginning to return, it may again cease to conduct if simply lowered a few degrees in temperature. By testing the nerve in this way for disappearance of conductivity at different temperatures, it is found that a tendency to cold rigor at relatively high temperatures persists for a considerable time after freezing, though, as time goes on, this passes off. In consequence of this exaggerated tendency to cold rigor, a second freezing of the nerve following closely upon a first is attended by no twitching of the attached muscle.

On numerous occasions now I have observed that some time after freezing, and especially after two acts of freezing, the muscle, which had been lying quite still, would suddenly begin to move convulsively, or pass into an irregular tetanus like a Ritter's tetanus. The result was in no way due to drying either of the muscle or of the nerve, for care was taken throughout to keep the preparation moistened with Ringer's solution; nor did any electrical stimulation act on the preparation. That the source of the disturbance lay in the previously frozen portion of nerve is indicated by the fact that, on one occasion, when the muscle was thus in active

contraction, the tetanus ceased at once when the nerve was snipped across with scissors just peripheral to the site of freezing. It would consequently seem that, under certain conditions, after freezing the nerve is in a state of highly unstable equilibrium and may easily pass into a state of violent commotion. On one occasion at least, after such a violent disturbance had occurred in the preparation, the conductivity of the portion of nerve affected by freezing was found to remain permanently absent although the muscle still responded to peripheral stimulation of the nerve.

One further phenomenon requires to be mentioned. When the nerve, after being thawed, has recovered to such an extent that tetani of the muscle follow on rapid stimulation, these tetani often tend to take at first a peculiar form. This is illustrated in fig. 2. (Although the figure illustrates more than the point immediately in question, a complete experiment has been shown, partly because it happens to demonstrate in small compass a number of points already described, partly because the conditions under which this peculiar form of tetanus is got are thereby rendered more clear.)

The tracing marked 1 shows the condition of the conductivity at the commencement of the experiment, the whole preparation being at room temperature. The muscle tetani corresponding to rhythmical stimulation (rate 144 per second) are smooth and elevated: in other words they are normal tetani. Tracing 2 shows a freezing tetanus followed by ultimate loss of conductivity. The nerve was then warmed by turning on fluid at room temperature at the spot marked "hot." Tracing 3, which illustrates the point immediately under discussion, shows a progressive and rapid return of conductivity, rhythmical stimulation at the same original rate (144 per second) being used throughout to test the condition of the nerve. The first three muscle responses are practically single twitches, the nerve being stimulated at intensities 30, 100, and 300, respectively. The next three are examples of the peculiar form of tetanus in question. In each of these the muscle first gives a single twitch and immediately thereafter relaxes. Then it begins to contract again, not in the rapidly summated fashion of the tetani in tracing 1, but more deliberately and slowly. With each successive series of stimulations the relaxation of the muscle after the preliminary twitch becomes less marked; this change is due, as other experiments have shown, to the rapid recovery going on in the nerve. Further, the preliminary twitches all show a certain regularity in height, thus indicating that the corresponding nerve processes are somewhat similar and of approximately the same magnitude.

A little later the tetani following rhythmical stimulation are free from irregularities (tracing 4), though the tetani are not so high as in tracing 1. The cold fluid was now made to run slowly through so as to cool the nerve gradually, and as a result the muscle responses become single initial twitches of approximately the same height as the preliminary twitches in tracing 3. These finally become abolished, and the nerve freezes again

with only one minute twitch to indicate what has occurred. This time, after warming, the conductivity did not return for at least half an hour,

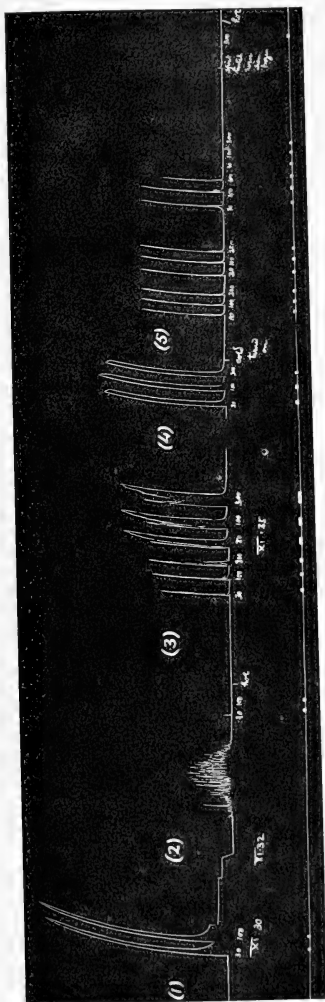


FIG. 2.—This figure illustrates a peculiar condition of the conductivity which often occurs during recovery of a nerve after freezing (see tracing (3)). Rate of stimulation 144 per second.

- Tracing (1) shows two normal tetani, the result of stimulation before cooling of the nerve. The nerve was then lowered on to the freezing tube.
- " (2) shows freezing tetanus. The nerve was thawed again at point marked "hot."
- " (3) shows more or less rapid recovery of the nerve after thawing. The last three series of rhythmical excitations are followed by the peculiar muscle response in question, indicating that the nerve conducts normally the first excitation of the rhythmical series, but immediately thereafter conducts imperfectly, while eventually it conducts well again.
- " (4) shows still more complete recovery: the nerve was now cooled again.
- " (5) shows ultimate disappearance of conductivity on cooling. Freezing occurs this time without muscular twitching.

though two hours later the muscle again responded by complete tetani to rhythmical central stimulation.

Throughout this experiment the drum was kept uniformly revolving, and the figure shows a record of eight minutes' duration.

As to the internal processes in the nerve corresponding to the kind of tetanus seen in tracing 3, all we can say is that the nerve readily transmits at least the first excitation of the series, but this preliminary effort is followed by temporary disablement which, as the stimulation proceeds, is gradually recovered from. That the preliminary twitch in the tetanus represents only one excitation is probable from its correspondence in height with the twitches seen in tracing 5, which, from other experiments, we know to correspond to one excitation alone. But why the first excitation of the series is followed by a temporary fall in conductivity while later excitations are not, is hard to say.

### III. NERVE FATIGUE AFTER FREEZING.

In a previous paper (2) I have shown that, after being in cold rigor, nerve may be readily fatigued during the stage of recovery of conduction due to subsequent warming, and that the fatigue then induced may be more or less long-lasting. The method adopted to produce such fatigue was sometimes by means of alternate central and peripheral rhythmical stimulation, sometimes by means of central stimulation alone. As somewhat similar results have been obtained after freezing of the nerve, I shall now describe them.

#### (a) Short-lived Fatigue.

On p. 87 it was pointed out that, during the recovery stage after freezing, the refractory period corresponding to different intensities of stimulation may sometimes be shortest when the intensity is greatest, while in other cases again the reverse is the case. It would follow from this that in certain cases during recovery the refractory period may be almost the same for all intensities of stimulation. The example shown in fig. 3 is apparently a case in point.

In the experiment of which this is a record, a minute portion of the nerve was frozen and the conductivity found to remain absent for some considerable time after thawing. The nerve was taken off the electrodes and put back into Ringer's solution for an hour and a half. At the end of that time it was found to have almost entirely regained its conductivity, the tetani on central stimulation being practically as high as those obtained on peripheral stimulation. The previously frozen part of the nerve was now gradually cooled to  $-1^{\circ}$  C., at which temperature cold rigor came on. On gradually warming the nerve again conductivity returned, at first imperfectly and then more and more completely. At a temperature of  $+3.5^{\circ}$  C. isolated make-and-break shocks at intensity 200 are seen to produce single twitches, while rhythmical stimulation at a rate of 144 per second produces "fatigue tetani" both at 200 and at 500 units of intensity.

On repeating the rhythmical stimulation, fatigue tetani of much the same form in each case are got at intensities 300, 400, 500, and 100.

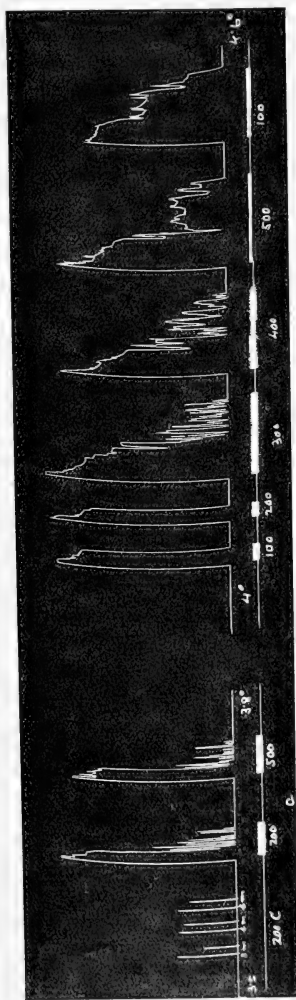


FIG. 3.—The figure shows "fatigue tetani" as the result of rhythmical stimulation (144 per second) of the proximal end of a nerve which had been frozen and thawed, and once again cooled until cold rigor set in. The tracings were taken during the stage of recovery from cold rigor. The tetanic response of the muscle in each case tends to disappear as the stimulation is continued.

This special form of tetanus is in no way due to fatigue of the muscle, for control experiments have again and again shown that peripheral stimu-

lation of the nerve under such conditions causes full and complete tetanus of the muscle. Furthermore, had peripheral stimulation been used here, the conductivity of the affected portion of nerve would thereafter have been abolished and the experiment temporarily stopped. The fatigue is in the nerve and in it alone.

From the improvement in the muscle response after each short rest from stimulation it is evident that the fatigue in each case is short-lived. This corresponds to the fatigue already demonstrated by Fröhlich (10) in anæsthetised nerve. Although in the present instance the nerve had been in cold rigor subsequent to the actual freezing process, still similar curves are often obtained during the stage of recovery that follows directly upon freezing and thawing.

#### (b) More or Less Lasting Fatigue.

The tracings seen in the next two figures (figs. 4 and 5) are more interesting. They are again from a preparation of which the nerve was frozen, the process of freezing having been accompanied in the case by more or less prolonged tetanus and much subsequent twitching of the muscle. On thawing conductivity remained absent for at least twenty minutes. The nerve was put away to rest in Ringer's solution, and its conductivity examined two and a half hours later at room temperature.

Rhythmical stimulation (rate 144 per second) of the proximal end of the nerve first produced the effect seen in fig. 4, tracing 4, viz. "fatigue tetani." Almost immediately afterwards the muscle responses were of the form seen in tracing 5, after which peripheral stimulation of the nerve was carried out—tracing 6. The smooth and elevated tetani which follow upon peripheral stimulation show that the muscle is in normal condition and not fatigued. After a rest of about five minutes, stimulation was now applied to the proximal end of the nerve, with the result seen in tracing 7. It can be seen that with each succeeding series of excitations the corresponding muscle responses, which are of the "fatigue tetanus" form throughout, become less and less vigorous, and tend eventually to die away.

Another rest of about five minutes was given, and series of rhythmical stimulations at a constant intensity of 500 units once more applied to the central end of the nerve, with an exactly similar result to the previous one (see fig. 5, tracing 1), only that this time the muscle responses died away completely in the end. A rest of four minutes was given, and another two tetani inscribed with central stimulation. Seeing that the same process of gradual extinction of the muscle response was evidently going to be repeated, the nerve was then stimulated peripherally so as to make absolutely sure that no fatigue of the muscle had occurred; the result of this peripheral stimulation, however, is that the nerve now refuses to conduct excitations applied at the central end. As explained in the above-mentioned paper (2), the result of strong peripheral stimulation, when the nerve is already in a fatigued condition due to rhythmical central stimula-



tion, is temporarily to abolish the conductivity. The excitations which start

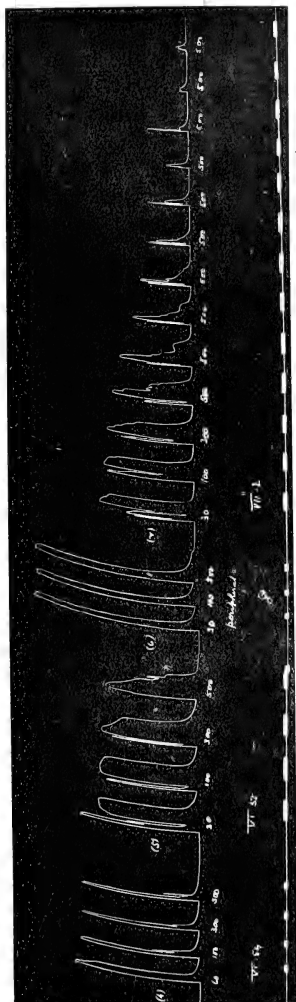


FIG. 4.—The figure shows the result of successive series of rhythmic stimulations (144 per second) applied to a nerve which had been frozen and then thawed again. Tracing 6 shows the result of peripheral stimulation. Tracing 7 shows the gradual falling off in height of the muscle tetani corresponding to successive series of rhythmic excitations, the falling off being due to gradual fatigue of the nerve.

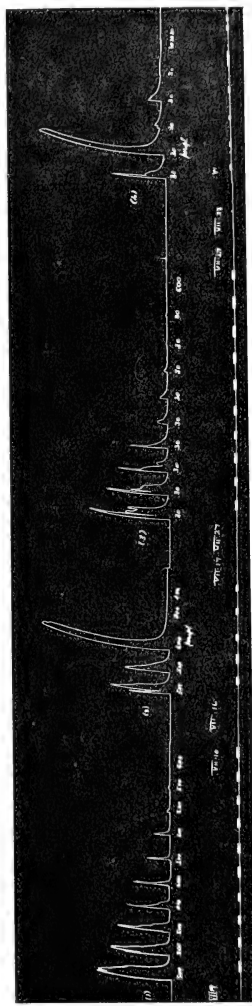


FIG. 5.—This figure shows a continuation of the experiment of which fig. 4 is a record. It shows gradual fatigue of the nerve with each successive series of rhythmic excitations applied to the proximal end of the nerve. Between the inscription of (1) and that of (2) an interval of 6 minutes elapsed; an interval of 10 minutes intervened between the inscription of (2) and (3), an interval of 4 minutes between (3) and (4). (This tracing is reproduced on a somewhat smaller scale than fig. 4.)

from the peripheral side run backwards along the nerve, and probably thus disable the fatigued portion.

A rest of ten minutes was now given, and the nerve once more subjected to regular series of centrally applied rhythmical stimulations. The intensity was much lower than that previously employed, being 30 units instead of 500, and yet the same effect as before was repeated, the nerve ultimately ceasing to conduct. A rest of four minutes suffices to restore the conductivity in some measure—tracing 4. Peripheral stimulation this time, however, does not absolutely abolish conductivity, possibly because the intensity of stimulation is low. The nerve, however, soon ceases to conduct when repeatedly stimulated.

Now, in this experiment we can throughout watch the gradual fatigue of the nerve as it is subjected to successive series of stimulations. With each more or less prolonged rest it recovers to a certain extent, to break down whenever regular series of stimulations are again applied.

Nerve which has been frozen and thawed again is readily fatigued by means of rapid rhythmical stimulation. As a rule the fatigue is short-lasting, and on cessation of the stimulation recovery occurs with great rapidity. In some cases, however, recovery may be delayed for a considerable period.

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ON PROTAGON: ITS CHEMICAL COMPOSITION AND PHYSICAL CONSTANTS, ITS BEHAVIOUR TOWARDS ALCOHOL, AND ITS INDIVIDUALITY. By R. A. WILSON and W. CRAMER. (From the Physiological Laboratory, University of Edinburgh.)

PROTAGON IS A PRODUCT OF A DEFINITE COMPOSITION.

WITHIN recent years the existence of protagon as a definite compound has been categorically denied by Gies and his collaborators, whose views have been endorsed by Rosenheim and Tebb, and positive assertions have been made concerning the part played by protagon in the history of neurochemistry, assertions which it is difficult to test by the exact methods of experimental investigation. Whether these authors are right or not, whether protagon is a mixture or not, there is one outstanding fact that must be clearly recognised: namely, that from brain a substance of a constant chemical composition can be extracted which has received the name protagon.

Gamgee, in his investigations on this substance, prepared a number of samples, which on analysis gave figures agreeing inter se, and which retained this composition after recrystallisation. From his analyses he gives the following figures for the composition of protagon:

C.	H.	N.	P.
66.39	10.69	2.39	1.06 per cent.

Gamgee's results were confirmed by a number of observers, who by identical and by different methods extracted from nervous tissues substances having the same composition as protagon, as will be seen from the following table:

	C.	H.	N.	P.	S.
	per cent.	per cent.	per cent.	per cent.	per cent.
Gamgee . . . . .	66.39	10.69	2.39	1.06	...
Baumstark . . . . .	66.48	11.12	2.35	1.02	...
Ruppel . . . . .	66.29	10.75	2.32	1.13	0.096
Lesem and Gies . . . . .	66.11	10.90	2.02	1.23	0.77
Cramer . . . . .	66.37	10.82	2.29	1.04	0.71

We have not here considered observations in which substances were prepared from brain, but only partially analysed, as was done, for instance,

by Gulewitch, Posner and Gies, Lochhead and Cramer. As a rule, only the phosphorus content was determined in these cases, which is, indeed, a fairly good indicator of the nature of the preparation. It must, however, not be forgotten that preparations have been obtained from brain by Kosse and Freytag, by Noll and by Cramer, which, although having the same phosphorus percentage as protagon, differ in their nitrogen or carbon contents.

By a method which is described at the end of this paper, we have extracted from brain, by means of boiling absolute alcohol, a substance giving the following analytical results:<sup>1</sup>

	C.	H.	N.	P.	S.
	per cent.	per cent.	per cent.	per cent.	per cent.
Sample B { 4th recryst. . .	66.64	10.92	2.41	0.92	...
{ 5th recryst. . .	66.57	10.98	2.39	0.93	0.73
Sample C, 4th recryst. . .	66.40	11.01	2.33	0.97	...
Sample A, 4th recryst. . .	...	...	...	0.99	...

Another sample was prepared by a slight modification of Gamgee's method (see p. 104), and gave on analysis:

	C.	H.	N.	P.	S.
Sample D, 3rd recryst. . .	66.40	10.71	2.55	1.02	0.68 per cent.

It is this constancy of the chemical composition which has induced many physiological chemists to consider protagon as a definite chemical compound; and, whether this view is right or not, we must insist that the name protagon cannot be applied to substances of a different chemical composition. As a rule, if a chemist tries to prepare a known substance by a given method and fails to obtain the same substance as previous workers, he is inclined to suspect his technique. Some workers on protagon, however, use that name for any substance that has been prepared according to a known method, irrespective of its chemical composition. If a substance of a composition different from protagon is obtained, this is taken as evidence for "the variability and indefiniteness of the protagon mixture." A recent paper on this subject by Rosenheim and Tebb illustrates very instructively the confusion which has arisen with regard to the use of the name protagon.

In order to bring forward evidence to support Thudichum's view that protagon is identical with cerebrote, a substance isolated from ox brains by means of alcohol extraction by Couerbe in 1837, Rosenheim and Tebb have repeated Couerbe's method and have analysed the substance obtained in this way. The analytical figures are given here:

<sup>1</sup> The nitrogen was determined by Dumas' method, the phosphorus by Neumann's method, the sulphur by Carius' method.

	C. per cent.	H. per cent.	N. per cent.	P. per cent.
Couerbe's cerebrote; crude product	67.82	11.10	3.40	2.33
Substance prepared by Rosenheim and Tebb; crude product . . . .	...	...	4.34	1.09
Thrice recrystallised . . . . .	...	...	2.08	0.65

Although the composition of Couerbe's cerebrote differs markedly from Rosenheim and Tebb's substances, they call their substances Couerbe's cerebrote, because they have obtained these substances by using Couerbe's method. A comparison of these figures with the analyses of protagon given above shows that not one of these "cerebrates" has the same composition as protagon. Nevertheless Rosenheim and Tebb conclude that protagon and cerebrote—it is not said which of the three cerebrates—are the same substance under two different names, because the same solvent has been used for their preparation and because there is some superficial resemblance.

It seems almost unnecessary to demand that investigations on protagon intended to demonstrate the composite nature of this substance should be made on material the identity of which with protagon is beyond doubt. But a critical survey of these observations will show that even here the same tendency to identify protagon by its method of preparation, against the evidence of the analytical results, is apparent, although not in such an exaggerated form as in the case just discussed.

The substance which Wörner and Thierfelder called protagon, and from which they isolated cerebron, was prepared according to Gamgee's method. The analytical results of the carbon, hydrogen, and nitrogen determinations show variations from 62.37 per cent. C. to 68.97 per cent. C., and from 2.39 per cent. N. to 3.39 per cent. N. The phosphorus was not determined.

Posner and Gies have obtained substances by means of Gamgee's method with a phosphorus percentage varying from 1.73 to 0.89. All their products they call indiscriminately "protagon." This is the more remarkable since Gies in his first investigations on this subject had worked with substances which had the same chemical composition as protagon, and since Posner and Gies themselves had obtained substances the phosphorus percentage of which was identical with that of protagon. Although these observers had the personal experience that a substance of the definite composition of protagon can be obtained, they do not hesitate to apply this name also to a substance of a different composition.

Rosenheim and Tebb even go so far as to apply the name protagon to substances which show an entirely opposite behaviour towards a certain solvent. On page 6 these authors state that acetone is a suitable solvent for protagon. On the next page they give the results of subjecting a sample of protagon twice recrystallised to fractional crystallisation from acetone.

The portion remaining insoluble is called "insoluble protagon," and is purified by recrystallisation from alcohol and then called "protagon thrice recrystallised." This protagon is therefore insoluble in boiling acetone. On page 11 a method for the preparation of protagon is described, which consists in extracting brain with boiling acetone. The substances crystallising out on cooling from the various extractions are alleged to be "typical protagon." We wish to point out that even if protagon is a mixture, it cannot be at the same time soluble and insoluble in one and the same solvent. We doubt whether the substances obtained by the method of acetone extraction are protagon. The evidence given by Rosenheim and Tebb on this point is neither very clear nor very conclusive. The nitrogen and phosphorus contents of the crude products only are determined. Although they differ from each other and partly from protagon, it is not said which product is considered to be protagon. No attempt is made to show that a substance of a constant chemical composition identical with protagon is obtained after repeated recrystallisation. Besides, in our experience, protagon is not easily soluble in acetone. When the experiments described in a previous paper by Lochead and Cramer were carried out, acetone was tried as a means of extracting protagon from brain and found not to be a suitable solvent. The samples of protagon which we have prepared are also not readily soluble in acetone. We believe that we have found an explanation of the apparent contradiction between these observations on the solubility of protagon in acetone, and shall refer to it later.

Here it may be sufficient to point out the inconsistency in applying the name protagon both to a substance soluble in acetone and to a substance insoluble in this solvent.

It is evident that many observations, on the basis of which the existence of protagon as a definite chemical compound has been denied, were made on material called protagon, which, however, was not identical with protagon, but at the best represented a crude product containing protagon, together with other substances.

This looseness of designation is, as we have seen, due to the fact that, contrary to the ordinary rules of chemical investigations, the chemical composition is not considered to be a criterion of protagon but its method of preparation. In this way the impression has gained ground that protagon is such a variable substance that it is a mere accident if a sample is obtained of the same composition as Gamgee's protagon. We believe that we have found an explanation of these failures to obtain protagon, and shall show later that protagon can be prepared without difficulty, if certain conditions are observed during its preparation and recrystallisation.

#### PROTAGON IS A PRODUCT OF DEFINITE PHYSICAL CONSTANTS.

We have spoken of the chemical composition as the only means of identifying protagon, because its physical characters, even its crystalline form, are neither very conclusive evidence of its identity nor do they allow

of a comparison of various samples by a quantitative method. We have therefore endeavoured to determine some physical constants of protagon, namely, the specific rotatory power and the refractive index of its solution. Some difficulty was experienced in finding a solvent which would dissolve a sufficient amount of protagon without the use of higher temperatures. We found that protagon was comparatively soluble in pyridine, even at room temperature. By making our determinations at 30°, a 3 per cent. solution could be used. The polarimeter was a Laurent apparatus. The determinations were made in a 10 cm. tube at 30° with sodium light. The refractometer was a Pulfrich instrument. A high density prism was employed. The pyridine used was Kahlbaum's pyridine, and had at 30° the refractive index 1.5062. The results are given in the following table:

Sample.	Specific rotation $[\alpha]_D^{30} =$	Refractive index of 3 per cent. solution at 30°.
A . . .	+6.66	1.5034
B . . .	+6.90	1.5033
C . . .	+6.61	1.5034
D . . .	+7.01	1.5032

The results for the specific rotatory power agree well with each other, especially if it is considered that the actual reading taken was very small. They stand in striking contrast to the observations of Rosenheim and Tebb, which were published shortly after our first preliminary communication on this subject had appeared. According to these authors, the specific rotatory power of protagon varies between 2.7° and 7.5°, and thus demonstrates again the composite nature of protagon. As Rosenheim and Tebb include, under the term protagon, the substance prepared according to Couerbe's method for cerebrote, substances insoluble in acetone and substances soluble in acetone, and as most of these substances differ in their chemical composition both inter se and from protagon, their physical properties could hardly be expected to show any agreement.

From our observations we conclude that different methods of extraction isolate from brain a substance, protagon, of a definite and constant chemical composition, which will retain this composition after repeated recrystallisation and possesses definite and constant physical properties. These facts are quite independent of the question to be discussed later, as to whether this substance, protagon, is a definite chemical compound or not.

#### THE ACTION OF WARM ALCOHOL UPON PROTAGON.

We have already seen that the plain postulate, that investigations on the nature of protagon should be made on protagon and not on some crude product possibly containing protagon, has not always been fulfilled. These observations need therefore not be taken into consideration. Nor can the

failure of some workers to isolate protagon from brain be considered as evidence against its existence, seeing that other workers have obtained this substance without difficulty. If we exclude all these observations, there are still a number of experiments which show that, by means of a certain process, which has been called a "process of fractional crystallisation," protagon is split up into substances varying widely in their phosphorus percentage and in their solubility in alcohol and ether. This was demonstrated conclusively by Gies and his collaborators. In a former paper by one of us it was suggested that the protagon of Lesem and Gies may have been contaminated with pseudocerebrin. But as the analytical figures of their preparations are identical with those of Gamgee's protagon, their material must be considered as representing typical protagon, and Posner and Gies are right in contending that, if their preparations were contaminated with pseudocerebrin, the same may be said of Gamgee's and Cramer's protagon. In order to remove any possible objection, Posner and Gies have recrystallised protagon ten times until the phosphorus percentage of the crystalline product and of the mother liquid was almost the same and identical with that of protagon. Even from this preparation different substances could be isolated when the so-called process of fractional crystallisation was applied.

This process consists in the treatment of protagon with a quantity of warm alcohol, insufficient to dissolve it, over periods lasting many hours (20-24 hours). After separating the soluble from the insoluble part, the solution is allowed to cool slowly, and the substances crystallising out at different temperatures are collected separately and then show the differences mentioned above.

Of the correctness of these facts there can be no doubt; it is only in their interpretation that we differ from Gies and his collaborators. In order to interpret these facts as proving conclusively the composite nature of protagon, it is of course essential that the prolonged treatment with warm alcohol does not effect any change in the protagon—in other words, that the process is really one of recrystallisation and not one of decomposition. This last possibility has, indeed, been considered by Lesem and Gies, who, in speaking of their results of fractional crystallisation, say: "They show that protagon is either a mixture of bodies or else a substance decomposing quite readily under the conditions of such experiments." Rosenheim and Tebb simply dismiss the second possibility by saying that the process of fractional crystallisation evidently cannot effect any serious chemical decomposition. A priori there is no reason why these results should not prove with equal force that the prolonged treatment with warm alcohol has induced a decomposition. Before the treatment protagon is, as we have seen, a substance of a constant composition retaining this composition after simple recrystallisation, which involves only a short contact with warm or boiling alcohol, the mother liquor and the crystalline product having an almost identical phosphorus percentage: in the course of its preparation it



has been subjected (in the precipitated stage) to a very thorough washing with cold ether. After the treatment a substance remains less soluble in warm alcohol than protagon, and almost phosphorus free; the substances crystallising out vary considerably in their phosphorus percentage from that of their respective mother liquids, the difference sometimes exceeding 1 per cent., and yield to ether a considerable quantity of a substance containing less phosphorus than protagon. If protagon is a mixture of all these substances, it must follow that in the process of simple recrystallisation these substances dissolve and crystallise out in constant proportions, as this process always leads to the same product—protagon.

The assumption that protagon is a mixture of these substances, so different in many respects, is therefore not without difficulty; and if the chemical individuality of protagon had not been under suspicion, these results would have been interpreted as proving the instability of protagon towards warm alcohol. The statements of previous workers on this question, whether protagon is decomposed or not by warm or boiling alcohol, are very contradictory. The reason for this is probably that any change that might take place would not be very obvious, and could only be demonstrated by an elaborate experiment. Indeed, if one had wished to study the action of warm alcohol on protagon, one could only have done so by experiments similar to those of Gies and his collaborators.

The determination of the physical constants and their constant value has made it possible for us to obtain direct and conclusive evidence on this question. The two samples of protagon, A and B, the physical constants of which had been determined, were treated with 80 per cent. alcohol at 44° for 22 hours, in the manner prescribed for the process of fractional crystallisation. The vessels were then cooled in ice and the alcohol evaporated in *vacuo*, no filtration having taken place. The remaining residue was dried in *vacuo* over concentrated sulphuric acid and its specific rotatory power and the refractory quotient were determined as before. As nothing had been removed by filtration the proportion of the elements must have remained the same as in protagon, so that on analysis the figures obtained would be identical with those for the composition of protagon. As a control a sample was dissolved in boiling alcohol, the solution was kept boiling for 1½ minutes, and the vessel cooled in ice. After evaporating the alcohol in *vacuo* without previous filtration, the residue was dried and its physical constants determined as before. The results are given in the following table:

Sample.	Treatment.	Rotation $\left[ \alpha \right]_D^{20}$	Refractive index of 3 per cent. solution.
A . .	85 per cent. alcohol at 44° for 22 hours	13.43	1.5041
B . .	" " " " " " " "	13.08	1.5038
C . .	Boiling " alcohol for 1½ minutes "	6.69	1.5034

The results show that a short contact with boiling alcohol is without effect on protagon, while prolonged treatment with warm alcohol produces a change. The product obtained after this treatment is not identical with protagon, as the physical constants show, although its analytical figures would agree with protagon. The results obtained by the so-called process of fractional crystallisation, therefore, do not give any conclusive indication as to whether protagon is a mixture or not, the method being an unsuitable one, but have to be interpreted as proving the relative instability of protagon towards warm alcohol. This conclusion again is not in any way dependent upon either the composite or the uniform nature of protagon: the products which are found after the treatment are, apart from unchanged protagon, decomposition products of either the definite compound protagon or of the substances constituting the mixture protagon.

The statement made in a former paper by one of us that "protagon is not decomposed by warm ether or boiling alcohol," must therefore be corrected.<sup>1</sup> In the case of boiling alcohol it is true only if the solvent is prevented from acting on protagon for some time. We have not investigated the effect of ether on protagon, but the instability of this substance towards warm alcohol makes it probable that other solvents are not without disintegrating effect. This is perhaps the explanation of the contradiction above referred to, that, although protagon is not readily soluble in acetone, a method of acetone extraction has been proposed for the preparation of protagon. As in this method the brain material is boiled with acetone for four hours, it seems doubtful, in view of our experiments, whether decomposition has not taken place, and whether the substances extracted are not decomposition products of protagon, possibly together with some unchanged protagon.

The relative instability of protagon towards warm alcohol is a fact which throws light on some obscure and controversial points of the protagon problem. It is clear that a prolonged treatment with warm alcohol, both in the preparation and in the recrystallisation of protagon, must be avoided; otherwise decomposition products are formed which are extracted together with protagon. This is probably the explanation of the lack of uniformity in the composition of products obtained by some workers, especially when Gamgee's method has been used. It is usually stated that Gamgee's method was followed in every detail. We have been unable to find in Gamgee's communications any definite information about the time of recrystallisation, and most other workers who have made use of this method do not give any detailed statement with regard to this point. The sample of protagon which we prepared by Gamgee's method, slightly modified, and which was identical in its chemical and physical properties with protagon,

<sup>1</sup> While freely acknowledging this error, I wish to point out that the statement in the same paper, that "choline is the only base formed" on hydrolysis by baryta water, has been misunderstood by Rosenheim and Tebb. If read in connection with the paper, this statement appears as the result of experiments intended to decide whether choline or neurine or both bases are formed. [W. C.]

was extracted for four hours with alcohol and three times recrystallised, the solution being effected in each case by warm alcohol in three-quarters of an hour. The products which Posner and Gies obtained by this method, and which had such an abnormally high phosphorus constant, were prepared by extractions lasting for twenty-four hours. In purifying the crude product by recrystallisation it was treated with alcohol for periods varying from five to fifteen hours.

In order to test the validity of our conclusion that it is the prolonged treatment with warm alcohol which is responsible for the failure to obtain protagon, we have applied Gamgee's method in the same way as before, only extending the extraction to twenty-four hours, and the treatment with warm alcohol for the solution of the crude product in the recrystallisation process to twelve hours. The specific rotatory power of the substance which had been prepared in this way was  $[\alpha]_D^{20} = 13.12$ , refr. index 1.5039. This substance was, therefore, not protagon, whatever the results of the chemical analysis would have been.

We can also understand the apparently paradoxical fact that a protagon sample, obtained after repeated crystallisation and identical in every respect with protagon, may appear after a further recrystallisation to be contaminated with pseudocerebrin or other substances. Formerly pseudocerebrin was held to be extracted from brain, together with protagon, from which it had to be separated. We know now that this substance (which is identical with cerebrin) is formed from protagon by the hydrolysing action of warm alcohol. If, therefore, in the process of recrystallisation the alcohol has been allowed to be in contact with protagon for a longer time than in the previous recrystallisation—and as the destructive action of warm alcohol has not been suspected previously, such a condition may have easily occurred—decomposition products will be formed, and after this recrystallisation the protagon may really be less pure than it was before.

#### METHOD FOR THE PREPARATION OF PROTAGON.

In order to shorten as much as possible the time during which the hot or warm solvent is in contact with the material in the preparation of protagon, we have adopted the following procedure, which we have found to be a most convenient method for the preparation of protagon.

The brain mass is made into a pulp and treated repeatedly with 96 per cent. alcohol in a wide-mouthed bottle. The extraction is accelerated by the use of a shaking machine, the material being kept afterwards in an ice chest. After three to four extractions ether is added instead of alcohol, and the treatment continued until lecithin and cholesterin are completely extracted. After removing the ether by filtration and drying the remaining mass by exposing it to the air at room temperature, a brown mass remains which can be made easily into a fine powder. To this powder any solvent can be applied directly. We have prepared protagon from it according to

Gamgee's method by means of warm alcohol (see p. 104) and by extraction with boiling absolute alcohol. In the latter case the boiling solvent is poured on the powder and the mixture kept boiling for one to two minutes in a water bath, moving the mixture all the time. The alcoholic solution is filtered through a hot-water funnel; the filtrate is allowed to drop into a vessel cooled in ice. The same process of extraction is repeated twice. The crude crystalline product is washed with ether and dried in vacuo.

Recrystallisation is effected by pouring boiling absolute alcohol on the sample of protagon. The solution is kept boiling for one minute and then filtered as before.

This method offers many advantages. Water and all the substances soluble in cold alcohol and cold ether are removed before the extraction begins, so that the bulk of the material is greatly reduced and less of the hot solvent is necessary. In this way even a large quantity of material, fifteen to twenty ox brains, can be worked up easily, while with other methods the bulk of the material and the volume of alcohol are so great that the manipulations cannot be carried out neatly and require a longer time.

#### IS PROTAGON A DEFINITE COMPOUND OR A MIXTURE OF PHOSPHATIDS AND CEREBROSIDES ?

In the preceding pages we have considered protagon simply as a substance prepared from brain, and having constant and definite chemical and physical properties. The question whether it is a definite chemical compound or a mixture we have left open, so that the facts which we have observed and the conclusions which we have drawn remain independent of this controversial subject.

Conclusive proof of the chemical individuality of protagon can only be brought by synthesis. Evidence to the contrary could be obtained by isolating the substances of which the mixture protagon is constituent and to reconstitute protagon from them. Rosenheim and Tebb hope to be able soon "to reconstitute a pure protagon" with a phosphorus percentage varying from 0.9 to 1.26, by making a mechanical mixture in certain proportions of substances nearly phosphorus free and substances containing about 3 per cent. phosphorus. We have no doubt that it is possible to obtain in this way a mixture of the same phosphorus percentage as protagon. But if that were to prove that protagon is a mixture, one might also prove that fat is a mixture of glycerine and fatty acids, because a mixture of these substances in certain proportions would have the same carbon percentage. If Rosenheim and Tebb wish to prove that this mixture of alcohol-soluble and alcohol-insoluble substances is identical with protagon, they will have to show that this mixture retains its composition after repeated recrystallisations and that it has the same specific rotatory power as protagon.

Until this question can be decided conclusively, either one way or the other, an objective interpretation of the known facts must be sufficient.

We have emphasised already the fact that protagon has a definite chemical composition and retains this composition after repeated recrystallisation. This substance has been obtained by various observers and by various methods. As we have pointed out in a former communication, this fact is evidence in favour of the view that protagon is a definite chemical compound. To this evidence we add further the fact of the constancy of its physical properties. The crystalline form of protagon we have never considered to be of much value in recognising the nature of protagon, as it is well known that mixtures of complex organic compounds frequently crystallise out together in a definite crystalline form. The weight of the analytical evidence has been admitted even by those who hold different views on the nature of protagon. Lesem and Gies discuss their analytical results of four samples of protagon as follows: "Much to our surprise, these results accord as well as many analytical series given for what are undoubtedly individual substances. Our data in this connection, considered by themselves, would seem to harmonise with the older view of the integrity of protagon."

Against this view the results of the so-called process of fractional crystallisation have been adduced as demonstrating that protagon is a mixture of substances differing widely in their solubility, differing widely in their chemical composition and constitution, and in their physical constants. These results appear in a new light, since we have been able to prove that they are due to a factor which has not been recognised before, namely, the instability of protagon towards warm alcohol. This fact changes the process of fractional recrystallisation into one of partial decomposition, and invalidates the conclusions which have been drawn from these experiments.

This property of protagon is responsible for the fact that, by following known methods for the preparation of protagon, substances have been extracted from brain which differ from protagon in their chemical composition. By calling all the substances protagon which were prepared according to an acknowledged method, these results have been used as additional evidence for the variability of the protagon mixture. In the only case in which the account of the technique employed was detailed enough to repeat the process, it was possible to show that the failure to obtain protagon was due to the prolonged treatment with warm alcohol.

Not only is it incorrect to interpret these results as evidence against the existence of protagon, but we must protest against such reasoning, which threatens to deprive the protagon problem of its very basis. Whatever protagon is, the name protagon has been given to a substance of a definite chemical composition, having, as we have seen, definite physical constants. Like every other chemical substance, protagon is identified by these properties and not by its method of preparation. Many of the

substances which figure in recent papers as protagon have no claim to this name.

There is therefore no evidence in favour of the view of Thudichum and his followers that protagon is a mixture of substances differing from each other in almost every respect. On the contrary, we must conclude that the substances found after the prolonged treatment with warm alcohol are, besides unchanged protagon, decomposition products of protagon. One of these decomposition products, the phosphorus free phrenosin (cerebron, pseudocerebrin), has indeed been found to be identical with cerebrin, which is obtained by hydrolysing protagon by baryta water. The phosphorised moiety of the protagon molecule, however, does not behave in the same way towards warm alcohol and towards baryta. This last reagent carries the hydrolysis to the ultimate constituents; of which choline, sphingosine, glycerophosphoric acid and fatty acids have been isolated. The action of alcohol does not go so far. This is quite clear from the investigations of Thudichum, who isolated numerous substances from brain by methods involving prolonged extraction with boiling alcohol and distillation of the alcoholic solutions regardless of any decomposition. These substances were hydrolysed further by means of baryta water. The treatment with warm alcohol, instead of being a means of separating the substances which are present in the mixture protagon, would appear therefore to be a useful method for the study of the more complex groups which enter into the composition of the compound protagon.

Seen in this light, the observations of Gies and of Rosenheim and Tebb, so far from being opposed to the view that protagon is a definite compound, are a valuable contribution to the study of the constitution of this substance.

The question has also to be considered whether the substances which have been isolated from brain, and which at the same time have been isolated from protagon hydrolysed by alcohol, such as, for instance, phrenosin (pseudocerebrin, cerebrin, cerebron), exist preformed in the brain or are formed only in the process of extraction.

Although our results support the view that protagon is a definite compound, we do not exclude the possibility, which has been considered already in a former paper, that several protagons exist just as several lecithins exist. The existence of such substances, differing perhaps only in the nature of the fatty acid radicle which they contain, would be no more evidence against the existence of protagon than the existence of several lecithins is considered to disprove the existence of a definite compound lecithin. Such protagons would be distinguished only by slight differences in the carbon and hydrogen contents, and would resemble each other very closely in every respect, so that they could not be separated easily. It is therefore possible that protagon is a mixture of such substances, although there is at present no evidence for it. Such a view, which is quite compatible with the idea that protagon is a definite compound, is

fundamentally different from the view that protagon is a variable and indefinite mixture of cerebrosides and phosphatids.

#### CONCLUSIONS.

1. Protagon is a substance of a definite chemical composition, retaining this composition after repeated recrystallisation.

2. Protagon is a substance with definite and constant physical properties. The specific rotatory power and the refractive index of several samples of protagon have been determined.

3. Protagon is identified by its chemical composition and by its physical constants. Many substances to which the name protagon has been given on account of their method of preparation, do not conform to these conditions, and therefore have no claim to the name protagon. Couerbe's cerebrote is not identical with protagon, but probably a mixture of substances of which protagon is one.

4. Protagon is decomposed by a prolonged treatment with warm alcohol. The so-called process of fractional crystallisation is therefore in reality a process of partial decomposition. The conclusions which have been drawn on the assumption that it is a process of recrystallisation are not valid. There is, consequently, no evidence for the view that protagon is a mixture of cerebrosides and phosphatids.

5. The constancy of the physical and chemical properties of protagon support the view that protagon is a definite compound. The substances isolated from protagon after prolonged treatment with warm alcohol, and formerly held to exist as such in the mixture protagon, must now be considered to be the constituents of the protagon molecule. They are the intermediate decomposition products of protagon.

6. Details of a method for the preparation of protagon are given, by means of which a prolonged contact with warm or boiling alcohol can be avoided as much as possible.

#### ADDENDUM BY W. CRAMER.

The paper by Lochhead and Cramer has called forth a polemical paper by Gies (*Journal Biolog. Chemistry*, iii. 4, p. 339) which is mainly a restatement of the views of Posner and Gies and does not adduce any new facts. Gies believes that our results support his view, and that we obtained "different mixtures by extracting brain with different solvents." He applies to our results a different standard from that which led him to state of the protagon samples of Lesem and Gies that the analytical results "accord as well as many analytical series for what are undoubtedly individual substances." The phosphorus content of the purified protagon samples of Lesem and Gies varies from 0.89 per cent. to 1.26 per cent., that of Lochhead and Cramer's purified products from 0.96 per cent. to 1.07 per cent. Even if our non-purified products are included, the

difference in the phosphorus percentage is only 0.03 per cent. more than in the samples of Lesem and Gies, the variation in these being 0.94 per cent. to 1.34 per cent.

Gies further states (in his recent paper on Paranucleo-Protagon, *American Journal of Physiology*, xx. 2, p. 379), referring to our results: "When the phosphorus contents of their protagon products were lowered by recrystallisation to the percentage amount that appeared to them to be about right, they arbitrarily discontinued in each case the recrystallisation process, in spite of the fact that repetition of it promised to decrease further the proportionate contents of phosphorus." I have looked in vain through our paper for any statement which could warrant this wanton suggestion.

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THE "FLY-CATCHING REFLEX" IN THE FROG. By J. A. GUNN.  
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ONE of the most conspicuous symptoms which result in the frog from the administration of toxic doses of yohimbine is the appearance of a fly-catching reflex. This symptom is interesting not only on account of the invariability of its occurrence, but also because it seems to illustrate the close resemblance which may obtain between the effects of toxic action and operative lesion on the central nervous system.

The following experiment will serve to show the general effects produced by a large sublethal dose of yohimbine in frogs, and also the relation which the symptom under consideration bears to other symptoms.

A healthy male frog (*R. temporaria*) weighing 29 grammes was used.

At 3.7 p.m. the throat respirations were 30 in 10 seconds, the heart-beats 8 in 10 seconds.

At 3.20, 0.7 cc. of a solution of yohimbine lactate (0.01 gm. in 5 cc.) was injected into the dorsal lymph sac. This was equal to 0.048 gm. per kilo, the minimum lethal dose being 0.05 gm. per kilo.

At 3.35 the normal respirations had entirely ceased, and were replaced by infrequent gulping movements. The head was lowered and the limbs not fully drawn up. The frog made no spontaneous movements, but when pinched jumped well, and when laid on his back recovered his usual posture quickly. The conjunctival and limb reflexes were acute. The lower eyelid covered half the eye. At 3.55 if pinched he did not jump, but moved forward on his abdomen by kicking. When laid on his back he recovered once, but when placed on his back a second time immediately after he was unable to do so.

At 4.15 when laid on his back he kicked vigorously, but could not turn over.

At 4.53 (one hour thirty-three minutes after injection) when laid on his back he made a few feeble movements and then lay still with legs extended. When a foot was now pinched the leg was drawn up quickly. Movements of any nature brought on fatigue very soon. When the animal's hand or nose was touched he snapped in the direction touched, extending his tongue as a frog does when catching a fly.

At 7.0 he jumped feebly when pinched. Though in jumping he could raise himself off the ground, his movements were badly co-ordinated: for example, one hind limb would get flexed behind the other.

At 9.0 when pinched he went into a convulsion, in which the hind limbs were rigidly extended with slight opisthotonus, followed by emprosthotonus, with the body flexed and the arms flexed under it. The fly-catching reflex was elicited as before.

At 7.20 a.m. next morning he still had when pinched convulsions similar to that described. The eyes were fully open. If a bright object was held near his eyes, he snapped at it, extending his tongue. He could not turn over when placed on his back.

During all this day he remained in nearly the same condition, with, however, some increase of co-ordinating power, for by the end of the day he could turn over, though still with some difficulty, when laid on his back.

On the second day after injection he sat with his limbs fully drawn up, made frequent spontaneous movements, could jump well, and turned over rapidly when laid on his back. When his hand or nose was touched he no longer snapped, and when a bright object was held near his eyes he moved away as a normal frog does. He appeared to have practically completely recovered, except that his respirations were only ten per ten seconds. Two days later, however, the rate of the respirations was the same as before injection.

When the earlier symptoms are analysed with a view to their explanation, it is apparent that the cessation of voluntary movements, the inco-ordination of movement when such movement is elicited by stimulation, and the loss of the power of jumping are symptoms which so much resemble those which result from operative destruction of the cerebrum, mid-brain and cerebellum as to lead one to infer that yohimbine early in its action abolishes the functions of these parts.

That the medulla oblongata, too, is involved is shown by the cessation of respiration, and by the inability of the animal to recover its normal posture when laid on its back. These effects are not the result of a peripheral paralysis, for, as I have ascertained from control experiments, the motor nerves and the voluntary muscles at this time react normally to electrical stimulation.

Since pinching the foot elicits withdrawal of the leg as promptly as before injection, yohimbine does not impair the functions of the spinal cord as it does those of the higher parts of the central nervous system.

Is there anything in the nature of the action of yohimbine on the central nervous system to explain the appearance of the "fly-catching reflex"?

In every one of a large number of experiments with doses of yohimbine lactate ranging from 0.22 gm. to 0.08 gm. per kilo subcutaneously, I have found this reflex elicitable at some time during the experiment. With the largest of these doses (0.08 gm. per kilo), which killed the frog in one and a half hours, it was obtained only once, namely, about an hour after injection, though it was tested for at regular intervals of a few minutes. With the smallest of these doses this reflex could be elicited for over twenty-four hours.

The frog poisoned by yohimbine snaps and generally extends his tongue if his hand or nose be touched; sometimes if a bright object be brought near his eyes. If the hand of a normal frog be touched the arm is drawn away; if the nose be touched the head is depressed and the eyes closed; while if a bright object be approached close to its eyes, the animal merely moves away or closes its eyes if it react at all. But Schrader (1) has shown that the snapping for food is a reflex from slight stimulation, and that a frog deprived of its cerebrum will catch flies under suitable circumstances, and given a long enough time for recovery from operation. He has also shown that if the brain is destroyed down to the fore part of the medulla oblongata there is developed a somewhat different snap reflex. In this case the frog snaps if its nose or hand be touched lightly, the head being directed as far as possible towards the place of stimulation.

The remarkable resemblance between these reflexes and those occurring after injection of yohimbine led me to conclude that yohimbine, by a paralyzing action on the upper part of the central nervous system, imitates the operative lesions and results of such lesions as Schrader describes.

The evidence which I have cited above shows that yohimbine does paralyse the functions of certain parts of the supraspinal portion of the central nervous system. Though the appearance of this fly-catching reflex has not to my knowledge been described hitherto as the result of the action of a toxic agent on the frog, still, as a consequence of such action, symptoms simulating the effects of operative lesions are well known to occur.

However, in the case of poisoning of an intact animal, when touching the muzzle or hand or bringing a bright object near the eyes evokes a fly-catching or snapping reaction, there is a possibility that this reaction is a voluntary one, abnormal it is true, but rendered elicitable, for example, by a depression of some normal inhibitory influence of the cortex cerebri. In order to decide this point I destroyed the cerebra of two frogs and administered yohimbine to one of them immediately after operation. The snap reflex occurred in a few hours in the poisoned frog, and in it alone. Since it occurs when voluntary impulses are cut off, it is a true reflex.

There is another possible explanation of the production of this reflex by yohimbine, still in keeping with the known method of action of drugs. There would seem to be in this condition a hypersensitiveness of the centre involved, or, what may be the same thing, a condition in which varied, and divergent afferent stimuli find a path of least resistance in reflexion through the centre for the apprehension of food. The action of yohimbine may therefore consist in "facilitation of the discharge of force already latently present, and the rendering of the liberating forces more effective tending to thwart inhibition" (2), and this action may be exerted especially on this centre in the bulb. Certain facts go to show that yohimbine may so act on the nervous system. The fact that small doses of yohimbine, by an action on the respiratory centre, induce an increase in the rate and amplitude of the respirations (3), may be explained by the same kind of

action. Also, later than the fly-catching reflex, there come on spinal convulsions. These may be due to the supposed action on the bulb—an action similar in nature to that of strychnine—spreading to the spinal cord.

A third circumstance may possibly have some bearing on the ease with which this snap reflex is elicited in a frog poisoned by yohimbine. "A point of general interest in the physiology of the great alimentary nerve centre in the bulb is the high degree to which it employs inhibition. Each subdivision of it is depressible by inhibitory fibres from some afferent nerve trunk, e.g. respiration by fibres in the superior laryngeal, deglutition by fibres in the superior and partly in the inferior laryngeal nerves" (4). I have noticed in the frog, poisoned either by yohimbine or by other substances which paralyse the respiration, that cessation of the normal respiratory movements is followed for a short time by gulping movements which appear to be movements rather of deglutition than of respiration. The swallowing movements are induced especially by slightly disturbing the frog. Is it possible, as a converse of the stimulation of the laryngeal nerves, that paralysis of the respiration removes some normal inhibitory effect, and so allows a more ready elicitation of the movements of deglutition, and also perhaps of the more complicated fly-catching reflex?

Whether yohimbine produces this reflex by a paralysing action on the upper part of the central nervous system (as by operation), or by an action on the medulla oblongata facilitating the elicitation of a latent reflex, or by both actions combined, the phenomenon is interesting as illustrating in a particular manner the close resemblance in the effects produced by operative lesion and toxic action on the nervous system of the frog, and on the other hand, as showing the selective action of a toxic agent on the nervous system, since there may occur in yohimbine poisoning a paralysis of the respiratory centre coincident with an exaggerated activity of the closely related centre for the apprehension of food.

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ON THE RESULTS OF HETEROPLASTIC OVARIAN TRANS-  
PLANTATION AS COMPARED WITH THOSE PRODUCED  
BY TRANSPLANTATION IN THE SAME INDIVIDUAL.  
By F. H. A. MARSHALL and W. A. JOLLY.<sup>1</sup> (From the Physiology  
Department, University of Edinburgh.)

(Received for publication 15th January 1908.)

IN a paper published in the Transactions of the Royal Society of Edinburgh we recorded the results of a series of experiments on rats in which the ovaries were removed from the normal position and transplanted beneath the skin or on to the peritoneum. In the present paper an account is given of certain further experiments, the results of which, on the whole, confirm and extend our previous conclusions. One of these experiments was upon a monkey, but the remainder were upon rats, the ovaries being transplanted on to the peritoneum or on to the tissue of the kidney.

In the case of the latter operation the technique adopted was as follows:—The ovaries were removed from the normal position. An incision was made on the external margin of one of the kidneys, either in the same or in another rat. One or both of the ovaries were then placed inside the incision so that they were in direct contact with the highly vascular cut surface of the kidney. The incision in the kidney with its contained ovary was then sewn up with a catgut stitch and the peritoneal cavity closed.

The following is an account of the separate experiments:—

(1) The ovaries were removed from the normal position and grafted together on to the peritoneum of the same rat (homoplastic transplantation) in the manner described in our previous paper. Fourteen and a half months afterwards the rat was killed, when the grafted ovaries were found in position. Microscopic sections showed that the ovarian tissue was normal, several corpora lutea being present. The uterus was also normal.

(2) The ovaries were removed from the normal position and transplanted on to the peritoneum of the same individual. At the time of the operation the uterus appeared somewhat distended. After thirteen months the rat was killed, when ovarian tissue was found in the position of the graft. Microscopic examination showed, however, that it had undergone partial degeneration. The uterus now appeared normal, both superficially and histologically.

(3) The ovaries were removed from a rat, and one of them was transplanted into the right kidney of another rat belonging to a different litter.

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When the last-mentioned animal was killed two and a half months subsequently, it was found that the graft, as far as was observed, had been absorbed, only a certain amount of scar tissue being left in its place. The rat's own ovaries had not been removed.

(4) This experiment was identical with the preceding one, the result being merely a persistence of scar tissue.

(5) The ovaries were removed from a young rat and grafted together into the right kidney of another rat belonging to a different litter. The latter animal's own ovaries were not removed. Two and a half months later it was killed, when it was found that the grafts had been absorbed, their position being occupied by connective tissue.

(6) This experiment was similar, but the grafted ovary was found to be entirely degenerated after a little more than one month.

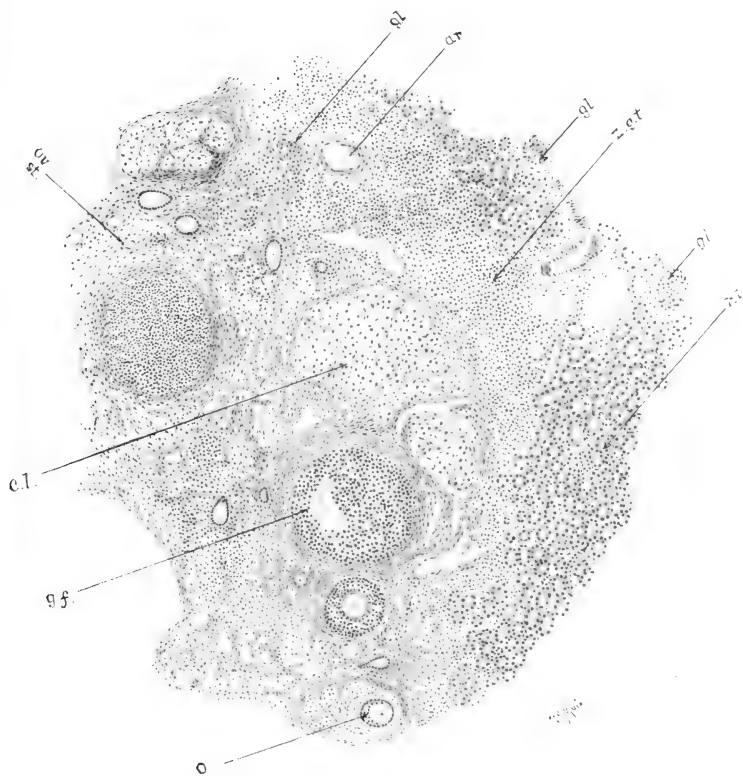
(7) In this experiment the ovaries of a young rat were grafted into the right kidney of a male. On the latter being killed it was found that the graft was degenerated and palpably in process of absorption.

(8) The ovaries were removed from a young rat and grafted into the right kidney of another female belonging to the same litter. About one and a half months afterwards this rat was killed, when it was found that the graft had taken perfectly, the ovaries containing normal follicles in various stages of development and at least two recently formed corpora lutea. Sections showed also that the ovarian tissue was in almost complete continuity with the kidney tissue in which it was embedded (see figure). The animal's own ovaries had not been removed.

(9) This experiment was similar to the last, the ovaries being grafted into the right kidney in a whole sister. After three months the rat with the transplanted ovaries was killed, the organs being found *in situ* in a state of partial preservation.

(10) An ovary from one female rat was grafted into the right kidney of another belonging to the same litter. The rat with the graft was killed three months afterwards. Microscopic sections through the kidney revealed ovarian tissue and follicles with ova in the periphery, but the central part was less well preserved. The animal's own ovaries had not been removed.

(11) The ovaries were removed from a white rat. About two months afterwards the ovaries were removed from a piebald rat, and one of them was grafted into the left kidney of the white rat previously castrated. After another six months (or eight months after the first operation) the white rat was killed, when it was found that the transplantation had been perfectly effected. Normal ovarian tissue in abundance, and containing numerous ova, was observed in sections through the kidney. The uterus showed little or no indication of degeneration. It was evident, therefore, that whatever degeneration this organ may have undergone during the first two months after the removal of the animal's original ovaries was arrested by the successful ovarian graft, the uterus being restored (or almost restored)



Section through part of kidney containing transplanted ovary. (Experiment S.)

*ar.*, artery; *cl.*, corpus luteum; *gl.*, glomerulus of kidney; *gf.*, Graafian follicle; *o.*, ovum; *ov. st.*, ovarian stroma; *ret.*, renal tubules; *z.g.t.*, zone of granulation tissue between the kidney tissue on the right and the ovarian tissue on the left of the figure.

to the normal condition. It is hardly possible, however, that anything more than a slight uterine atrophy could have occurred in so short a period as two months, but our previous observations have shown that the degenerative process may be far advanced after six months' castration. The white rat into which the ovary was grafted did not belong to the same litter as the castrated piebald rat, and so far as known was not a relative of it.<sup>1</sup>

In another experiment the ovaries were removed from a monkey and grafted on to the peritoneum of another monkey (heteroplastic transplantation). At the same time the ovaries of the latter were removed from the normal position and also grafted on to the peritoneum (homoplastic transplantation). About two months later the monkey with the grafted ovaries was killed, when it was found that the heteroplastic ovaries had been absorbed, while the homoplastic ovaries were still in position but had undergone a certain amount of fibrous degeneration.

#### CONCLUSIONS.

As a result of these experiments, taken in conjunction with those described in our former paper, the following conclusions may be drawn:—

(1) Greater success attends transplantation of the ovaries into the kidney than on to the peritoneum, probably on account of the greater vascularity of the kidney.

(2) Homoplastic transplantation of ovaries is very considerably easier to perform successfully than heteroplastic transplantation. This fact can scarcely be ascribed to differences in the technique of the two operations, since this was identical in each experiment, the two animals being operated upon simultaneously in the case of the heteroplastic transplantations.

(3) Heteroplastic transplantation of ovaries is apparently easier to perform successfully when the two animals employed in the experiment are near relatives of each other. In our experiments there were few exceptions to this rule.

(4) The presence of an animal's own ovaries does not seem to exert any inhibitory influence on the successful attachment and growth of additional ovaries obtained from another individual.

(5) The presence of a successfully grafted ovary in an abnormal position in the body, whether obtained from the same or from another individual, is sufficient to arrest the degenerative changes which habitually take place in the uterus after the complete extirpation of the ovaries, as other experiments have shown. It may be concluded, therefore, that the ovarian influence on the uterus is chemical rather than nervous in nature.

<sup>1</sup> It is possible that the two rats employed in this experiment might have been sisters belonging to different litters, since they were obtained from the same breeder.



## LITERATURE.

An account of the literature of ovarian grafting down to the beginning of 1907 is given in our previous paper. Since its publication a few further cases have been placed on record. Guthrie in a preliminary note has described certain experiments on heteroplastic transplantation of ovaries in fowls. The ovaries are stated to have become successfully attached, and in some instances to have afterwards given rise to ova which were fertilised in the ordinary way. Moreover, the chickens so produced are supposed to have inherited some of their characteristics from their "foster mothers"—that is to say, from the hens into which the ovaries were grafted.

Some experiments on ovarian transplantation are reported in the "Münchener medizinische Wochenschrift," but very few details are given. Foges records a case of ovarian grafting into the spleen of a hare, which appears to have been partially successful; and Bucura, in commenting on Foges' results, states that he successfully transplanted ovaries and testes from guinea-pigs into a castrated female rabbit.

Pankow reports nine cases of ovarian transplantation in the human subject, seven of them being homoplasts and two heteroplasts. In the former the menstrual periods are said to have started again at intervals of from three to six months after the respective operations, but there was no evidence that the heteroplastic transplantations were successful.

The following case recorded by Krönig was omitted from our previous account of the literature:—In a woman suffering from osteomalacia the ovaries were removed from the normal position and transplanted on to the peritoneum. The result was beneficial, but with the return of menstruation, which occurred about two months afterwards, the symptoms of the disease reasserted themselves. The inference is, therefore, that the grafts were successful, but there was no direct evidence of the fact.

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THE HISTOLOGICAL APPEARANCES OF THE MAMMALIAN  
PITUITARY BODY. By P. T. HERRING. (From the Physiology  
Department, University of Edinburgh.)

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INTRODUCTION.

THE structure and significance of the pituitary body have long been objects of much speculation. Erroneous conceptions of its structure are responsible for some of the many theories which have been advanced with regard to its functions. The pituitary, indeed, derives its name from the old idea that it was a gland which discharges a secretion—pituita—into the nostrils.

Rathke (32) discovered the double origin of the pituitary, and on developmental grounds classed it among glands. Other observers looked upon it as part of the brain. Luschka (23) called it a "nerve-gland" in which the two parts are separated from one another by pia mater. Ecker (8), on the other hand, held the view that both portions of the pituitary combine to form a unit of the nature of a "blood-vessel gland."

Burdach (4), Luschka (23), and Virchow (46) regarded the posterior lobe as the anterior terminal end of the cerebro-spinal canal, a "filum terminale anterius," resembling in structure the filum terminale of the spinal cord. Virchow also compared the anterior lobe to the thyroid gland, and described in it vesicles containing colloid material which show a striking resemblance to the follicles of the thyroid. Rogowitsch (34), H. Stieda (43), Schönemann (39), and others have attached great importance to this resemblance, and ascribe similar functions to the two glands. Removal of the thyroid is, according to their observations, followed by a compensatory hypertrophy of certain parts of the glandular lobe of the pituitary.

In 1886 Marie drew attention to a relationship between changes in the pituitary and the disease acromegaly or gigantism. Clinical and pathological experiences have led to the theory which assigns to the pituitary the rôle of regulating the normal development of the body, more especially of the extremities and bones. The nature of the change that the pituitary undergoes in acromegaly is uncertain, and before any light can be thrown upon its pathology it is necessary that the significance of the various histological elements that constitute the normal pituitary should

be understood. Moreover, it appears that acromegaly may occur without any apparent change in the pituitary, and that tumours of the pituitary are not always attended by acromegaly. A feature as constant as acromegaly in affections of the pituitary is the occurrence of polyuria with or without sugar in the urine (Hansemann (16), Sternberg (41)).

Oliver and Schäfer (28) in 1895 described the presence of a substance in saline extracts of the pituitary, which, when injected intravenously, produces a rise of blood-pressure. Howell (18) showed that this substance is only present in the posterior lobe. Magnus and Schäfer (24) in 1901 noticed that intravenous injection of saline extract of the posterior lobe is followed by a marked increase of urine flow. Schäfer and Herring (37) confirmed this observation, and showed the striking parallelism which exists between the suprarenal capsules and the pituitary in development, structure, and functions. In each there are two parts, one of which, a highly vascular epithelium, yields no active extract, while the other, of neuro-ectodermic origin, gives an extract which has a remarkable physiological effect upon the heart and arteries. The view was conjectured that in the epithelial part of each organ the material which is to furnish the active agent of the secretion passes through certain stages of formation, and that its production is merely completed in the neuro-ectodermic part, in which part alone the full activity of the secretion is acquired. That the posterior lobe of the pituitary should furnish an active secretion is difficult to reconcile with the usual views held on its structure. The older anatomists, W. Müller (27), Schwalbe (40), and Toldt (45), looked upon it as a mass of connective tissue cells and fibres which during development have destroyed all trace of the original nerve tissue. Berkley (2), on the other hand, describes in it a complex arrangement of nerve cells and nerve fibres, besides neuroglia and ependyma cells. Kölliker (19) takes up an intermediate position, and believes that there are no true nerve cells, but neuroglia and ependyma, a view similar to the one held by Virchow. Peremeschko (30) first recognised that the posterior lobe has an epithelial investment. Osborne and Swale Vincent (29) state that extracts of the central part of the posterior lobe are more active than extracts of the margin of the lobe, and believe that the epithelial investment would be found to be inactive if it could be properly isolated.

The pituitary body is found in all vertebrates, and, although differing widely in structure and in the arrangement of its component parts, possesses many features common to all. In fishes, the posterior lobe has a complex vascular structure of a glandular nature, which was called the "saccus vasculosus" by Gottsche (12). L. Stieda (44) proved that the saccus vasculosus communicates with the brain cavity, and Rabl-Rückhard (31) named it an infundibular gland. Their researches have been confirmed by Kupffer (21). The function of the saccus vasculosus is unknown, but its secretion, if it is a secretory gland, apparently mixes with the fluid contents of the ventricles of the brain. According to Kupffer, the

posterior lobe of the mammalian pituitary in its early development retains for a time a glandular structure. In the adult mammal the epithelial investment of the posterior lobe is regarded by Kölliker as the representative of an infundibular gland. B. Haller (14) states that in mammals—as a type of which he takes the mouse—and in all other classes of vertebrates the anterior lobe of the pituitary and epithelial investment of the posterior lobe form a gland, the tubules of which open by a small median and ventral mouth into the space between the pia and dura mater. Haller believes that the pituitary in all vertebrates secretes directly into the subdural space. Edinger (9) denies that this is true of the human pituitary, Salzer (36) could find no opening in the pituitary of the rat and mouse, and Sterzi (42) found none in the pituitary of *Petromyzon*.

There are other views on the structure and functions of the pituitary body. Boeke (3) and Gemelli (11) describe appearances in the posterior lobe of fishes which they regard as indicative of sense organs. Cyon (6) looks upon it as an organ which regulates the amount of blood passing to the brain. Guerrini (13) and others believe that the pituitary produces a secretion which has a vague antitoxic action.

Our knowledge of the structure of the pituitary body is, therefore, far from exact, and is inadequate to account for the physiological effects which follow intravenous injection of extracts, especially of the posterior lobe. Even the important question as to whether the glandular portion secretes directly into the subdural space is still unsettled. The work, the results of which are given in this paper, was begun with the intention of investigating the physiological histology of the posterior lobe, but the two portions of the pituitary were found to be so closely associated that no part would be complete without careful consideration of the other. The development and comparative anatomy of the pituitary body have been examined, but are only touched upon in this paper where reference to them throws light upon the particular point considered.

#### MATERIAL AND METHODS EMPLOYED.

The cat furnishes some of the best material for the study of the pituitary body, for in this animal the posterior lobe retains throughout life its original cavity in free communication with the third ventricle of the brain. The structure of the posterior lobe in the cat is thus rendered simpler because the arrangement of the cells which line the cavity persists in the adult in much the same manner as obtains in the developing organ. The parts which are derived from the buccal mucous membrane form an almost complete investment for the nervous portion, and the original lumen of the epithelial pouch also persists throughout life in the form of a well-marked cleft. The so-called colloid cysts are also prominent features in the pituitary of the cat.

The pituitary of the monkey more closely resembles that of man, and is a type in which greater fusion of the original elements from which it is developed has taken place. The posterior lobe is solid throughout. Its investment by the epithelial portion is not so complete as it is in the cat, and only a small cleft remains as the representative of the original buccal pouch.

The pituitary of the dog offers in some respects a type which is intermediate between that of the cat and that of the monkey. The posterior lobe is solid, but the cavity of the third ventricle of the brain is continued downwards and backwards towards the neck of the posterior lobe. The epithelial investment is very complete, and the cleft in it well developed as in the cat. The colloid cysts are more numerous than in the pituitary of the monkey, and their arrangement and structure present features which distinguish them from those of the cat's pituitary. The morphology of the pituitary bodies of the cat, dog, and monkey will be described briefly, and the structure of the various parts more minutely detailed in the cat.

For the investigation of the finer structure of the pituitary body Flemming's fixative gives the best results; a 10 per cent. solution of formol and saturated corrosive sublimate have also been employed. Sections have been cut serially in a vertical antero-posterior plane; these show the relations of the various parts of the pituitary to one another better than do sections cut in other directions. Most of the material has been cut in paraffin, but the freezing microtome has also been used, and the Golgi preparations cut by hand.

The structure of the anterior lobe is shown to the best advantage by staining with eosin and methylene blue, or by the employment of some of the many methods devised for the staining of blood films. Many preparations were made by Cajal's silver reduction method, which is especially valuable for showing the fibrils of the neuroglia, and the ependyma cells of the posterior lobe. Cox's modification of Golgi's method was also adopted for the investigation of the nervous elements. Fresh tissues have been teased out and examined in salt solution and in osmic acid, and chromic acid fixed preparations have been cut by the freezing microtome. The blood-vessels were also injected from the common carotids with carmine gelatine, and the vascular supply of the pituitary body studied in thick sections.

A word must be said about the removal of the pituitary body for purposes of examination. In order to investigate the question raised by B. Haller as to the presence of an opening on the median ventral aspect connecting the epithelial cleft with the subdural space by means of a lymph space, it is almost essential to remove the sella turcica and part of the brain from below, to decalcify the bone and cut sections of the pituitary in situ. This can be more readily done in the young animal. For most purposes it is sufficient in the adult animal to dissect the bone piecemeal from the dura mater, which forms an envelope to the pituitary, thickened

at certain points, especially behind. Great care must be taken not to rupture the thin layer of epithelium which in the cat is continued backwards from the anterior lobe, to be reflected at the place where the blood-vessels enter the posterior lobe, to form a closely fitting investment over the ventral aspect of the latter. Removal of the pituitary from the cranial cavity by raising the brain and dissecting from above is almost invariably followed by rupture of the neck of the posterior lobe. The dura mater should always be preserved intact without being pulled upon, and the best way to do this is to dissect off the bone from below, disturbing

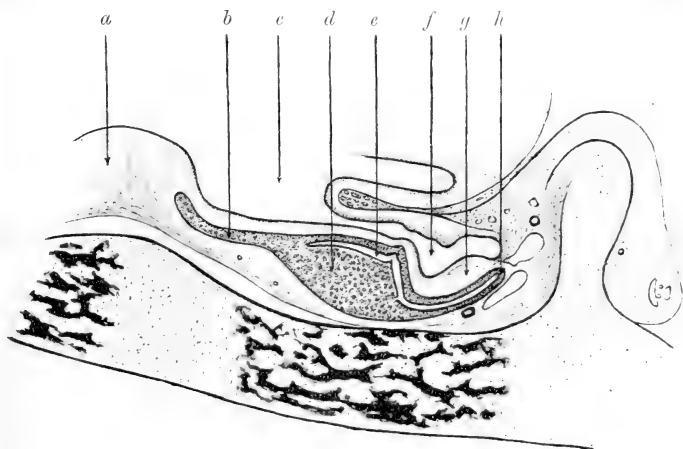


FIG. 1.—Mesial sagittal section through pituitary body and sella turcica of new-born kitten. (Semi-diagrammatic.)

*a*, optic chiasma; *b*, tongue-like process of pars intermedia; *c*, third ventricle; *d*, anterior lobe proper; *e*, epithelial cleft; *f*, central cavity of posterior lobe; *g*, nervous substance of posterior lobe; *h*, posterior reflection of epithelium.

the base of the brain as little as possible. A portion of the brain can then be cut out with the pituitary attached, and the piece trimmed after hardening.

#### MORPHOLOGY.

The relations of the anterior and posterior lobes of the pituitary to one another, and to their immediate surroundings, can be most readily appreciated by reference to the comparatively simple pituitary of the new-born kitten. Fig. 1 is a diagram of a mesial sagittal section through the pituitary and sella turcica of a new-born kitten. The infundibulum cerebri is a continuation of the brain backwards and slightly downwards, and consists of a comparatively thin wall of brain substance

enclosing a cavity which is a continuation of the third ventricle. The infundibulum has a funnel-shaped origin from the base of the brain, narrowing as it passes backwards to a tubular neck, then expanding to form a hollow club-shaped body which makes up the larger portion of the posterior lobe. The central cavity also enlarges behind the neck of the infundibulum.

The anterior lobe, composed of epithelial cells, lies below, and its thickest portion is in front of the infundibulum. It extends for some distance anteriorly, forming a tongue-shaped projection which reaches to the under surface of the tuber cinereum. The anterior lobe also spreads further laterally, and enfolds the sides of the infundibulum, the neck of which is encircled completely, so that, as in the figure, a portion of the anterior lobe appears above it. In some kittens the wrapping of the epithelium round the posterior lobe is more complete, and the only part of the lobe which is never covered by epithelium is a small part behind where the blood-vessels make their entrance. A narrow and somewhat S-shaped space lies inside the epithelium close to and following in its outline the under surface of the nervous portion of the posterior lobe, but separated from it by several layers of epithelium. The space or cleft is, as Kölliker (20) pointed out, the remnant of the cavity of the pouch of buccal epithelium from which the anterior lobe is derived. The layer of epithelium which lies between the cleft and the part of the posterior lobe developed from the brain is comparatively thin, and very closely applied to the nervous substance, thus forming an investment to it which is more or less complete according to the degree in which the anterior lobe has grown round the posterior. The cleft extends laterally, and in some cases almost surrounds the body of the posterior lobe. The posterior lobe as separated from the anterior by the cleft is therefore a composite body derived from the brain and from buccal epithelium, and it is to this structure of elements derived from two sources that the name of posterior lobe is usually applied, although strictly speaking the epithelial investment belongs developmentally to the anterior lobe. The cleft is sometimes more complicated, and branches of it may run into the substance of the anterior lobe. Serial sections show no opening below such as has been described by B. Haller (14), nor does there appear to be an opening at any point; the cleft is a closed cavity in the kitten, but very great care has to be taken in the removal and preparation of the pituitary to prevent rupture of the thin layer of epithelium which is continued backwards from the anterior lobe.

The greater portion of the anterior lobe is a solid structure made up of columns of cells and wide blood-channels. Granules are present in many of the cells of this part, but are not so marked a feature of the anterior lobe in the new-born kitten as they are in the adult cat. Colloid cysts are not found in the pituitary of the new-born kitten.

The relation of the pituitary body to the sella turcica is shown in fig. 1.



The gland lies on the body of the sphenoid bone, and is separated from it by the dura mater, which is thickened in front and behind, and contains blood-vessels, and what appears to be a lymph space. This space, which was described by B. Haller, is not a marked feature in the kitten, but is more pronounced in the foetus of the ox, where, at an earlier stage in development, it penetrates for some distance into the body of the sphenoid bone. Ossification of the bone at this point is delayed by the gradual disappearance of the epithelial stalk which connects the anterior lobe of the pituitary with the buccal epithelium. No trace of this connection is

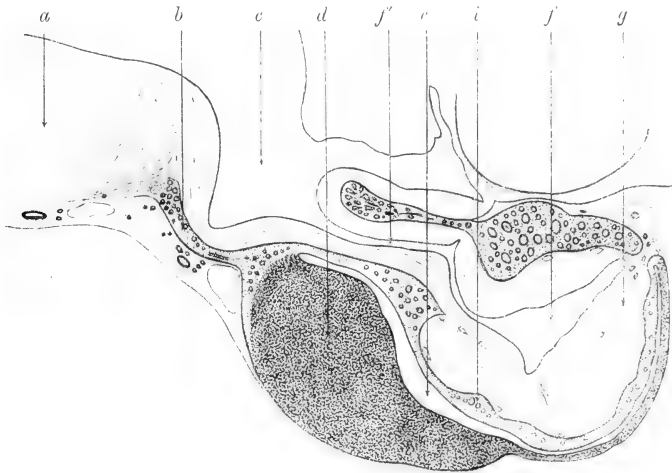


FIG. 2.—Mesial sagittal section through the pituitary body of an adult cat. (Semi-diagrammatic.)

*a*, optic chiasma; *b*, tongue-like process of pars intermedia; *c*, third ventricle; *d*, anterior lobe proper; *e*, epithelial cleft; *f*, cavity of posterior lobe; *f'*, cavity of neck of posterior lobe; *g*, nervous substance of posterior lobe; *i*, epithelial investment of posterior lobe.  
The dark shading indicates the distribution of the characteristic cells of the anterior lobe; the lighter shading shows the position of epithelium belonging to the pars intermedia.

found in the new-born kitten, and there is no evidence of any opening of the glandular tubules or cleft of the pituitary into the lymph space either in the foetal ox or kitten.

The pituitary body of the adult cat is very similar in structure to that of the kitten, but presents several important modifications. In mesial sagittal section the posterior lobe appears larger than the anterior; the latter is, however, the larger, and extends further laterally, embracing the posterior lobe. The central cavity of the posterior lobe persists, and a tapering process of it runs upwards and backwards towards the place of entry of the blood-vessels into the infundibulum (fig. 2). This process is

always present, and frequently runs up to the epithelial investment. The neck of the infundibulum is narrow and its lumen small.

The anterior lobe and epithelial investment of the nervous portion of the posterior lobe are separated by the cleft, which, as a rule, persists in its entirety. Occasionally the cleft is closed up to a large extent, especially in its posterior part, and the two layers of epithelium are more or less fused, but a space always remains between the main part of the anterior lobe and the epithelial investment of the neck of the infundibulum. The main mass of the anterior lobe in front of the cleft contains cells holding granules which stain deeply with eosin. These granular cells are not present in the epithelial investment of the posterior lobe, nor are they found in the tongue-like process of the anterior lobe which runs forwards towards the optic

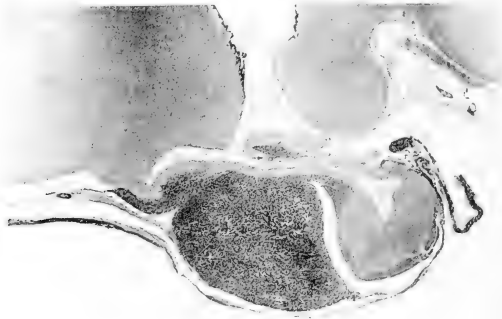


FIG. 3.—Mesial sagittal section through pituitary body of an adult cat. (Photograph.) Compare with fig 2.

chiasma. The epithelial investment of the posterior lobe is well marked and thickened, especially round the neck of the infundibulum, where there are many layers of cells. The cells are frequently arranged in groups round a central lumen which contains a colloid material. These colloid vesicles are for the most part small and scattered at intervals; they are especially well developed round the neck of the infundibulum and in the tongue-like process, where they show a resemblance to the vesicles of the thyroid gland. They are rarely found among the eosinophil cells of the anterior lobe, and appear to be placed never far distant from the nervous portion of the pituitary.

Considerable variations occur in different cats in the relative size and arrangement of the parts described. This is especially the case with the eosinophil cells of the anterior lobe, which are sometimes continued far backwards over the posterior lobe, but separated always from it by the

cleft. At other times they end abruptly, and there is nothing but a thin layer of connective tissue with occasional epithelial cells in it extending backwards from the posterior margin of the anterior lobe to the reflection on to the posterior aspect of the infundibulum. The thinning and partial disappearance of epithelium in this situation in the adult cat may possibly allow a communication between the cleft and the subdural space, but there is no direct opening to be seen, and where the epithelium persists, as it often does, serial sections show that the cleft is completely closed by it. The readiness with which rupture may take place here is easily appreciated

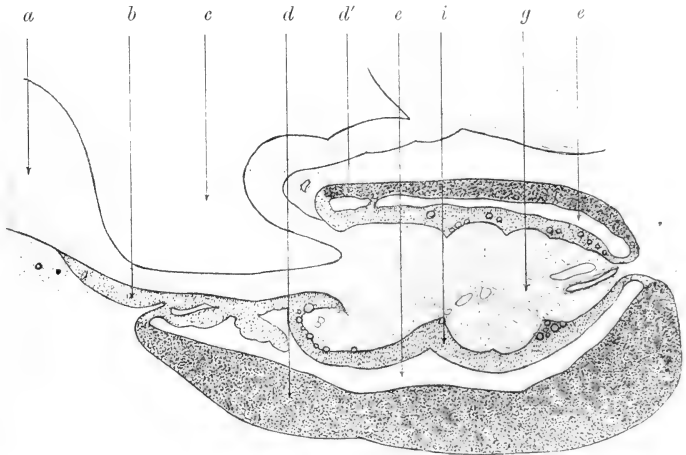


FIG. 4.—Mesial sagittal section through the pituitary body of an adult dog. (Semi-diagrammatic.)

*a*, optic chiasma; *b*, tongue-like process of pars intermedia; *c*, third ventricle; *d*, anterior lobe proper; *d'*, part of anterior lobe appearing above; *e*, epithelial cleft; *g*, nervous substance of posterior lobe; *i*, epithelial investment of posterior lobe. The dark shading indicates the anterior lobe proper; the lighter shading shows the position of the epithelium of the pars intermedia.

from the appearance in fig. 3, which is a photograph of an actual specimen of the cat's pituitary.

The pituitary body of the dog (fig. 4) presents further differences in the structure and arrangement of its parts. The body of the posterior lobe is solid, but a cavity occurs in its neck which opens by a comparatively wide mouth into the third ventricle of the brain.

The attachments of the pituitary body to the base of the brain are very similar in the cat and dog. A thin lamina of brain substance runs forwards from the neck of the infundibulum for some distance to merge with the tuber cinereum. This lamina is closely invested below by the tongue-shaped process of epithelium which runs forward from the anterior lobe.

The posterior lamina, after leaving the neck of the infundibulum, is sharply bent back upon itself, and appears in sagittal section as a long thin strip of brain substance tapering as it passes backwards until it joins the septum between the corpora mamillaria. The bend in the lamina encloses epithelium which is continuous with the epithelial investment of the posterior lobe, and which has in the cat a distinctly tubular character in this situation. In coronal section the opening of the neck into the third ventricle is not so wide, and the lateral laminae are shorter. The neck is really funnel-shaped, but compressed from side to side. It is completely invested by epithelium.

In the dog the anterior lobe almost completely embraces the posterior, but the main mass of the lobe containing eosinophil cells lies below it and at its sides. Prolongations of the anterior lobe pass over the dorsal aspect of the posterior lobe to unite with one another. The epithelium is reflected at the neck, and at the postero-superior extremity of the posterior lobe, to form an investment which covers the nervous portion of the lobe. The reflected portion of the epithelium is separated from the outer covering by the cleft, which is extremely well developed in the dog's pituitary. Finger-like processes of the epithelium which invests the posterior lobe frequently project into the cleft, and sometimes join with the outer layers of epithelium, forming strands across it. The cleft is a closed cavity in the dog, but the epithelium bounding it is very thin at the posterior reflection, and consequently liable to rupture there in course of preparation.

The investment of the posterior lobe is thick, and portions of it pass deeply into the nervous substance. It contains no eosinophil cells, but numerous colloid vesicles. The vesicles are larger in the dog than in the cat, and occur in groups which are for the most part situated in the deeper layers of the epithelium, and are not infrequently found in the adjacent nervous substance.

The pituitary body of the monkey (fig. 5) presents a very different type. The posterior lobe is solid and the cavity of the third ventricle is not even prolonged into its neck. The attachment of the pituitary to the brain is by a narrow solid stalk of nervous substance, which is surrounded by a thin layer of epithelium continuous with the anterior lobe.

The anterior lobe lies in front of the posterior, and is partly separated from it by the cleft. The main mass of the anterior lobe lies in front of the cleft, and is made up of columns of cells, many of which stain deeply with eosin, and of blood-channels. The epithelial investment of the posterior lobe is moderately thick behind the cleft, and contains no eosinophil cells. It is continued round the posterior lobe and completely invests the neck, spreading on to the adjacent parts of the brain, but is usually deficient towards the middle line on the posterior aspect of the nervous lobe. The main blood-vessels enter and leave the posterior lobe in this situation, and in all animals examined the epithelial investment stops more or less short of this place. The epithelium frequently dips

into the nervous substance, and strands of it may pass quite deeply into it, and even into the brain tissue in the neighbourhood of the floor of the third ventricle. The cleft is here again a closed cavity, and is not nearly so well developed as in the pituitaries of the cat and dog. In some cases very little of it remains, but the epithelium which lies between it and the nervous substance of the posterior lobe is always distinct from that of the main mass of the anterior lobe and contains no eosinophil cells. The same

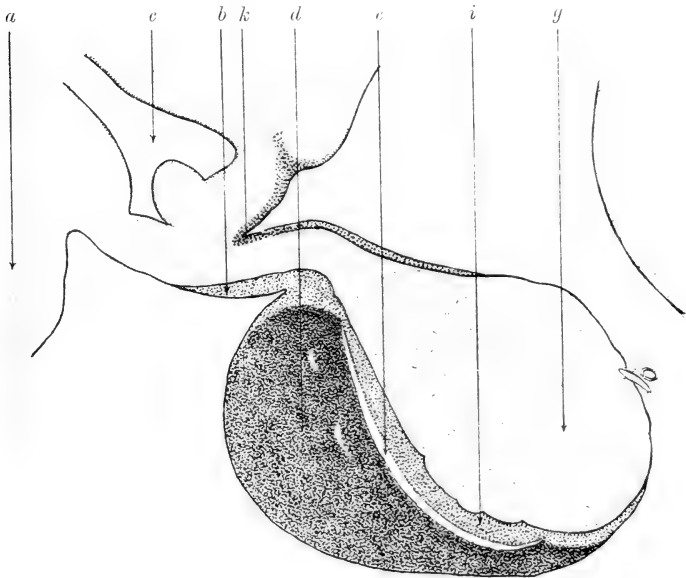


FIG. 5.—Mesial sagittal section through the pituitary body of an adult monkey. (Semi-diagrammatic.)

*a*, optic chiasma; *b*, tongue-like process of pars intermedia; *c*, third ventricle; *d*, anterior lobe proper; *e*, epithelial cleft; *g*, nervous substance of posterior lobe; *i*, epithelial investment of posterior lobe; *k*, epithelium of pars intermedia extending over and into adjacent brain substance. The dark shading indicates the anterior lobe proper; the lighter shading shows the position of the epithelium of the pars intermedia.

is true of the epithelium which invests the neck and sides of the posterior lobe. In this respect the epithelial investment of the nervous portion resembles that of the pituitaries of the cat and dog, and like them, too, may contain colloid-holding vesicles, but they are comparatively scarce in the monkey, and are not always present.

In the pituitary body of the monkey there is, then, a very complete fusion of the tissues derived from the buccal mucous membrane and from

the brain. The cleft is rudimentary and may be almost completely closed, though great differences in this respect occur in different individuals. The pituitary of the monkey closely resembles that of man. The reactions to certain staining reagents, such as hæmatoxylin and eosin, differentiate it into three parts: an anterior glandular, which constitutes the bulk of the epithelial lobe and which contains eosinophil cells; a posterior lobe of nervous origin; and an intermediate portion (Edinger (9)), which is composed of epithelial cells closely investing the nervous portion. The intermediate portion, although derived from the same source as the main anterior lobe, differs from it in adult mammals in that it contains no eosinophil cells, and may exhibit the presence of vesicles resembling the colloid vesicles of the thyroid gland. The pituitary bodies of the ox, pig, and rabbit, also belong to the third type. Traces of a central cavity are sometimes found in the neck of the posterior lobe, but in general the pituitary bodies of these animals conform to the type illustrated in fig. 5.

#### STRUCTURE OF THE ANTERIOR LOBE.

The anterior or glandular lobe as seen from below when attached to the brain is the more prominent portion of the pituitary body, surrounding, as it does, a large part of the posterior lobe; it also makes up the greater bulk of the organ. For the sake of description, however, it is convenient to consider as the anterior lobe only that portion of it which has already been distinguished from the "pars intermedia." The part thus designated as the anterior lobe is separated by the cleft and by the pars intermedia from the nervous portion of the pituitary. It is continuous with the epithelium of the pars intermedia above in the region of the neck of the infundibulum, and behind with the thin layer of epithelium which passes backwards to be reflected on to the body of the posterior lobe. It is a solid structure made up of columns of cells separated from one another by large and numerous blood-vessels and a small amount of connective tissue. The distinguishing histological feature of this lobe is the presence in it of two main kinds of cells, one of which has a marked affinity for certain staining reagents.

The occurrence of two kinds of cells in the anterior lobe of the pituitary was recognised by Hannover (15) in 1844, but little attention was bestowed upon them until the researches of Flesch (10) and Dostoiewsky (7), appearing independently of one another in 1884, definitely established their existence. Both Flesch and Dostoiewsky described one kind of cell possessing a large, round, or polyhedral body full of big granules, which retain a deep red colour when treated with eosin and hæmatoxylin, and differentiated in alcohol. These cells are called "chromophil" cells by Flesch. Lothringer (22) states that they are probably identical with the "Mutterzellen" of Luschka. The other

kind of cell is small, contains a large nucleus and little protoplasm, which is decolourised by the same method of procedure. This variety is the "chromophobe" cell. The two kinds of cells occur together in strings or clumps, sometimes the one preponderating, sometimes the other. The clumps are surrounded by a basement membrane, and vary in size according to the number and character of the enclosed cells. The distribution of the two kinds varies in different animals. Dostoiewsky says that in man and the ox the clear cells are chiefly grouped together in the central part of the gland, while in small animals, rat, cat, and rabbit, they are more scattered throughout the lobe. The gland is extremely vascular, and the blood-vessels are of the nature of wide channels. Rogowitsch (34) calls the "chromophobe" cell of Flesch the "Hauptzelle," recognises the "chromophil" as a distinct cell, and states that a third variety exists in the form of nucleated masses of embryonic tissue. H. Stieda (43) comes to a similar conclusion and describes as "Kernhaufen" masses of embryonic tissue full of closely packed nuclei, having little protoplasm which behaves like that of the "Hauptzellen" to stains, and no cell borders. Schönemann (39) goes still further, and believes that most of the so-called "Hauptzellen" have no real borders, and that they are to be regarded as "kernreiches Protoplasma." According to Rogowitsch, Stieda, and Schönemann, the changes in the pituitary which follow removal of the thyroids are confined to the cells of the anterior lobe. Their results have a certain general agreement, but differ considerably in detail. Rogowitsch finds colloid in the "chromophil" cells, and states that it passes directly from them into the blood-vessels, both of which observations are strongly combated by Stieda. The latter believes that thyroidectomy is followed by increase in size of the "Hauptzellen," and that no formation of colloid takes place. Rogowitsch describes hypertrophy of the "Kernhaufen" with vacuolisation and colloid formation. Schönemann is of the opinion that "chromophil" cells are not a prominent feature of the healthy pituitary, that their development after thyroidectomy is a degenerative process, and, further, that they undergo colloid change, accompanied by proliferation of connective tissue and blood-vessels.

Saint-Remy (35) in 1892, after careful examination of the pituitary bodies of many vertebrates, came to the conclusion that there is only one kind of cell in the anterior lobe, and that the varieties previously described are merely the expressions of different functional stages of the same cell. The "chromophil" cell is really a "Hauptzelle," or principal cell, in the protoplasm of which deeply staining granules have accumulated. The granules are probably transformed into some product of secretion and eliminated from the cell, which then becomes a smaller body recognisable as a principal cell. All stages between these extreme forms may be recognised in the normal gland.

Claus and Van der Stricht (5) came to similar conclusions. Benda (1)

has more recently confirmed Saint-Remy's views. He distinguishes three main forms showing transitional stages. The small, poorly granular cell is the young form, while the large, deeply-staining granular cell marks the acme of functional development. A third variety is the large cell devoid of granules, which he regards as a cell the function of which is temporarily or permanently interrupted. Benda pointed out that there is no evidence of any of the cells being the products of degenerative changes as supposed by Schönemann, and further that the granules bear no

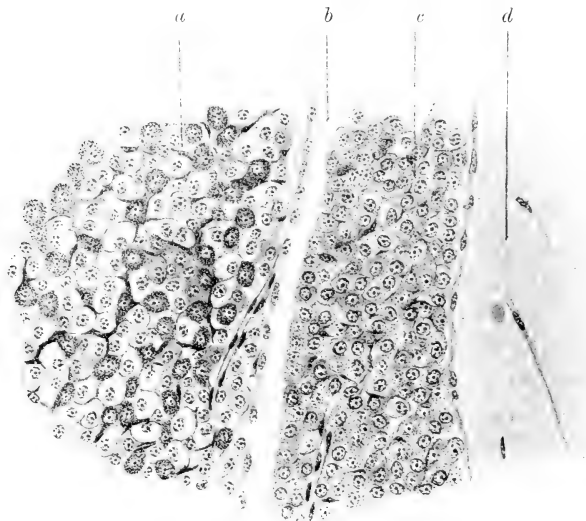


FIG. 6.—Mesial sagittal section through part of the pituitary body of an adult cat.

*a*, anterior lobe showing different forms of epithelial cells: the blood channels are collapsed and their position indicated only by endothelial cells; *b*, epithelial cleft separating anterior from posterior lobe; *c*, epithelial investment of posterior lobe—"Epithelsaum" of Lothringer; *d*, nervous substance of posterior lobe.

relation to the formation of colloid material. He believes that the granules break down into a secretion which passes directly into the blood-vessels by diffusion through their thin walls.

My own observations are to a large extent confirmatory of the views expressed by Benda. In the anterior lobe of the cat's pituitary there exist three main varieties of cells: a small polygonal cell with large nucleus and little protoplasm, containing few or no granules; a larger cell with similar nucleus and protoplasm, which may be clear, but frequently shows a diffuse arrangement of fine granules; and, lastly, cells which are full of deeply-staining material. The latter kind of cell is sometimes smaller than the diffusely granular form (cf. fig. 7), but this is not always the case, and



the deeply-staining cell may be quite as large. In fig. 7 it looks as though there were two kinds of cells: a large, clear, and diffusely granular kind, and a smaller, deeply-staining cell. The picture presented by these cells varies according to the method and depth of staining, and differs in different parts of the lobe in the same section. The fixative employed has also a great influence on the staining reactions, and with formalin or corrosive sublimate fixation there appear to be only two kinds of cells: the granular and clear. Occasionally a cell is seen which is diffusely granular in most of its body, but contains around its nucleus protoplasm of the deeply-staining variety. It is extremely difficult to decide whether these appearances indicate

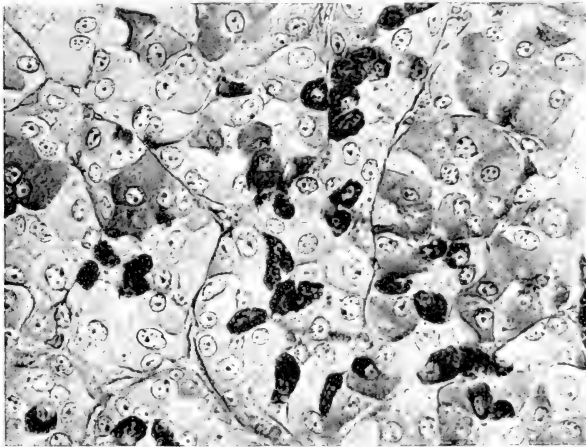


FIG. 7.—Photograph of part of anterior lobe proper of an adult cat,  $\times 500$ . Fixed in Flemming's solution, stained with hot alcoholic eosin, differentiated in alkaline alcohol, and counterstained with picro-fuchsin.

Shows clear cells and granular cells. The latter appear to be of two varieties—one kind consists of large cells whose protoplasm takes on a diffuse stain; the other of smaller cells which are full of deeply-staining granules. The blood-channels are collapsed and their position is indicated by the dark lines.

distinct forms of cells, or whether they are merely expressions of different functional stages of one and the same kind of cell.

The different cell forms are distributed fairly uniformly throughout the lobe; the clear cells sometimes predominate in certain localities, but are not constantly distributed in these positions. Where anterior lobe blends with the epithelium of the pars intermedia, a gradual transition is sometimes seen between the two kinds; at other times the dividing line is sharply marked (cf. fig. 8). The cells typical of the anterior lobe may spread right round the neck of the posterior lobe, or backwards over the body of the posterior lobe to the posterior reflection of epithelium. The general appearances of the relation of the cells of the anterior lobe to the cleft and

epithelium of the pars intermedia is shown in fig. 6, which is a drawing from a sagittal section through part of both lobes of the pituitary of the cat. In this specimen and in the one from which fig. 7 is taken, the blood-channels are collapsed and their position is indicated by endothelial cells. The cells are arranged in solid columns, between which run thin-walled blood-channels. The columns show no central lumen, nor is there any colloid met with either in the cells or between them. Where colloid is present it lies among the clearer cells of the pars intermedia, and never in relation to the characteristic granular cells of the anterior lobe.

The cells of the anterior lobe and of the pars intermedia are derived from the same origin and become differentiated during foetal life. In the kitten the cells of the anterior lobe do not show such marked differences in size and the possession of granules as they do in the adult. The clear cell and the granular cell are recognisable, and there are transitional forms. While it is almost impossible as yet to settle the exact nature of these cells, I am inclined to believe that all the varieties represent varying stages of functional activity of one and the same kind of cell, and that the deeply-staining material is the product of the cell destined to be poured as an internal secretion into the blood-vessels.

The blood-vessels of the anterior lobe are extremely numerous and wide. When injected with carmine gelatine from the carotids they are seen to form wide channels resembling to some extent the sinusoids of the liver. The endothelial cells are closely applied to the epithelial cells without intervening connective tissue cells. In this respect also they resemble the sinusoids of Minot. There is, however, no evidence of any intracellular canalisation of the epithelial cells, such as is found in the liver (17). A fine reticulum of connective tissue is present in most places, resembling the "Gitterfasern" of the liver lobules. Whether lymphatics exist or not is doubtful; the sinusoidal character of the blood-vessels and the closely fitting endothelial cells render their presence unlikely in many parts of the anterior lobe. In certain situations near the cleft and pars intermedia true capillaries and connective tissue are found, and lymphatic vessels appear to exist in these situations.

The anterior lobe in the cat is usually separated from the cleft by a single layer of flattened cells, which are larger than endothelial cells, and are continuous at the anterior and posterior ends of the cleft with the cells of the epithelial reflection (fig. 6).

In the dog the anterior lobe is permeated by extraordinarily large, thin-walled blood sinuses. Lothringer (22) compared the structure of the anterior lobe in this animal to cavernous tissue. In the monkey, too, the blood-vessels are in the form of wide, thin-walled sinuses running more or less parallel to one another in an antero-posterior direction.

The changes in structure of the anterior lobe which have been alleged to follow thyroidectomy in the rabbit require further investigation. The normal variation in structure and arrangement of the cells varies within

wide limits. Different methods of fixation and staining give very diverse pictures. The most useful method for showing the finer structure of the pituitary body as a whole is Flemming's fixative followed by Muir's eosin and methylene blue stain. Some of the clear cells of the anterior lobe occasionally seem wanting in outline, but careful staining shows that they are not "Kernhaufen." Cajal's silver reduction method leaves no doubt that they are cells, and their outlines are readily seen when this method is employed.

The anterior lobe of the pituitary is evidently an important glandular body, and probably furnishes a secretion which passes directly into the blood-vessels; in this sense it is a blood-vessel gland, as was surmised by Ecker. Its function is unknown; extracts of it, when injected into the blood-vessels, have no immediate physiological action beyond that common to most glandular extracts. It is possible that this part of the pituitary has something to do with the regulation of the growth of the body, but in the meantime there is not evidence enough to form a basis for any definite statement.

#### STRUCTURE OF THE INTERMEDIATE PART OF THE PITUITARY.

The intermediate part of the pituitary body has its origin in common with the anterior lobe. It arises from the epithelial pouch which grows inwards from the buccal mucous membrane, being a development of that portion of its wall which is closely applied to the nervous portion of the pituitary. It is separated from the anterior lobe by the cleft throughout a large part of its extent in the cat, but is continuous with it, in front round the neck of the infundibulum, and behind at the posterior reflection. The connection between it and the nervous portion is very intimate. The portion which surrounds the neck of the infundibulum shows a structure differing somewhat from the part which covers the body of the posterior lobe. In the cat the epithelium surrounding the neck of the infundibulum is distinctly tubular, but the lumen is not a continuous one. The cells are arranged round a central lumen, which frequently contains a colloid material. The tubules are continued forwards in the tongue-like process already mentioned. Between them are numerous large blood-vessels; this portion of the gland is very vascular. Fig. 8 shows the structure of the tongue-like process of the pituitary of an adult cat, and its line of separation from the granular cells of the anterior lobe.

The tubules do not appear to open into the subdural space, and are probably columns of cells in which lumina only appear at intervals where the colloid material accumulates between the cells. In the region of the anterior part of the cleft the tubules sometimes appear to open into the latter, but their lumina are frequently interrupted. Colloid material has been noted by many observers in the cleft, and may enter it in this

manner, but the occurrence of colloid in the cleft is, in my experience, rare, and where found histologically has been in large, thin-walled cysts belonging to the epithelium of the neck of the posterior lobe.<sup>1</sup>

Connective tissue and lymphatics are found between the cell columns, and the whole structure is closely united to the under surface of the brain, into which blood capillaries freely penetrate.

The epithelial cells do not show the regular arrangement which is so characteristic of the thyroid vesicles; the walls are irregular and may be composed of one or several layers of cells. The cells are small and clear; fine granules may be present in their protoplasm. The colloid

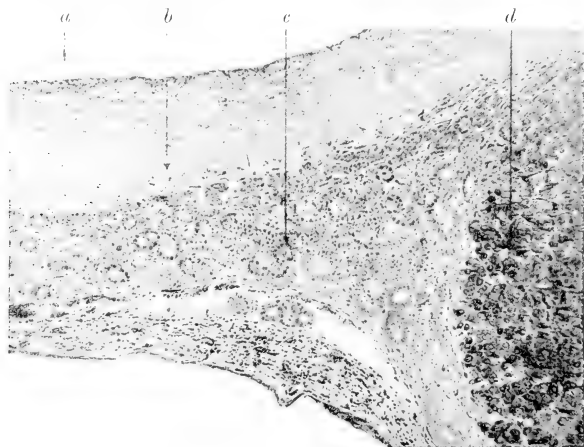


FIG. 8.—Mesial sagittal section through tongue-like process of pars intermedia and adjacent part of brain and of anterior lobe proper of an adult cat. (Photograph  $\times 100$ .)

*a*, third ventricle; *b*, portion of anterior lamina of neck of posterior lobe; *c*, tongue-like process of pars intermedia, consisting of epithelial cells in form of solid columns and tabules; many of the latter contain colloid; *d*, granular cells of anterior lobe proper.

material varies in amount in different animals; it does not stain deeply, and has a somewhat different appearance from the colloid met with in the thyroid. Whether it is the same kind of material or not is open to question. Schnitzler and Ewald (38) state that thyreo-iodine occurs in the pituitary.

The junction of the intermediate portion with the anterior lobe is

<sup>1</sup> A pituitary body from an apparently healthy female cat, that had had a litter of kittens a short time previously, showed a large mass of colloid substance surrounding the neck of the infundibulum and occupying a large part of the cleft. The material did not lie free in the cleft, but was surrounded by a single layer of flattened epithelium. The cyst was nearly as large as the posterior lobe, and originated from the epithelium surrounding the neck of the infundibulum. The substance in the cyst was not of a homogeneous nature, but consisted of irregular solid masses lying among a clearer material.

usually well defined by the occurrence of coarsely granular cells in the latter. The cells of this part are very like the clear cells of the anterior lobe, and are closely packed together in solid columns; occasionally they spread downwards a little over the front of the anterior lobe (see fig. 2). Behind they are continuous with the epithelial covering of the posterior lobe, the cells of which they closely resemble.

The epithelial covering of the posterior lobe was described by Peremeschko (30), who gave it the name of "Markschicht." He found it to vary in thickness in different situations, and to be firmly attached to the nervous substance. He also noted the presence of colloid vesicles in the "Markschicht" of the dog's pituitary, and that the cells are unlike those of the "Korksicht" or anterior lobe. Lothringer (22) gave it the name of "Epithelsaum," a term which is used by Retzius (33) and later observers. Peremeschko was the first to point out that the cleft lies in the part of the pituitary which is composed of epithelium, and that it does not separate epithelial from tissue of nervous origin, as had previously been thought. In addition to the ordinary epithelial cells which are found in the "Epithelsaum," Lothringer described marginal cells which lie between the others and reach the free border, or are arched back upon themselves. Similar cells have been figured by Retzius (33) in Golgi preparations, and compared by him to neuroglia cells. According to Retzius they are, for the most part, small and thread-like, and reach through the whole border. Other cells do not pass right through, but are branched. The cell nuclei are often placed near the outer end, while the inner end widens to a three-cornered foot, which is placed against the nervous tissue of the posterior lobe. The structure of the epithelial border is shown in fig. 9, which is taken from a section of kitten's pituitary prepared by Cajal's silver method. Long, thin nucleated cells of a spindle shape are numerous, and take a vertical course through the epithelium. They appear to be of ectodermic origin, and act as supporting cells. Similar cells are found in the adult, but are better seen in the young animal. A section through the epithelial covering of the adult pituitary gives appearances shown in fig. 6. The cells are arranged in several layers over the greater part of the body of the posterior lobe, but are much thicker in some places than in others. In the cat a great accumulation of epithelial cells is found in the region of the lower part of the neck of the infundibulum, forming a thick mass between the cleft and nervous substance. Colloid material may be present in rounded spaces between adjacent cells in any part of the covering, but the vesicles are largest and most numerous in the thicker parts round the neck. In some cats there is a great development of the epithelium at the sides of the posterior extremity of the nervous lobe. In this situation there are distinct tubules which open into the cleft. The cells forming the walls of the tubules are clear and devoid of granules, and there is an absence of colloid material. This tubular arrangement is not always present.

and appears to be the result of proliferation of the epithelium at the end of the cleft, a lumen being retained in the outgrowths continuous with the original cavity.

In the dog large vesicles are frequently seen in the epithelium; they are larger than those of the cat's pituitary, and often occur in groups. They are for the most part deeply situated and often separated from the nervous substance by a single layer of flattened cells, and even these may be deficient, so that the colloid material is partly bordered by the nervous substance. In the monkey the vesicles are less numerous, and occur for the most part in the epithelium at the ends of the cleft.

The cells which make up the greater part of the epithelial border are well-defined polygonal cells resembling somewhat the clear cells of the

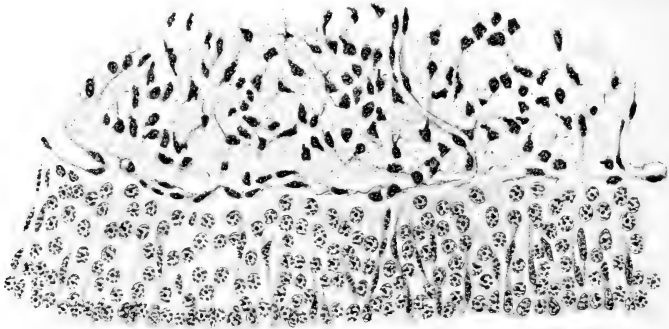


FIG. 9.—Vertical section through epithelial investment and nervous substance of posterior lobe of new-born kitten. Prepared by Cajal's method.

The epithelial investment contains numerous spindle-shaped cells, the marginal cells of Retzius; the nervous substance is composed of neuroglia cells and granular-looking matrix. Numerous blood capillaries are found in the nervous substance, but do not penetrate into the epithelium.

anterior lobe, but they stain rather more deeply and their protoplasm is more granular. Occasionally deeply-staining cells are met with, but they never show the eosinophil granules of the cells of the anterior lobe. The colloid material appears to be a product of the epithelial cells, and accumulates between adjacent cells, giving rise to the appearances of vesicles. The spaces thus formed may communicate, but are for the most part closed.

The epithelial investment of the posterior lobe presents another notable feature which distinguishes it from the remainder of the epithelial part of the pituitary—it rarely contains blood-vessels. In the pituitary of the cat no vessels occur in the thinner portions, and where the epithelium is much thickened a few capillaries only enter it from the adjacent nervous substance. Large blood-vessels, chiefly veins, and capillaries are very

numerous at the junction of the epithelium with the nervous portion (cf. fig. 9). In the dog, capillaries are rather more numerous. In the monkey they are absent. A very striking difference is thus afforded between the highly vascular epithelium of the anterior lobe and the non-vascular epithelium which covers the posterior lobe. This peculiarity indicates a difference between the two parts, if not of function, at any rate of the mode of absorption of the products of the epithelial cells. Another significant feature relates to the position of the colloid material. This occurs for the most part in extra-vascular situations, and never far distant from the nervous portion of the pituitary. If it is a product of secretion utilised by the animal, it must pass inwards to the nervous tissue and be carried away by the numerous blood-vessels situated immediately under the epithelium, or pass into lymph spaces in the nervous portion. This point will be discussed later in connection with the structure of the nervous portion. In the other place, where colloid material is common in the cat, viz. in the tongue-like process (fig. 8), the epithelium containing it is surrounded by connective tissue, blood-vessels and lymphatics, and even here a close relation exists between the epithelial cells and adjacent brain substance. The structure of the intermediate part of the pituitary body shows such marked differences from the structure of the anterior lobe that it is quite probable it has a different function.

#### STRUCTURE OF THE NERVOUS PART OF THE PITUITARY.

On no part of the pituitary body have there been so many and different opinions expressed with regard to structure as on the portion derived from the brain. Virchow (46) regarded it as a "filum terminale anterius," consisting of ependyma cells, networks of white fibres, and finely granular masses in which cells appear. He could find no nerve cells in it. Peremeschko (30) described a feltwork of connective tissue fibres and spindle-shaped cells, with a few ganglion cells among them. The latter, he says, lie mostly two or three together surrounded by connective tissue, and differ from ordinary ganglion cells in that their protoplasm is scantier and their nuclei flattened. W. Müller (27) and Mihalkovics (25), who studied the development of the pituitary, agreed that with the ingrowth of blood-vessels into the nervous portion, a proliferation of connective tissue cells accompanying them destroys the original brain tissue, converting the infundibulum into a connective tissue appendage of the brain. W. Müller (27) compared the arrangement of the connective tissue cells to the appearances shown by a spindle-celled sarcoma, a simile which has been frequently employed since by authors of text-books of anatomy. Lothringer (22) described the bulk of the organ as made up of bundles of fibres, with nuclei resembling those of plain muscle fibres, crossing one another at sharp angles, and holding in the meshwork thus formed round, angular, or polygonal cells. He believed it to be chiefly neuroglia tissue, and poor in nerve cells.

Berkley (2) examined the nervous lobe of the pituitary of the dog, employing Golgi and other methods. He gives a description and diagram of the posterior lobe of the dog's pituitary, and divides the organ into three parts, all of which are microscopically dissimilar in appearance. "There is first an outer lamina of slightly irregular ependymal cells, three or four deep, arranged after the manner of the cuticular epithelium, separated into divisions by thin processes extending from the fibres of the surrounding capsule and terminating at a definite line where a separation from the more internally lying elements occurs; then follows a more internal zone of varying depth, containing epithelial cells of a secretory type, which in places along the posterior and inferior border of the lobe, as well as occasionally in the more central regions, are arranged into distinct closed acini lined with a low variety of cylindrical epithelial cell, and often hold in their lumen collections of a colloid substance." Berkley in this passage clearly describes as ependymal cells the long thin cells of the epithelial investment, the nature of which has already been discussed. Berkley further states: "The secretory region gradually merges into a central region of small, rounded, and polygonal cells separated by extensive connective tissue partitions, carrying blood-vessels, and scattered widely among these cells are others of larger dimensions. Some of the latter are of spindle form, others of pear or rounded shape, and still others of very irregular form with their borders ill-defined; all having a finely granular appearance, with here and there larger granules scattered among them, that are tinged by osmic acid a blackish colour. In the region of the neck of the infundibulum the epithelial elements, except the outer ependymal row, become segregated into groups of mainly oval and pear-shaped cells separated by a fine stroma, with small nuclei here and there in it, and are now easily recognised as nerve cells. A certain number of the spindle cells are very long as well as broad, and probably correspond to those described by Krause as spindle cells of uncertain function, but they are undoubtedly nerve cells." Berkley gives a correct description of the structure of the lobe, but with his statement that the epithelial cells become nerve cells, I am quite unable to agree. He finds several varieties of neuroglia cells, chiefly of the moss and spider type. No less than six varieties of nerve cells, including large, medium, and small pyramidal cells, are described by him as occurring in the posterior lobe of the pituitary. The axis cylinders have a general tendency to pass upwards and forwards. Berkley concludes that "the pituitary gland retains in the dog, as one of the highest orders of vertebrates, its double rôle of secretory and nervous functions, intact; the former perhaps modified, the latter, the original special sense organ, probably lying quiescent, not atrophied, and only changed in so far as to admit of a slightly different arrangement of its constituent elements." Berkley is quoted at length, because it is on the result of his work that the belief in the presence of true nerve cells in the pituitary is mainly based. Retzius finds no true nerve cells, and no medullated fibres, and inclines to



the opinion that, although nerve fibres may be present, the bulk of the posterior lobe consists of neuroglia cells and fibres, and ependyma cells. Kölliker (19) takes up the same position, and holds that the apparent nerve cells are really neuroglia and ependyma; many of the fibres of the latter run for a considerable distance in a longitudinal direction, and form thick bundles. Kölliker agrees with Retzius, that, in man and the higher mammals, there are no true nerve elements in the infundibular lobe, and that the occurrence of glandular structures in it betokens the formation of an infundibular gland in the sense of Kupffer.

The posterior lobe of the pituitary body, when fixed in formalin, corrosive sublimate, or other fixing agent in common use, and stained in thin sections by hæmatoxylin and eosin, presents in its interior a structure which has a general resemblance to connective tissue. There are numerous flattened, spindle-shaped, and branching cells, and a matrix large in amount, finely granular, and holding numerous ill-defined fibres resembling white fibrous tissue. Blood-vessels are fairly numerous, especially small arteries and capillaries, and the tissue of the nervous lobe frequently appears to be arranged in whorls around them, a layer of condensed matrix lying next the vessels, and outside that a lighter zone anastomosing with similar layers around adjacent vessels. This disposition of the matrix is very well brought out by the iron-alum-hæmatoxylin method of staining. A somewhat similar arrangement is seen in the neck of the infundibulum, where there are two very distinct layers surrounding the central cavity, an inner layer in which run longitudinally placed fibres, and an outer layer, which is finely granular and apparently traversed by much finer fibres, having for the most part a vertical direction.

The cells in the posterior lobe do not resemble true nerve cells, and when the sections are stained by Nissl's method nothing of the nature of Nissl bodies is found in them. In sections prepared by Cox's modification of Golgi's method, the nervous substance is seen to be composed of neuroglia cells and fibres (fig. 10).

The neuroglia cells are numerous, and their branches interlace, forming a dense network of fibres throughout the body of the infundibulum. Many of the branches end in relation to the blood-vessels, which they frequently bend round; others run to the periphery of the lobe, but do not penetrate into the epithelial investment. In addition to the neuroglia cells and fibres, there are cells lining the central cavity, continuous through the neck of the infundibulum with the cells that line the cavity of the third ventricle. They are undoubtedly ependyma cells, and are extremely well developed in the pituitary. The fibres from the cells take various directions, according to the position of the cell bodies, but most of them assume a longitudinal direction, and pass into the neck of the infundibulum. In this situation they at first run in the internal layer, then turn into the outer layer, and, breaking up into extremely fine fibrils, take a course at right angles to their previous direction, and end at the margin of the neck in proximity to the

epithelial columns surrounding it. The arrangement of these fibres is not easily made out in Golgi preparations, but can be more readily followed in thinner sections prepared by Cajal's reduced silver method. Cajal's method shows that the posterior lobe is pervaded by a dense network of fibres which agree in their manner of disposition with the arrangement revealed by the Golgi method, but are more uniformly stained than by the latter. The ependyma fibres are large and thick in the adult cat, especially near their origin from

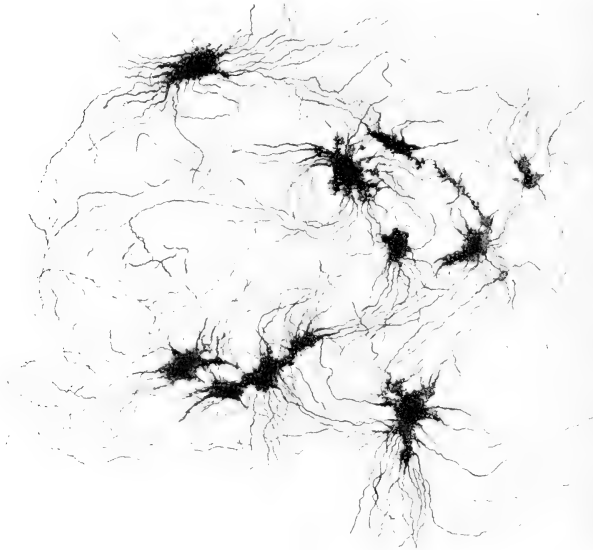


FIG. 10.—Section of part of nervous substance of posterior lobe of the pituitary body of an adult cat, showing neuroglia cells and fibres. Prepared by Cox's modification of Golgi's method.

the cell. They appear to begin abruptly in the cell protoplasm, often by several rootlets, which soon fuse to form a single process or remain separate.

In the body of the posterior lobe the ependyma fibres take various courses. Some run outwards and are lost in the neuroglial network. At the posterior end of the lobe where the central cavity approaches the epithelial covering, fibres may be traced outwards to end immediately under the latter. Most of the ependyma fibres, and especially those of the anterior part of the lobe and the neck, take a longitudinal direction, as though passing upwards and forwards into the brain. The two layers already mentioned as occurring in the neck of the infundibulum are particularly well defined by Cajal's method. Fig. 12 is a drawing of a longitudinal section through part of the

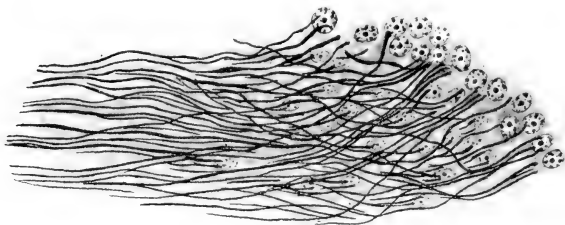


FIG. 11.—Diagram showing arrangement of the ependyma cells lining the central cavity of the posterior lobe of an adult cat. The ependyma cells send their processes forwards into the neck of the posterior lobe. Drawn from a section prepared by Cajal's method.

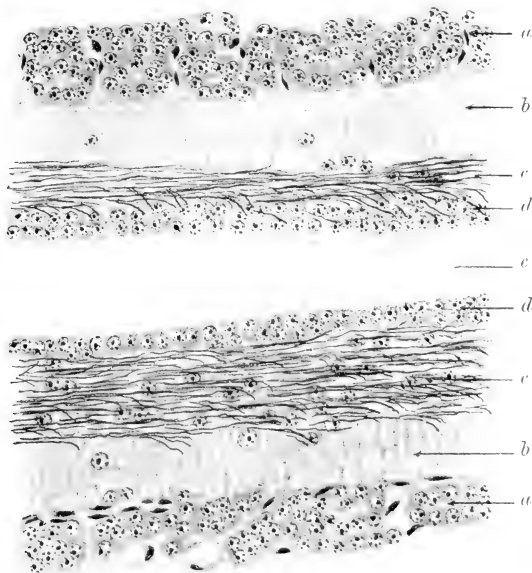


FIG. 12.—Mesial sagittal section through neck of the posterior lobe of a kitten. Drawing from a section prepared by Cajal's method. *a*, epithelial cells of pars intermedia surrounding neck; *b*, outer layer of nervous substance of neck; *c*, inner layer of ependyma fibres; *d*, ependyma cells lining neck; *e*, central cavity of neck.

neck of the posterior lobe of a kitten in which the two layers are shown, surrounded by the epithelial columns of the intermediate portion of the pituitary.

The distinction between the two layers begins at the junction between body and neck of the posterior lobe, and is continued throughout the laminae which connect it to the brain. In mesial sagittal section the appearances of a Cajal preparation are very striking. The finely granular, clear outer layer extends anteriorly to the tuber cinereum, increasing in size as it passes forward. The inner layer of longitudinally running fibres diminishes in thickness, but is quite well marked where it fuses with fibres and cells of the tuber cinereum. In the posterior lamina the two layers are equally distinct, but the longitudinal fibres are fewer in number, and both layers

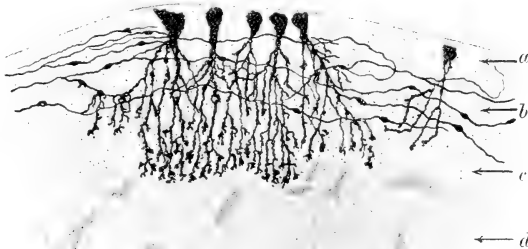


FIG. 13.—Mesial sagittal section through anterior lamina of nervous substance in floor of third ventricle of adult cat. Drawing from a section prepared by Cox's modification of Golgi's method.

*a*, ependyma cells; *b*, inner layer of horizontal fibres; *c*, outer layer of vertical fibres from ependyma cells; *d*, position of cells of pars intermedia of tongue-like process. Cf. fig. 8.

become very thin towards their junction with the brain substance in the region of the corpora mamillaria. The cells which line the cavity and neck of the infundibulum vary somewhat in appearance, according to the methods of fixation and staining employed. In the kitten, as revealed by Cajal's method, there may be more than one layer of rounded cells from which, as in fig. 12, fibres arise. None of the cells are ciliated in the cat. In the adult there is one layer of cells, but others often lie among the fibres, and appear to give origin to some of the latter. The anterior lamina in front of the neck of the infundibulum is lined by cells, which, when prepared by Golgi's method, have the appearance shown in fig. 13. They are well-developed ependyma cells, and their branching processes pass outwards, giving rise to the vertical striation which in Cajal and other preparations is characteristic of the outer layer of the neck of the infundibulum. In Golgi preparations these cells appear to give off processes which

run with the other longitudinal fibres in the inner layer. Similar appearances were described by Berkley, but it is almost impossible to decide whether longitudinal fibres arise from these cells or not; they may be fibres from cells further back, which cross the cell processes in this situation.

The layers of the neck of the infundibulum have been described by several authors. Mihalkovics (25) noted them in the developing pituitary, but believed that the inner layer is composed of connective tissue fibres arising from cells which enter the posterior lobe with the blood-vessels. Lothringer (22) described them, and stated that the layers are prolongations of the tissue of the tuber cinereum into the posterior lobe, neuroglia fibres increasing and nervous elements diminishing from before backwards. According to Lothringer, the longitudinal fibres have their origin in the tuber cinereum. This view has been adopted by most subsequent authors except Berkley, who, on the other hand, regards them as nerve fibres arising from cells in the posterior lobe, and passing upwards and forwards to the brain. Cajal (quoted from Kölliker's "Gewebelehre," Bd. ii., S. 604, 1896) found in two-days-old mice nerve fibres originating in the tuber cinereum, and passing downwards into the body of the posterior lobe. In the lobe itself he described one of the thickest plexuses of nerve fibres known, and cells of a doubtful nature.

The fibres are not medullated nerve fibres, and do not stain as such with osmic acid in fresh teased preparations. They are much thicker than ordinary non-medullated fibres, and are devoid of nuclei along their course. Many of them resemble white fibrous tissue, but they are stained by Cajal's method, although not so deeply as the nerve fibres in the adjacent brain substance. The posterior lobe of the pituitary body of the cat, owing to the persistence throughout life of its original cavity, is a particularly good subject for the elucidation of the question of the origin of these longitudinally running fibres. In this animal the fibres clearly arise from cells lining the cavity of the body and neck, and pass forwards and upwards towards the brain. They are the fibres of ependyma cells. After a course of varying length, they break up into fine fibrils which enter the outer layer and terminate on its external surface. The origin of the fibres can be traced in the developing organ, and their elongation and oblique course explained. The cell bodies, from which the fibres proceed, move downwards and backwards with the growth of the infundibulum, but the outer ends of the fibres remain attached to the junction with the epithelial part, and do not participate in the movement. New fibres arise during growth, some of them apparently taking origin from cells which lie deeper and not lining the cavity. In the front portion of the anterior lamina most of the ependyma fibres run vertically, and have only a very short course (fig. 13). There are numerous cells in the tuber cinereum, and fibres pass into or out of this body; it is difficult to tell where their cells of origin lie. Some fibres may arise in the tuber cinereum, but they resemble the ependyma fibres, and probably belong to ependyma or neuroglia cells.

Similar bundles of fibres are found in the solid neck of the posterior lobe of the monkey's pituitary. Here, too, the cells of origin lie below, and the fibres run upwards; they must be regarded as ependyma cells which have become enclosed in the body of the organ.

In the body of the posterior lobe the ependyma and neuroglia fibres make up a very thick network. The presence of true nerve cells in the pituitary is very doubtful. In preparations made by Cox's modification of Golgi's method the cells stained resemble neuroglia cells and are all of the same type. That this is not an accident is shown by the uniformity of the results in different specimens, and by the fact that when the sections include brain substance the true nerve cells in the latter are well and characteristically shown. The neuroglia cells, like those of adjacent parts of the brain, are spider cells with very numerous processes. In Cajal preparations the fibres run through the cell, the body of which is often difficult to distinguish.

The nerve supply of the glandular portion of the pituitary body is stated by Berkley to be derived from the sympathetic system. Branches enter the gland with the blood-vessels, and end among the epithelial cells. Cajal's method shows fine fibres entering amongst the cells of the epithelial investment, especially in its thicker portions near the neck of the infundibulum. The fibres come from the nervous portion, where they are closely associated with the blood-vessels. I have not been able to trace their origin, but for developmental reasons am inclined to believe that they accompany the blood-vessels into the gland, and are, as Berkley states, derived from the sympathetic system. Neither by Golgi's nor Cajal's method have I been able to find nerve fibres in the anterior lobe, but I have not specially investigated this point.

The nervous tissue of the posterior lobe of the pituitary appears, then, to have the structure assigned to it by Virchow in 1857, and to be made up of neuroglia and ependyma cells and fibres. There are, however, other very important elements present. The epithelial covering is in contact with the nervous portion from an early stage of development, and grows around it. But the relation becomes still more intimate, for the epithelial cells invade the nervous portion. This ingrowth may take place at any part of the lobe, but, in the cat's pituitary, is most marked in the region of the neck of the infundibulum, and at the posterior reflection of epithelium. In the latter situation the epithelial cells frequently accompany the blood-vessels for some distance into the lobe. Strands of cells retaining their connection with the epithelial investment are often seen passing into the nervous substance; at other times cell islets of a similar nature are found at variable distances from the epithelial covering. The islets consist of well-defined epithelial cells, and in preparations fixed in Flemming's solution are very distinct. They react to stains in a manner which identifies them with the cells of the epithelial covering. In the adult cat they are often very numerous, especially in the neck of the

infundibulum; but they may occur at any part, and are not infrequent in the middle of the posterior lobe, lying partly in the cavity. The islets vary in size from a few cells to twenty or more, and are usually compact, but in the neck of the infundibulum have a looser structure, and individual epithelial cells occur among the fibres of the nervous portion. These are probably the cells referred to by Berkley, but they are not nerve cells; they are derived from the epithelial investment. Occasionally a pituitary body is met with in which the posterior lobe is quite transformed by the ingrowth of epithelium. One of the most remarkable features about the epithelial investment of the pituitary is the property it possesses from a very early stage of development of spreading over and around the structures with which it comes into contact. It also tends to invade them, and may even spread for some distance into the base of the brain in some animals. In the monkey (cf. fig. 5) the cells often penetrate towards the cavity of the third ventricle behind the neck of the infundibulum. The same is true in the case of other animals; and it is not confined to mammals—a similar invasion is common in birds. The extent of the ingrowth shows great variations in different individuals of the same species. Careful and good fixation of the tissue is necessary to show it; for, if any shrinkage takes place, the epithelial cells are more difficult to distinguish from the other cells of the posterior lobe, and are easily overlooked. When the islets are large and deeply placed they are readily seen.

In well-fixed preparations, and especially after fixation by Flemming's solution, the posterior lobe is found to contain small hyaline bodies, highly refractive when unstained. These bodies lie scattered throughout the nervous substance of the lobe, and stain indifferently with eosin or methylene blue. They are not so prominent in formalin fixed preparations, but can be seen. They are not found in the pituitary of the new-born kitten, but in adult pituitaries are invariably present, and in all animals examined. They occur in the body of the lobe and in the neck, extending for some distance upwards towards the brain, but not into the brain itself. Their appearance suggests that of cells which have undergone hyaline degeneration; no nucleus is present, the outline is often irregular, and no structure can be made out in them. Sometimes, however, they have a distinctly granular appearance, and are not unlike the granules of the cells of the anterior lobe, but do not stain so deeply. The significance of these bodies is difficult to determine. They seem to be of the nature of a secretion, and are not unlike diluted colloid material. In some situations the substance lies in what look like lymph spaces lined with endothelium. The question naturally arises as to whether this material represents the physiologically active principle of the posterior lobe; its situation certainly agrees with the position in which that is found. The substance often lies between the ependyma cells near the central cavity, and may possibly be a secretory product of these cells, in which case the nervous part of the pituitary might be regarded as a glandular structure, but it is not always

confined to the nervous part, and occurs occasionally among the cells of the epithelial investment, especially where that is thickened just below the neck of the infundibulum. This fact points strongly to its being a product of the cells of the epithelial investment. The material is most abundant in the neck and around the central cavity and in the neighbourhood of the epithelial islets.

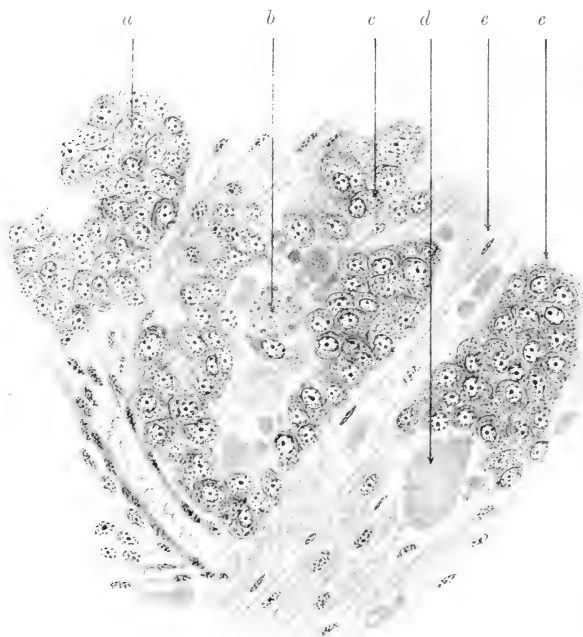


FIG. 14.—Drawing of part of the posterior lobe of the pituitary body of an adult cat. From specimen fixed in Flemming's solution and stained with eosin and methylene blue.

*a*, cells of epithelial investment; *b*, granular body; *c, c*, islets of epithelial cells similar in character to those of the epithelial investment; *d*, colloid or hyaline body; *e*, ependyma and neuroglia fibres. The drawing is from part of the section in the neighbourhood of the neck of the posterior lobe. Granular and colloid bodies occur in close proximity to the epithelial cell islets.

The accompanying figure (fig. 14) is a drawing of the appearances seen in a portion of the posterior lobe of an adult cat near the neck of the infundibulum. Several epithelial islets and scattered cells lie among the neuroglial and ependyma fibres. In close relation to the cells are seen masses of a hyaline or granular character. The substance often stains very



like the so-called colloid material, and may be of that nature; but is unlike in many respects the colloid of the thyroid gland. In the posterior lobe of the dog's pituitary somewhat similar appearances present themselves, but in this case the epithelial islets frequently consist of a number of cells which group themselves round a central cavity containing colloid material. Isolated cysts, the walls of which are composed of a single layer of cells, are not uncommon, and were noted by Lothringer. In the dog, too, the larger cysts of the epithelial investment are not always complete, and the contained material may abut against the neuroglial tissue. The colloid substance, when completely or partly enclosed by epithelial cells, varies considerably in its staining properties in different parts of the same pituitary. As a rule it takes on little depth of colour with stains, and is unlike the colloid of the thyroid in this respect; it has a hyaline rather than a colloid appearance. Occasionally, however, the substance is denser and takes on a deeper stain, and in the dog sometimes looks as though it were a swollen cell with disintegrating nucleus. The hyaline material of the nervous portion of the posterior lobe also varies in appearance, and in its staining properties; most of it might be of the same nature as the so-called colloid of the epithelial investment and of the intermediate part generally, but in a diluted form. Some of this material must be the product of the epithelial cells, for it occurs in places where no other kind of cell is present. The universal occurrence of this material in the nervous substance, often at considerable distances from epithelial cells, is difficult to explain, unless we can suppose it to be carried from them by lymphatic vessels. The substance often does lie in distinct spaces lined by what appears to be endothelial cells. The general tendency of the direction of the material seems to be towards the neck of the infundibulum in the cat, and it increases in amount towards this situation. Large masses are sometimes seen lying among the ependyma cells, and similar material may be present in large amount in the central cavity, communications between the two being evident in places.

In fig. 15 a typical portion of the neck of the infundibulum of a cat's pituitary is seen. The central cavity is lined by ependyma cells, outside which are cells with large nuclei and little protoplasm. Occupying the central cavity is a mass of hyaline material, and masses of a similar substance lie beneath the ependyma cells and between the ependyma fibres. In other places in the posterior lobe the material is distinctly present in lymph channels accompanying the blood-vessels. Evidence strongly points to the probability that the material is on its way to the central cavity, and so into the ventricles of the brain. In this sense the posterior lobe of the pituitary is a gland which pours its secretion into the third ventricle of the brain. It is possible that the ependyma and neuroglia cells have also a secretory function, but improbable that they secrete the material described. They may, however, have some influence upon it. The most likely supposition is that the ependyma and neuroglia cells

form a scaffolding for the posterior lobe of the pituitary, upon which is built up a covering of epithelial cells.<sup>1</sup> The relations of the two structures become very intimate; their blood supply is derived from arteries which enter the nervous substance posteriorly; the veins begin immediately below the epithelium, and return through the nervous substance. Interchange of material between blood-vessels and cells must be through the medium of lymph, seeing that most of the epithelial investment is extra-vascular. Cells from the investing layer grow into the nervous framework, giving rise to epithelial columns and cell islets. Secretion goes on either by an emptying of material from the cells into the lymph, or possibly by a breaking down and destruction of the whole cell. The latter indeed is

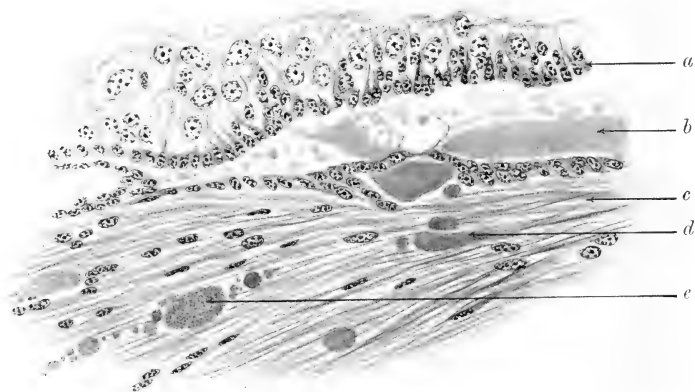


FIG. 15.—Section of part of neck of posterior lobe of the pituitary body of an adult cat. From specimen fixed in Flemming's solution and stained with eosin and methylene blue. Shows colloid material lying among ependyma fibres and in the central cavity of the neck. *a*, ependyma cells lining central cavity; *b*, colloid material in central cavity; *c*, ependyma fibres; *d*, colloid material; *e*, granular body lying among ependyma fibres.

the more probable fate of isolated epithelial cells, and seems to occur at times in the epithelial investment itself. The material known as colloid substance, which has been supposed by many to be identical with the colloid of the thyroid gland, occurs in comparatively large amounts in the pituitary of the cat, an animal which cannot long survive thyroid extirpation. Further chemical and experimental research is necessary to prove

<sup>1</sup> In the posterior lobe of a Belgian hare, a ganglion with large ganglion cells and medullated fibres was found. The ganglion occupied a large portion of one side of the posterior part of the lobe, and the fibres appeared as though entering it obliquely from the side and not through the neck of the lobe. Red bone marrow was also present, and the ganglion may have been part of one of the Gasserian ganglia, which, in the rabbit, are very close to the pituitary body. A large part of the nervous portion of the lobe was destroyed by it. The intermediate portion was large in amount, and its cells thickly massed around the neck. The animal was a healthy adult.

whether the colloid substance of the pituitary is identical in composition, or in its physiological action, with the colloid of the thyroid gland. The physiological action is apparently quite different, but this may be due to the presence in the lobe of other substances. Any secretion formed in the pars intermedia of the pituitary must pass into the adjacent substance of nervous origin either by blood-vessels or lymphatics. There is a possible exception to this rule in the tongue-like process of the cat's pituitary, but even here blood-vessels from it pass into the adjacent anterior lamina connecting the neck of the infundibulum with the tuber cinereum, and the tubules are surrounded by connective tissue containing lymphatics, the course of which is unknown. They may possibly accompany the blood-vessels. The epithelial cells do not resemble in staining properties the cells of the medulla of the suprarenal capsule; they have no affinity for chromic acid. Nor do they resemble them in their relations to blood-vessels, the absence of which is so characteristic of most of the pars intermedia.

A question of importance arose in the early investigations of the structure of the pituitary. Peremeschko (30) described the cleft of the epithelial part as being continuous with the central cavity of the neck of the infundibulum, and so with the third ventricle of the brain. If this were the case it would furnish a proof of Kupffer's view (21) that the epithelial portion represents a "palæostoma" or old mouth of an ancestral form of vertebrate, and there would be in the mammalian embryo a communication between the neural canal and buccal cavity. Subsequent observers have denied the accuracy of Peremeschko's observations. In the pituitaries of the pig and man, in which Peremeschko described the continuation, there is obviously no such thing, for the body of the infundibulum is solid in both cases. Nor is there any indication of it in the embryo of either pig or man, so far as I have been able to see. It is far more likely to occur in the pituitary of the cat, in which the central cavity of the infundibulum is well developed and prolonged far backwards. In the adult cat the epithelium, as already stated, frequently invades the posterior end of the cavity, so that epithelial cells may even form part of its lining. This peculiarity affords some support for Kupffer's view. In the adult cat I have never been able to find a direct communication between the cleft and the cavity of the infundibulum, although many specimens have been examined with this object. In one embryo kitten, however, I have found such a communication. Cleft and cavity in this specimen are undoubtedly in direct continuity at the postero-superior angle of the posterior lobe. In other kittens, at a comparatively late stage of embryonic life, a direct continuity is occasionally seen between tubular epithelium at the end of the cleft, and the ependyma cells lining the central cavity of the infundibulum. In the kitten this coming together of the two portions occurs some time after the epithelial duct between buccal mucous membrane and epithelial portion of the pituitary has

disappeared, so that there is never a direct continuity between the neural canal and the exterior. The observations, nevertheless, give support to Kupffer's views on the morphological significance of the pituitary.

#### VASCULAR SUPPLY OF THE PITUITARY BODY.

The arrangement of the blood-vessels in the pituitary body has already been described along with the structure of its several parts, but a general

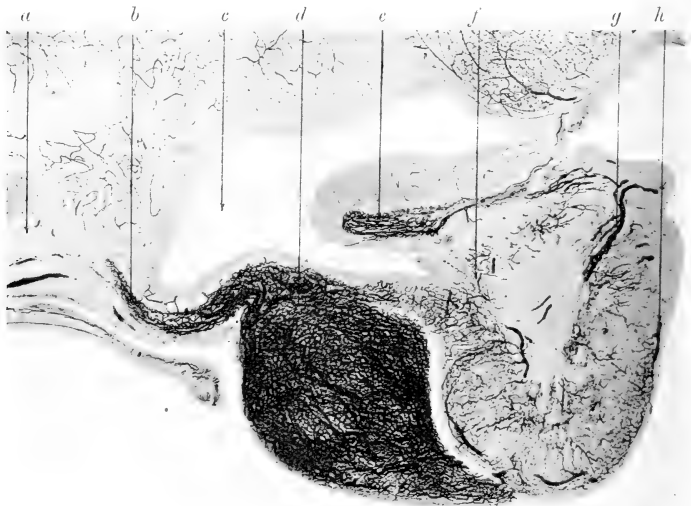


FIG. 16.—Mesial sagittal section of pituitary body of adult cat; blood-vessels injected with carmine gelatine. (Photograph.)

*a*, optic chiasma; *b*, tongue-like process of pars intermedia; *c*, third ventricle; *d*, anterior lobe; *e*, pars intermedia lying above neck of posterior lobe; *f*, posterior lobe; *g*, central artery entering posterior lobe at its postero-superior angle; *h*, large vein lying between nervous substance and epithelial investment of posterior lobe.

survey of the vascular distribution in the cat's pituitary may be given. The most noticeable feature of the injected organ is the difference in vascularity of the two lobes. The anterior lobe is filled with wide channels, making it one of the most vascular structures of the body; the posterior lobe, on the other hand, resembles in the number and arrangement of its vessels the adjacent white matter of the brain. A large blood sinus is found on either side of the pituitary body below. The anterior lobe is supplied by arteries, apparently from the internal carotid, which enter it at the sides of the infundibulum above, and break up immediately into large, thin-walled.

vessels. The posterior lobe is supplied by a median artery which enters it at its postero-superior extremity; branches run forwards near the central cavity, and break up into capillaries. The veins of the posterior lobe are situated immediately beneath the epithelial investment, and converge towards the place of entrance of the artery; leaving the lobe in this situation, they turn outwards to join the large lateral sinus on either side. Some of the veins from the anterior lobe appear to take a similar course, passing through the epithelial investment of the neck of the infundibulum to run in the nervous portion; others leave the anterior lobe at its neck and pass outwards into the lateral sinus. The presence of so many large arteries and veins in the immediate vicinity of the pituitary body would render the operation of removing it an extremely difficult one, even were the organ so situated as to be convenient of access. Minot (26) states that the blood-vessels of the anterior lobe of the pituitary will probably be found to have a sinusoidal development. This appears to be partly true at any rate of the anterior lobe of the pig's pituitary.

#### CONCLUSIONS.

We may conclude from the histological appearances of the mammalian pituitary body that it is an organ of physiological importance. It may be divided into two parts, which show structural differences probably indicative of distinct functions.

The anterior lobe, consisting of large granular cells and numerous blood-vessels, is a gland producing an internal secretion which is poured directly into the blood. It is a blood-vascular gland, the function of which is undetermined, but which may exercise an influence on growth. The careful examination of the pituitary body in cases of acromegaly may throw some light upon this question; at present any statement as to its probable functions must be purely speculative.

The posterior lobe is made up of two structures. Of these, the part developed from the brain and consisting of neuroglia and ependyma cells and fibres acts as a framework. It is more or less surrounded and invaded by epithelium, which probably furnishes its active part. There is histological evidence of a secretion produced by the epithelial cells, which apparently passes into lymph-vessels, and is destined to enter the ventricles of the brain. The posterior lobe of the mammalian pituitary is a brain gland, not by virtue of tissue of brain origin, but by the growth into it of epithelial cells of ectodermic origin. Extracts have the property of producing marked effects on cardiac and plain muscle fibres comparable in some respects to the action of the medulla of the suprarenal capsule. They have also a selective action upon the kidney, causing dilatation of the renal blood-vessels and diuresis. Disturbances of the posterior lobe of the pituitary are probably responsible for the occurrence of the diabetic conditions

which have been so frequently recorded at some time or other in the history of cases of acromegaly and of affections and lesions associated with the base of the skull.

#### SUMMARY.

Three types of mammalian pituitary body are recognised. In one, e.g., the cat, the posterior lobe is hollow and its cavity is in free communication with the third ventricle of the brain, while the epithelium of the anterior lobe affords an almost complete investment for the posterior lobe; in the second type, e.g., the dog, the body of the posterior lobe is solid, but the neck is hollow, and communicates with the third ventricle: the posterior lobe is here again almost completely surrounded with epithelium; in the third type, e.g., man, monkey, ox, pig, and rabbit, the body and neck of the posterior lobe are solid, although traces of a cavity are occasionally found in the neck; in this type the epithelium does not invest the posterior lobe so completely, but is aggregated around the neck and spreads over and into the adjacent surface of the brain.

The epithelial portion of the pituitary body is differentiated into two distinct parts: an anterior lobe proper, consisting of solid columns of cells, between which run wide and thin-walled blood-channels; and an intermediate portion, which lies between the anterior lobe and the nervous tissue of the pituitary, forming a closely-fitting investment of the latter.

The anterior lobe contains cells which are clear or hold in their protoplasm varying amounts of deeply-staining granules. They are probably different functional stages of one and the same kind of cell, and the granules give rise to a secretion which is absorbed by the blood-vessels.

The intermediate portion consists of finely granular cells arranged in layers of varying thickness closely applied to the body and neck of the posterior lobe and to the under surface of adjacent parts of the brain. The part of it which is separated from the anterior lobe by the cleft is almost devoid of blood-vessels. In the cat the portion lying in front of the anterior lobe has a tubular appearance and is very vascular. Colloid material occurs between the cells of the *pars intermedia*, and in most situations appears to pass into the adjacent nervous substance, to be absorbed by blood-vessels or lymphatics.

The nervous portion of the pituitary body is made up of neuroglia cells and fibres. Ependyma cells line the central cavity in the cat and send long fibres forwards and upwards towards the brain, most of which terminate in the outer part of the neck. There are no true nerve cells and the nerves supplying the pituitary probably reach it through sympathetic fibres accompanying the blood-vessels (Berkley). The nervous portion is invaded to a large extent by the epithelial cells of the *pars intermedia*. Columns of epithelial cells grow into it, especially in the region of the neck, and islets of these cells are frequently found throughout the posterior lobe;

in the pituitary of the cat epithelial cells may even grow into its central cavity.

A substance histologically resembling the colloid of the thyroid gland, but probably of a different nature, occurs in large quantities in the nervous portion of the posterior lobe. It appears to be a product of the epithelial cells, and, in the cat at any rate, to be carried by lymphatics into the central cavity, and so into the third ventricle of the brain. In this respect the posterior lobe of the pituitary is an infundibular gland. Whether this substance is modified by its passage through the nervous substance or not is unsettled. Its distribution corresponds with the site of the tissue, the extracts of which have active physiological results when injected into the blood.

The anterior lobe of the pituitary is extremely vascular and its circulation sinusoidal. The posterior lobe is supplied for the most part by a central artery which enters it at its postero-superior angle and runs forward giving off branches; the veins begin immediately below the epithelial investment and run backwards in this situation, to emerge near the entry of the artery. The veins of both lobes enter large blood sinuses lying close to the sides of the pituitary body.

Histological evidence is against the statement of Bela Haller that the anterior lobe is a tubular gland which pours its secretion directly into the subdural space.

I am indebted to Mr Richard Muir for the care with which he has executed the drawings and photographs accompanying this paper.

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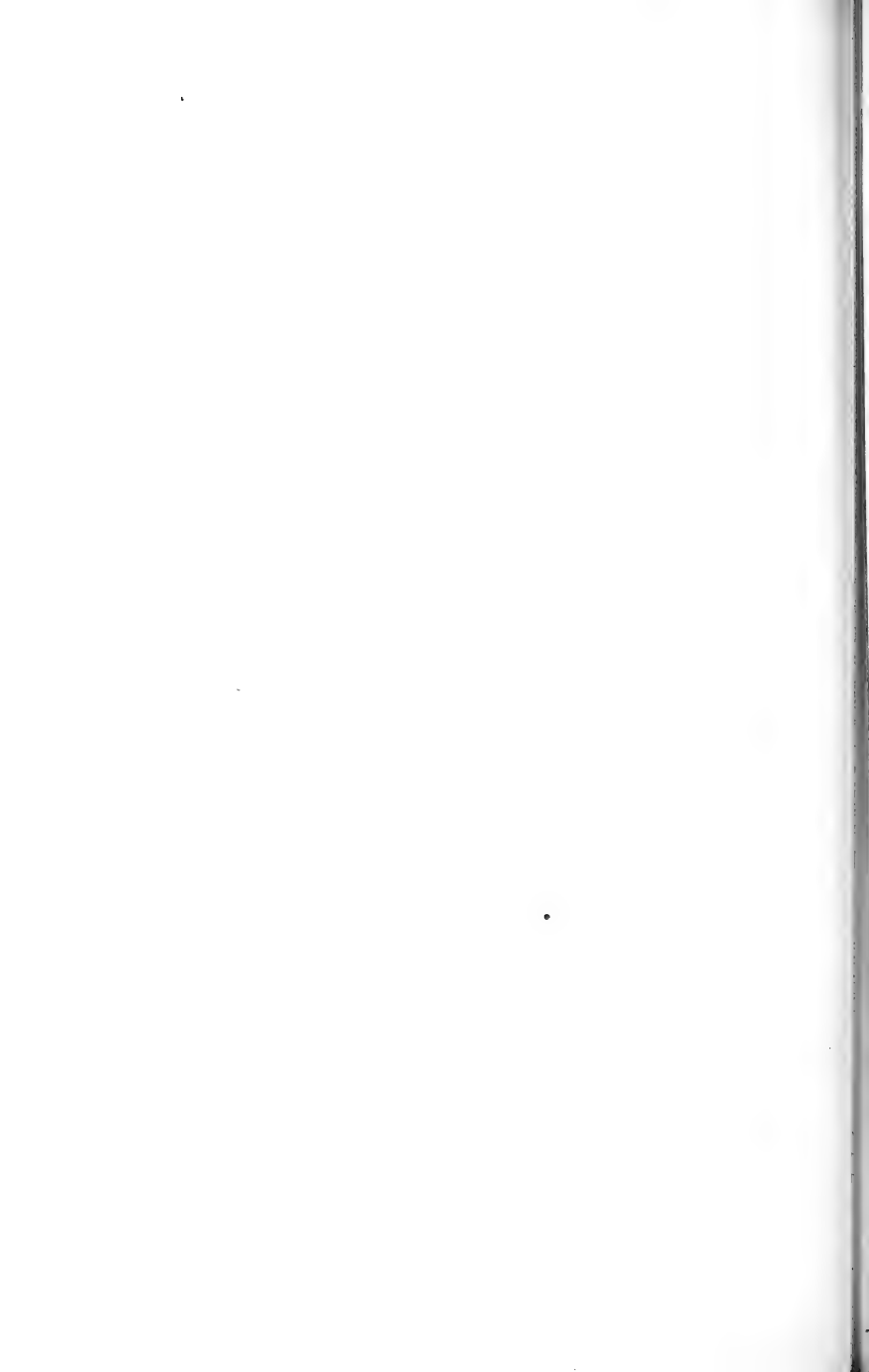
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THE DEVELOPMENT OF THE MAMMALIAN PITUITARY AND ITS  
MORPHOLOGICAL SIGNIFICANCE. By P. T. HERRING. (From  
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INTRODUCTION.

THE development of the pituitary body has been a favourite subject of research by embryologists. Its position in the embryo, forming as it were a meeting-point for the anterior end of the neural canal, buccal invagination, archenteron, and notochord, gives to the pituitary an importance, the significance of which has been the object of much speculation. Some authorities have looked upon its relations to these structures as more or less accidental; others have attached great weight to them. Kupffer, indeed, regarded the pituitary body as an important key to the phylogeny of the vertebrate head. The morphological significance of the pituitary is also of interest from a physiological point of view, and some of the theories which have been advanced regarding it will be briefly discussed in this paper.

Nearly all the work that has been done on the development of the pituitary body has been concerned with its mode of origin and with the early stages of its growth. The later stages, although probably of greater physiological importance, have been comparatively neglected. The differentiation of the epithelium of the anterior lobe, the relations of epithelium to the nervous tissue of the posterior lobe, and the extraordinary differences in the vascularity of its several parts are all features which need investigation. Its development in mammals has been followed chiefly in animals in which the posterior lobe of the pituitary becomes a solid structure at a comparatively early stage. In the cat, this lobe remains hollow throughout development, and presents peculiarities of morphological interest which are not found in the pituitaries of other animals. The structure of the posterior lobe in the cat is also of a simpler character, and the nature and arrangement of the cells found in it can be interpreted more readily than in the case of those animals which possess a solid lobe. For these reasons the pituitary body of the cat forms the basis of the description in this paper. The embryos of man, ox, and pig, which furnish a different type of pituitary, have also been examined, and some of the more important features presented by them receive attention.

## HISTORICAL.

The pituitary body was at one time thought to be wholly derived from the brain, but Rathke (26) in 1838 described the invagination of mucous membrane which is now known as Rathke's pouch. Rathke rightly assigned to this pouch the origin of the epithelial portion of the pituitary, but was mistaken in believing it to be derived from the entoderm of the fore-gut. His view was not at once accepted. Reichert (28) failed to find the invagination, and put forward the theory that the epithelial portion of the pituitary is a structure of mesodermic origin derived from the anterior end of the notochord. His (14) lent additional support to Reichert's view, but made no special investigations of the subject himself. Both Rathke (27) and Reichert (29) subsequently changed their opinions, the latter believing the anterior lobe to arise from a proliferation of the cells of the pia mater. Dursy (8) sought to unite the original view of Rathke with that of Reichert and His, and described the origin of the epithelium of the pituitary from the fore-gut, and the origin of its vascular stroma from the tissue of the head of the notochord. W. Müller (23) demonstrated that the anterior lobe of the pituitary is derived from Rathke's pouch, but fell into the same error as Rathke and Dursy in believing it to be of entodermic origin. The later researches of Götte (12) and Balfour (3) showed that the pouch described by Rathke is derived, not from the fore-gut, but from the epithelium of the buccal cavity immediately in front of the oral plate. The pouch is now recognised as an ectodermic structure.

The posterior lobe of the pituitary was at first believed to represent the anterior extremity of the brain (v. Baer (2)). Götte (12) showed that in amphibians this is not the case, the infundibulum being a later formation. The researches of Mihalkovics (21), van Wijhe (35), Kupffer (20), and others have demonstrated that the infundibulum cannot be regarded as the representative of the anterior end of the brain axis; it is an outgrowth of the "Zwischenhirn" or thalamencephalon.

The proximity of the anterior end of the notochord to the developing pituitary body led to the belief, not only that the notochord enters into the structure of the pituitary, but that it also exercises a mechanical influence upon the formation of the infundibulum. Both His and Dursy considered that a close union between the notochord and the wall of the cerebral vesicle is the dominating factor in the development of the infundibulum, but were not agreed as to the exact manner in which this is brought about. W. Müller believed that the head of the notochord anchors a portion of the wall of the brain, and that with the growth of the surrounding tissues the rest of the brain is carried forwards, leaving a diverticulum of its wall, the infundibulum, attached to the notochord. The attachment is subsequently dissolved by a proliferation of connective tissue cells. Mihalkovics (21) and others showed that the head of the notochord does not come into

immediate relationship with the brain, and cannot therefore act upon it in this manner.

The most complete account of the early development of the pituitary body is that given by Mihalkovics (21), who investigated the subject in rabbit and chick embryos. Mihalkovics found that the anterior lobe is developed from Rathke's pouch, which, in mammals as in amphibians, is of ectodermic origin. The beginning of the pouch or hypophysial angle lies in front of the oral plate, where the epidermis bends round the base of the brain to the nasal mucosa. In the rabbit, Mihalkovics states that the end of the notochord is in contact with the epidermis at the back of Rathke's pouch. When the oral plate ruptures, its upper stump, containing in its upper part the head of the notochord, bends forward and narrows the mouth of the epithelial pouch, leading to the formation of a definite sac—the hypophysial sac. The wall of the sac presses upon the base of the anterior brain vesicle, giving rise at its upper extremity to a fold in the wall of the brain which becomes the primitive infundibulum. Mihalkovics denied that the end of the notochord is ever united to the wall of the fore-brain; it does not enter into the formation of the infundibulum at all, but has some influence upon the hypophysial sac, by preventing this from extending backwards. The primitive infundibular process comprises the surrounding tissue of the tuber cinereum as well as the origin of the infundibulum, and the true infundibulum is formed at a later stage by its own growth from a portion of the primitive infundibular process. Mihalkovics made a careful investigation of the relations of the notochord, and found that its head touches the lower part of the posterior wall of the hypophysial sac in rabbits, but is placed at a higher level in birds; it exercises no traction upon the sac in either, and, beyond presenting a barrier to the backward growth of the sac, takes no part in the formation of the pituitary body.

The main conclusions of Mihalkovics' researches have been confirmed by Kölliker (16), Kraushaar (18), Minot (22), Kupffer (19), Salzer (33), and others. Kupffer described an additional origin of part of the anterior lobe of the pituitary from the entoderm of the fore-gut. According to Kupffer, the pituitary body of amphibians is built up from three separate sources: part of the epithelial lobe is derived from Rathke's pouch, and part from the anterior end of the fore-gut, while the infundibulum is of brain origin. In mammals, e.g. the sheep, the hypophysial pouch appears behind the "Riechplakode." Behind this and ventral to it is a swelling, the "Haftscheibe," which is an important larval organ in *Lepidosteus*. Then comes the double-layered oral plate ("Rachenhaut"), and behind this an outgrowth of entoderm directed dorsally and forwards, known as Seessel's pouch. The third portion of the pituitary, the cerebral, appears later, after the disappearance of the oral plate and median "Riechplakode." In the next stage the growth of entoderm increases, but is cut off from Seessel's pouch; no cavity is to be found in it, and the end of the notochord

remains in contact with it. The infundibulum now begins to grow. In the older embryos, e.g. 11-mm. sheep, the entodermic part degenerates and appears as a string-like appendage of the notochord; it eventually disappears, and does not enter into the formation of the adult mammalian pituitary.

Kupffer came to the conclusion that the intimate relationship between infundibulum, mouth, and intestine is not an accidental one, but denotes an ancestral communication between the brain tube and the anterior part of the intestinal canal. A structure resembling in many respects the early stages of development of the vertebrate pituitary is found in Ascidians, and is known as the subneural gland. Julin (15) in 1881 pointed out that this gland is probably homologous with the hypophysis of higher vertebrates, and since then it has been frequently spoken of as the Ascidian hypophysis. Kupffer believes that the direct ancestors of vertebrates showed the same relations as are seen to-day in the tailed Ascidian larva. In a scheme of the ancestral vertebrate he describes the mouth ("Palæostoma") opening dorsally in front of the brain. The brain tube is in communication with the anterior part of the intestine by a canal running through the base of the anterior brain vesicle. This canal has developed upon it a subcerebral gland. Ventral to the palæostoma is the "Haftorgan" on the anterior pole of the body. In the course of development the new vertebrate mouth (Neostoma) is formed, in agreement with Dohrn's hypothesis, from a pair of gill-clefts below the "Haftorgan." The part of the intestine between the old and the new mouth, or preoral intestine, is reduced, but persists to a certain extent in some vertebrates. The palæostoma is lined by epidermis, and its representative in vertebrates is Rathke's pouch; it also forms the outer part of the nasal duct ("Nasenrachengang") of *Myxine*, and the entire nasal duct of *Petromyzon*. The remains of the canalis neurentericus anterior, with its appertaining glands, are to be seen in the infundibular process and saccus vasculosus. In mammals, the only representative of the preoral intestine is the transitory appearance of the solid mass of cells formed from Seessel's pouch, but in amphibians it persists as part of the anterior lobe of the pituitary.

Kupffer's views on the morphological significance of the pituitary body have not met with general acceptance. Willey (36) states that the present relation of the hypophysis to the infundibulum in the craniates, however intimate it may be in some cases, is, nevertheless, incidental and secondary. Willey believes that the hypophysis arose in connection with a functional neuropore. B. Haller (13) criticises Kupffer's results and differs from him in many important particulars. He believes the nasal duct of *Cyclostomata* to be a secondary structure and not related to the origin of the hypophysis. He also states that the anterior lobe of the pituitary of mammals and other vertebrates is a tubular gland which pours a secretion into the subdural space. The latter statement has not been confirmed by subsequent observers. Gaskell (9) quotes Haller's results in support of the

theory that the glandular hypophysis was originally the coxal gland of Arthropoda.

Kupffer's description of the threefold origin of the pituitary body has received support from observations by J. Nusbaum (24) and Saint-Remy (31). Nusbaum found that in dog embryos of 9 mm. Seessel's pouch is well developed, and its anterior extremity abuts against the posterior wall of Rathke's pouch. In 80 per cent. of older embryos examined it gives rise to a column of cells which unites with the epithelium of Rathke's pouch, and thus enters into the formation of the anterior lobe. In the remaining embryos no such appearance is seen, and the anterior lobe is entirely ectodermic in origin.\* Traces of a lumen were noticed by Nusbaum in the column of cells growing from the fore-gut, but not a definite communication between the interior of the buccal invagination and the fore-gut. The connection is not preserved for long, and the entodermic cells disappear, with the exception of a few which join the posterior wall of Rathke's pouch. What further part these cells play—if any—in the formation of the anterior lobe of the pituitary Nusbaum did not determine.

Saint-Remy (31) described a budding of Seessel's pouch in the embryo chick towards the seventieth hour of incubation. The bud acquires a fine lumen, and, reaching Rathke's pouch, affords a direct communication between the interior of the latter and the fore-gut. The connection lasts a little, then disappears, the cells of Seessel's pouch never actually uniting with those of Rathke's pouch. Saint-Remy agrees with Kupffer that the entodermic origin is rudimentary in birds and mammals, and does not enter into the formation of the adult pituitary body. It is, however, of morphological importance, and betokens the existence in lower forms of vertebrates of a communication between the intestine and the buccal invagination.

Dohrn (7) looked upon the pituitary as the remains of a preoral gill-cleft. Salvi (32) has brought forward evidence in support of this view, and states that in reptiles part of the pituitary is developed from the walls of the premandibular cavities, which he believes to be the representatives of gill-clefts. Valenti (34) describes the origin of the anterior lobe in amphibians from an invagination of the fore-gut arising some distance behind Seessel's pouch. The invagination, he considers, has not the significance attributed by Kupffer to Seessel's pouch, but is rather to be regarded as the representative of a gill-cleft. Valenti therefore supports Dohrn's theory. Dohrn's view was based chiefly upon the assumption of a bilateral origin of the anterior lobe of the pituitary. Dohrn himself described a bilateral origin in Hippocampus, and Gaupp (10) found something similar in reptiles. Gaupp, however, described a median origin in addition to lateral ones, and believes all to be formed from the buccal cavity.

Yet another interpretation of the significance of the pituitary has been put forward by Beard (4), who believes the anterior lobe to be homologous with the permanent mouth of Annelids.

## AUTHOR'S OBSERVATIONS.

My own results are confirmatory of those of Mihalkovics and Kupffer. In a 4-mm. cat embryo, the youngest I have had the opportunity of ex-

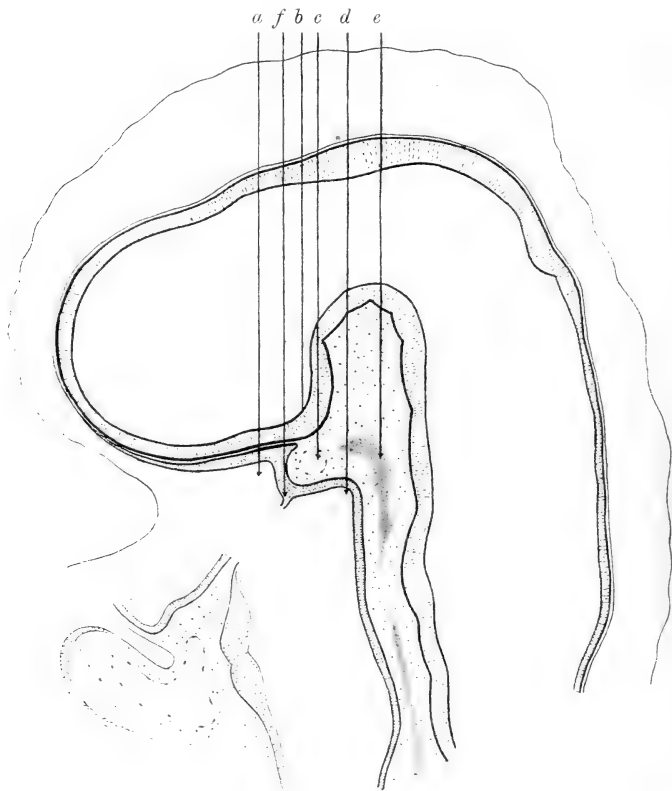


FIG. 1.—Mesial sagittal section through head of 4-mm. kitten. (Diagram.)

*a*, hypophysial angle formed by buccal mucous membrane; *b*, depression in wall of cerebral vesicle where the infundibulum is formed; *c*, blood-channel; *d*, anterior end of fore-gut or Seessel's pouch; *e*, head of notochord; *f*, upper stump of ruptured oral plate.

amining, the appearance is that indicated in fig. 1. The oral plate (*f*) between buccal invagination and fore-gut has just ruptured. Immediately in front of the oral plate is the hypophysial angle described by Mihalkovics. The anterior limb of the angle is composed of buccal



epithelium, which in this situation is closely adherent to the wall of the anterior cerebral vesicle. The posterior limb of the angle, also composed of buccal epithelium, leaves the wall of the brain and bends downward to form the anterior layer of the upper stump of the oral plate. At this stage there is no invagination of the wall of the cerebral vesicle to form the infundibulum, but its site is indicated by a definite depression. The anterior end of the notochord does not touch the posterior limb of the

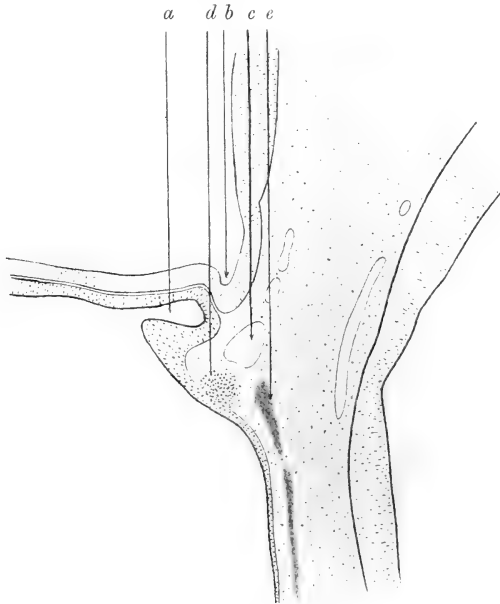


FIG. 2.—Mesial sagittal section through part of head of a 6-mm. kitten.  
*a*, buccal invagination or Rathke's pouch; *b*, beginning of invagination of wall of cerebral vesicle to form the infundibular process; *c*, blood-channel; *d*, clump of cells derived from anterior end of fore-gut; *e*, head of notochord.

hypophysial angle, but is separated from it by a large blood-channel (*c*). Behind the oral plate is a small dorsal invagination of the wall of the fore-gut, which is the only indication of anything resembling Seessel's pouch. Its wall is not thickened, and there is no evidence of any entodermic origin for the pituitary in this specimen.

In a 6-mm. cat embryo (fig. 2), the remains of the oral plate have disappeared. The hypophysial angle has become a definite sac (*a*), Rathke's pouch. This change appears to have been brought about by a bending forwards of the upper stump of the oral plate and a proliferation of the

cells in its wall. The pouch is widening out behind the neck, and the latter is found to be constricted when the sections next in series to it are examined. The anterior wall of Rathke's pouch is closely applied to the wall of the cerebral vesicle, and at the dorsal extremity of the pouch an invagination of the wall of the cerebral vesicle is forming the primitive infundibulum.

The head of the notochord bears no immediate relation to Rathke's pouch, and is separated from it by a clump of cells which is continuous with the epithelium of the fore-gut, and appears to be formed by a proliferation of the cells of the latter. A large blood-vessel (*c*) is also seen in this specimen, lying between Rathke's pouch and the head of the notochord.

At this stage it is difficult to determine where ectoderm ends and entoderm begins: the upper stump of the oral plate has disappeared as such, and its representative is uncertain. There is no indication of a pouch in the fore-gut, but the clump of cells appears to be derived from the wall of the latter. Minot (22) makes the fold of epithelium at the posterior margin of Rathke's pouch homologous with the upper lip of Petromyzon. If this is the case, the fore-gut must begin behind this fold. The close relation between the head of the notochord and the cell clump makes it likely that the latter is derived from the fore-gut, for, in the 4-mm. embryo, the head of the notochord is some distance behind the oral plate, and the epithelium opposite it is that of the fore-gut. The clump of cells is the only structure which resembles the proliferation of entoderm described by Kupffer. It is not found in any of the older embryos that I have examined, but the amount of suitable material at disposal for this purpose has been limited. Rathke's pouch is the only part that enters into the formation of the anterior lobe of the pituitary; it is single and median in origin, and there is no indication in the embryos of the cat and the pig of any other "Anlage" for the anterior lobe. I have not found any communication between the epithelium of Rathke's pouch and that derived from the fore-gut, as described by Nusbaum in the dog, but cannot say that this does not occur. In the specimens I have examined there is nothing to indicate in the slightest degree that Rathke's pouch is reinforced by epithelium from the fore-gut. The epithelial proliferation of the latter disappears as stated by Kupffer, and takes no part in the formation of the pituitary.

One of the most important characteristics of the developing pituitary is the close union maintained between buccal and cerebral portions from the earliest stage. Minot (22) emphasised its importance in mechanically keeping the two parts together, and thus explaining their intimate relations. Salzer (33) also noted it, and states that he could find no connective tissue between the infundibular process and hypophysial sac. With these observations I thoroughly agree. The buccal epithelium in the anterior part of the hypophysial angle is intimately connected with the epithelium of the cerebral vesicle, without the interposition of connective tissue. In the further growth of the embryo this close union is preserved, but in other parts connective tissue develops and separates the buccal epithelium from

the wall of the cerebral vesicle. No doubt this process is contributory to the formation of Rathke's pouch and infundibulum, but it is probably of morphological significance as well, and betokens the existence in an ancestral vertebrate of a communication between buccal cavity and neural canal.

A later stage of development is shown in fig. 3, which is taken from a pig embryo of 12 mm. The buccal mucous membrane is now widely separated

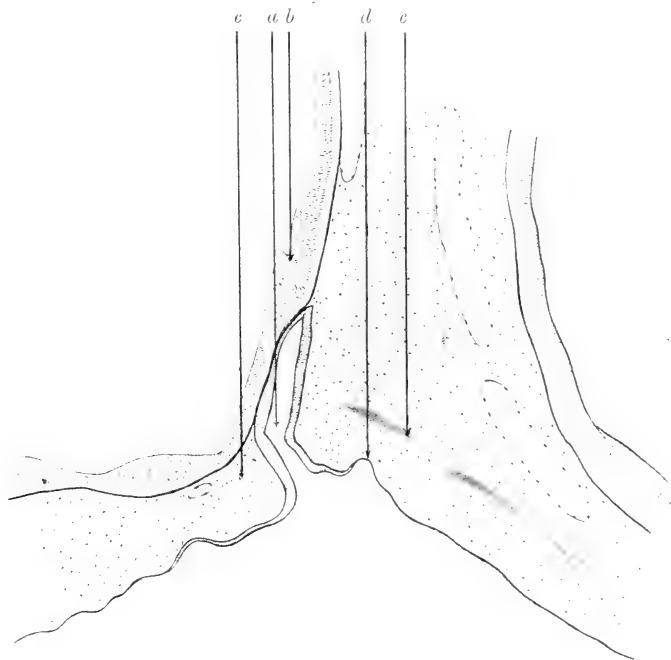


FIG. 3.—Mesial sagittal section through part of head of a 12-mm. pig embryo.  
*a*, Rathke's pouch; *b*, beginning of infundibular process; *c*, blood-channel; *d*, remnant of Seessel's pouch (?); *e*, notochord.

from the wall of the cerebral vesicle, except at that part where the anterior wall of Rathke's pouch closely adheres to it. The infundibulum is only beginning to form, and the cells lining the cerebral vesicle at this point have proliferated and elongated, and look more like ependyma cells. There is no indication of any proliferation of cells of the fore-gut. Rathke's pouch is median in situation, its neck is constricted, and serial sections show that there is no lateral origin of the pituitary. The notochord persists, but has no immediate relation to Rathke's pouch. It takes

no part mechanically or otherwise in the formation of the pituitary. Its situation, nevertheless, is not without significance; its arrest behind the anterior end of the brain tube allows the latter to communicate with the buccal epithelium and possibly with the fore-gut in some animals. Had the notochord grown further forward, a median origin for the anterior lobe of the

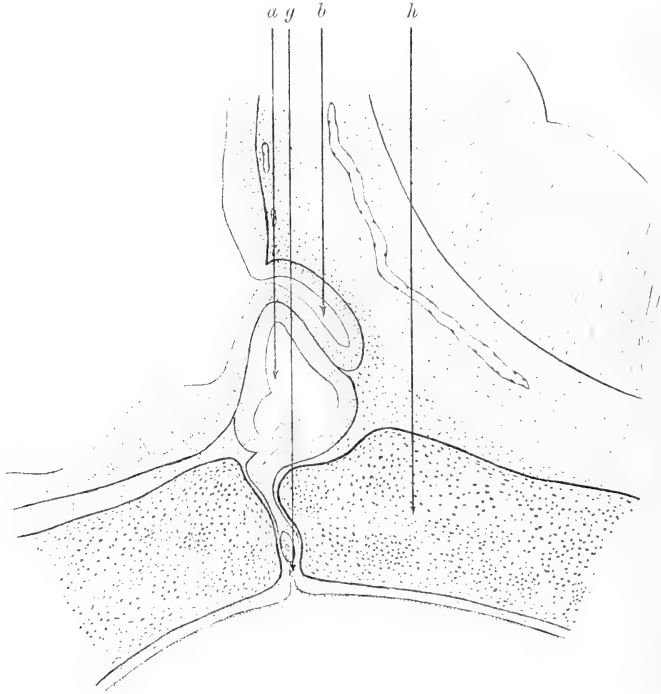


FIG. 4.—Mesial sagittal section through part of head of an 18-mm. kitten.  
*a*, hypophysial sac now closed below; *b*, infundibular process; *g*, neck of sac connected with nasal mucous membrane; *h*, cartilage of sphenoid bone.

pituitary would have been impossible. The median origin of the pituitary, or rather the ancestral condition which this implies, may indeed explain why the head of the notochord has been arrested in this situation.

In an 18-mm. cat embryo considerable changes have taken place (fig. 4). Rathke's pouch has become a closed sac, but its wall is still connected by a stalk of epithelium with what is now becoming nasal mucous membrane. The narrowing of the neck of the sac, its closure and ultimate disappear-

ance are due to the strong growth of connective tissue around it and the development of the sphenoid bone. It is unnecessary to attribute the change to the pressure exerted on the neck of the sac by the carotid arteries and their growing adventitia, as did W. Müller. The above explanation of Mihalkovics is probably the right one. The hypophysial sac is now of considerable size and extends laterally, its anterior wall being still in close connection with the wall of the cerebral vesicle. A well-marked invagination of the wall of the latter constitutes the infundibular process; its anterior wall is also closely invested by the epithelium of the wall of the hypophysial sac. The infundibular process becomes the nervous portion of the posterior lobe of the pituitary, while the part of the wall of the hypophysial sac adhering to it constitutes the epithelial covering of the posterior lobe or "Epithelsaum" of Lothringer. Epithelium and nervous tissue have been in close contact with one another from their first appearance. The cavity of Rathke's pouch persists throughout life as the epithelial cleft which partly separates the anterior from the posterior lobe. In its lateral extension the sac is beginning to envelop the sides of the infundibular process. Its walls are composed of cylindrical cells which closely resemble those of the infundibular process. They are thickened in the anterior part of the sac in the region of its neck, where there is a distinct fold; the thickening in this situation is the beginning of the anterior lobe proper of the pituitary. Its cells are not as yet differentiated from the cells of the remainder of the sac.

During subsequent development the pituitary body is removed further and further from the nasal mucosa by the growth of the sphenoid bone. Ossification in the latter is delayed for some time by the persistence of a cord of epithelial cells connecting the anterior lobe of the pituitary with the nasal mucous membrane. This connection is still found in cat embryos of from 35 to 40 mm., but is then imperfect and soon after disappears, allowing the opening in the bone to close up. Differentiation between anterior lobe proper and the pars intermedia now begins to take place. The anterior lobe is formed by a proliferation of the cells of the lower part of the anterior wall of the sac just above its neck. Solid columns of cells are formed in this situation, and invade the cavity of the sac so as gradually to fill it, leaving only a narrow space or cleft between them and the epithelium covering the posterior lobe. The anterior lobe also grows forward and laterally. The neck of the sac retains a tubular character for some time, and becomes somewhat convoluted. One of these convolutions (fig. 5, *k*) applies itself to the under surface of the brain and gives rise to the tongue-shaped process which extends forwards from the anterior lobe towards the optic chiasma.

The structures which enter into the formation of the pituitary are closely related to large blood-vessels from their earliest appearance. In figs. 1 and 2 a large blood-channel is seen lying immediately behind Rathke's pouch, in front of the notochord. Dursy, indeed, as already

stated, sought to derive the origin of the blood-vessels of the pituitary from the tissue of the head of the notochord. The exact manner in which the blood-vessels of the anterior lobe of the cat's pituitary are formed is somewhat difficult to make out, but in the pig embryo their origin is partly sinusoidal. The cell columns of the anterior lobe grow into large blood-

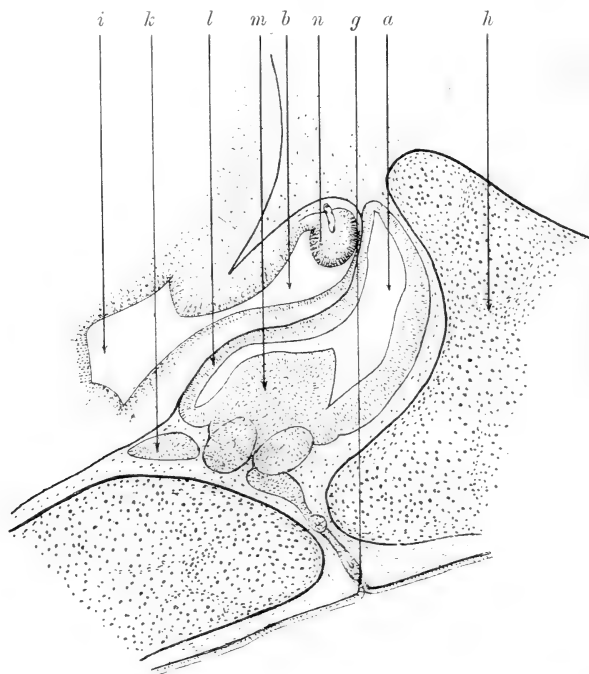


FIG. 5.—Mesial sagittal section through part of head of 35-mm. kitten.

*a*, remains of cavity of Rathke's pouch now recognisable as the epithelial cleft of the pituitary; *b*, central cavity of infundibular process; *g*, remnant of epithelial duct connecting hypophysial sac with the nasal mucous membrane; *i*, third ventricle of brain; *k*, part of epithelial duct which becomes the tongue-like process of pars intermedia; *l*, cells of anterior wall of hypophysial sac (pars intermedia); *m*, anterior lobe proper; *n*, vascular knob covered with ependyma cells projecting into cavity of the infundibular process.

sinuses, pushing the endothelial lining before them. In a pig embryo of 60 mm. the appearance of the anterior lobe is very like that of the developing liver. It is not, however, entirely sinusoidal, and some ingrowth of blood-vessels with accompanying connective tissue takes place, the latter being always small in quantity. This method of development of the blood-vessels of the anterior lobe was first pointed out by Gaupp (10) in reptiles.

Gaupp found large blood-spaces of a venous nature, and states that the epithelium grows into them, passing through their walls. Minot also inferred from the structure of the anterior lobe of the pituitary that the development of its blood-vessels is partly sinusoidal. While the anterior lobe and tongue-like process of epithelium are extremely vascular, that part of the wall of the original sac which is applied to the brain remains devoid of blood-vessels. Its cells proliferate and spread round the nervous substance of the posterior lobe, forming a covering of epithelium of varying thickness.

The nervous portion of the posterior lobe has meanwhile grown in length and expanded to form a definite body, which, in the cat, retains a large central cavity. The neck is constricted, but remains hollow. In a 35-mm. cat embryo (fig. 5) the epithelium lining the central cavity is composed of ependyma cells with thin processes, the nervous tissue is small in amount, contains few cells, and is chiefly made up of the processes of the lining cells. At the postero-superior angle of the lobe there is frequently seen a knob-shaped body (*n*) of large and deeply staining ependyma cells, behind which are blood-vessels. This vascular knob appears to be growing into the central cavity, and marks the entry of blood-vessels into the posterior lobe. The thickening of epithelium does not persist, but disappears; the blood-vessels, however, grow into the lobe in this situation. The appearance is not a constant one, but when it occurs the deeper staining of the ependyma cells and the vascularity of the tissue behind them are striking features. The blood-vessels of the posterior lobe of the pituitary are, in the cat, almost entirely derived from an ingrowth in this situation; true capillaries are formed in the lobe and are accompanied by a small amount of connective tissue. The latter is never present in large quantities, and the posterior lobe of the pituitary does not become a connective tissue appendage of the brain, as stated by W. Müller and many others; there is remarkably little connective tissue in the posterior lobe. The appearance which W. Müller likened to a spindle-celled sarcoma is very marked in the older pituitary. It is, however, not due to the presence of connective tissue fibres and cells, for when the pituitary is prepared by Cajal's silver method it is found that the appearances described by W. Müller are caused by the presence of large numbers of ependyma- and neuroglia-cells and fibres, chiefly the former. In the developing pituitary there is never any sign of true nerve cells. The ependyma cells lining its central cavity are at first like those lining the third ventricle of the brain. Their fibres run vertically and end at the outer surface of the lobe. As the posterior lobe elongates the peripheral ends of the fibres remain attached, the cells become more numerous, and are moved further and further from the points of attachment of their fibres. In this way the ependyma fibres of the neck of the posterior lobe acquire an oblique direction, and finally run almost longitudinally, their cells of origin being situated much further back than the outer ends of their fibres. In the posterior lobe of the kitten, especially in the region of the neck, the ependyma fibres become very numerous, and

their arrangement, as shown by Cajal's method, is very complex. In the adult cat the individual fibres are much thicker, and the cells fewer in proportion to the size of the lobe. Neither by Cajal's nor Golgi's method have I been able to find true nerve fibres entering the posterior lobe of the cat's pituitary through the neck. The ependyma fibres take on a lighter stain by Cajal's method than the nerve fibres in the brain. Fibres can be seen in the fully developed pituitary which are thinner and stain more

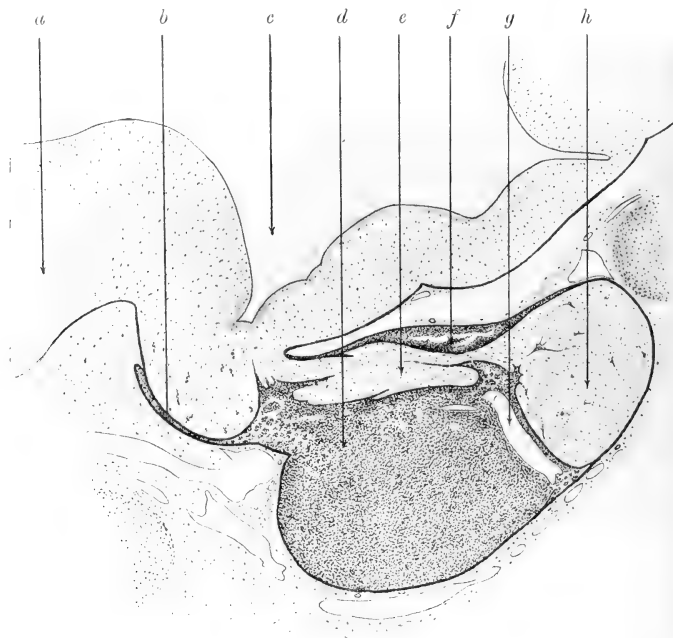


FIG. 6.—Mesial sagittal section through developing pituitary body of a human fetus (fifth month). Drawing from a photograph.

*a*, optic chiasma; *b*, tongue-like process of epithelium; *c*, third ventricle; *d*, anterior lobe; *e*, neck of posterior lobe; *f*, epithelium surrounding neck; *g*, epithelial cleft; *h*, posterior lobe.

deeply. Some of them enter the epithelium round the neck of the posterior lobe and ramify there. They are probably true nerve fibres which have entered with the blood-vessels, and, as Berkley (5) states, derived from the sympathetic.

In the embryos of man, ox, and pig the posterior lobe is solid from an early stage, and is relatively smaller than the anterior lobe. There is the same close connection between the epithelium and the nervous tissue. In fig. 6 a mesial sagittal section through the pituitary body of a human



embryo (5th month) is illustrated. The anterior lobe is a compact structure of columns of epithelial cells devoid of lumina. Many of its cells are granular in character, and are beginning to differ from the clearer cells, which are in closer relation with the nervous tissue. There is a well-marked cleft in the epithelium, which, in this specimen, is carried right round the neck of the posterior lobe. The cells of the pars intermedia, or that portion of epithelium derived from the anterior wall of Rathke's pouch, which is closely adherent to the wall of the cerebral vesicle, are

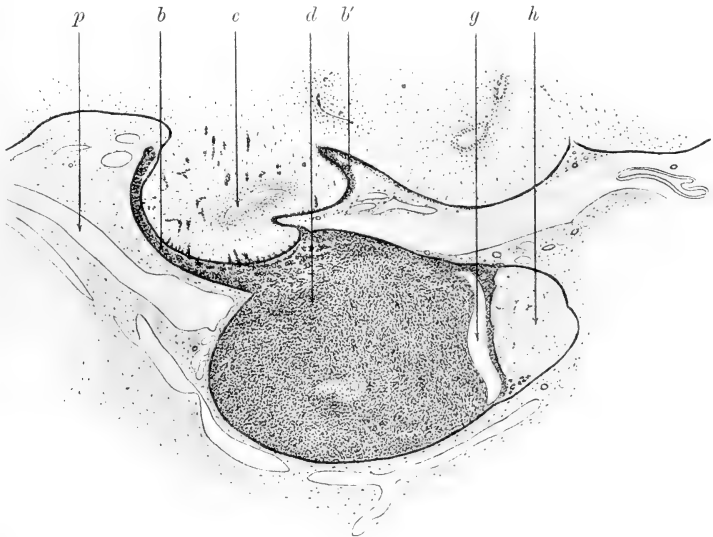


FIG. 7.—Sagittal section through same pituitary as shown in fig. 6, but further to one side.  
Drawing from a photograph.

*b*, tongue-like process of epithelium spreading forward; *b'*, epithelial cells spreading backwards over surface of the brain; *c*, third ventricle; *d*, anterior lobe; *g*, epithelial cleft; *h*, posterior lobe; *p*, lymph space.

widely spread over the surface of the neck and body of the posterior lobe; they also tend to break up the neck of the lobe by passing into its substance along with blood-vessels, and extend forwards in a thin layer for some distance over the tuber cinereum. Fig. 7 shows the appearances presented by the same pituitary in a section further from the middle line. The anterior lobe is rather larger here than it is in the mesial plane. The posterior lobe is small, and its neck, which is a very thin one, is not seen. The cleft is still seen, but the epithelium covering the posterior lobe is small in amount and is confined to that part of it which borders the cleft. In the human embryo at this stage the intermediate part of the pituitary

covering the neck and part of the posterior lobe is relatively smaller than it is in the cat embryo. But the intermediate part is not really reduced; it has changed its position, and in the human embryo is found to extend further over the surface of the brain. A thin layer (fig. 7, *b*) is prolonged forwards and backwards (*b'*) over the brain substance adjacent to the neck. The cells are arranged in columns, which may be a single cell thick, no lumen being found in them. Blood-vessels accompany this layer, and pass freely inwards into the brain substance, often carrying with them cells of the *pars intermedia* for a short distance along their course. The intimate relation of the cells of the *pars intermedia* to *pars nervosa*, and their differences in structure from the cells of the anterior lobe proper, appear to indicate that they are physiologically as well as anatomically connected with the brain. In the cat they are aggregated around the neck and body of the posterior lobe, which are hollow and in communication with the third ventricle. In animals which have a solid posterior lobe they are disposed more in relation to the brain substance adjacent to the neck, and in the monkey may spread inwards almost to the floor of the third ventricle.

In experiments which have been made on the physiological action of extracts of the posterior lobe, the material has usually been taken from the pituitary of the ox, on account of its size. An illustration (fig. 8) is given of the developing pituitary of the ox. In this animal the posterior lobe is a thin, solid, elongated structure. The epithelium of the intermediate part spreads widely over its anterior surface, as seen in the figure, but embraces it laterally as well, and passes for considerable distances in the form of columns of cells into the substance of the body of the lobe. Its epithelium is therefore closely bound up with the nervous substance of the lobe during development, and forms an important element in its composition. The question of the derivation of the active physiological principle of extracts of the posterior lobe has been discussed in a previous paper, and reasons have been given for regarding it as in great part derived from the epithelium of the *pars intermedia*. It is of interest, therefore, to find that in the development of the pituitary of the ox epithelial cells pass freely into the substance of the posterior lobe.

The disposition of the cells of the *pars intermedia* is such as to bring them into close relation with the neural canal in the region of the third ventricle. This of course follows from the history of the mode of development of the pituitary, but the spreading of epithelium over the surface and into the brain itself seems to indicate some further connection between the two. In fishes, e.g. the cod, the epithelium of the anterior lobe appears to have the same intimate connection with the nervous tissue of the posterior lobe which obtains in mammals and birds. The posterior lobe is hollow and has connected with it a large *saccus vasculosus* lined with folds of columnar epithelium. The *saccus vasculosus* is said by all who have worked at its development to be derived from the

brain, and its secretion, if it is a secretory structure, passes into the third ventricle through the infundibulum. For this reason Rabl-Rückhard (25) called it an infundibular gland. No such structure is found in mammals, but Retzius (30) has described in them a slight swelling in the form of a clover leaf appearing on the surface of the brain behind the infundibulum, which he called the eminentia saccularis, and believed to be the homologue of the saccus vasculosus of fishes. Retzius described its external appearance only. In section, it is found to be a

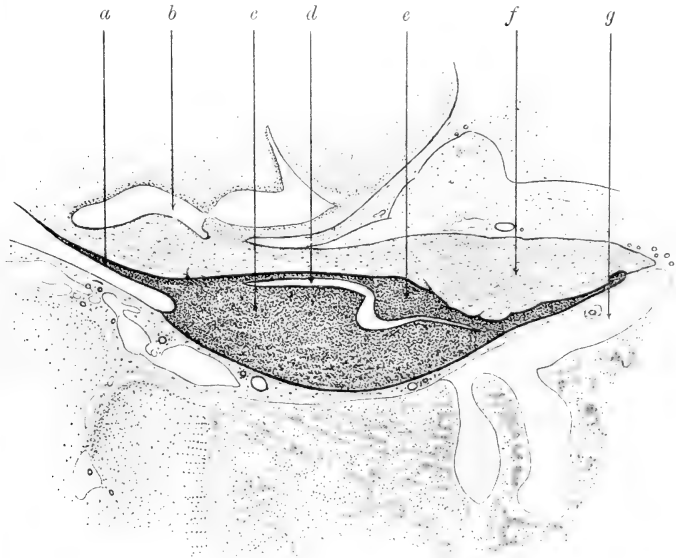


FIG. 8.—Mesial sagittal section through developing pituitary body of ox. Drawing from a photograph.

*a*, tongue-like process of epithelium spreading forwards; *b*, third ventricle; *c*, anterior lobe; *d*, epithelial cleft; *e*, epithelium of pars intermedia; *f*, posterior lobe; *g*, large lymph space extending into body of ossifying sphenoid bone.

thinning of brain substance in the floor of the third ventricle in front of the corpora mamillaria. It is doubtful if this eminence is really homologous with the saccus vasculosus of fishes; it is not in the position one would expect, and should be sought rather in the postero-superior angle of the posterior lobe of the pituitary, where the blood-vessels enter, and where there is frequently in the cat, during development, the vascular knob already mentioned.

Although the mammalian brain has no saccus vasculosus, the posterior lobe of the pituitary possesses an investment of epithelium which differs

from that of the anterior lobe, and it is because of the occurrence of colloid vesicles in this situation that the mammalian posterior lobe has been termed by Kölliker (17) an infundibular gland. Whether the epithelium of the pars intermedia of the mammalian pituitary has a similar function to that of the saccus vasculosus of fishes is a difficult question to answer. Extracts of the saccus vasculosus of the cod do not appear to have the same physiological action as extracts of the posterior lobe of the mammalian pituitary when injected into the blood; but my investigations into the comparative physiology of the vertebrate pituitary are not sufficiently advanced to make any conclusive statement on this point. The presence of epithelial cells of the pars intermedia in the interior of the cavity of the posterior lobe of the cat's pituitary renders it probable that they furnish some material which passes in the direction of the brain. There must be some significance in the fact that in all craniate vertebrates the cerebrospinal canal has in close proximity to its anterior end, and intimately bound up with it, a glandular organ connected with the mouth. In the Ascidian larva a subneural gland or hypophysis cerebri occupies the same position. Andriezen (1) described in the *Ammocoete* and larval *Amphioxus* a subneural gland, a duct lined with ciliated epithelium affording a communication between the buccal and the neural cavities, and a group of nerve cells around and at the back of the upper opening where the duct widens into the ventricular cavity; an arrangement, in fact, which is very similar to that found in the larval Ascidian. Andriezen believed that the buccal ventricular duct serves as an inlet for oxygenated water to the spinal cord, the nerve cells acting as a sensory mechanism to test the quality of the water admitted. In higher animals the water vascular duct disappears and the posterior lobe gradually loses its nerve substance. The anterior lobe or subneural gland alone remains functional, but its secretion is carried to the brain by lymphatics and blood-vessels. Sensory structures have been described in the infundibular region by Boeke (6) and by Gemelli (11) in fishes. There are, as already stated, no appearances indicative of such in the posterior lobe of mammals. Andriezen's view is to some extent similar to the one expressed by Kupffer, but his anatomical data do not agree with the description of the larval *Amphioxus* as worked out by Willey and other authorities.

If the anterior lobe of the pituitary body is to be regarded as the remnant of an old mouth into the neural canal, it is possible that such a connection will occasionally show itself in the course of development. In one cat embryo I have met with a communication between the epithelial cleft and the central cavity of the posterior lobe. The opening between the two is at the postero-superior extremity of the posterior lobe, and it has been rendered possible by the spreading of Rathke's sac further backwards than usual. The cleft or remnant of the original lumen of the buccal invagination is in open continuity through the infundibulum with the cavity of the third ventricle. The opening is a median one, and

consists of a narrow canal lined by ependyma cells passing backwards from the wider lumen of the cavity of the body of the posterior lobe to meet with the fold of buccal mucous membrane and pass through it into its cleft. The ependyma cells cease where the canal meets epithelium, and the

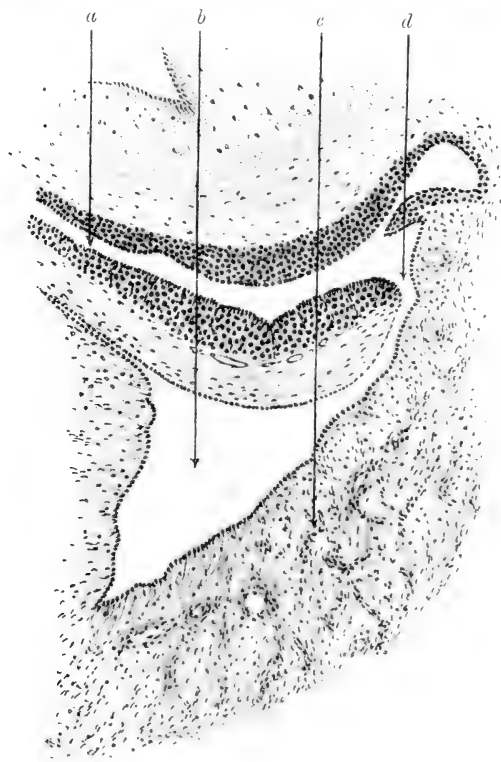


FIG. 9.—Mesial sagittal section through postero-superior angle of the posterior lobe of the pituitary of a kitten near full time. Drawing from a photograph.

*a*, part of epithelial cleft; *b*, central cavity of posterior lobe; *c*, nervous substance of posterior lobe; *d*, canal from cavity of posterior lobe opening into cleft.

lining of its actual opening into the cleft is formed by the epithelium of the buccal invagination. The opening takes place at a comparatively late stage in development, after the buccal invagination has become separated from its origin by the growth of the sphenoid bone. I have examined many

cat embryos, but have not met with another specimen showing actual continuity of both cavities, although it is not unusual to find the appearances shown in figs. 10 and 11, where a somewhat similar condition exists; a canal runs backwards from the central cavity of the posterior lobe to meet the epithelium formed from the buccal invagination. In some kittens

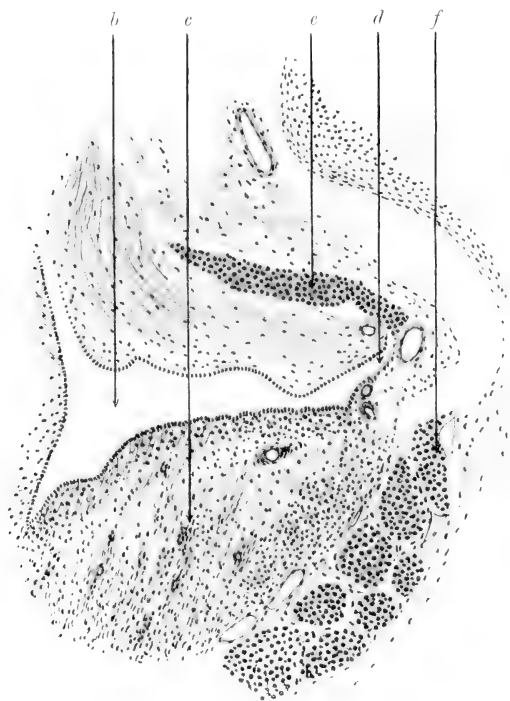


FIG. 10.—Mesial sagittal section through postero-superior angle of the posterior lobe of the pituitary of another kitten near full time. Drawing from a photograph.

*b*, central cavity of posterior lobe; *c*, substance of posterior lobe; *d*, canal from cavity of posterior lobe running backwards into *e*, epithelial cells of the pars intermedia; *f*, cells of pars intermedia investing posterior lobe below.

there is an indication of a tubular character of the epithelium of the pars intermedia in this situation, and the central cavity of the posterior lobe appears to run into it. In older animals epithelial cells often invade the tissue of the posterior lobe in the position in which the canal is indicated in the figures, and may come to lie in the central cavity. In one adult

cat the nervous tissue of the posterior lobe is almost entirely destroyed by an overgrowth of epithelial cells of the pars intermedia. These observations and the occurrence of epithelial cells in the brain substance of the floor of the third ventricle in the adult monkey seem to point to some physiological connection between epithelial cells and cerebro-spinal canal.

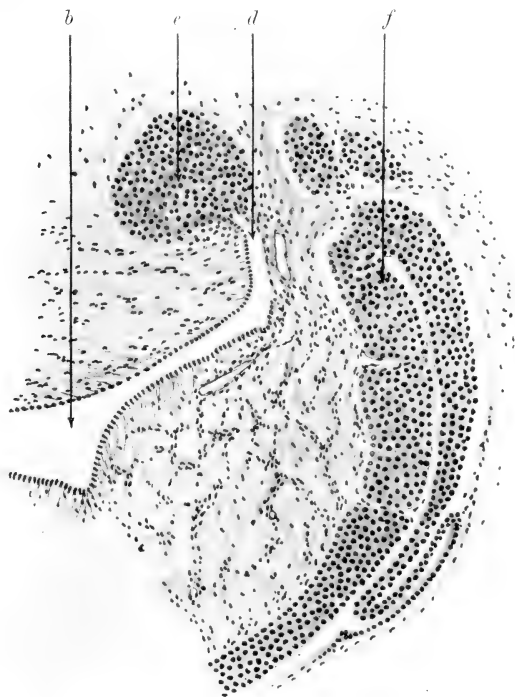


FIG. 11.—Mesial sagittal section through postero-superior angle of the posterior lobe of the pituitary of a third kitten near full time. Drawing from a photograph.

*b*, central cavity of posterior lobe; *d*, canal leading from this cavity into *c*, epithelial cells of pars intermedia; *f*, cells of pars intermedia and cleft lying below the posterior lobe.

Since the intimate relations that exist between the two from the earliest stages of development are not only maintained but emphasised in the adult mammalian pituitary, it is unlikely that they are accidental, and Kupffer's hypothesis as to the significance of the pituitary body appears to have many facts that support it. The nature of the connection between epithelium and cerebro-spinal canal from a physiological point of view awaits an

explanation. It is possible that the epithelial cells secrete some substance which is necessary for the brain. Andriezen's view that the secretion of the cells of the anterior lobe of the pituitary is carried by lymphatics and blood-vessels to the brain is unlikely, owing to the vascular arrangements in the lobe; but it is probable that something of the kind occurs with the secretion of the cells of the pars intermedia. B. Haller regards the anterior lobe as a tubular gland which provides a secretion for the membranes of the brain and spinal cord. It is remarkable that there should be so large a cleft in the pituitaries of the dog and cat, unless it has some function. An external opening of the cleft is frequently seen, but it may quite well be an artificial one, and in carefully prepared specimens I have been unable to find it. The anterior lobe is not a tubular gland, and the only cells that can pour a secretion into the cleft are those of the pars intermedia. On the other hand, the cleft is not always well developed even in the cat's pituitary, and may be almost entirely closed by fusion of the anterior lobe with the cells of the epithelial covering of the lobe. In the pituitary of the monkey there may be little remnant of the cleft, and certainly no opening from it into the subdural space. It is rare to find any histological evidence of a secretion into the cleft, and where colloid has been present it has been enclosed in a thin-walled cyst and not lying free in the cleft. In the rabbit's pituitary it is not uncommon to find the cleft filled with red bone-marrow and fat cells. Lymph spaces in the dura mater below the pituitary body are frequently seen, but there is no evidence that they are specially connected with it: they probably belong to a system of lymphatics present at the base of the brain.

#### CONCLUSIONS AND SUMMARY.

Development of the pituitary body begins very early in embryonic life. In mammals the epithelial portion is derived entirely from the ectodermic wall of the buccal invagination known as Rathke's pouch. Its origin is single and mesial. The epithelium is differentiated at an early stage into two parts, which show differences in arrangement, structure, and vascularity. One of these, which has been termed the pars intermedia, is closely adherent to the wall of the cerebral vesicle from its earliest appearance, and remains attached to it throughout life. It forms a layer of cells of varying thickness over body and neck of the posterior lobe and adjacent parts of the brain, and tends to arrange itself in positions where it can approach as near as possible to the cerebro-spinal canal. The cells of the pars intermedia are further characterised by the absence of deeply staining granules from their protoplasm, by their tendency to form a colloid substance in the adult organ, and by their comparatively poor supply of blood-vessels. Its relation to the nervous part of the pituitary and to the adjacent wall of the brain tends to become even more intimate as development proceeds, by the ingrowth of its cells into these structures.



The other portion of buccal epithelium gives rise to the anterior lobe proper. The lower portion of Rathke's pouch, which is not adherent to the brain, forms a solid mass of cells which grow into surrounding blood-channels and into the cavity of the pouch itself. Its cells become filled with deeply staining granules and form columns without any lumen, separated from one another by blood-channels of a sinusoidal character. The original cavity of Rathke's pouch persists as a narrow cleft separating the anterior lobe proper from the epithelial investment of the posterior lobe. The cleft remains a closed cavity, which varies in extent in different species and in different individuals of the same species. In the cat embryo there is evidence of some proliferation of cells of the anterior end of the fore-gut; these soon disappear, and do not enter into the formation of the adult pituitary.

The infundibulum is an invagination of part of the wall of the thalamencephalon which is adherent to the anterior and upper wall of Rathke's pouch. It therefore possesses an epithelial covering derived from the latter. The infundibular process grows backwards, and, in the cat, retains its central cavity. It is lined by ependyma cells which during development become elongated, so that ependyma fibres run obliquely in its neck. The body of the lobe consists of ependyma and neuroglia cells and fibres; no true nerve cells are present in it, and there is very little connective tissue. The posterior lobe of the pituitary is, from the first, a composite structure of epithelium of the pars intermedia and of neuroglia and ependyma, and the relations between the two tissues become more and more intimate. Its vascular supply is derived from a different source from that of the anterior lobe; blood-vessels grow into it at its posterior-superior angle and form true capillaries in the lobe.

The intimate nature of the connection between the wall of Rathke's pouch and the cerebral vesicle, and the maintenance of a close relationship between the cells of the pars intermedia and the cerebro-spinal canal, render it probable that the pituitary body of mammalia is to be regarded as the representative of an old mouth opening into the canal of the central nervous system. Such an arrangement exists in its simplest form in the Ascidian larva. A connection between Rathke's pouch or original mouth-cavity and the interior of the infundibulum is sometimes seen in the developing cat, and in the adult cat it is not uncommon to find epithelial cells, derived from the buccal cavity, lying inside the posterior lobe in communication with the third ventricle of the brain. The relations between epithelium and nervous tissue are not accidental in the mammalian pituitary. The latter may have arisen, as Willey stated, from a functional neuropore, but is more likely to have been produced in the manner indicated by Kupffer. There is less probability of Dohrn's view being a correct solution of the problem. The question is one of great interest, and is by no means settled. The anterior lobe proper is a gland whose secretion must enter the blood directly, and so pass into the general circulation. The pars

intermedia, on the other hand, appears to secrete into the brain tissue, and must be regarded as a brain gland. The nature of these secretions, and the question as to whether that of the pars intermedia is modified by its passage through brain substance, await further investigation.

I have to express my indebtedness to Mr Richard Muir for the care with which he has executed the accompanying illustrations. The expenses of the research have been defrayed by a grant from the Earl of Moray fund for the prosecution of research in the University of Edinburgh.

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THE PHYSIOLOGICAL ACTION OF EXTRACTS OF THE PITUITARY BODY AND SACCUS VASCULOSUS OF CERTAIN FISHES. Preliminary Note. By P. T. HERRING. (From the Physiology Department, University of Edinburgh.)

(Received for publication 10th March 1908.)

IN certain fishes, elasmobranchs and teleosts, the infundibular region of the brain is distinguished among other things by the presence of an extremely vascular gland—the saccus vasculosus. In elasmobranchs, e.g. the skate (*Raja batis*), the saccus vasculosus is large and paired, and its lobes open by a common median passage into the infundibulum, and so into the third ventricle of the brain. In teleosts, e.g. the cod (*Gadus morrhua*), the saccus vasculosus is single and situated in the middle line between the *lobi inferiores*; it opens into the infundibulum immediately behind the posterior lobe of the pituitary. In both the skate and the cod the saccus vasculosus consists of a complicated sac lined by a single layer of columnar epithelium which is separated from numerous large and thin-walled blood-vessels by a thin basement membrane. The wall is thrown into frequent folds, especially in the cod, thereby reducing the size of the cavity, but increasing the surface area of its interior.

Extracts of the saccus vasculosus made by boiling it in Ringer's fluid have no marked physiological action when injected into the blood-vessels of a cat. Whether taken from the skate or the cod they do not produce a rise of blood pressure, but rather a slight fall; kidney volume is a little increased, but there is no effect upon the secretion of urine. The results are practically those of an injection of Ringer's fluid. The saccus vasculosus of fishes does not yield the active principles which are characteristic of the posterior lobe of the mammalian pituitary. Gentes,<sup>1</sup> on anatomical grounds, believes that the saccus vasculosus is a ventral choroid plexus.

The pituitary body of the skate, and, according to Gentes, of elasmobranchs generally, has no posterior lobe. Neither does it possess the granular cells of the anterior lobe of higher vertebrates. It is, nevertheless, a large body, and presents the features of an internally secreting gland. Extracts produce in the cat a slight fall of blood pressure, a dilatation of

<sup>1</sup> Gentes, "Recherches sur l'hypophyse et le sac vasculaire des vertébrés," Soc. scientif. d'Arcachon, Station biologique, Travaux des laboratoires, p. 268, fasc. 1. Bordeaux, 1907.

the kidney, and some increase in urine flow. The blood pressure raising substance is apparently absent, or is in such small amount that its action is entirely masked by the depressor effects of the glandular extract. Extracts of the lobi inferiores and adjacent brain substance produce the well-marked fall of blood pressure and diminution of kidney volume which are characteristic of extracts of the central nervous system.

In teleosts the pituitary body consists of an anterior lobe proper characterised by the presence in it of deeply-staining granular cells, an intermediate part of smaller clear cells, and a nervous portion. The latter is surrounded and invaded by the cells of the pars intermedia. Extracts of this portion of the pituitary body, pars nervosa and pars intermedia, produce in the cat the typical effects of extracts of the posterior lobe of mammals, viz. rise of blood pressure, dilatation of the kidney, and increase of urine.

NOTE ON THE ACTION OF PITUITARY EXTRACTS UPON THE  
 ENUCLEATED FROG'S EYE. By W. CRAMER. (From the  
 Physiology Department, Edinburgh University.)

(Received for publication 5th March 1908.)

EXTRACTS of the posterior lobe of the pituitary body of the ox produce a distinct dilatation of the pupil of the enucleated frog's eye.

By using strong extracts made from the desiccated posterior lobe the action on the pupil becomes apparent within an hour or two. The following experiment, in which a solution made from 0.4 g. desiccated pituitary in 3 c.c. of Ringer's solution was used, may be taken as an example. The size of the pupil, which in complete contraction is slit-like, was measured by determining the length of the short diameter by means of a pair of compasses.

Time.	Left eye + pituitary extract.	Right eye + Ringer's solution.
	Size of pupil.	Size of pupil.
12.30 p.m. (before the experi- ment)	1 mm.	1 mm.
2   "           . . . . .	3 mm.	1 mm.
3   "           . . . . .	4 mm.	1 mm.
4   "           . . . . .	4.5 mm.	0.75 mm.

The action of a solution of adrenalin (hemisin) 1 : 10000 is more rapid but not so lasting, as will be seen from the following simultaneous experiment.

Time.	Left eye + adrenalin 1 : 10000.	Right eye + Ringer's solution.
	Size of pupil.	Size of pupil.
12.30 p.m. (before the experi- ment)	2.5 mm.	3 mm.
2   "           . . . . .	4 mm.	2.5 mm.
3   "           . . . . .	4 mm.	2 mm.
4   "           . . . . .	3.5 mm.	2 mm.

## 190 Action of Pituitary Extracts upon the Enucleated Frog's Eye

If more dilute pituitary extracts are used the effect only becomes visible after twelve or more hours. After that time the pupil of a frog's eye immersed in Ringer's solution is, as a rule, completely contracted. In the following experiment 3 c.c. of a 2 per cent. pituitary extract were used.

Time.	Right eye and pituitary extract.	Left eye and Ringer's solution.
	Size of pupil.	Size of pupil.
Before the experiment . . . . .	2.5 mm.	2.5 mm.
After 16 hours . . . . .	3 mm.	0.75 mm.

A frog's eye placed in Ringer's solution together with the fresh posterior lobe of a cat's pituitary body shows a distinct dilatation after sixteen hours. If the anterior lobe is used instead of the posterior lobe the pupil is completely contracted after sixteen hours.

The experiments of Schäfer and Herring<sup>1</sup> have shown that the effects of pituitary extracts on the kidney and on the circulation are independent of each other, and probably due to different substances. The question arises which substance is concerned in the action of the pituitary extracts on the pupil. Our observations tend to show that the substance acting on the pupil is not identical with the substance which stimulates renal activity. For some of the desiccated preparations after having been kept for several months proved inert when tested against a frog's eye; their intravenous injection, however, still produced a marked effect on the flow of urine, while at the same time their action on the blood-vessels was very weak. But any preparation which produced a dilatation of the pupil gave, on intravenous injection, a typical effect both on the kidney and on the circulation.

<sup>1</sup> Schäfer and Herring, *Phil. Trans., Series B*, vol. excix., 1906, p. 1-29.



THE ACTION OF YOHIMBINE ON MEDULLATED NERVE, WITH  
SPECIAL REFERENCE TO FATIGABILITY. By JOHN TAIT  
and JAS. A. GUNN. (From the Physiology Department, University  
of Edinburgh.)

(Received for publication 21st February 1908.)

EXPERIMENTS carried out in recent years by Gotch and Burch (1), Boycott (2), Boruttan (3), and F. W. Fröhlich (4), have shown that when medullated nerve is thrown into activity by an external stimulus a certain short period of time must elapse before it can function again. This period of inexcitability, or refractory period, is normally very short—not more than .002 second for the sciatic nerve of the frog—but can be much prolonged by subjecting the nerve to special conditions. Thus low temperature (1) (2), anaesthesia (3) (4), and asphyxia (4), all greatly prolong the refractory period.

The readiest method of demonstrating the existence of this refractory period is to excite the nerve of a nerve-muscle preparation by two successive maximal stimuli separated by a very short interval of time. By the response of the attached muscle it is then possible to tell whether the nerve has conducted two excitations or only one. If the muscle response is a summated one it is evident that both excitations have been transmitted; if summation does not occur, then the second stimulus must have been in some way ineffective. Absence of summation occurs only when the interval of time between the two maximal stimuli is sufficiently short. In such cases it has been shown by means of the capillary electrometer (1) that the block to the second excitation is seated in the nerve.

The length of the refractory period would seem to be dependent on the intensity of the preceding excitation. Generally speaking, it has been found that the stronger the stimulus applied to the nerve the longer does the nerve take to recover its functional capacity. By combining powerful stimulation with anaesthesia or asphyxia or cooling of the nerve, one would therefore expect to get a maximal refractory period, and indeed Fröhlich was able to prolong it to .1 second (4).

Besides the method of applying two successive stimuli it is obvious that a series of rapid recurring stimuli might be applied to the nerve; and if a sufficiently high rate of excitation could be attained, one would expect that at least some of the excitations would be ineffective.

Experiments with a rapid rate of stimulation have been carried out on numerous occasions. Neglecting in the meantime those in which the enormously high rates afforded by the discharge of Leyden jars, etc., have been used, where the interval between the individual stimuli is of a different order of magnitude from the refractory period, we shall mention experiments where the rate of stimulation has been over 400 a second and yet not greater than tens of thousands.

Bernstein (5), using a rate of 500 a second, obtained only a single initial contraction of the muscle. Roth (6), with a rate of 1000-5000, obtained tetanus. Langdon and Schenck (7), with a rate of 1800-2000 per second, also got tetanus. Kronecker (8), using a special device whereby he claimed to attain a rate of 20,000 per second, found tetanus, which on repetition became an initial twitch and subsequently failed. From the want of uniformity in these results it was for a time difficult to draw any general conclusion.

Of late, however, thanks to the work of Wedensky (9) and of Fröhlich (4), it has become possible to reach a definite generalisation. Wedensky, who happened to combine the method of rapid stimulation with anæsthesia of the nerve, found that strong excitations at a rate of about 100 per second applied to the proximal end of a nerve whose middle portion is deeply anæsthetised, produces simply a single twitch of the muscle. Fröhlich pointed out that this twitch is of the same height as the twitch evoked by one single maximal excitation, and that the result occurs only when the successive stimuli are separated by an interval less than the corresponding refractory period of the anæsthetised nerve. When, on the other hand, the stimuli succeed each other at an interval greater than the refractory period, then tetanus of the muscle occurs.

The fact that one initial maximal twitch follows upon repeated stimulation of the nerve shows that only the first excitation of the series has taken effect; this excitation prevents the second from being effective, the second prevents the third, and so on; consequently when once the excitations are applied at a sufficiently rapid rate the nerve refuses to conduct any more than the first excitatory process.

Such an effect, though at first sight suggestive of fatigue of nerve, is not necessarily fatigue. It is conceivable that a conducting mechanism built on simple physical principles might give the same result. Nevertheless, by a closely analogous method Fröhlich succeeded in showing that the nerve does actually become fatigued when subjected to rapid stimulation. When during anæsthesia of the nerve he selected a rate of stimulation which just about coincided with the definite refractory period corresponding to the intensity of stimulation used and to the given degree of anæsthesia, he obtained, not a single twitch, but a tetanus of peculiar form. This tetanus, instead of gradually climbing, after the normal fashion of a muscle tetanus, attained its maximum almost immediately, and then rapidly fell off in height until in the space of a second or so the muscle ceased to contract altogether.

Interruption of the rhythmical stimulation but for a second sufficed to restore the conductivity of the nerve to its previous condition.

Now, on the assumption that the degree of anæsthesia does not increase during the short period of observation, this peculiar form of tetanus points to a progressive lengthening of the refractory period due to the continuous activity of the nerve, and any change in the direction of depression of function which is solely due to activity is fatigue. That the gradual prolongation of the refractory period is not due to a temporary and coincident deepening of the anæsthesia is sufficiently clear from the consistent regularity with which the effect occurs even when the anæsthesia is passing off.

Fröhlich's work, while establishing the fact that nerve can be fatigued—a fact of fundamental importance in regard to our views as to the nature of the nerve impulse—serves at the same time to emphasise the high powers of restitution possessed by the structure. Even when anæsthetised almost to the point of complete absence of conductivity, the nerve still required to be stimulated uninterruptedly in order to maintain the fatigued condition. Interruption of the rhythmical stimulation for a fraction of a second left time for an apparently complete recovery.

The anæsthetic agents which were found by Wedensky and Fröhlich to prolong the refractory period of nerve include most of the common anæsthetics known to medicine (ether, chloroform, cocaine, phenol, etc.). In spite of the chemical differences between these substances, the kind of change produced in nerve by means of all of them seems to be virtually the same. Further, this change corresponds identically with that caused both by asphyxia (4) and by cooling (9), so that one can recognise a common element in the action of all of these things.

The present paper deals with the changes produced in nerve by means of yohimbine. This substance, which is an alkaloid derived from the bark of the Yohimbehe tree (10), was shown by Magnani in 1902 to be a local anæsthetic (11). We have investigated its action on the sciatic nerve of the frog, availing ourselves of the method of Wedensky—i.e. rapid rhythmical stimulation, in order to show changes in conductivity. The response of the attached gastrocnemius muscle was used as an index of the condition of the nerve.

The investigation has shown:—

(1) That fatigue changes in nerve may be demonstrated more readily by the application of this substance than by any method known to us.

(2) Yohimbine seems to differ somewhat in action on nerve from other anæsthetics.

For our experiments we used a 2 per cent. solution of yohimbine lactate in Ringer's fluid, which we applied to the middle portion of the dissected nerve. In order to keep the solution in contact with this part of the nerve, strips of blotting-paper (usually about 3 centimetres long) moistened with the solution were laid under and over the middle portion.

Thus the proximal and distal ends of the nerve were left unaffected by the solution, and to each of these parts a pair of electrodes was applied. To ensure that the solution should not run along the uncovered parts of the nerve and affect either the proximal or distal ends, the middle portion was kept at a slightly lower level than the two ends. The whole nerve-muscle preparation was kept in a moist chamber. The electrodes were connected by means of a Pohl commutator from which the cross wires were removed with a standard Kronecker coil (original pattern), in the primary circuit of which was an accumulator charged to  $4\frac{1}{2}$  volts.

The result of soaking the nerve for a number of hours (the time varied from two to three hours in our experiments) is to abolish conductivity in

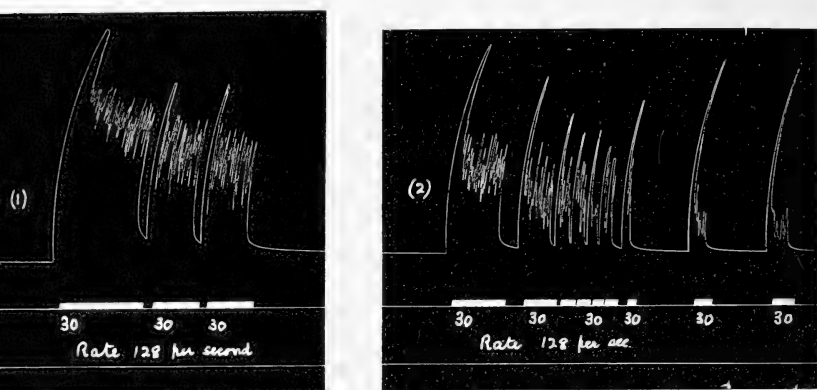


FIG. 1 (reduced to two-thirds).—Yohimbine lactate, 2 per cent., applied for 1 hour to 3 cm. of nerve. Proximal stimulation, rate 128 per sec. Intensity, 30 Kronecker units. Rate of drum, 1 mm. per sec. Tracing (2) taken 40 secs. after tracing (1).

Tracing (1) shows (i.) irregularity of tetanic responses; (ii.) diminution in height of successive tetanic responses. Tracing (2) shows (i.) diminution in extent of successive tetanic responses with diminishing intervals between stimulations; (ii.) subsequent improved responses with increased intervals of rest between stimulations.

the part affected by the yohimbine solution. As is the case when other anæsthetics are applied to nerve, the abolition of conductivity does not occur abruptly but comes on gradually, so that long before the nerve has actually lost the power of conduction, changes can be detected which indicate a depression of function.

Thus when the proximal end of the nerve is stimulated at some fixed rate lying between 100 and 200 excitations per second, the tetanic responses of the muscle begin to undergo a change; instead of being smooth-topped, they become irregular in form, and the muscle, instead of remaining in continuous contraction, ultimately twitches more or less spasmodically (see fig. 1). On the other hand, the muscle response to distal stimulation is a smooth and regular tetanus, showing that the irregularity

of the muscle tetanus in the former case is not due to fatigue of the muscle or of the nerve ends in the muscle. In this respect Yohimbine resembles in its action other anæsthetics.

At a somewhat later stage the abnormality in the muscle response is clearly seen to be of a definite type. To any given series of continuously applied rhythmical excitations the immediate response of the muscle is a summated tetanus which quickly begins to decline in height, and finally becomes feeble and irregular, or ceases altogether. Thus the general form of the tetanus approaches that of the "fatigue tetanus" described by Fröhlich. (See fig. 1, tracing (1), and fig. 2.)

In the case of other anæsthetic agents applied to nerve it was found by Wedensky that the muscle response is largely dependent on the intensity of stimulation used. Wedensky showed (9) that at any given stage of anæsthesia, provided the rate of stimulation is kept constant, there is one definite intensity of stimulation (optimum of intensity) which produces a maximal height of tetanus; intensities either above or below this optimum cause a less height of tetanic response. In other words, when the nerve is anæsthetised, say by ether or cocaine, weak rhythmical stimulation produces a tetanus of submaximal height, stimulation at some moderate intensity causes maximal height of tetanus, while strong stimulation produces again submaximal tetanus. It is found, too, that tetani of the form which Fröhlich calls "fatigue tetani" are more readily obtained with strong stimulation. Furthermore, with deep anæsthesia and strong rhythmical stimulation, the muscle response, as already mentioned, is a single twitch of the same height as the twitch evoked by one single maximal excitation (4). The same is the case when nerve is asphyxiated (4). A similar effect has been shown by one of us (Tait) to occur when nerve is cooled. All these facts indicate that under these conditions the refractory period of the nerve corresponding to strong stimulation is longer than that corresponding to weak.

Yohimbinised nerve does not conform in this regard to nerve subjected to these other influences. At almost all stages of yohimbine anæsthesia in which alterations of the muscle response to rhythmical stimulation can be detected, this response takes the form of a "fatigue tetanus"—i.e. the last part of the tetanus is at least markedly lower than the first. Furthermore, the highest and best sustained muscle response is not produced by stimulation at moderate intensity, but in every case strong stimulation is more effective than any moderate stimulation as regards both the height and duration of the corresponding tetanus. (See fig. 2.) Thus it is evident that the refractory period of yohimbinised nerve does not increase with the intensity of the stimulation. If anything, the contrary would seem to be the case.

An examination of the tracings in figs. 1 and 2 shows that when series of rhythmical stimulations are applied in closely succeeding sets or groups to the proximal end of yohimbinised nerve, the successive muscular

responses tend to fall off in height with repetition of the successive series of stimulations. Thus in fig. 1, tracing (2), it is very clear that the responses to the first six series of stimulations become progressively lower and lower. This effect may be due either to rapidly deepening anaesthesia of the nerve or to fatigue. That it is due to some fatigue condition and not to progressive and rapid anaesthesia, is indicated by the fact that

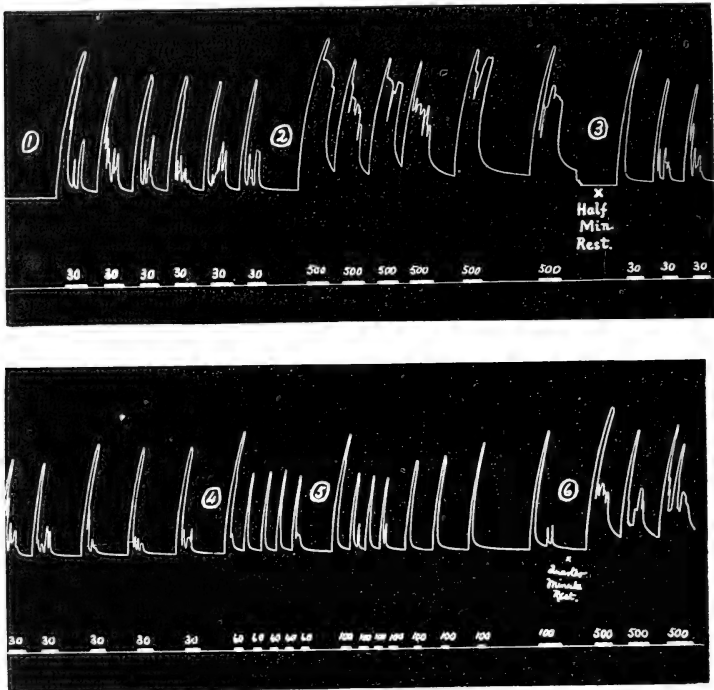


Fig. 2 (reduced to about one-half).—Length of nerve anaesthetised, 3 cm. Duration of application of yohimbine, 40 minutes. Rate of stimulation, 144 per sec. Rate of drum, 1.5 mm. per sec. Six series of responses are shown, corresponding to intensities varying from 30 to 500 Kronecker units.

Note (i.) the tetani are all of the "fatigue" form; (ii.) the responses corresponding to strong stimulation are more marked than those corresponding to weak; (iii.) in any given series with constant intensity of stimulation the height of the responses varies as the duration of the period of rest between stimulations.

if longer intervals of rest are allowed between the successive sets of excitations, the effect does not occur. Further, the effect is most marked when the intervals of rest between successive series of stimulations are made progressively less and less, as is the case in the first six responses of fig. 1, tracing (2), or in the middle series of responses in fig. 3.

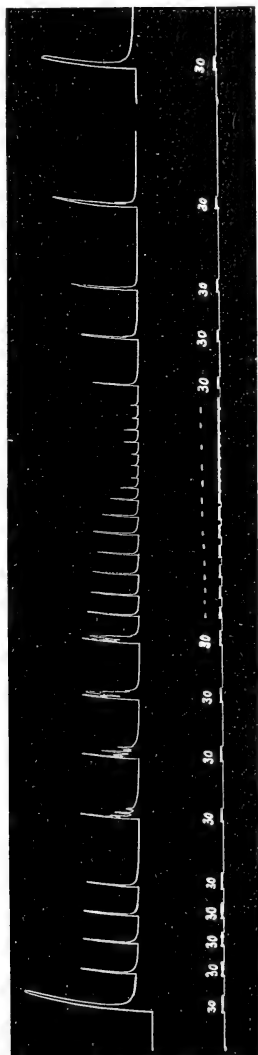


FIG. 3 (reduced to one-half).—Length of nerve anaesthetised, 1 cm. Duration of application, 1½ hours. Rate of stimulation, 144 per sec. Intensity, 30 Kronecker units. Rate of drum, 1 mm. per sec.

The tracing shows that the general extent of the muscle responses varies as the duration of the interval of rest between stimulations

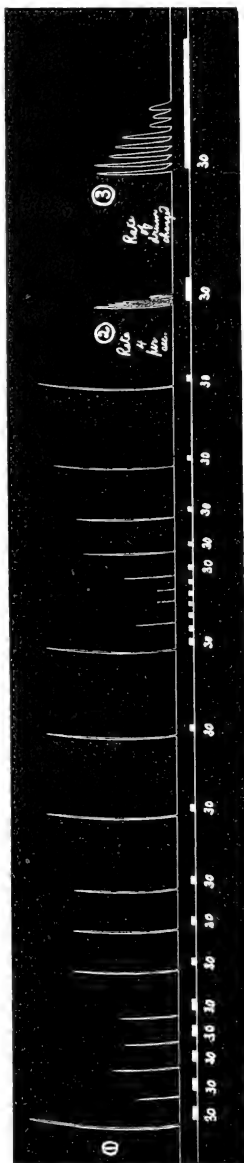


FIG. 4 (reduced to about two-thirds).—Length of nerve anaesthetised, 2·7 cm. Duration of application, 1¼ hours. Rate of stimulation in tracing (1), 128 per sec.; in tracings (2) and (3), 4 per sec. Rate of drum in the case of tracings (1) and (2), 1 mm. per sec.; in the case of tracing (3), 10 mm. per sec.

Tracing (1) shows summated muscle twitches varying in height with duration of interval of rest between stimulations.

Tracings (2) and (3) show rapid diminution in height of successive muscle twitches on repetition of stimulations, and final abolition of conductivity of nerve.

On the other hand, when the successive muscle responses have become less and less marked as a result of a steady diminution in the intervals of time between the sets of stimulations, they gradually increase in height again when the intervals of rest between sets of stimulations are made longer and longer. (See the last three responses in fig. 1, tracing (2), or the last five responses in fig. 3.) Provided the same short interval of time elapses in each case between the successive sets of stimulations, the second, third and fourth, etc., responses of the muscle may be all of about the same magnitude, whereas the first response of any series—beginning after an adequate interval of rest—is more marked. (See generally the tracings in figs. 2 and 3.) At any given stage of anaesthesia, therefore, the efficiency of the muscle response induced by stimulation of the proximal end of the nerve is directly proportional to the interval of time during which the preparation has rested from activity. This is clearly a fatigue phenomenon.

During the later stages of anaesthesia with yohimbine the muscle responses to rapid rhythmical stimulation tend to resemble simple muscle twitches rather than tetani (see fig. 4), and this is the case whether strong or weak stimulation is used. These seeming simple twitches are, however, in reality summated muscle responses. This is readily seen when one compares the height of the muscle contractions evoked on the one hand by rhythmical stimulation, and on the other by single maximal break shocks applied to the proximal end of the anaesthetised nerve (care being taken in each case to examine the preparation after an adequate interval of rest). In every instance the effect of rhythmical stimulation is to produce a much higher muscle response than that produced by a single maximal excitation. In this respect the action of yohimbine is once again different from that of other anaesthetics, for in the later stages of anaesthesia with, say, ether or cocaine, rapid stimulation, especially when strong, produces indeed a muscle twitch, but this twitch is of the same height as the response to one single maximal excitation of the nerve.

If we come now to the interpretation of this phenomenon we must conclude that when a series of excitatory processes are made to travel in rapid succession from a normal portion of nerve into a portion deeply anaesthetised with yohimbine, probably the first few excitatory processes succeed in traversing the anaesthetised part, but the passage of these unfits the affected portion of nerve for the immediate transmission of further excitatory processes. Only after an adequate interval of rest is the nerve able to function again, and an examination of the tracings in fig. 4 will show that this interval must be spread over many seconds to restore the conducting mechanism to exactly the same degree of functional capacity as before. From the fact that even with a relatively rapid rate of stimulation (between 100 and 200 per second) the deeply anaesthetised nerve is at the start able to transmit more than the first excitatory process, while after the passage of a few excitations it temporarily ceases to function, we infer that the refractory period of yohimbinised nerve is dependent, not so much



on mere degree of anaesthesia by itself as on the extent to which the anaesthetised nerve is within any given short period of time thrown into activity. It is activity during the anaesthesia rather than the anaesthesia itself which causes the prolongation of the refractory period.

The progressive impairment of function of the nerve with activity is equally well shown if during this stage of deep anaesthesia the nerve is stimulated at a slow rate (4 per second), with break shocks of a strength that is just maximal. (See fig. 4, last two tracings.) Then the individual twitches of the muscle consistently decline in height with each repetition of the excitation and finally, after a certain small number of responses have occurred, die away entirely. A rest of a considerable number of seconds (not more than thirty) suffices to restore the nerve to its previous condition, when the same process can be repeated again by rhythmical stimulation at the same slow rate. On the other hand, if the interval of rest be not sufficiently long (say only two to five seconds), the process of recovery is not so complete, and the next set of muscle responses are fewer in number and of less height. Meanwhile, if the nerve is stimulated at a part distal to the anaesthetised portion, the muscle responds by a continuous series of maximal twitches. (See fig. 5.)

Such experiments demonstrate in striking fashion not only the existence of fatigue in yohimbinised nerve, but also the gradual nature of the recovery from fatigue. In every case after a period of continuous activity the nerve becomes exhausted and requires a rest of a considerable number of seconds before it has regained its previous state of functional efficiency. Nevertheless, by stimulating the nerve after a shorter period of rest it can be shown that the recovery process, though incomplete, has still gone on to a certain extent. Further, the fact that in every case recovery does occur after fatigue indicates that nerve is characterised not so much by non-fatigability as by the possession of an extremely efficient mechanism for repair after fatigue.

How far the refractory period of nerve may be prolonged when the nerve is under the influence of yohimbine anaesthesia we have not determined exactly. Much depends on the signification in which the term "refractory period" is used. If we take the term to mean that period of time which elapses before one maximal stimulus following upon another of equal intensity can be fully effective, then the refractory period has been prolonged to at least .25 second. If, however, we extend the definition to include the period of time necessary for complete recovery of the nerve after the application of a series of stimuli applied in rapid succession, then the refractory period has been prolonged to a number of seconds (more than five).

In the later stages of yohimbine anaesthesia when the proximal end of the nerve is stimulated by isolated maximal shocks at long intervals, the corresponding muscle responses are definitely lower in height than those produced by similar stimulation of the distal end. This points to a diminution

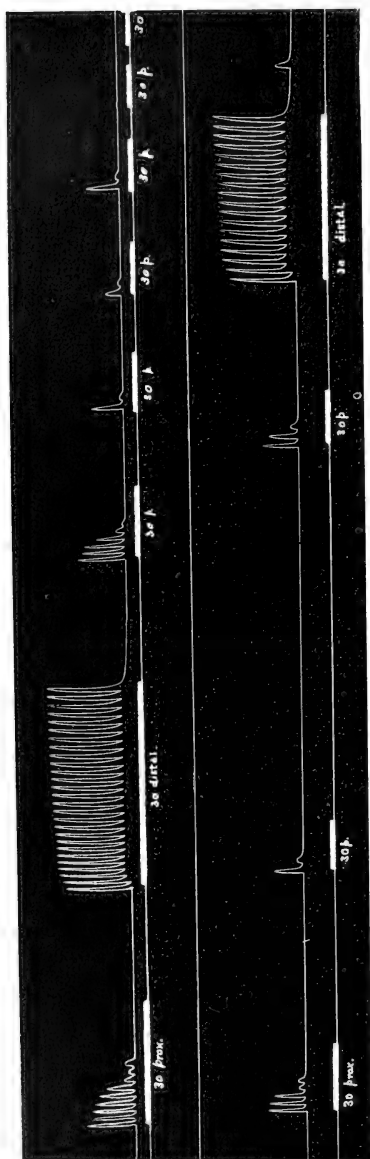


FIG. 5 (reduced to one-half).—From same preparation as in fig. 4. Rate of stimulation, 4 per sec. Intensity, 30 Kronecker units (break shocks alone effective). Rate of drum, 10 mm. per sec.

Tracing in upper line, taken during the same stage of anesthesia as in fig. 4, shows—

- (1) Result of proximal stimulation of nerve.
- (2) " " distal
- (3) " " subsequent repeated proximal stimulation of nerve.

Tracing in lower line, taken at a somewhat later stage, shows—

- (1) The difference in result according as distal or proximal stimulation is used.
- (2) Partial recovery of the distally stimulated nerve with increased interval of rest.

in amplitude of the excitatory processes as they traverse the yohimbinised area. When the conductivity by deepening of the anaesthesia is just about to disappear, these responses become minimal. In this respect the anaesthetic action of yohimbine corresponds with the action of cooling, of asphyxia, and of anaesthesia produced by means of the more commonly used anaesthetics.

Before concluding, we ought to say that the fatigue effects which we have ascribed to the action of yohimbine can be due only to a change in the nerve itself. In a normal nerve-muscle preparation, strong stimulation, especially of the distal end of the nerve, may so alter the nerve-endings in the muscle that subsequent stimulation of the nerve may produce effects which might be erroneously ascribed to fatigue of the nerve. In all the experiments carried out by us which show fatigue phenomena this fallacy is excluded by the fact (1) that the intensity of the stimulation applied to the nerve was at no time more than just maximal; (2) that the fatigue phenomena in each case appeared before distal stimulation was used.

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1. A two per cent. solution of yohimbine lactate has been applied to the middle portion of the sciatic nerve of a frog's gastrocnemius preparation, and alterations in the conductivity of the nerve observed by means of the muscular response to rhythmical stimulation applied proximally to the alkaloid-affected portion. The rate of stimulation varied between 144 and 4 per second.

2. In its action on nerve, yohimbine resembles in many respects the already known action of other anaesthetics, of low temperature, and of asphyxia. It ultimately abolishes conductivity. The process of abolition of the conductivity is gradual, and is characterised by a progressive diminution in the amplitude of excitatory processes which traverse the affected part of the nerve, and by a prolongation of the refractory period of the nerve. By means of it, too, fatigue changes may be shown to occur in the nerve.

3. On the other hand, the action of yohimbine differs in important respects from that of asphyxia of low temperature, and of anaesthesia with ordinary agents. The tetanic responses of the muscle corresponding to rapid rhythmical stimulation of the proximal end of a yohimbinised nerve are always of one type, and resemble the "fatigue tetani" described by F. W. Fröhlich. In stages of deep anaesthesia it is not easy to demonstrate the occurrence of initial non-summated maximal twitches as a result of rapid rhythmical stimulation of the nerve. The duration of the refractory period does not seem to vary directly with the strength of the stimulus applied to the nerve, and is more

clearly dependent on the amount of previous activity than is the case when other agents are used to depress the function of nerve.

4. The anaesthetic action of yohimbine lactate as applied in solution to the outside of a dissected nerve is characterised by great evenness and regularity. Partly for this reason, and partly because of the unusual prolongation of the refractory period due to yohimbine, it has been shown that nerve is a very convenient tissue on which to study the process of fatigue and recovery from fatigue.

5. In spite of the ready fatigability of yohimbinised nerve, complete restoration of function seems in every case to follow the katabolic changes due to activity.

6. The refractory period of nerve has been prolonged to .25 second; while fatigue changes lasting for more than five seconds have been demonstrated.

The expenses of this research were defrayed by a grant from the Carnegie Trust.

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NOTE ON THE MULTIPLICATION AND MIGRATION OF  
NUCLEOLI IN NERVE CELLS OF MAMMALS. By W. PAGE  
MAY and C. E. WALKER. (With Two Plates.)

(Received for publication 29th February 1908.)

METHODS.

THE tissues mainly employed in the observations here described were the Gasserian and cerebro-spinal ganglia of rats, rabbits, cats, monkeys, and chimpanzees. Other nerve cells were also similarly examined, notably those of the red nucleus, cerebral cortex, and other large motor and sensory cells throughout the central nervous system, and similar phenomena to those described in the present communication were also found to obtain in them. Further details bearing on this subject we hope to bring forward later. The animals were, with one or two exceptions, young adults.

Absolutely fresh material was fixed in Flemming's fluid (strong formula), or a modification of Zenker's fluid (G. Arnold's). It was dehydrated by increasing the percentage of alcohol by 10 per cent. at each stage, and the greatest care was taken to prevent any possibility of maceration by shortening the time between removing the material from the fixative and getting it into 70 per cent. alcohol. Imbedding was carried out at a temperature of 45° Cent., and this process did not occupy more than an hour and a half; thus any undue exposure to heat was avoided. The manipulation used in mounting and staining was according to the strictest cytological methods. Various staining methods were employed and all gave similar results, though it was found that particular methods rendered certain points in the observations clearer than others. The main processes adopted were:—

- A. Basic fuchsin, followed by methylene blue and Unna's orange tannin.
- B. Saffranin, followed by methylene blue and Unna's orange tannin (Breinl method).
- C. Thionin counterstained with Bordeaux red, etc.

In the present communication the term "nucleolus" is used in its strictest sense, i.e. it is restricted to the structure which has sometimes been called the "true nucleolus," whilst such bodies as the so-called "chromatin nucleoli" contained in the nucleus are definitely excluded.<sup>1</sup>

<sup>1</sup> Cf. Wilson, "The Cell in Development and Inheritance," p 34; Macmillan, London and New York, 1904. Walker, "The Essentials of Cytology," pp. 12 and 13; Constable, London, 1907.

The nucleolus here dealt with is generally spherical in shape, occasionally oval. It is bounded by a definite membrane, and the contents are usually homogeneous or finely granular in structure. As compared with the chromatin present with it in the nucleus, its staining reaction is acid. Small aggregations of chromatin are almost invariably found lying upon the outer surface of the membrane of the nucleolus. These small collections of chromatin are often continuous with the chromatin granules in the lining of the nucleus, and are so closely applied to the outer surface of the membrane that it is only in specimens specially stained for the purpose that their actual position can be ascertained. Without this examination it is also difficult to be sure as to the staining reaction of the nucleolar material proper, as this is masked by the chromatin lying upon the surface.<sup>1</sup>

Several nucleoli are frequently found in the nuclei of nerve cells, indeed the present observations indicate that more than one is usual. Frequently there are four or five, or more,<sup>2</sup> which may vary greatly in size.

In many cases a small excrescence may be seen at one point at the margin of the nucleolus, and perhaps this is more usual where only one large nucleolus is present (figs. 1 and 2). Generally, if not always, one or more of the chromatin aggregates already mentioned as being observed upon the nucleolar membrane are found to be present upon the outer surface of these excrescences, in sections specially stained for the purpose (figs. 1 and 2). Other cells are found where the excrescence has apparently increased in size and travelled away from the nucleolus, being still attached to it by a process of the membrane. This process seems to persist until what was originally the excrescence has grown to a considerable size (figs. 3 to 5). Cells are also frequently to be found which exhibit traces of what was evidently the connection between the nucleolus and the excrescence, and where the excrescence has attained a size which approaches or equals that of the nucleolus (figs. 6 and 7). A careful examination of the appearance of the nucleolus and of the excrescence up to the time when, according to the present interpretation, they are separated from each other, shows that both are exactly similar in structure, and that there may be several similar nucleoli undergoing similar processes in the same nucleus. It is, in fact, easy to find, in the same slide, every stage between the single nucleolus with one or more small excrescences, and two distinct nucleoli, which are almost exactly similar to each other. From this it is equally easy to pass on to cells with four and five nucleoli, or even more (fig. 8). The structure of these bodies is so definite that there is no possibility of mistaking them, in a properly preserved specimen, for any other nuclear constituent, such as a mass of chromatin.

Very rarely a nucleolus may be seen dividing by a process analogous to amitosis, or to the division of a drop of viscous fluid into two (fig. 9).<sup>3</sup>

The many observations with regard to the migration of the nucleoli

<sup>1</sup> Method C.

<sup>2</sup> We have counted as many as nine in one nucleus.

<sup>3</sup> We have only observed this twice in many hundreds of cells.

into the cytoplasm of the cell<sup>1</sup> seemed to indicate the destiny of these bodies, which are apparently continuously produced in the nerve cells of the animals here investigated, and a particular study of this phenomenon was therefore made. It has been claimed that the alleged migration of the nucleoli is due to some mechanical force such as the dragging or pressing of the edge of the microtome knife in the process of cutting sections, or to the action of gravity.<sup>2</sup>

In the present observations nucleoli partly extruded through the nuclear membrane and in the cytoplasm clear of the nucleus were frequently found. In the great majority of these cases the apparent extrusion of the nucleolus was undoubtedly due to the action of the knife. Such nucleoli were always in an exactly similar position in relation to the nucleus and cytoplasm in every section in a series. Thus, if one were found half-way through or outside the nuclear membrane to the lower right-hand side of the centre of the nucleus, the other nucleoli would be displaced in exactly the same direction in other cells, not only in the same section, but in the other sections of the series. Furthermore, the nucleolus thus displaced carried with it a considerable amount of linin with its contained chromatin granules, and a large empty space in the nucleus with the broken ends of the strands of linin round it could be found in all these cases (fig. 10). Also the nuclear membrane was always definitely ruptured, and there was never any sign of its being reconstructed, which one would expect to find, and, as will be seen, actually was found, when the nucleoli were extruded normally; for nuclei, even when they contain several nucleoli, are usually found to possess a membrane without any large ruptures, and fresh nucleoli are apparently constantly being produced. An interesting fact is, however, indicated by this occurrence. The nucleoli must be of a highly dense and resistant structure as compared with the rest of the cell, as the edge of the knife, when it happens to catch one of them, does not cut through it as it does through the other cellular constituents, but carries it bodily along for a considerable distance.

In a, comparatively speaking, few cells, however, a true migration of the nucleolus was observed, and the phenomenon is here described in some detail, as it appears to differ in some respects from what has been stated to occur by other authors: some points seem to have escaped notice altogether.

The staining reaction of the nucleoli when inside the nucleus was very marked with certain combinations used in the present investigation. Thus with Method A the nucleolus stains blue or violet, while with Method B it stains brilliant scarlet.

The way in which the passage of the nucleolus from the nucleus occurs is apparently as follows:—A nucleolus lies for some time against the

<sup>1</sup> Montgomery, Rhode, Hatai, and others.

<sup>2</sup> Herrick, "Movements of the Nucleolus through the Action of Gravity," *Anatom. Anz.*, Bd. x. 95.

nuclear membrane. The nuclear membrane is often seen to protrude considerably at this point (fig. 11), and then the nucleolus passes through into the cytoplasm (figs. 12 and 13). Sometimes this protrusion is very large compared with the size of the nucleolus, and then it seems to be depressed in the middle in a form not unlike the crater of an extinct volcano, the nucleolus lying at the bottom of the crater. When seen under the microscope, this formation gives the appearance of two protrusions, one on either side of the nucleolus. This is due to the fact that the crater formation is seen in actual or in optical section. The nuclear membrane is re-formed very quickly, and is, as far as can be ascertained, always re-formed long before the nucleolus leaves the outer surface of the nuclear membrane. That the nucleoli remain contiguous to the nuclear membrane for some time is rendered highly probable, if not actually certain, by the fact that in every slide examined a very large number were seen in this position. That the passage of the nucleolus is brief, is rendered probable by the fact that nucleoli in the act of passing through are very rarely to be found in comparison with those in any other position either inside or outside the nucleus. That the nuclear membrane is re-formed before the nucleolus leaves its outer surface is rendered almost certain by the fact that nucleoli are comparatively frequently found lying adjacent to or upon it, but no breach in the neighbourhood was ever observed. In the latter case, the surface of the nucleolus that is touching the nuclear membrane being concave, a comparatively large area of membrane is covered by it (figs. 14 and 15). Whether the extruded nucleolus always remains thus attached to the membrane, however, appears doubtful, as it has been found in this position comparatively seldom. More often it is found adjacent to the nucleus, but not compressed upon its membrane (fig. 16). No breach in the nuclear membrane has been found that could legitimately be connected with the passage of a nucleolus at any other time than during the actual process of passing through.

The nucleoli that migrate appear to be usually, if not always, among the largest found in the nuclei of the nerve cells. After they have passed into the cytoplasm they increase in size, often to a considerable extent, and the contents seem generally to become definitely granular.

One of the most remarkable facts in connection with this migration of the nucleoli is that as they pass into the cytoplasm their staining reaction alters. Thus with Method A the nucleoli inside the nucleus are blue or violet. Those passing through are purple or red. Those definitely outside are bright red or pink, and those which have travelled away from the nuclear membrane are pink or red. With Method B the nucleoli inside the nucleus are brilliant scarlet. Those passing through are reddish orange, and those which have passed through a pale orange or yellow. Nucleoli that have been artificially forced out of the nucleus by the knife of the microtome stain exactly as do those that are found in the nuclei. This suggests strongly that some important chemical or physical change



takes place in the nucleolus when it passes into the cytoplasm. It also seems to offer a simple and obvious means of judging at once whether a nucleolus has been extruded naturally or otherwise.

The pseudopodial processes observed in the nuclei of the nerve ganglion cells in adult and young animals seem frequently to be intimately connected with the phenomenon of the migration of the nucleoli. The protrusion of the nuclear membrane described above seems to persist in many cases long after the nucleolus has left the neighbourhood of the nucleus. This is particularly noticeable in cases where the nuclear membrane has been depressed in the middle, as already described. In optical section this gives the appearance of two protrusions with the concavity between them directed towards the extruded nucleolus. As to whether these protrusions are always the protrusions formed in connection with the passage of the nucleolus which have persisted in cases where the nucleolus does not remain attached to the nuclear membrane, or whether they represent maybe a separate phenomenon, the present observations do not appear to give any definite suggestion. In any case, the occurrence of the extruded nucleoli and the protrusions in certain definitely relative positions seem to be too frequent to be due to a mere coincidence (figs. 17-19).

Whatever may be the case with regard to the nerve ganglion cells in embryos, it has been found absolutely impossible to demonstrate centrosomes or astral rays in the material used in the present observations.<sup>1</sup>

After its extrusion from the nucleus, the nucleolus travels towards the periphery of the cytoplasm of the cell (figs. 17, 18, and 19). When it reaches the periphery of the cell it sometimes passes bodily through the surface membrane and is set free among the surrounding cells (fig. 20).

This is, however, the sequence of events in the case of some only among the nucleoli. In other cases the nucleolus may be seen lying on the inside of the surface membrane of the nerve cell in the immediate neighbourhood of a leucocyte or in the case of cerebro-spinal ganglia of the nucleus of a capsular cell. Here the substance of the nucleolus seems to pass piecemeal through several small openings, and to be absorbed into the cytoplasm of the neighbouring cell. The absorbed material seems to lie close to the nucleus of the cell that has absorbed it (figs. 21 and 22).

Those nucleoli that pass out of the nerve cells without being disintegrated, seem sometimes to be taken bodily into the cytoplasm of a capsular cell or of a leucocyte, where they are probably disintegrated (figs. 23 and 24).

In studying cerebro-spinal ganglia, while it is quite easy in very many cases to say definitely whether a particular cell is a leucocyte or a capsular

<sup>1</sup> The term "centrosome" appears to have been used somewhat loosely upon some occasions. The sense in which it is used here is that generally accepted by cytologists, i.e. minute structures, oval or bean-shaped, generally two in number, sometimes surrounded by an archoplasm or attraction sphere, which is usually contiguous to the nucleus. We think it possible that the extruded nucleolus lying upon the nuclear membrane, as shown in figs. 14 and 15, may very probably have been mistaken for the archoplasm, particularly as the nucleolar contents are granular in appearance at this stage.

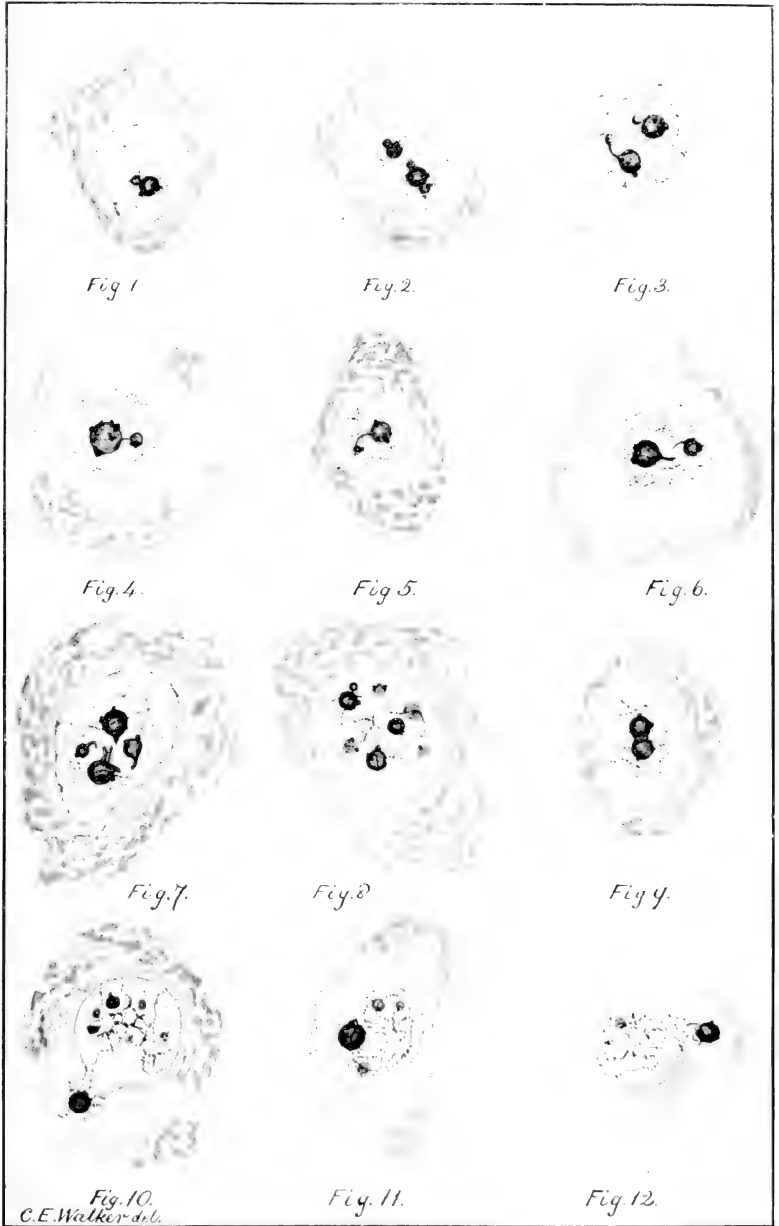
cell, other cells intermediate in character between these two very different types were found during the present investigations. The nucleoli seemed to be taken into the cytoplasm of the capsular cells, of the intermediate forms, and of the leucocytes indifferently.

The present observations do not seem in accordance with the interpretation of Flemming or of Oscar and Richard Hertwig, as regards the function of the nucleolus. These observers held that the nucleoli supply nutriment which contributes to the formation of the chromosomes during the process of mitosis, and this interpretation is perhaps strengthened by the fact that the nucleolus, when present, seems then to disintegrate. As, however, the phenomenon of mitosis has not hitherto been observed among the nerve cells of the adult mammal, and apparently does not occur, the function of the nucleoli here dealt with must be something entirely independent of the formation of chromosomes as they appear during mitosis. That the nucleoli are constantly being increased in number, even in adult life, by the process described in this paper, proves that they subservise some important function, but whether this is in the nature of an excretion, secretion or some other form of cell phenomenon, there does not as yet appear to be any direct evidence.

#### CONCLUSIONS.

The conclusions arrived at from the observations here described are—

1. That the nucleoli of the nerve cells described multiply continually, generally by a process of budding, more rarely by an equal division in bulk of a pre-existing nucleolus.
  2. That the nucleoli pass out of the nucleus, and in the process their staining reaction changes.
  3. After passing out of the nucleus the nucleoli become granular in appearance, and increase in size. The increase in size would appear to be due to the lessened density of the contents, rather than to an increase in substance.
  4. The nucleoli sometimes pass bodily out of the nerve cell (peri-karyon), and are taken into the cytoplasm of leucocytes or capsular cells. At other times the substance of the nucleoli seems to pass in small portions from the cytoplasm of the nerve cell into that of a capsular cell or a leucocyte.
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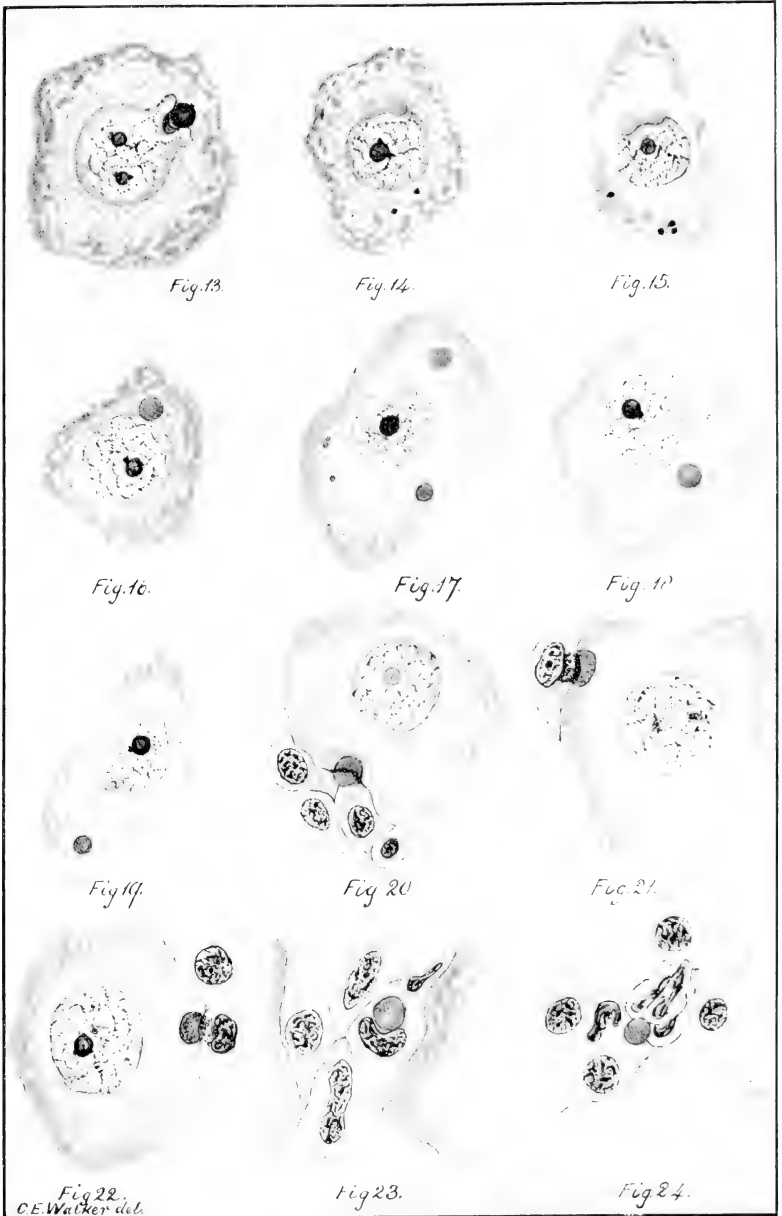


Fig. 13.

Fig. 14.

Fig. 15.

Fig. 16.

Fig. 17.

Fig. 18.

Fig. 19.

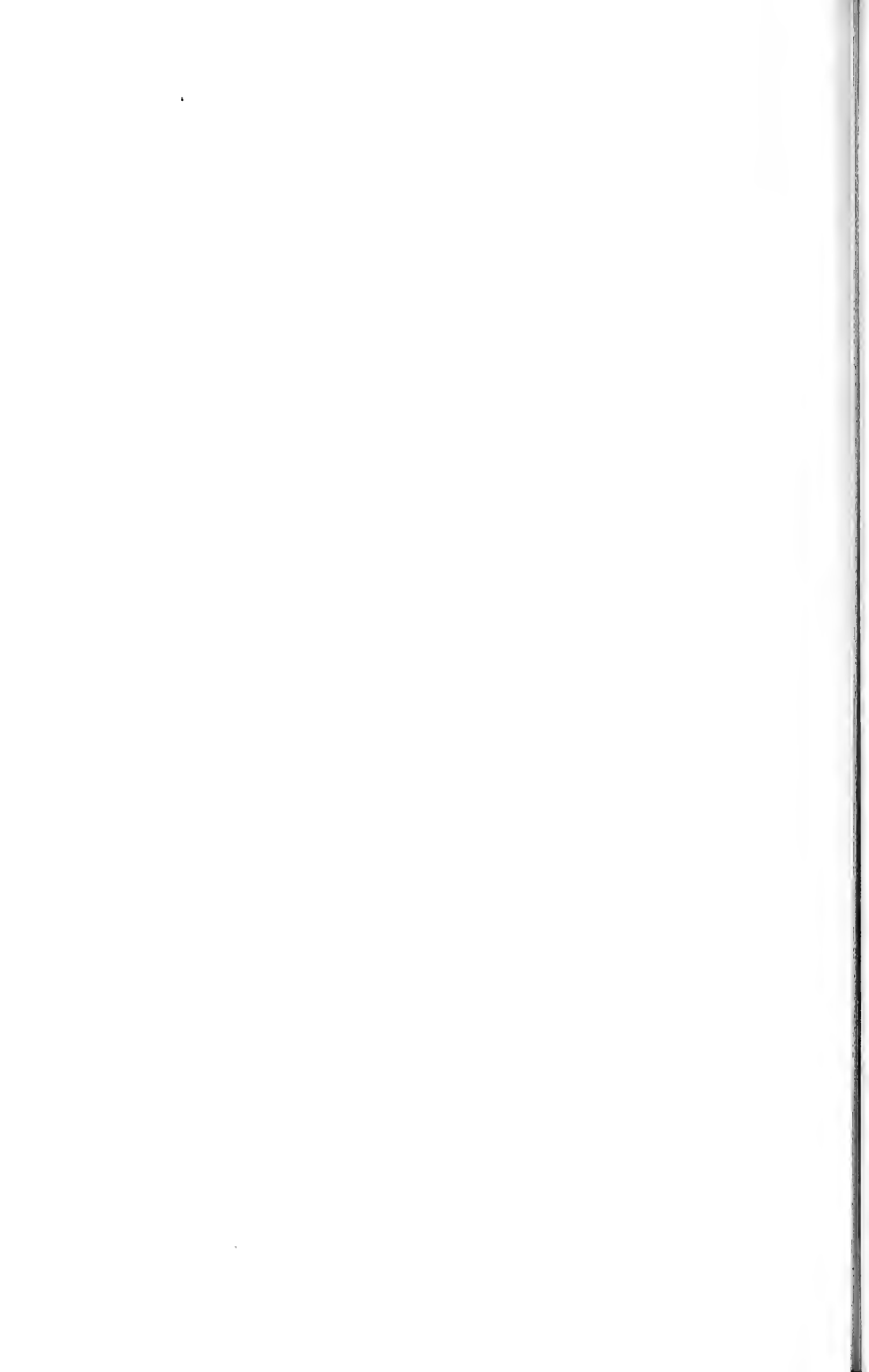
Fig. 20.

Fig. 21.

Fig. 22.  
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Fig. 23.

Fig. 24.



DESCRIPTION OF PLATES.

Fig. 1. Cell from Gasserian ganglion of a rabbit, showing an early stage in the budding of the nucleolus, and some chromatin aggregates upon the membrane of the nucleolus.

Fig. 2. Ditto.

Fig. 3. A cell from the same ganglion, in which a bud has travelled some distance from the nucleolus, but is still attached to it.

Fig. 4. Ditto.

Fig. 5. Ditto.

Fig. 6. A cell from the same ganglion, in which the attachment of the bud to the nucleolus is broken.

Fig. 7. A cell showing the same phenomenon, with several buds separated off from the nucleolus.

Fig. 8. A cell from the same ganglion, showing several nucleoli.

Fig. 9. A rare mode of nucleolar division.

Fig. 10. A cell from the same ganglion, showing a nucleolus that has been pushed out of the nucleus by the action of the microtome knife. There is an empty space in the nucleus, and the nuclear membrane shows no signs of being regenerated. Here the staining reaction of the nucleolus is the same as in the case of nucleoli contained in the nucleus.

Fig. 11. A cell from a spinal ganglion of a cat, showing the nucleolus pressing out the nuclear membrane.

Fig. 12. A cell from the same ganglion, showing the nucleolus passing through the nuclear membrane. Here the staining reaction of the nucleolus is changing.

Fig. 13. A cell from the same ganglion, which shows the appearance, in optical section, of the crater formation of the protrusions of the nuclear membrane described in the text. The nuclear membrane has re-formed behind the nucleolus.

Fig. 14. A cell from the same ganglion, in which the extruded nucleolus is adherent to the nuclear membrane. The nucleolus has increased considerably in size, and has become definitely granular. Here its staining reaction is quite different from what it is in the case of the nucleoli still contained in the nucleus.

Fig. 15. Ditto.

Fig. 16. An extruded nucleolus still adjacent to the nuclear membrane (spinal ganglion of cat).

Figs. 17, 18, and 19. Nucleoli passing towards the periphery of the cell after being extruded from the nucleus (spinal ganglion of cat). The pseudopodial processes of the nucleus are interpreted as being the remnants of the protrusions produced by the passage of the nucleolus.

Fig. 20. A nucleolus passing bodily out of a nerve cell (spinal ganglion of cat).

Figs. 21 and 22. The contents of nucleoli being taken from the nerve cells into the cytoplasm of adjacent cells (spinal ganglion of cat).

Fig. 23. A nucleolus derived from a nerve cell in the cytoplasm of a capsular cell.

Fig. 24. A nucleolus derived from a nerve cell surrounded by leucocytes.





THE ELECTRICAL RESPONSE OF MUSCLE TO VOLUNTARY, REFLEX, AND ARTIFICIAL STIMULATION. By FLORENCE BUCHANAN. (From the University Museum, Oxford.)

(Received for publication May 29, 1908.)

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THINKING that the action currents of human muscle in voluntary contraction might throw light upon the nature of the normal stimulus to skeletal muscle, I had several times made the attempt to record such action currents with the capillary electrometer, but had not succeeded in doing so until August of last year. As the records did not seem to me to give the required information, I was meaning to content myself with merely drawing attention to the fact that they can be obtained, when there appeared a paper by Dr Piper of Kiel (1) in which were reproduced a number of records of human muscle in voluntary contraction, taken with the string-galvanometer, which seemed to him to give the solution to the problem.

Electrophysiology is so much beset with the difficulty of culling, from the instrumental effects observed, the underlying physiological phenomena, and of excluding everything for which the recording instrument alone may be responsible, that the observation of the same phenomenon with different recording instruments is always likely to be of value. It is the more so when, as apparently in this instance, the records do not show the same thing, and when the conclusions drawn from them by two observers are diametrically opposed.

Dr Piper's kindness in informing me of results contained in his two later papers (2), (3), before their publication, and his readiness to let me examine several of his still unpublished records there referred to, make me feel sure that he is as anxious as I am myself to come to a true understanding of the physiological significance of what we are both recording, and that he will prefer a criticism of his views to undisputed acquiescence in them. I trust, therefore, that he will not take amiss any criticism which appears in the following pages.

He has recorded, with different patterns of Einthoven's string-galvanometer, the electrical responses of several different muscles in man to their normal stimulus. He finds in each record one particularly prominent rhythm (see note on p. 241) which he regards as constant for each muscle or group of muscles. This rhythm, he thinks, indicates the rate at which successive stimuli are sent to the muscle from the central nervous system. He concludes, for instance, that the flexor muscles in the lower arm, which are the muscles which both he and I have investigated most, are normally supplied by impulses arriving with a frequency of 47 to 50 per second (1), (2). He bases his conclusion—to a large extent—on the fact that the galvanometer record obtained from the same muscle, when the median nerve is artificially stimulated by induction shocks of about this frequency, has a similar character. As will presently appear (pp. 228 and 241), the records given by my electrometer of the responses of the same muscle under the same two conditions exhibit very little resemblance to one another; but even were it much stronger than it is, and in the records of both instruments, the fact that an effect can be imitated in one particular way affords, of course, no evidence whatever that there is no other way in which the effect may

have been produced. It has, as a matter of fact, been shown both by Garten (6) and by myself (4) that a rhythm of a frequency of from 50 to 100 per second may be observed in the electrical response of excised frog's muscle when either it or its motor nerve is subjected to a continuous stimulus (whether actually so, or consisting of instantaneous stimuli recurring in such rapid succession that the whole may be so regarded) or to a variety of other stimuli of short duration which have nothing discontinuous in their nature. The frequency of this rhythm varies (as we have each of us shown) with the condition of the muscle much more than with the particular nature of the exciting stimulus. The central stimulus which provokes a normal contraction in any animal might just as well partake of the nature of any of these non-discontinuous stimuli as of that of the particular discontinuous one which Piper found to produce an effect on his galvanometer of a similar character. I had, indeed, laid stress on the fact [(4), p. 149] that the central stimulus intervening in the reflex response of the muscle of a frog in certain stages of strychnine poisoning is not to be regarded as of the nature of a series of instantaneous stimuli, although its effect on the muscle can be imitated (as in photo. 35, on pl. vii.) by that of a series of such stimuli, recurring with a frequency of, say, 50 per second, interrupted at intervals. The view then expressed was afterwards put to the test and confirmed. The experiments which confirm it deserve more than the passing mention they received in the following year. (5), and since the results obtained have so distinct a bearing on the subject to be chiefly discussed in the present paper, namely, on the significance to be attached to the rhythm observed in the electrical response of a muscle in voluntary contraction, I propose to begin by giving some account of them and to reproduce a few typical records.

## II. THE TWO KINDS OF RHYTHM EXHIBITED IN THE REFLEX ELECTRICAL RESPONSES OF FROG'S MUSCLE IN STRYCHNINE SPASM.

For the sake of clearness I shall henceforward designate those undulations which occur in the records with a frequency of from 40 to 100 per second as wavelets, the curves recurring with a frequency of from 3 to 14 per second (on which the wavelets may be superimposed) as waves.

The independence of waves and wavelets is shown by the fact that either may be present without the other. I have already reproduced records of frog's muscle in strychnine spasm showing (a) wavelets exclusively or almost exclusively [(4), pl. vii., ph. 37; pl. ix., ph. 45, 46, 47]; (b) waves exclusively [(4), pl. viii., ph. 39, 40, 41; pl. ix., ph. 50]; and (c) the two side by side [(4), pl. vii., ph. 38; pl. ix., ph. 48, 49]. The records I have now to reproduce are to bear witness to what was stated in 1902, namely, that what I now call wavelets depend for their frequency upon something in the muscle, that the waves depend for theirs upon something in the spinal cord.

Experiments in which the temperature of the recording muscle was varied, while that of the rest of the frog remained constant.

For these experiments the gastrocnemius was chosen as recording muscle, and was prepared so that it remained in connection with the rest of the preparation by its sciatic nerve only. The nerve ran through an aperture in the wall dividing two moist chambers, in the one of which was the muscle, in the other the decerebrate frog. The temperature of the muscle chamber was varied by placing over it now a tray of iced water, now one of warm water.

In the experiment to which fig. 1 relates the iliac artery had been ligatured on the left side and 5 minims of 1 per cent. curare injected into the dorsal lymph sac; then, when the curare had taken effect, 1 minim 0.1 per cent. strychnine acetate had been injected and half an hour later the left gastrocnemius prepared and arranged in the way just described. Its tendon end was connected in the usual way with the mercury of the electrometer, a spot on its dorsal surface with the acid. The muscle was excited each time by a single break induction shock applied to the skin of the back of the frog. The temperature of the moist chamber in which the body of the frog was lying remained at 12° C. throughout the experiment. Twelve records were first taken with the muscle also at 12° C. None of these exhibit more than a single wave, on the rise of which are wavelets which in nine of the records have a frequency of  $100 \pm 3$  per second, in two of them (the fifth and the sixth) one of about 90 per second, and in one (the first) one of 110 per second. The twelfth record is reproduced in fig. 1, A.

The muscle chamber was then cooled, and when its temperature was 10° C. the record reproduced in fig. 1, B, was taken. The frequency of the wavelets is 81 per second. A second record, taken after the muscle had been cooled to 9° C., showed a wavelet frequency of 70 per second.

The preparation was now left for an hour and a half, the muscle chamber remaining covered by the ice-tray, and its temperature being 7° C. at the end of the time. The only muscle which contracted when the skin was stimulated was, as before, the recording gastrocnemius, which had been protected from the influence of the curare. The contraction was now no longer twitch-like as it had been in the morning, but was, each time, a long and steady spasm. All the electrical responses were now serial, waves as well as wavelets being seen in the records when the plate was travelling at a sufficiently slow rate to show them. Five records of the reflex response were taken with the muscle at 7° C., of which the last is reproduced in fig. 1, C. The muscle chamber was then allowed to return to the temperature of the room and the record reproduced in fig. 1, D, was taken. It was then warmed beyond the temperature of the room and five records were taken with the temperature of the muscle rising to 16° C. The last

four records were taken while the muscle was being cooled again to 9° C. The table on the following page gives the period of the first wave in each record, and of a second when a second complete one was on the plate, in thousandths of a second ( $\sigma$ ). It also gives the frequency of the wavelets on the first wave in as many of the photographs as they were distinct enough to be counted.

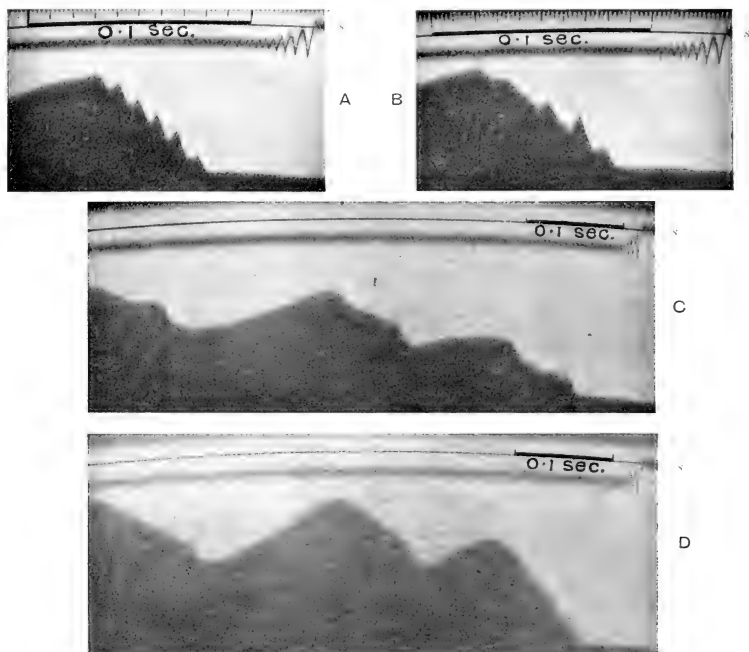


FIG. 1.—Reflex electrical responses of frog's gastrocnemius (strychnine). Spinal cord at 12° C. in all. The muscle at 12° C. in A, at 10° C. in B, at 7° C. in C, at 12° C. in D. Recording surface describing an arc. Rate of movement indicated by 500 fork tracing above. It was considerably slower in C and D than it was in A and B. *s* is the signal key, the break of which produced the excitation.

The results obtained from each experiment of this kind were so uniform that only six of them, not all on curarised frogs, were made. In all the wavelet frequency changed when the temperature of the muscle was changed, while that of the waves remained unaltered, i.e. the wave-length varied no more than it usually does when a succession of responses are recorded all with the muscle (as well as the cord) at the same temperature, and it did not vary in any definite direction with the temperature of the muscle. In only one, as it happens, out of this set of experiments were

there no waves in the records, these being of the type which in my previous paper [(4), p. 146] I called "type i," and presenting a long series of wavelets only (lasting for some 0.3 second) the frequency of which again varied directly with the temperature of the muscle. This series of experiments shows, therefore, that the frequency of the wavelet rhythm is dependent

(Gastrocnemius of strychnine frog, D 82. Apr. 7, 1902. (Stimulation by break induction shock to skin of the back, throughout.) Temperature of spinal cord constant (12° C.).

Temperature of muscle.	Total reflex-time.	Duration of waves.		Frequency of wavelets on 1st wave.
		1.	2.	
7° C.	52σ	172σ	198σ	54 per sec.
7° C.	52σ	184σ	210σ	41 "
7° C.	52σ	183σ	...	40 "
7° C.	51σ	180σ	...	?
7° C.	52σ	180σ	195σ	41 "
12° C.	51σ	174σ	...	90 "
13° C.	59σ	no 2nd wave	...	96 "
14° C.	50σ	188σ	...	100 "
15° C.	47σ	183σ	209σ	96 "
16° C.	46σ	184σ	217σ	106 "
16° C.	47σ	184σ	...	110 "
11° C.	47σ	180σ	...	?
10° C.	50σ	175σ	210σ	70 "
9° C.	50σ	174σ	186σ	?
8½° C.	47σ	187σ	...	?

on the muscle, but does not, of course, show that it is independent altogether of the cord. This however is, I think, shown by the second series of experiments.

Experiments in which the temperature of the spinal cord was varied, and that of the recording muscle kept constant.

A few of these were made in 1902, and several more have been made within the last year. The temperature of the cord was altered by running water at different temperatures through a glass tube passing under or over the back of the frog, and so shaped that it went no nearer to the recording muscle. The muscles used to record in these experiments were: the gastrocnemius, the sartorius, the biceps and the triceps femoris, and the semitendinosus. I shall have to refer later (p. 237) to differences of wavelet rhythm characterising these different muscles. Here I will only give the details of one typical experiment and reproduce two typical records. In the experiment to which fig. 2 refers the triceps was the recording muscle, the distal leading-off electrode was on the tendon end and connected with the mercury of the electrometer, the proximal one was about one centimetre away from it, and so on that part of the muscle in which there is no

external evidence of its threefold origin. The temperature of the moist chamber in which the whole preparation lay remained throughout the experiment at 12° C. Through the glass tube lying over the back of the frog iced-water at 4° C. had been running for nearly half an hour before the experiment was begun. After five responses had been recorded, water at 22° C. was passed through the tube for two minutes, and three more responses were recorded. Then water at 5° C. was run through for three minutes and three more responses were recorded. Finally, water at 24° C. was once more run through for three minutes and three more records were taken. The responses in each case were to the excitation of three different afferent nerves, to the brachial and sciatic of the opposite side, and to the

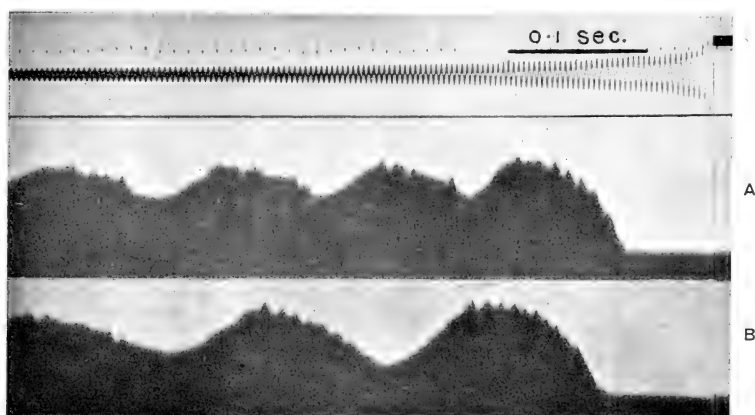


FIG. 2.—Reflex electrical responses of frog's triceps femoris (strychnine). Muscle at 12° C. in both. Water running through glass tube over spinal cord at 22° C. in A, at 5° C. in B. Recording surface moving horizontally, rate indicated by 100 fork tracing above the signals.

sciatic of the same side. Enlargements of the first part of two photographs, taken when the opposite sciatic was stimulated by a break induction shock, are reproduced in fig. 2. When A was taken the temperature of the cord was about 22° C., and it had been subsequently cooled to about 5° C. when B was taken. The table on p. 218 shows the frequency of the wavelets on the first wave in all the responses in which it could be determined, and the time duration of each of the first four waves.

In the records of this as in those of most experiments in which the electrical responses of frog's muscle in strychnine spasm were recorded, whether the cord were cooled or not, there is a good deal of variation in the frequency of the wavelets, not only in successive responses, but also at different times in one and the same response. When the record is serial the wavelet frequency shows that the rhythm usually remains the same for

the time indicated by the ascent of any one wave, so that in cord-cooling experiments one can compare the frequency in corresponding waves in the successive responses as in the experiment to which the table refers. When there is little or no indication of waves in a record, as in the one reproduced in fig. 3, one frequency may give place to another abruptly, and in cord-cooling experiments it is more difficult to select the corresponding parts. But in the records obtained in several of such experiments made on frogs in which the action of strychnine was not at its height and the electrical reflex response non-serial, I have not been able to discover any definite relation between the frequency of the rhythm and the temperature

L. triceps of strychnine frog, F 160. Feb. 24, 1908. (Stimulation by break induction shock to the L. sciatic, to the R. sciatic, or to the R. brachial nerve.) Temperature of muscle constant ( $12^{\circ}$  C.).

Temperature of water passing over the back of the frog.	Nerve stimulated	Total reflex-time.	Duration of waves.				Frequency of wavelets on 1st wave.
			1	2	3	4	
4° C.	R. br.	100σ	180σ	160σ	175σ	198σ	88 per sec.
	R. sci.	87σ	190σ	160σ	180σ	215σ	90 "
	L. sci.	50σ	215σ	165σ	185σ	230σ	100 "
	R. sci.	87σ	183σ	172σ	183σ	210σ	92 "
	R. br.	95σ	180σ	155σ	170σ	230σ	97 "
22° C.	R. br.	72σ	115σ	100σ	115σ	120σ	94 "
	R. sci.	62σ	115σ	105σ	120σ	130σ	100 "
	L. sci.	40σ	125σ	100σ	120σ	115σ	90 "
5° C.	R. br.	100σ	150σ	130σ	155σ	170σ	95 "
	R. sci.	75σ	165σ	148σ	170σ	no 5th wave	100 "
	L. sci.	48σ	182σ	135σ	175σ	172σ	100 "
24° C.	R. br.	87σ	115σ	105σ	120σ	100σ	100? "
	R. sci.	60σ	117σ	100σ	113σ	165σ	94 "
	L. sci.	40σ	124σ	106σ	115σ	no 5th wave	100 "

of the cord, when this was alternately raised and lowered. The wavelets frequently are more marked when the cord is cool, i.e. the ascent and descent of each wavelet is steeper (as is often the case also in the serial responses), but there is no constant lowering of frequency in any part of the record with cold.

The waves, on the other hand, in all experiments I have made in which the responses have been serial, vary in frequency in a very definite way with the temperature of the cord. Thus in the experiment already quoted we find that in the first five records, taken when the cord was at a temperature of about  $4^{\circ}$  C., the frequency of the waves (estimated from the four which alone appeared on each plate) was from 5.6 to 5 per second. In the next three, in which the cord was nearly at  $22^{\circ}$  C., the frequency (again estimating from the first four waves only, although there were more on the



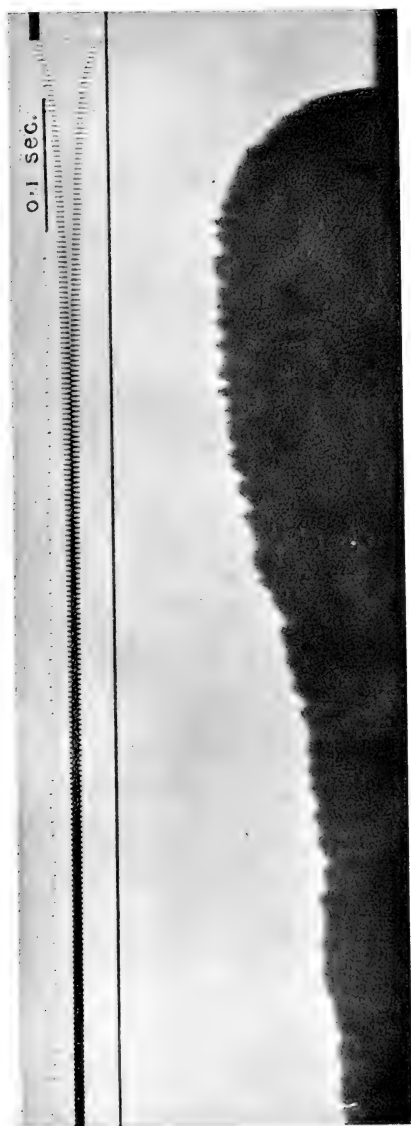


FIG. 3.—Reflex electrical response of frog's semitendinosus. The frog had very nearly recovered from the effect of a very minute dose of strychnine nitrate (0.02 mgr.) administered the day before.

plate) was from 8.8 to 8.5 per second. In the next three, with the cord cooled again, it was from 6.6 to 6.0 per second; and in the last three, with the cord warmed again, it was either 9.0 or 8.0 per second, and would have been 9.0 in all three if the estimation had been made from the first three waves only in the second response.

It would appear, therefore, from these two sets of experiments, that in the electrical responses of frog's muscle to a stimulus which comes, in the last instance, from the central nervous system, two kinds of rhythm may manifest themselves in capillary electrometer records. The one of them, that of the wavelets, which is very rarely absent in the responses of muscle in strychnine spasm, gives us no positive information as to the nature of the stimulus which immediately provokes the response; but, from the conditions under which the frequency may be modified and from those which fail to modify it, we learn that the existence of a rhythm of this kind in the response is no indication of the existence of a similar rhythm in the stimulus, and since a single wavelet has a duration similar to that of a response to an instantaneous stimulus, we may infer that the central stimulus is not of the nature of a series of such stimuli.

The other kind of rhythm, that of the waves, which is much more frequently absent from the records, and the absence of which seems to be determined by the extent to which the animal is affected by the drug at the time the records are taken (I say advisedly "the animal" and not "the central nervous system"), does apparently, when present, tell us the rate at which stimuli were coming from the cord at the time. The form of each wave also tells us that the effect of each stimulus was reduced, sometimes even to zero, before the effect of the next began, and suggests (but this is by analogy only at present) that each stimulus had a duration corresponding only to that part of the wave in which wavelets are present. Moreover, the different forms of wave which occur in different experiments suggest that this stimulus ("Zeitreiz" of v. Kries) sometimes rises to its maximum strength quickly and sometimes slowly. My records, in which waves are altogether absent, suggest, as did v. Kries's observations (7) and (8), that a single central stimulus may have quite a long duration—not infrequently one of a third of a second—sometimes even lasting for a whole second [see (4), pl. ix., ph. 46]. It is, therefore, by a study of what occurs in this 3 to 14 per second rhythm, of what occasions its absence or presence, and of the conditions which modify it, that we can alone hope, it seems to me, to come to any satisfactory conclusions as to the nature of the normal stimulus, and to ascertain whether or not it is rhythmical. For this purpose we must use animals in which it is possible to modify conditions for any one part independently of other parts; and, since temperature is the easiest condition to alter, it may be that polikothermal animals will give us most information. But the experiments will have to be repeated with warm-blooded animals before we are prepared, when we meet with a rhythm in the effect of so complicated a

thing as the electrical response which accompanies the normal contraction of skin-covered muscles in man, to say anything as to what it indicates. Only if such experiments gave positive results—e.g. if it could be demonstrated that the rhythm obtaining in the electrical reflex response of the gastrocnemius of the rabbit, which, according to Piper [(1), p. 332], has a frequency of 50 per second like that of the lower-arm flexors of man, can be definitely reduced in frequency by cooling any part of the central nervous system while the temperature of the muscle remained constant—only then should I feel inclined to admit Dr Piper's conclusion that the rhythm he has observed in his records is also that of the normal stimulus. Until I have any evidence of this sort, I cannot help thinking that the rhythmical effect which both he and I have recorded in human muscles contracting to the normal stimulus, partakes of the nature of the wavelets seen in my frog records rather than of that of the waves. But before giving my reasons for so thinking, I must give some account of my own experiments on such muscles, in which the electrical response was recorded with the same instrument or with one having the same properties as the one used for the frog experiments I have just described.

### III. THE RHYTHM EXHIBITED IN CAPILLARY ELECTROMETER RECORDS OF THE RESPONSE OF MUSCLE TO VOLUNTARY EFFORT IN MAN.

I have taken records of the response of the lower-arm flexors with about twenty different people, with some of them on more than one occasion. I am indebted to about a dozen Oxford undergraduates and to a few other friends (four of them women, and two of them children) for their kindness in acting as subjects for these experiments.

Methods.—As leading-off electrodes I have, as a rule, used sponges soaked in salt solution, and tied up with muslin to prevent possible alteration of area of contact when the muscle contracted. They were applied to the skin, the one over the particular part of muscle required to give its record, the other to that over some other part of muscle or other tissue. Outside each sponge was a zinc rod with binding screw. I have also used, in certain experiments, non-polarisable electrodes similar to those used by Piper, or others made by interpolating a zinc-sulphate-containing sponge between the sponge which touched the skin and the zinc. There is not the same necessity for using non-polarisable electrodes with the capillary electrometer as there is with the galvanometer, and therefore, when I found that it made no difference to the result (the record) which of these three kinds of electrode I employed, when trying them all in turn on the same person, I felt justified in continuing to use the simplest and most convenient kind which I had used in the first instance. The electrodes were each held in position by elastic bands, the positions being, with each person to begin with, the same as those chosen by Piper. The arm rested supine on an insulating support, and in the hand was a dynamometer in

the form of a compressible steel ellipse with scale. That electrode which was over the part of the muscle felt to become most tense when the dynamometer was squeezed was, to begin with, always connected with the acid of the electrometer. The image of the meniscus was projected through a slit on to a photographic plate moving at a known and equable rate inside a dark box. There being no eye-piece to the microscope, the image was inverted. The distance of the plate from the image was such as to magnify it with the objective used about three hundred times in all cases. The rate of movement of the plate was shown by the vibrations of a spring in unison with, and driven by, a 100 tuning fork. The trolley carrying the photographic plate broke a key when it began to pass the slit which left a record on the plate of the moment at which it was broken. The subject was, as a rule, told to contract the muscle that was being investigated—to clench his fist or his jaws, for instance—only when he heard this key break, so that while he was reacting to sound the meniscus might inscribe its resting position on the plate.

The effect observed. Evidence that it is not to be attributed to inequalities of pressure of the skin on the electrodes.—Fig. 4 is typical of the effect produced upon the electrometer by the flexores digitorum. After recording it for the first time, I made the following experiment for the purpose of ascertaining whether or not what I had recorded could be due to variations in the pressure of the skin against the sponge, for such variations there must undoubtedly be when the muscle suddenly becomes tense. A piece of dry bandage was tied loosely round the arm of the subject, electrode and all, at the place where the tension of the muscle becomes greatest when the fist is clenched. A second person, holding the ends of the bandage, pulled them as hard as he could, so as to tightly press the zinc rod and intervening sponge against the arm of the subject while the plate was passing the slit, the subject himself being passive. The plate was passed through under such conditions alternately with other plates which were passed through while the subject was actively clenching his fist, beginning to do so at the sound of the signal. While all photographs taken with the muscle contracting at the given signal showed an effect of the kind reproduced in fig. 4, all those taken with the muscle passive, but the pressure on the skin increased by the second person, showed a meniscus tracing as smooth throughout as that seen during the reaction time in the alternate photographs. This experiment makes the presumption strong that the effect observed when the muscle contracts actively is really a muscular effect.

What strikes us most in all the records is the great irregularity of the undulations which indicate electrical variations, irregularity both in duration and in the steepness which in capillary electrometer records indicates amount, or strength, of variation. The irregularity is of the same kind and the variations have the same sort of frequency as have the wavelets in records obtained with many frog's muscles in strychnine spasm

(see fig. 3, p. 219) or in frog's muscle made to give a persistent contraction by the application of a descending constant current to the motor nerve, by breaking an ascending constant current running through the nerve, or by the stimulation of either the nerve or the muscle itself by a series of instantaneous stimuli following one another in such quick succession that they are unable each individually to produce a distinct effect. So great is the irregularity of the excursions in the records obtained with these arm muscles in most people, that it is very difficult to say what their frequency is. One frequency may prevail for a few hundredths of a second, then another, then the first back again, or yet another. In most of the records a frequency of 100 to 120 per second manifests itself somewhere, also one of about 60 to 80 per second. It was only very rarely that one as low as 50 per second also appeared, only, in fact, in four of all the people with whom I have so far taken records; in one of whom, however, it even at times became as low as 40 per second (see table on p. 227). The effect, although it varies greatly in different people, does not, so far as my present experience goes, seem to be altered in any definite way either by sex or by age. The jags on the curve are often—and in the records taken with some people more than in those taken with others—thrown into groups which recur with a frequency of from 14 to 30 per second, but the curve outline is so irregular that I can lay but little stress upon the presence of these groupings. It may, however, be worth mentioning that groupings of the same sort of frequency occur in records taken with the masseters, where, as we shall presently see, the excursions which fall into groups have a much greater and more constant frequency than they have in the arm muscles.

In the hope of getting more information as to what it was that my records represented, I have, with one or more of the subjects, attempted to alter the effect by varying certain conditions.

(a) The contacts.—The connections with the electrometer were usually such that the part of the flexors which became most tense when the fist was clenched was connected with the acid. The first excursion which the meniscus made from its resting position was then always adostial (upwards in the records), not only when the second electrode was distal to it, as it usually was, but also when it was on the elbow or on the shoulder. Thus the spot chosen as being mechanically the most active one always becomes galvanometrically negative before any other spot, whether or not these also become negative afterwards. When the connections with the electrometer were reversed the first movement was always abostial. This is, of course, only what was to be expected on the assumption that what we are recording is essentially a muscular phenomenon, and the fact therefore helps to support the assumption. The records give however, as a rule, little, if any, evidence of the way in which the connections had been made except at the start, so that unless this appears on the plate I doubt whether anyone could tell with confidence from the records which way the connections had been made.

This means that it is difficult to find any place on the arm which, to judge from the records, remains wholly electrically inactive when the fist is clenched. I tried to do this by varying the position of the second leading-off electrode, leaving the one applied to the most active part of the flexors and connected with the acid of the electrometer, in the same position. Records were always first taken with the second electrode on the skin over the tendons of the flexors near the wrist (position i.). It was then sometimes moved so as to be also on the contracting muscle at a place about 10 cm. distal to the other electrode, i.e. presumably on a spot of nearly the same activity (position ii.); or it was placed (iii.) either on the back of the elbow or on the shoulder, i.e. on a spot likely to remain inactive when the flexors contracted. So long as the two electrodes made contact with equal areas of the skin, the records taken with the second one in positions i. and iii. gave as little evidence that the effect represented related to what was happening under the first electrode only, as did those taken with it in position ii. The amplitudes of the excursions were, however, less when the second electrode was in position ii. than when it was in position i. It was in position i., in the records reproduced in figs 4, 5, and 6. There is, so far as I can see, no way of assuring oneself of the neutrality of any particular spot in the arm of a living person while it is performing any voluntary action. It would be easier to assure oneself of the neutrality of some quite other part of the body so far as mechanical action is concerned, but no other part of the body save the limb to which the fixed electrode is attached will serve the purpose when it is the electrical action which is concerned, because the changes due to the heart's electrical action are apt to appear whenever the two electrodes are far apart on the body and add further complication to the record (see note on p. 241).

By reducing the size of the area of contact made with the second electrode I have, however, obtained records which, to anyone accustomed to the interpretation of them, would suggest that the spot under one electrode was recording alone, or to a much larger extent than the other. Fig. 5 shows the records of the voluntary response given by the flexors when, on the one hand (A), the two electrodes (in position i.) were each 3 to 4 cm. in diameter; and when, on the other (B), the one of them (that connected with the mercury) was reduced so that it made contact only with an area of skin  $1\frac{1}{2}$  cm. in diameter. The rounded summits of the individual excursions in B contrast strongly with the pointed summits in A. The capillary electrometer shows the same sort of difference between the action currents (*Einzelschwankung*) of a frog's sartorius when, on the one hand, the tendon end (connected with the mercury) is devitalised, and when, on the other, it is sound.

All the records furnished by the flexors of the same individual as those reproduced in fig. 5 were far more regular than were those supplied by any

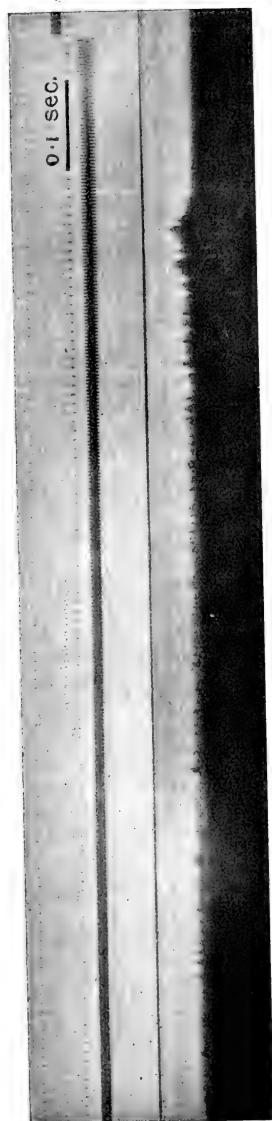


FIG. 4. Voluntary electrical response of Flexores digitorum in man. The signal to contract for the muscle to contract is seen on the right-hand side on the line s below the 100 fork tracing.

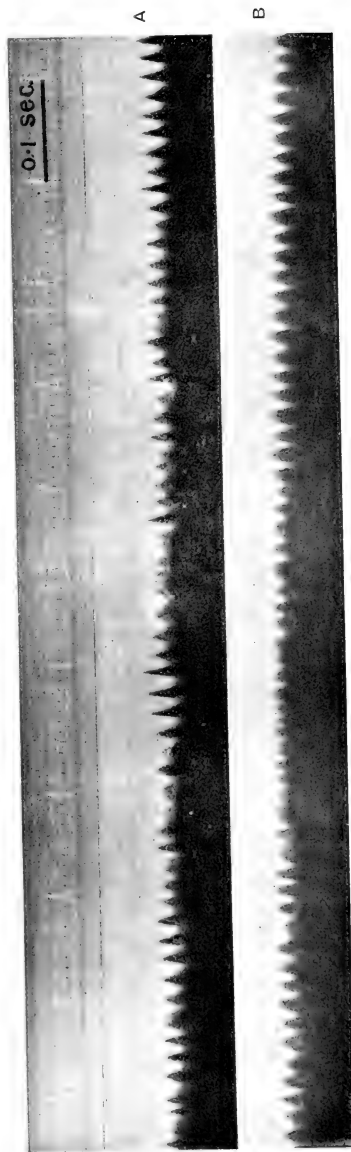


FIG. 5.—Voluntary electrical responses of the Flexores digitorum in man. The signal to contract had been given about a second before the plate began to move across the slit, so that the response had begun before it began to be recorded. Tracing to be read like the others, from right to left.  
 A, The two skin surfaces led-off-from of equal size.  
 B, The one over the tendons (under the electrode connected with H<sub>2</sub>) much smaller than the one over the most active part of the muscle.

other individual I have so far tried, and this was true on each of three separate occasions. Similar records have since been obtained with one other person (see Addendum). All the experiments in which the position of the leading-off electrodes was altered were made upon him also. When most regular the frequency was slower than it is in most people, and each individual effect was stronger. The strength of the mechanical effect was not in any way remarkable (see p. 227).

When both electrodes were small the ascents as well as the descents of the separate excursions were less steep, i.e. the effects, as Piper has shown, are weaker. I attribute the difficulty I had experienced until lately in recording the electrical response of voluntary muscle in man to the fact that I had previously tried to obtain it by leading off from surfaces too small for such negativity as might prevail under them to be able to affect the electrometer.

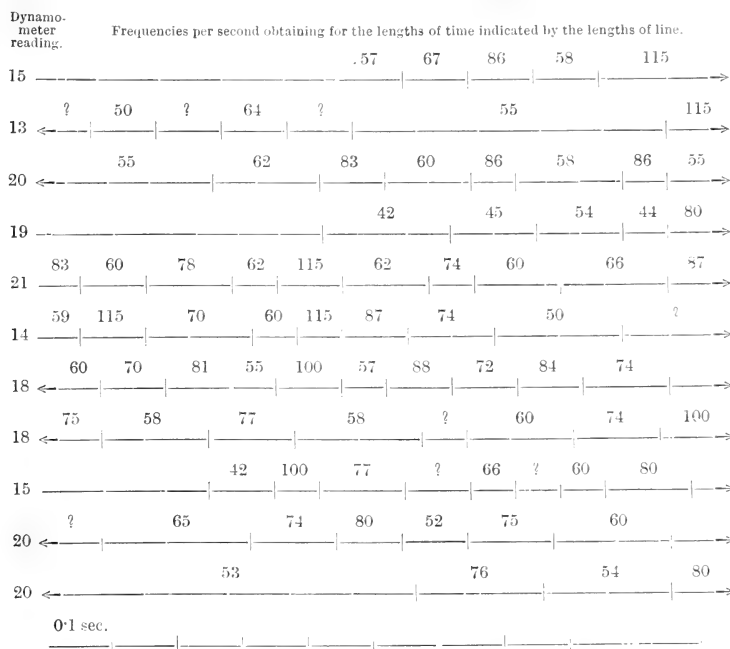
(b) The recording muscle.—Besides the muscles in the lower arm, I have used those in the hand and those in the jaw. In the hand muscles a response frequency of 100 to 140 per second appears more often than any slower one. Records taken with the masseters show a series of very fine teeth the frequency of which is more uniform than it usually is in records obtained from the arm muscles. It is always high, frequencies of 170 to 180 or even 200 per second being met with. I shall have to refer again to the difference of frequency obtaining in different muscles (p. 237).

With one subject an attempt was made to alter the temperature of the fibres, and records were taken when the recording arm was now at room temperature ( $13^{\circ}$  C.), now in an incubator kept at  $35^{\circ}$  C. Although the records were good they exhibit the usual irregularity, so that I should hesitate to draw any conclusions from them. I hope to repeat the experiment with the subject who gave the fig. 5 records.

(c) The voluntary effort.—With each person records were taken when he was grasping as hard as he could, and the dynamometer scale was read. With some people records were also taken when they were purposely not exerting their full strength, but such as to make the scale read about half or two-thirds of their maximum. The difference in the strength of the effect, i.e. in the steepness of the ascents and descents of the undulations on the curve, was very marked in any two such records taken with the same person, just as the difference of amplitude of the swings of the fibre of the string-galvanometer was marked in Piper's records. The whole response was, however, of the same type, i.e. the outline of the curve was equally irregular in the two cases with most people; and it was just as regular when the contraction was weaker as when it was strong in the flexors of the individual with whom the fig. 5 records were taken, although the jags were sometimes so small that they were difficult to count. To ascertain whether or not the frequency of the rhythm varies with the strength of the effect, the records obtained with this individual are the most useful.



The following table shows the frequencies which obtained, and the relative length of time for which each obtained, in eleven successive records, seven of which were taken when he was squeezing the dynamometer as hard as he could, the four others when he was purposely doing less:—



As his reaction time was extraordinarily long (3 to 5 tenths of a second), he was given the signal to clench before the trolley had quite reached the break key when all but three of these particular records were taken. The effect however, must have only just begun when the plate began to pass the slit. In two of the records which show the start the frequency is certainly lower than it ever seems to be subsequently, i.e. during the length of time the plate took to pass (a little over a second). I have noticed such a slow beginning in records taken with a few other people, but it is not universal, nor did the attempt to give a slow instead of a sharp contraction to the maximum make it appear when it was not otherwise so. In the present instance, as in most others, the attempt was made to bring the pointer of the dynamometer up to whatever was intended to be its maximum reading as quickly as possible after the signal had been given. I can find no evidence in the measured records of any definite relation between strength

of mechanical effect and frequency, so that I am inclined to agree with Piper that the amount of the voluntary effort does not affect the response frequency, even though I find this to be so very far from constant. The two records reproduced in fig. 5 were the third and the last, in both of which the squeeze was maximal.

When we come to compare the mechanical with the electrical effect of a voluntary effort in different people, we find that the stronger mechanical effect is by no means always accompanied by the stronger, or even by the more regular, electrical effect. Thus the maximal reading of the dynamometer produced by the person just referred to as giving the strongest and most regular electrical response was 21, and on most occasions when he was trying to do his utmost the reading fell short of this and was only 18, 19, or 20. Almost immediately afterwards the experiment was repeated (with exactly the same recording arrangements) with the flexores digitorum of another subject who brought the reading up to 33 while the electrical response was being recorded. The records showed that the separate excursions of the meniscus were less strong (ascents less steep) and also less regular, although more regular than in a good many people. It would, I think, be premature to do more than call attention to this fact at the present moment. It is illustrated by a comparison of figs. 4 and 6 A with fig. 5 A, the scale reading 25 with each of the two people whose flexors gave figs. 4 and 6 A. So far as the strength of the electrical effect is concerned, since we know that in the same person this varies with the strength of the mechanical effect, we must look to something outside the muscle and the voluntary effort to account for the differences in different people. Variation in conductivity of tissue (fat and skin) interposed between muscle and electrodes is what first suggests itself, but I have as yet made no attempt to measure this.

#### IV. CONTRAST BETWEEN THE CAPILLARY ELECTROMETER RECORD OF THE VOLUNTARY RESPONSE AND THAT OF THE SAME MUSCLE TO ARTIFICIAL EXCITATION OF ITS NERVE BY A SERIES OF INDUCTION SHOCKS OF A FREQUENCY OF ABOUT 50 PER SECOND.

Below the record (fig. 6 A) given by the flexor muscle in response to the will there is reproduced (fig. 6 B) the record (on a plate moving at the same rate) given by the very same muscle immediately before (when the leading-off electrodes and the surface to which they were applied were precisely the same) in response to a series of induction shocks applied to the skin over the median nerve where it comes nearest to the surface just under the lower end of the biceps. The artificial stimulus was supplied by the vibrator of a Kronecker inductorium with secondary coil right up and no core in the primary coil. It produced a strong sensation. Contact was made and then broken 44 times a second. Whereas the subject was exerting himself in order so to compress the steel ellipse that

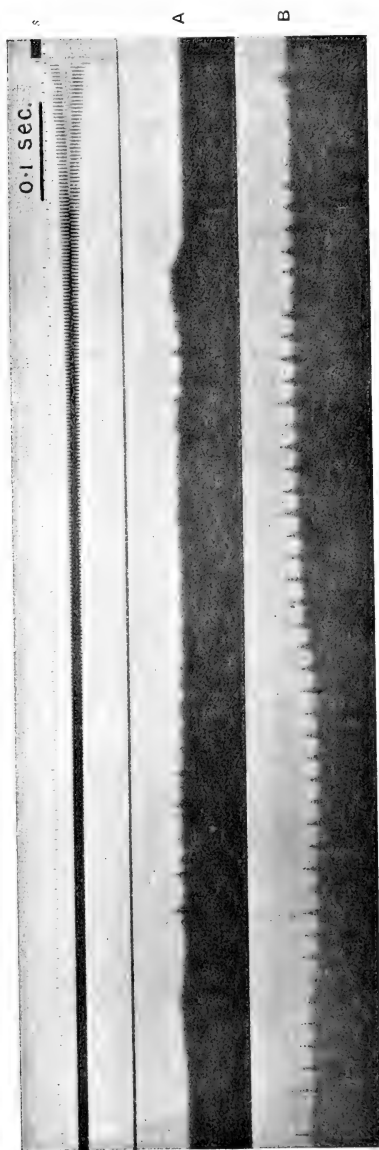


FIG. 6. — Electrical responses of Flexor digitorum in man.

A, Voluntary. A short strong grasp when the signal was heard.

B, Involuntary. Induction shocks 44 per second began to act on the median nerve when the signal key was broken.

the pointer might give a high reading when Photo. 6 A was taken, the artificial stimulus used when Photo. 6 B was taken only caused his fingers to bend over so as to touch the dynamometer, but hardly to compress it at all, and not enough to make the pointer move. The electrical response began about two-hundredths of a second after a short-circuit to the induction shocks had been broken by the break-key inside the dark box, and appears as a series of separate meniscus excursions succeeding one another at regular intervals of time (corresponding to that between each two breaks), not all precisely alike but each being the expression of a short and strong electrical variation beginning with relative negativity at the proximal contact and ending with relative negativity at the distal contact. To begin with each contact seems to have become in turn negative to the other by the same amount, but after about half a second the relative negativity of the distal contact (made with the skin over the tendons of the muscle) seems to have become greater than had been the relative negativity of the other contact (made with the skin over the most actively contracting part of the muscle) immediately before. The same indication (greater steepness of descent than of ascent) of the relative negativity becoming greater at the contact made with the skin over the tendons, appears in many of the records obtained when the will supplies the stimulus, but only when, as here, the distal electrode made as large a surface of contact as the proximal (e.g. fig. 5 A). It must be remembered that negativity of a contact with an active spot of muscle to one with a spot known to be inactive is often followed by positivity to it (*Journ. of Physiol.*, xxiii, p. 335), so that when two possibly active spots are recording, the relative negativity of the last one to become active may be the sum of the negativity of its own contact and of the positivity of the proximal contact. I have never noticed greater steepness of descent in the records (greater velocity of the abostial than of the adostial movement of the meniscus) when both contacts have been made with skin directly over the muscle itself, i.e. presumably with nearly equally active parts of the muscle.

The steepness of each ascent and descent in the artificial tetanus record presents a contrast to that observed in any of the undulations which can be recognised as distinct in the record obtained when the very same muscle (or group of muscles) was contracting in response to the will, and producing a far greater mechanical effect. The meniscus always came to rest between each two excursions; and it did so also, although for a shorter time, when the core was in the primary coil, as is shown by two records taken under such conditions. When the exciting electrodes were not near enough to the median nerve to provoke contraction of the muscle (i.e. movement of fingers or of wrist or of both), the photographs showed that the meniscus remained at its resting position throughout, even when the shocks were distinctly felt down the arm as well as up it (see Addendum, p. 241).

V. CONCLUSIONS TO BE DRAWN FROM THE FOREGOING EXPERIMENTS.  
FURTHER EXPERIMENTS WHICH FAVOUR THEM.

The conclusions that I have come to from my own experiments, comparing the records obtained with human muscles with those before referred to as obtained under simpler experimental conditions in the frog, and having regard in each case to the difference of character between the electrical responses to artificial discontinuous stimulation, by means of induction shocks at the rate of about 50 per second, and to a central stimulus, are:—

(i.) There is no certain indication that the central stimulus is discontinuous at all (unless interrupted by other stimuli), but if it be so, the separate stimuli are likely to be each what v. Kries has called a *Zeitreiz*. Whatever its nature it is certainly not a series of instantaneous stimuli recurring with a frequency of 50 per second.

(ii.) The rhythm which appears in electrometer records of human skin-covered muscle in voluntary contraction is as purely muscular in origin as is that proved by experiment to be so in the bared muscle of the frog thrown reflexly, or in a variety of other ways, into persistent contraction; its frequency may vary in different muscles, and in different kinds of fibre in the same muscle, between 50 and 200 per second.

Piper's records, which lead him to conclusions so diametrically opposed to these, differ from mine chiefly in exhibiting one particularly dominant rhythm, which he thinks is constant in frequency for each muscle, so that when a muscle is artificially excited by stimuli of the frequency denoted by this rhythm, he finds great resemblance where I find great contrast when a comparison is made with the voluntary response.

The first thing which, of course, suggests itself in trying to account for the discrepancy is the difference of recording instrument, or rather of the particular pattern of each instrument, that we have been respectively employing. In order to judge of the suitability of a particular instrument for recording electrical changes of unknown, but of possibly high, frequency, and of unknown, but of probably varying, strength, we must in the first place assure ourselves of the quickness with which the recording part of the instrument can come back to its position of rest after it has been disturbed, and ascertain that this is independent of the amount by which it has been disturbed. To show to what extent the particular capillary electrometer I have been using is suitable for the purpose, I therefore reproduce here not a comparison curve (*Aichungscurve*) but what seems to me to be more to the point, the photographic record obtained when a series of break and make induction shocks of a frequency of about 50 per second are allowed to escape into a dead muscle, which is connected with the two terminals of the electrometer. In order to be able to measure the intervals between the successive effects, the photograph (fig. 7) was taken on a much more rapidly

moving plate than are any of the others introduced into this paper. It shows that, when the vibrator of the primary coil was breaking and making a contact 54 times a second, each induction shock was producing a separate excursion of the meniscus, the one in the one direction the other in the other. The break effect was strong (ascent, considering the quick rate of movement of the plate, steep), the make effect was a good deal weaker, but each had the same duration, namely,  $1.5\sigma$ . Between each break and make effect, and again between make and break effect, there was an interval lasting respectively about 6 and 5 times as long as either of the excursions themselves, i.e. about  $9\sigma$  and  $8\sigma$ , during which the meniscus resumed its resting position. I conclude, therefore, that the instrument I have been using would be capable of registering as distinct each of a succession of effects produced immediately by instantaneous electrical changes whatever their strength, and whether all of equal strength or not, provided their

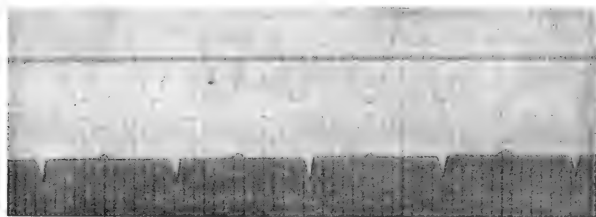


FIG. 7.—Diagram showing the effect produced upon the capillary electrometer when four make alternating with five break induction shocks were allowed to escape into it. Quick rate of plate, as shown by 100 fork tracing above.

frequency was below 700 per second. When the effects are not produced immediately by such changes, the highest frequency with which they can appear as distinct depends, of course, also upon the duration of each intermediate electrical change. When the electrical variation of the flexor muscle was interposed between the induction shocks and their effects on the electrometer, we may gather from the photographic record reproduced in fig. 6 B that the duration of the muscle effect was becoming shorter and shorter to successive stimuli. To begin with, it outlasted the stimulus producing it by nearly 0.02 second; before two-tenths of a second were over it was outlasting it by only about 0.01 second, and later by an even somewhat shorter time still; but whatever it was, the time taken by the instrument remained but a small fraction of it.

One would like to know, for each of the three string-galvanometers which were used by Piper, the time taken by the "string" (quartz fibre or platinum-tungsten wire) to come to rest, when disturbed by the immediate action upon it of a single induction shock. If we may judge by the escapes which are represented in the records reproduced in his second paper [(2),

pl. i., figs. 1, 2, 3], preceding the response of the flexors to a single break induction shock applied to the median nerve, it would appear that the fibre then in use took about, or nearly, three times longer to regain its resting position (i.e. 4 to  $5\sigma$ ) than did the meniscus of my electrometer, and, moreover, that the greater the excursion caused by the shock the longer was the time it took to regain it.

A record which Dr Piper was kind enough to send me at my request, of a series of very weak induction shocks of a 50 per second frequency, led into the fibre of the galvanometer he was using in his first set of experiments, showed that the fibre hardly halted (if it did so at all) at its resting position while passing it in either direction. The only record reproduced of the response of the flexors to artificial excitation of the median nerve by induction shocks of this frequency [(1) pl. i., fig. 3] was taken with the same fibre, and shows that it then never halted and that the swings were all precisely identical.

Knowing from Einthoven's work (9) how beautifully the string-galvanometer can be adapted to record accurately any kind of small electrical changes, I do not for a moment doubt that a fibre of (i.) such normal sensitiveness, (ii.) of such length, (iii.) of such resistance in proportion to that of the electrodes used and of the skin and the tissues lying between the electrodes and the muscle, (iv.) of such tension that its excursion is shorter than that, not only of some, but of all the variations it is likely to have to record, and yet (v.) so damped that it has no periodicity of its own—that such a fibre could be chosen and placed in such a magnetic field that it would reproduce the electrical variations which occur in human muscle in voluntary contraction better than the capillary electrometer can ever hope to do. Yet just because it has to be adapted to what it has to record, it seems to me that the string-galvanometer can never take the lead in giving information about electrical changes the strength of which and the frequency of which are unknown. A fibre of such kind and so arranged (as Piper says his was) as to reproduce accurately the electrocardiogram of man, is by no means therefore fitted to reproduce accurately electrical variations, seldom of much greater amount and some of them of smaller amount, recurring with a much greater frequency. It seems to me that it is not until a quick capillary electrometer has shown the nature of the variations to be registered that the time has come for the string-galvanometer to help us to understand better than we can from its own records what these mean. That it can then do it better follows from the fact that it records directly the relative difference of potential at each moment, whereas capillary electrometer records have always first to be interpreted in order to show this; and although it is easy enough for anyone accustomed to dealing with them to roughly interpret them at a glance, to do so accurately involves a great deal of time and labour.

Until I know, therefore, that the special fibres used by Piper were so chosen and so arranged that they had no periodicity of their own, and that

an excursion produced by a single induction shock was of as short a duration as it was with my capillary electrometer, and that it was equally short whatever the amount of the excursion, I cannot accept any evidence given by his records which is not in accordance with that given by mine. I cannot, that is to say, grant that for the lower-arm flexors the rhythm has a constant frequency of 47 to 50 per second, as he says it has in his first two papers, or even one varying only between 47 and 58 per second [(3), p. 510]. With regard to the masseters it may be noticed that he himself came to different conclusions as to their response frequency when he was using different instruments. With the first [(1), p. 332] he found it to be very irregular and varying between 60 and 80 per second; with the second [(2), p. 412] he found it to be 60 to 64 per second; with the third [(3), p. 509] 88 to 100 per second. Judging from the unpublished masseter records which he has been kind enough to let me see, taken, I believe, with the third instrument, I should myself have said that the response was characterised by greater regularity than was obtained with any other muscle, and that, when most regular, frequencies of 96, 100, 115 or 120 per second prevailed, although elsewhere it was sometimes 70 to 80 per second. My own records also show a more regular response with this muscle than is, as a rule, obtained with the flexores digitorum, although the frequency in the one person whose masseter response I have recorded was as high as from 170 to 200 per second. Some of Piper's other records, referred to, but not published, in his third paper, taken with several different muscles for a few seconds at a time, would also seem to me to show, as my own records do, that now one response frequency obtains, now another. With the gastrocnemius, for instance, I should have said that the records he sent me showed that frequencies of 73, 87, and 100 per second prevailed rather than the 44 which he estimates it at; with the extensor pollicis brevis 63 per second rather than 47 to 50; with the quadriceps femoris I would agree with him that one of the prevailing frequencies is about 44 per second, but I find others in the same, or in other records, of 52, 66, and 76 per second. I am, of course, counting something different from what he is, but the fact that two people may come to such different estimates with regard to the frequency from the same records shows the need for something more striking than he has yet given us, in the way of evidence that the muscles supplied from any one special centre in the cord or in the medulla oblongata all exhibit the same response frequency [(3), p. 516].

To my second conclusion (p. 231), that the rhythm observed originates in the muscle rather than in the central nervous system, Piper raises two objections [(1), p. 328], namely:—

(i.) That if the rhythm were not due to the central nervous system, we should expect to see in the records indications of another, and slower, rhythm as well, and that we do not. I do not see the necessity for expecting it. It by no means always appears, as I have shown (both in 1901 and in the present paper) in reflex responses of frog's muscle, even when in



strychnine spasm. Granting for the moment that a series of *Zeitreize* are coming from the cord, their frequency would only manifest itself in the records if the interval between one *Zeitreiz* and the next was so long that the effect of the first was over before that of the next began. It very frequently becomes so, or can be made to become so, in the strychnine frog, and then the rhythm of from 3 to 14 per second, already described, manifests itself in the records, with the quicker rhythm of 40 to 120 per second superimposed upon it. On the other hand, I am not at all sure that it is true that we have no evidence of a slower rhythm as well as of one varying between 40 and 120 per second, in the lower-arm flexors of man contracting at will. Many of my records show, as I have said (p. 223), a grouping of the undulations on the curve, the groups occurring with a frequency of from 14 to 30 per second. The beginning of such a group is always a particularly steep ascent, which means a particularly strong variation, such as I have shown (1901) to be frequently present at the beginning of each wave in the strychnine spasm record of the frog.

(ii.) Piper's second objection to my view is that, "keeping to physiological conditions of experiment" (by which, I suppose, he means avoiding the use of drugs such as strychnine) a muscle never responds with rhythmical oscillations to a single stimulus. This is true perhaps with most muscles, if the single stimulus is instantaneous. But, as I have recently shown [(10), fig. 2], even to a single instantaneous stimulus the gastrocnemius of the frog may give more than one oscillation in the record of the electrical response; and it and other muscles certainly do so when excited by break ascending galvanic currents [(4), pl. vii., ph. 33, 34]. Moreover, with a stimulus of somewhat more appreciable duration, such as that given by Garten's guillotine [(6), p. 340], oscillations seem always to occur. So far as I am aware, no records have been taken with a quick capillary electrometer of the response of muscle excited by v. Kries's *Federreonom* (7), and the oscillations in question are far too quick to be observed by the eye.

The view which I am now upholding, that the rhythm of the electrical response accompanying voluntary contraction is mainly of peripheral origin, is that of Wedensky [(11), p. 260]. I was unable to accept it in 1901 [(4), p. 152], partly because I had then no records of the voluntary response to compare with my records obtained with frog muscles, and partly because I was not always able to follow, as I then said (p. 139), his descriptions of the sounds he heard with the telephone.

The fact that the frequency is not altered by the strength of the central stimulus provoking it—a fact insisted upon by Piper and not contradicted by anything in my records—seems to me, as far as it goes, to furnish evidence in favour of this view, or rather against the view that the response frequency represents the innervation frequency. I have already shown [(4), p. 135] that when artificial stimuli of a frequency capable of impressing itself upon the muscle, are applied to the nerve of the frog's

sartorius, the muscle responds by a lower frequency if the stimuli are weak [(4), pl. vi., ph. 30 and 31]. Experiments made soon afterwards (in 1902), but of which no account has yet been published, showed what I was then not expecting, namely, that when stimuli of a frequency so great as to be incapable of impressing itself upon the muscle were applied to the nerve, however much their strength was reduced, the response frequency was not reduced; that is to say, although the response frequency differs in different records, as it always does when it bears no relation to the exciting frequency, it does not vary with the strength of the stimulus. Fig. 8 A is a record of the response of a sartorius muscle the nerve of which was excited by the vibrations of a 500 fork acting upon a telephone transmitter, with a whole Daniell in the primary circuit of the telephone transmitter. The undulations are seen to recur with a frequency of about

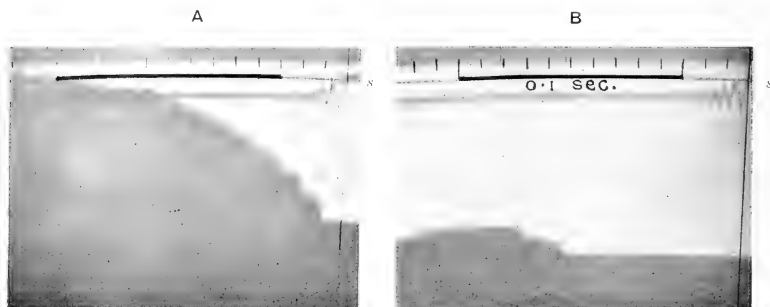


FIG. 8.—Electrical response of frog's sartorius to excitation of the nerve by currents of high frequency (to a tuning fork giving 500 d.v. per second, sounded in front of a telephone transmitter). Recording surface describing an arc. Rate of movement indicated by a 500 fork.

A, a whole Daniell in primary circuit. B, only a third of a Daniell in primary circuit.

88 per second. Fig. 8 B is the record obtained when only one-third of the Daniell was in the primary circuit. The muscle gave a much smaller contraction, and all four times that the electrical response was recorded with the strength of the stimulus so much reduced, it began, as in the record reproduced, a much longer time after the currents began to act on the nerve, i.e. after the break of a short circuit by the signal *s*. When the response began, however, the undulations had always a frequency as great as in any of the records taken with a stronger stimulus. In all four it was about 100 per second, whereas in the eleven records which were taken with the stimulus stronger the frequency varied between 65 and 100 per second. A stimulus therefore, which may be regarded as a continuous one may produce the same rhythm in muscle whatever its strength, whereas a series of stimuli of a frequency low enough for the muscle to follow, as one of 50 per second would be, and is, may fail to be all of them effectual when their strength is reduced.

Granting that the rhythm in voluntary contraction is, or is mainly, of peripheral origin, how are we to account for the differences in frequency which prevail in my records of the responses of one and the same muscle, and for those which both Piper and I find to exist in different muscles? To anyone who has taken records of the electrical response of the same and of different muscles in a large number of strychnine frogs, there is nothing surprising in the amount of variation which occurs in the frequency of anything which may be compared to the wavelets. As I have said elsewhere [(4), p. 148], the frequency of these in the frog's sartorius in strychnine spasm may vary in different preparations from 40 to 100 per second. Although I did not actually state for the strychnine reflex responses that the frequency is generally greater in the gastrocnemius than in the sartorius, the records reproduced [(4), pl. vii. ph. 36 (sartorius) and 38 (gastrocnemius)] showed it, and they were, and are, typical. I did, as a matter of fact, draw attention to the difference of behaviour of the two muscles in this respect when the response was to a continuous stimulus, or rather to one of very high frequency [(4), p. 139]; or when it was to the break of an ascending current through the nerve [(4), p. 142, and pl. vii. ph. 33 (sartorius) and 34 (gastrocnemius)].

In the reflex responses of strychnine a wavelet frequency of about 100 per second is most often met with in records taken with the frog's gastrocnemius, and the frequency is much more constant (whether there are waves or not) than it is with any of the other muscles I have used. In records taken with the sartorius a wavelet frequency of 50 to 60 per second more usually presents itself, either by itself or side by side with a quicker rhythm. Recent experiments have shown that with the triceps femoris as with the gastrocnemius a quick rhythm is more usual, while with the biceps femoris and the semitendinosus two or even three different frequencies are apt to prevail in turn in one and the same response, so that they behave more like the sartorius.

With regard to the significance of these differences of rhythm in the different muscles of the frog, I would suggest that they are due to the differences which Bonhöffer (12) has shown to exist in the kind of fibre composing the different muscles, and in the proportion of one kind to another in a single muscle. In view of the fact that it is the thick, "quick" fibres (of Grützner), those which are poor in sarcoplasm, which are present almost exclusively in the gastrocnemius and the triceps, it would be these fibres which exhibit a response frequency of about 100 per second to any kind of stimulus which is not of such nature as to impress a rhythm of its own upon them. In the sartorius and biceps there are as many, or even more, thin, "slow" fibres, rich in sarcoplasm, present. Bonhöffer says nothing about the semitendinosus. My experiments suggest that in it also the two kinds of fibres would be found.

I have not been able to find any description of the histological structure of the masseters of man compared with that of the flexores digitorum

(or other arm muscles), but my records of the electrical voluntary response, taken with the two muscles—those with the one giving principally a rhythm of over 170 per second, those with the other giving one that varies between 40 and 120 per second—suggest that histological investigation would show that the fibres composing the masseters are much more like one another than are those of the flexores digitorum, and that they are all of the "quick," thick variety. I do not see why it should be necessary to assume, as Piper does [(1) p. 331], that waves must travel with the same speed along all the fibres of the flexors. My recently obtained records lead me to expect to find fibres of different kinds in the limb muscles of man just as much as they are known to occur in those of the frog. By the same token I can now no longer doubt that the rhythmical electrical effects so often observed in frog's muscle responding to continuous stimulation, are normal, a point upon which I hesitated to express an opinion in 1901 [(4), p. 138]. The occasional absence of rhythmical effects with the three kinds of non-discontinuous stimulation I then employed, and its universal absence in veratrinised muscle, may be accounted for in a way which I hope to discuss elsewhere.

#### VI. THE REFLEX ELECTRICAL RESPONSE IN MAN.

When the median nerve is stimulated by a single strong break induction shock while the flexor muscles in the lower arm are connected with the capillary electrometer in the usual way (p. 221), the meniscus begins to inscribe a curve on the plate about  $5\sigma$  later. It indicates that the proximal contact and then the distal became each in turn negative to the other, and that at the end of about a hundredth of a second, although there was slight persistent relative distal-contact negativity, the effect of the direct stimulus was nearly over; and that then, some five or six hundredths of a second later, the proximal contact and then the distal each became negative in turn, three or four times in succession, but by a very much smaller amount than before. In view of the records obtained when the intact mixed nerve supplying the gastrocnemius of the frog is stimulated by a break induction shock, which I have recently published [(10), figs. 1, 2, 3], I cannot help regarding the second effect as a reflex effect, i.e. as the effect produced by the artificial stimulus on afferent fibres, and only on the muscle after the intervention of the central nervous system. Fig. 9 shows one such response. The second effect, lasting some three or four hundredths of a second, only made its appearance when there was a core in the primary coil, but it then appeared (in the one person so far who has been always successful in applying the small exciting electrodes used, to the only place on his arm which will serve to produce the direct response, and therefore the same person who supplied the two records reproduced in fig. 6) with the secondary coil at 7000, there being as usual only one Daniell in the primary circuit. The six records in which the second effect is seen, some of them taken on one day and some on another, all show that it occurred at almost precisely the

same moment after the stimulation of the median nerve, i.e. after  $72$  to  $75\sigma$ . If I am right in my interpretation of it, and if we allow from  $20$  to  $30\sigma$  for the time taken by the impulse to travel the distance of about a metre along the nerve from the place of stimulation to the neck and back again, and if we deduct this and the  $5\sigma$  taken by the impulse to reach the first recording spot of the muscle along the motor fibres, from the  $75\sigma$ , we should arrive at the conclusion that  $40$  to  $30\sigma$  had been spent in crossing the synapse, or the synapses, in the part, or parts, of the central nervous system concerned in such a simple reflex.

If we examine the records taken by Piper of the same thing [(2), pl. i., figs. 1, 2, 3], we see also, some  $86\sigma$  after the first effect is over, and thus some  $115\sigma$  after the excitation, a very small second effect followed a little

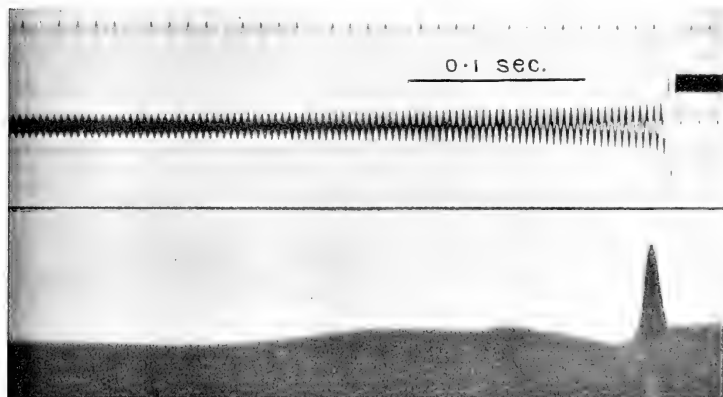


FIG. 9.—Direct and reflex electrical response of the flexores digitorum in man to a single break induction shock applied to the median nerve.

later by others of the same kind. He has just drawn attention to its presence on p. 399. I would suggest that it also is a reflex effect.

The contrast between the direct and the reflex effect in fig. 9 seems to me to be only an exaggeration of the contrast between the direct response to artificial stimulation recorded in fig. 6 B and the voluntary response recorded in fig. 6 A. The one effect is strong and single, stronger than any of the effects (produced without the core in the primary coil) in fig. 6 B; the other effect is very weak, weaker than the voluntary response, but prolonged. This difference between the electrical effects produced when, on the one hand, the motor nerve is excited by an instantaneous stimulus, and, on the other, by a stimulus coming (proximately) from the centre, together with the fact brought out so plainly in my experiments (pp. 228 and 241) that the mechanical effect produced by the central stimulus may be so very much greater than that of a series of artificial direct stimuli, suggests that

the mechanical effect depends for its strength not so much on the strength of the stimulus as on its duration. An artificial instantaneous stimulus or each of a series of such produces a greater electrical variation than is, in the same muscle of the same person, produced by a central stimulus, but it lasts so much shorter a time that it is unable to effect what a more prolonged, even though weaker, stimulus may do. In this connection I was interested to learn recently from Dr Waller that he had long ago recorded the reflex mechanical effect of the extensors of the leg in man to a single instantaneous stimulus to the nerve, and that he had found it to be much stronger in proportion to the direct effect than my records show to be the case for the reflex electrical effect.

#### VII. SUMMARY.

In the electrical response of human muscles to their normal stimulus a rhythm presents itself which for the flexores digitorum has no constant frequency but may vary during one and the same response between 50 and about 120 per second, but which for the masseters is more uniform and of a higher frequency (170 to 200 per second).

This rhythm is to be regarded as of peripheral origin, because it is of the same order and subject to the same sort of variation as is a rhythm which experiments on frogs have proved to be of such origin.

As the corresponding rhythm may be produced in the response of frogs' muscle to various kinds of artificial stimuli which are not discontinuous, the rhythm obtaining in the electrical response of human muscle to the will has as yet given us no information as to whether the natural stimulus is rhythmical. Such information can only be gained by further experiments upon animals (not only upon cold-blooded, but on warm-blooded ones), in which it is possible to simplify and control the conditions to a greater extent than is possible in man.

#### VIII. NOTE ON THE EFFECT ON THE ELECTROCARDIOGRAM OF THE CONTRACTION OF THE VOLUNTARY MUSCLES IN MAN.

If, while the electrocardiogram is being inscribed by the meniscus of the capillary electrometer, the subject having his two hands in two basins of salt solution connected with the two terminals, the one fist is clenched, a number of fine teeth appear on the curve which before was smooth, both on that part of it which corresponds to the systole and on that which corresponds to the diastole. It was the observation of this effect which suggested the experiments on arm muscles described in this paper. The teeth do not appear when muscles of other parts of the body alone are put into action. They have a frequency of from 100 to 140 per second, which I take therefore to be that of the electrical response of the muscles of the hand. But there is another effect produced by clenching one fist on the electrocardiogram which is equally striking, namely, the sudden and

immediate shortening of the time between each two periods of activity of the heart. Even so slight an action as this, which does not affect the respiration, may considerably reduce the pause between the next two systoles, and within the next five seconds it may become half what it was just before when the whole body was at rest. The quickening is not maintained for long, so that in counting the pulse for one minute the increased frequency is less striking. There is, of course, the same sudden temporary quickening when other muscles are put into action or when a deep breath is drawn.

#### IX. NOTE ON THE MISUSE OF THE WORD "RHYTHM."

I tried in my former paper (4) to avoid the use of this word for a succession of changes characterised by not recurring at regular intervals. I still think it a misnomer to so apply it, but the word is so convenient, and has been adopted by other people, e.g. Garten (6) and Piper (1), regardless of any exception which might be taken to it, that I have now myself applied it and its derivatives to phenomena which recur with approximate, although not absolute, regularity.

The working expenses involved in making all the experiments referred to in this paper, with the exception of those made in 1902, have been defrayed out of a grant from the Government Grant Committee of the Royal Society.

I have to thank Professor Bourne and Professor Osler for providing me with the accommodation necessary for carrying on this and other experimental work.

#### ADDENDUM.

Since the above paper was written, another medical undergraduate has enabled me to record the electrical response of his lower-arm flexors to a series of artificial stimuli applied to the median nerve. It contrasts with the response to the will in the same way as that to which fig. 6 B refers contrasts with that recorded in fig. 6 A, but the records are of exceptional interest because the voluntary electrical effect was exceptionally strong and regular, and the involuntary electrical effect accordingly a great deal stronger and more regular, although accompanied as before by a very much smaller mechanical effect. Records of the voluntary response show that the electrical effects recurred at different times with frequencies of 100, 77, 56, and 52 per second. In fig. 10 A, part of a record is reproduced which exhibits chiefly a frequency of 56 per second. Fig. 10 B is the record of the very next response of the same muscle (with the contacts in nowise altered), not to the will, but to a series of 35 double induction shocks succeeding one another at the rate of 50 per second—the secondary coil being right up, but no core in the primary. The dynamometer was still in the same position in the hand, but while the voluntary effort producing the effect recorded in A made the pointer move to 37, the strong artificial excitation producing the effect recorded in B made the pointer move only

to 2 on the scale. Fig. 10 B gives the same evidence as fig. 6 B, that the first few shocks produce effects which outlast them by a longer time than the later shocks, and that between the successive effects, when these are each short, there is an interval during which there is little or no difference of potential between the leading-off contacts. Owing, however, to the

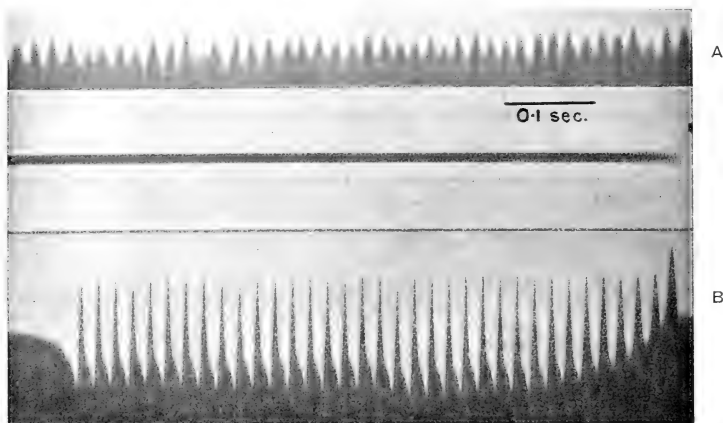


FIG. 10.

strong positive after-effect (relative distal negativity) at the end of each individual response, the absence of a fresh change at the proximal contact is denoted not by the meniscus being at rest as it was in the intervals when fig. 6 B was recorded, but by its striving to return to its zero position in the way which it eventually does when all stimulation has ceased.

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ON VAGUS CURRENTS EXAMINED WITH THE STRING GALVANOMETER. By W. EINTHOVEN (in collaboration with A. FLOHIL and P. J. T. A. BATAERD). (From the Physiological Laboratory of Leyden.)

(Received for publication 6th July 1908.)

THE demarcation current which may be derived from the peripheral end of a divided vagus nerve shows a negative variation whenever the lungs are expanded. This phenomenon was first demonstrated by Lewandowsky<sup>1</sup> with the aid of a Deprez-d'Arsonval galvanometer; later Alcock and Seemann<sup>2</sup> studied it more in detail with the capillary electrometer. In a research made by means of the string galvanometer we have been able completely to confirm the results of the above-mentioned authors, and also to obtain evidence of the presence of another source of stimuli

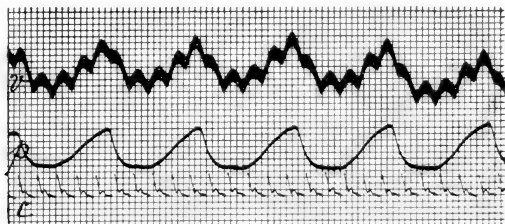


Figure showing the electrical changes in the vagus nerve which accompany the respiratory and heart movements.

v, electrovagram; p, respiration record (ascent of curve, inspiration; descent, expiration); c, pulse record.

situated in the heart—the vagus shows rhythmic action currents which synchronise with the heart-beats.

A vagus nerve in the neck of an anaesthetised dog is isolated for a considerable distance and divided at a spot high up in the neck. The current is led off from the peripheral cut end of the nerve by means of a pair of non-polarisable electrodes, one of which is brought in contact with the cross-section, the other with the surface, the space between the electrodes being

<sup>1</sup> Max Lewandowsky, "Ueber Schwankungen des Vagusstromes bei Volumänderungen der Lunge," Pflüger's Arch. f. d. ges. Physiol., Bd. lxxiii., S. 288, 1898.

<sup>2</sup> N. H. Alcock and John Seemann, "Ueber die negative Schwankung in den Lungenfasern des Vagus," Pflüger's Arch. f. d. ges. Physiol., Bd. cviii., S. 426, 1905.

about 1.5 cm. The demarcation current of the nerve is compensated in the usual way, and the electrodes are connected with the galvanometer in such a manner that a decrease of the demarcation current, or an action current, causes the image of the string to move in an upward direction. In the accompanying figure, 1 mm. of the abscissa corresponds to 0.2 sec.; 1 mm. of the ordinates, to 2.7 microvolts. The upper curve (*v*) represents the action currents of the vagus nerve, and may be called the "electrovagogram"; the middle one (*p*) reproduces the respiratory movements of the animal in such a way that every inspiration corresponds to an ascent and every expiration to a descent of the curve. This pneumogram is obtained by causing the dog to breathe into a large bottle and transmitting the oscillations of the pressure of the air in the bottle to a suitably placed recording Marey's tambour. The lower curve (*c*) indicates the sphygmogram of the crural artery.

It can be seen that the electrovagogram exhibits undulations having a double rhythm, viz.: (1) synchronous with the respiratory movements, (2) synchronous with the heart-beats of the animal. That these undulations are caused by the real action currents of the nerve and not by current escape from other organs, can be proved in different ways.

In the first place we may rely on the method employed, which does not appear subject to this source of error. We have further repeatedly submitted it to the usual proofs of control. Thus, if the nerve is ligatured peripherally to the electrodes or is killed by means of a drop of ammonia, the rhythmic oscillating image of the string is brought completely to rest.

In the dog, the respiratory oscillations of the right vagus are larger than those of the left one; while, on the other hand, the heart-beat oscillations of the left vagus surpass those of the same nerve on the right side. In the rabbit the vagus nerve shows exclusively respiratory oscillations, the depressor nerve solely heart-beat oscillations.

The excitatory state of the vagus endings in the lungs is determined by the volume of these organs and not by the intrapulmonary pressure. If during the cessation of the natural respiratory movements of the animal the lungs are insufflated artificially, the electrovagogram shows an undulation in the same direction as if the animal is making a natural inspiration. Nevertheless the insufflation is accompanied by an increase, the natural inspiration by a decrease of the intrapulmonary pressure.

The experiments which we have performed on the effects of insufflating and deflating the lungs have given evidence of the presence of two kinds of pulmonary vagus fibres: those having expiratory and those having inspiratory effects. The latter act more weakly, are sooner tired, and are killed more easily by injuries than the former, so that the two kinds of fibres can be separated from each other as to their action. Especially is it easy to isolate the effect of the expiratory fibres. The theory of Hering<sup>1</sup>

<sup>1</sup> E. Hering, "Die Selbststeuerung der Athmung durch den Nervus Vagus," Wiener Sitzungsberichte, 2 Abt., Bd. lvii., S. 672, 1868.

and Breuer<sup>1</sup> on the self-regulation of the respiratory movements obtains in this way fresh support.

The connection of the respiratory and the heart-beat undulations in the electrovagogram also throws light on the association which virtually exists between the heart's action and the respiratory movements. This association finds *inter alia* its expression in the constant ratio between the number of respiratory movements and the number of heart-beats per minute. It is well known that this ratio usually remains unaltered with variations in pulse frequency. Since we now learn that the beating heart sends rhythmic stimulations along the vagus to its centre, we may reasonably ask whether these stimulations may not reach the respiratory centre and there cause with each variation of the pulse frequency a proportional variation of the frequency of the respiratory movements. In this way an automatic regulation of the respiratory movements would be promoted by the heart's action.

Yet another automatic regulation may be pointed out, viz. that of the heart's action by itself. We must in all probability look to this regulation as furnishing the cause of the vagal tonus, this tonus being thus maintained automatically by the action of the heart itself.<sup>2</sup>

<sup>1</sup> J. Breuer, *ibid.*, Bd. lviii., S. 909, 1868.

<sup>2</sup> A more detailed description of our experiments will appear in *Pflüger's Archiv f. d. ges. Physiol.*



A SIMPLE METHOD OF OBSERVING THE AGGLUTINATION OF  
THE BLOOD CORPUSCLES IN GAMMARUS. By JOHN TAIT.  
(From the Laboratory of Physiology, Edinburgh University.)

*(Received for publication 12th July 1908.)*

If the antennæ of *Gammarus marinus* are observed with a low power of the microscope the circulation of the blood is readily seen. *Gammarus* has two pairs of antennæ of approximately equal length. The terminal part in each consists of a many-jointed filament which gradually tapers towards the free end; it is in this filament that the circulation is best seen. The anterior antenna is better suited for observation than the posterior, which is covered with brushes of setæ that obscure the view of the main stem.

In carrying out observations, the animal, after being washed in two or three changes of filtered sea-water, is laid on a glass slide and a drop of sea-water, free from any suspended particles, so placed as to wet the glass all round the antennæ. Trouble may be experienced on account of the tendency of the creatures to jerk about and move out of the field, but if a sufficient supply of the animals is at hand some will always be found to lie quiet for a minute or two at a time. If it is necessary to keep the animals absolutely still for a prolonged period, a simple plan is to use specimens which have become asphyxiated by being immersed along with many others in an insufficient supply of water. If a dozen are kept in a small dish of water overnight some will be found in the morning to be apparently dead, having ceased to swim or to execute breathing movements. Many of such apparently dead animals are alive, their heart continuing to beat, and the circulation can now be watched with ease, for the animals continue long in this narcotic condition.

The blood flows in a very definite course in the antennary filament. A main artery leads down the filament; this forms communications by means of cross capillaries (one of which occurs opposite each joint) with a returning vein, the system resembling a ladder in which the two main vessels form the supporting poles while the connecting capillaries correspond to the rungs. The existence of these vessels or channels is inferred from the very definite path traversed by the blood corpuscles. It is only exceptionally that the outline of the vessels themselves can be seen.

From this arrangement it follows that the blood-flow is more rapid and

abundant in the proximal part of the filament than in the distal part, where the corpuscles can be seen to travel slowly and in single file. In the vein, and still more so in the artery, the movement of the corpuscles is not uniform but jerky, each jerk corresponding to a heart-beat. This can readily be verified by observations on a young specimen (preferably cooled to slow the heart), in which the pulsating dorsal heart is easily visible. In the capillaries again and in the longitudinal vessels at the top of the antennæ the corpuscles are carried slowly and uniformly along, sometimes sticking at one spot and then being knocked away again by the impact of succeeding corpuscles. Sometimes in a narrow vessel a row of corpuscles may be piled up each one in contact with its neighbour: every time a new corpuscle strikes the proximal end of the column one becomes detached from the distal end and floats away. From the fact that the capillary flow is not intermittent one might infer that the venous pulse is due to direct lateral transmission of pressure from the arterial vessel, the whole system being enclosed within a relatively rigid tube formed by the calcareous rings of the filament.

Except where the corpuscles accidentally come in contact they float at a wide distance from each other in a relatively large volume of plasma. They vary both in size and in form. Generally speaking, they are flattened and somewhat irregular in shape, though showing no processes that might be called pseudopodia. They roll along as they travel, now presenting their narrower edge to view and again being seen on the flat. When removed from the animal and examined with a high power the largest of them are seen full of highly refractile granules which stain deeply with eosin. Other smaller ones contain either basophil granules or clear protoplasm. In none is the nucleus lobed as is the case in some of the white corpuscles in vertebrates. In all, four or five different varieties of corpuscles can be made out by staining.

If now, during the time that the circulation in an antenna is being observed, one amputates the terminal portion with a sharp knife, the rate of flow in the artery is greatly increased, while the flow in the vein may cease altogether. The blood meantime pours out at the amputated end, the corpuscles being washed to a distance in the water surrounding the antennæ. If the animal is breathing these corpuscles get washed away in the stream of water continually flowing past the antennæ owing to the breathing movements. If the animal is in a narcotic condition and not breathing they settle down on the slide all around the amputated end. Occasionally the plasma is brown in colour, in which case it can be seen pouring out in the surrounding sea-water like smoke from a funnel.

Some of the corpuscles, however, from the start begin to adhere to the amputated end. At first they stick round the edge of the divided filament, but soon increase in number and form a clump or mass all around the end. The formation of this clump does not immediately stop the flow of blood, for the central part is still kept tunnelled by the rush of outflowing corpuscles,

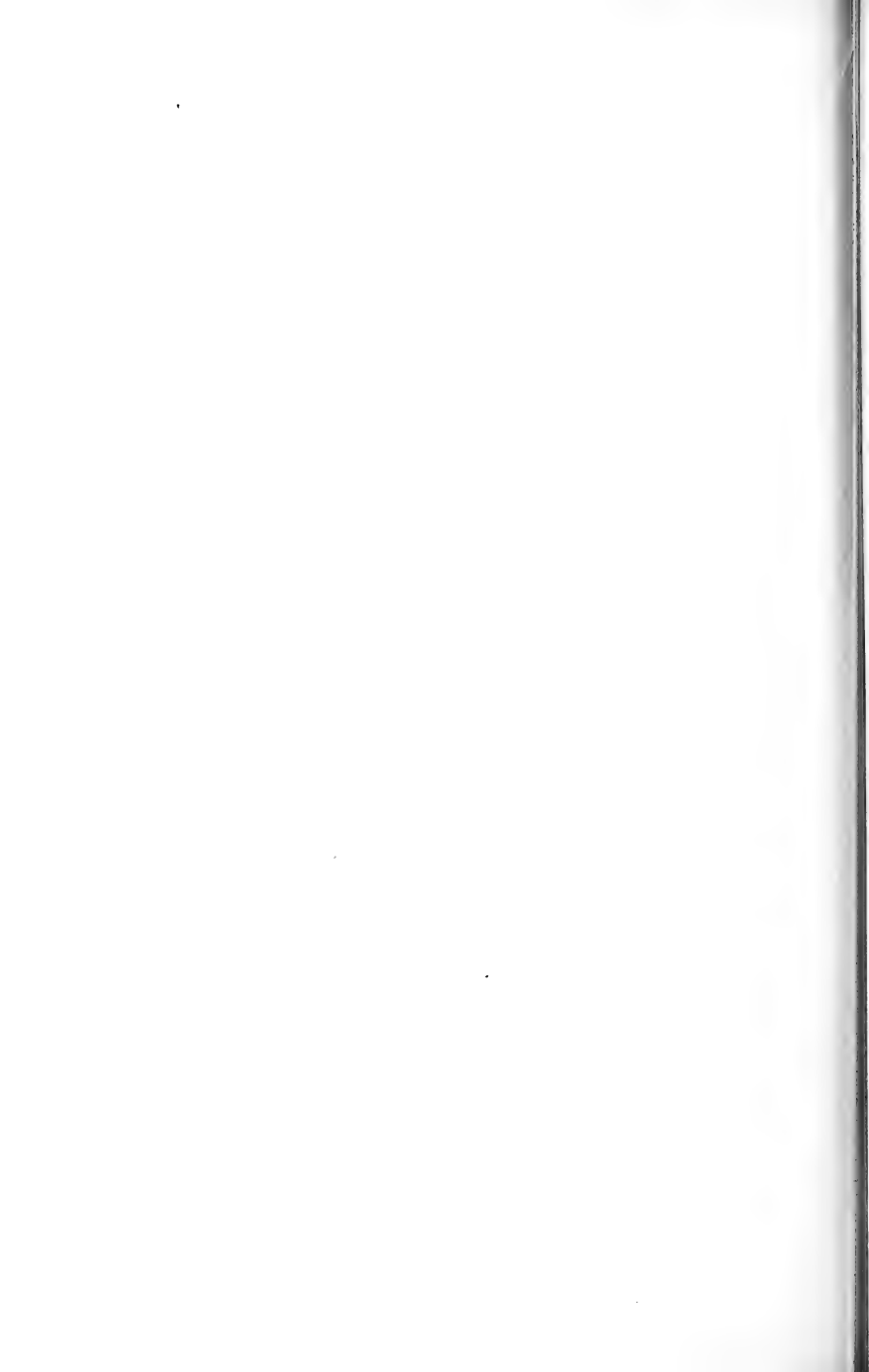
which causes vibrations and upheavals of portions of the clump. The appearance at this stage calls to mind that of an active volcano belching forth stones and cinders, some of which get piled up round the crater, while every now and then the whole mass is shaken and torn by the eruption of material from the centre. No portion of the adhering mass of cells gets torn away, however. When once the cells stick in the mass they remain firmly attached.

This flow from the central tunnel soon ceases, and the clot becomes entirely closed in by the adhesion of the cells all around. The mass which they form is at first porous and still allows of the escape of plasma, though the corpuscles which are carried down the filament to the spot are held back and become piled in a column inside the portion of the filament near the wound. The piling up may extend back over one or two segments, but not as a rule very far, for a way of escape for the corpuscles is found in one of the cross capillaries, and now the venous circulation begins again as if nothing had happened.

This whole process takes from a minute to two or three or more minutes according to the thickness of the portion amputated. A wound in the very end of the filament is naturally closed up sooner than one nearer the proximal end. Once the internal plugging of the vessel has occurred, the terminal clot may be removed without any further risk of bleeding. Often enough animals may be found in which for some reason or other a whole antennary filament has become filled with a mass of corpuscles. Amputation of such a filament produces no bleeding.

The cells which form the terminal clump do not long retain their rounded shape. During the time that the blood is still escaping they undergo changes in shape, becoming at first wrinkled and still later losing their outline and fusing with neighbouring cells. This fusion may have taken place in the cells which lie nearest the cut surface of the filament even at the time when other corpuscles are still escaping from the end. When the bleeding stops it is not long before the whole clump becomes felted into one irregular mass in which no outline of any cell is visible. At the same time the mass is seen to have become somewhat shrunken. Whether it is one special form of cell among the white blood corpuscles that contributes to the formation of the clot, or whether all forms get caught, I have not yet determined.

This method of observing the agglutination of the cells in bleeding is simple and requires little manipulative skill: it might well be adopted for class demonstration. The animals are readily procured, and the process may be repeated two or three times on the antennæ of the same specimen.





ON THE TIME TAKEN IN TRANSMISSION OF REFLEX IMPULSES IN THE SPINAL CORD OF THE FROG. By  
A. D. WALLER.

*(Received for publication 14th July 1908.)*

THE observations of Miss Buchanan on the time taken in transmission of reflex impulses in the spinal cord of the frog have reminded me of some experiments I made on this subject in 1884, the conclusions from which are in some respects similar to those drawn by Miss Buchanan. In other particulars the facts respectively observed by Miss Buchanan and by myself supplement each other, obtained as they have been by different methods, and altogether independently.

Miss Buchanan used the capillary electrometer, so that the reaction in only one limb at a time could be recorded; I used a double myograph, so that the reactions in two limbs to the same stimulus were simultaneously recorded.

The two methods are indeed complementary of each other, each affording information of its own not afforded by the other. I must say, however, that as regards one principal item—the comparison of delays of various kinds of reflexes—it appears to me that the electrical method followed by Miss Buchanan is distinctly inferior to the mechanical method of which I made use. By the former plan the comparison has to be established between successive individual effects, and individual differences of time are not out of the question, whereas by the latter it is easy to obtain for comparison the simultaneous effects of two different kinds of reflex contraction.

The two points in Miss Buchanan's results that have most aroused my interest in connection with my own results are:

1. The prolongation of reflex time in consequence of the action of strychnine on the spinal cord.

2. The fact that the cord delay is roughly about twice as great for a crossed as for an uncrossed reflex, and the inference therefrom that there are normally two spinal synapses interposed in a crossed path and a single synapse in any uncrossed path.

In my own experiments the points that seemed to me to be most noteworthy were:

1. The excessive prolongation of reflex times after strychnine injection.
2. The consequent dissociation that can be distinguished of the component parts of which crossed and uncrossed reflexes are made up.

The inferences which I then drew and still draw differ from those of Miss Buchanan: naturally, at that time I did not think or speak in terms of "synapses." Speaking in terms of the afferent and efferent aspects of spinal cells, I inferred from the facts under my observation that the delay of reflex action, which increases as the toxic effects of strychnine deepen, is the index of a gradually increasing block of transmission in the spinal cord, and that this block of transmission occurs at first exclusively and later chiefly at the junction of the afferent nerve with the cord.

I have reviewed this conclusion by a re-examination of the facts upon which it rested, and in the light of Miss Buchanan's experiments, in order to see whether it could be expressed in terms of synapses, and whether the conclusion so expressed was in harmony with Miss Buchanan's conclusion.

But, firstly, as regards the facts:—I found, as Miss Buchanan did, that the normal time of a simple reflex was approximately  $\frac{1}{100}$ th sec., and that of a crossed reflex was approximately  $\frac{2}{100}$ ths. (My actual figures were 0.008 and 0.016.) But, unlike Miss Buchanan, I found that this proportion of 1 to 2 was not maintained in the prolonged times of simple and crossed reflexes observed in strychninised frogs. The times (fig. 3) were, e.g., simple 0.038, crossed 0.046, difference 0.008; i.e. the difference of  $\frac{1}{100}$ th sec. attributable to transverse transmission, or, let us say, to the interposition of a second synapse in the reflex arc, remained unaffected in the presence of a prolongation of time as regards the first synapse of approximately  $\frac{2}{100}$ ths over and above the normal delay of  $\frac{1}{100}$ th.

The case of longitudinal transmission lends itself to a similar argument. Fig. 4 of an experiment in which muscular contractions of the arm and of the leg of a strychninised frog were recorded, stimulation (by a single break induction shock) being applied to the skin of the leg, gives the times: simple = 0.060, transmitted = 0.068, difference = 0.008; and this normal difference of approximately  $\frac{1}{100}$ th sec., attributable to longitudinal transmission, or, let us say, to the interposition of a second synapse in the reflex arc, remains unaffected in the presence of a prolongation of time as regards the first synapse of approximately  $\frac{2}{100}$ ths over and above the normal delay of  $\frac{1}{100}$ th.

I have expressed values in hundredths of a second for the sake of clearness, and for the sake of further clearness I will set out in detail in hundredths, and in terms of the synapse conception, what I consider to be the components of the total delays exhibited in fig. 4.

	Simple contraction.	Transmitted contraction.
	Muscular delay and nerve transmission.	Muscular delay and nerve transmission.
1st hundredth		
2nd "	} First synapse	} First synapse.
3rd "		
4th "		
5th "		
6th "		
7th "	... Second synapse.	

I must confess, however, that this analysis in terms of synapse delay does not give me much satisfaction, for it would require us to say that the spinal delay caused by strychnine occurs in the first and not in the second synapse. And I still prefer, in the absence of further guidance, to limit myself to the conclusion I drew in 1885,<sup>1</sup> to the effect that the prolongation of reflex time by strychnine is principally caused by a block or functional obstruction at the junction of afferent fibre with spinal cell.

I have said "principally" where it might have appeared permissible to say "exclusively." On attentive consideration of the analysis of fig. 4, the whole added delay appears to belong to the first synapse, or—as I prefer to say—to the afferent side of the centre, and there is no appreciable retardation attributable to the second synapse; i.e. the excitatory process

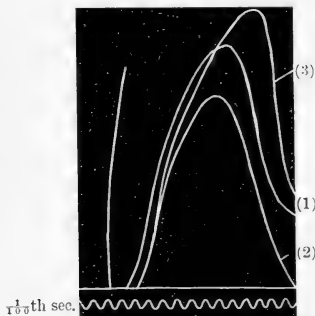


FIG. 1.—Normal.

- (1) Direct muscular contraction. The lost time is 0·008 sec.  
 (2) Muscular contraction caused by a single break shock to upper part of the spinal cord. The lost time is 0·016 sec.  
 (3) Muscular contraction (reflex) caused by a single break shock applied to the skin of the arm of the same side. The lost time is 0·016 sec.

passes from cell to cell within the cord with normal rapidity. But in extreme cases of delay where, as I have observed, the reflex time may reach  $\frac{1}{5}$ th sec., the ordinary  $\frac{1}{100}$ th sec. occupied by transmission in the cord by one cell to another (or by one synapse to another) is considerably exceeded.

Thus in the case of fig. 30 of my 1885 paper the values are as follows:—

Simple reflex, 0·200.

Crossed reflex, 0·228.

Analysis in this case (which I may mention in passing gives the highest values I ever observed) gives in place of the normal  $\frac{1}{100}$ th sec. delay for the first cell (or synapse) the enormous value of nearly  $\frac{2}{100}$ ths sec., and in

<sup>1</sup> British Medical Journal, "Report on Experiments in the Process of Fatigue and Recovery," 25th July 1885.

place of the normal  $\frac{1}{100}$ th for the second cell (or synapse) nearly  $\frac{3}{700}$ ths. It is on account of this and similar observations that I have not said above "exclusively," but only "principally."

The difference between (1) the direct and (3) the reflex contraction on the same side gives 0.008 as the value of the corrected reflex time.

The coincidence of commencement of response to (2) excitation of the upper part of the spinal cord and to (3) cutaneous excitation on the same side indicates that in both cases the mechanism of the delay is very probably of similar character. The longitudinal transmission delay of 0.008, like the corrected reflex time of 0.008, is probably occasioned by one similar nerve station. Lost time in nerve-fibres, at, say, 30 to 50 metres velocity per second, is in this connection negligible.

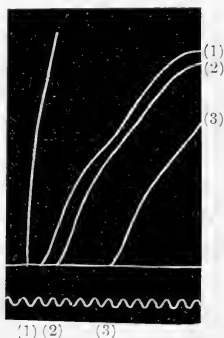


FIG. 2.—After strychnine.

(About 0.1 milligramme injected.)

- (1) Direct muscular contraction, 0.010 sec.  
 (2) Spinal muscular contraction, 0.020 "  
 (3) Reflex muscular contraction, 0.056 "

The identical values of the lost times in the two cases (2) and (3), in which the cell station at the origin of motor fibre is aroused by a cord fibre (2) and by a skin fibre (3) respectively, so that the commencements of the two contractions normally coincide, are altered in consequence of the action of strychnine. The delay of (2) is very slightly increased; that of (3) is very considerably increased. The augmentation of (2), as of (1) the direct muscular contraction, was so small, 0.010 as compared with 0.008, as to be within the limits of experimental error; but the augmentation of (3) the reflex time, by no less than 0.040 sec., was clear and unmistakable: it is a measure of the delay suffered by the centripetal stimulus at the junction between afferent nerve and spinal cord.

Miss Buchanan used much lighter doses of strychnine than I did, viz.: 1 min. of 0.01 per 100 liq. strychn. to 2 mins. of 0.02 per 100 liq. strychn., while I used 2 mins. of 1 per 1200 liq. strychn.

In Miss Buchanan's observations the prominent effects of strychnine on cord times were a slight diminution of time for the simple reflex and a considerable diminution of the additional time for the crossed reflex, e.g.,

	Simple.	Crossed.
Normal . . . . .	0·012 to 0·022	0·010
Strychnine . . . . .	0·009 to 0·020	0·004

while I got considerable augmentation of the simple reflex time with little or no alteration of the additional time, e.g.,

	Simple.	Crossed.
Normal . . . . .	0·010	0·010
Strychnine . . . . .	0·030	0·010

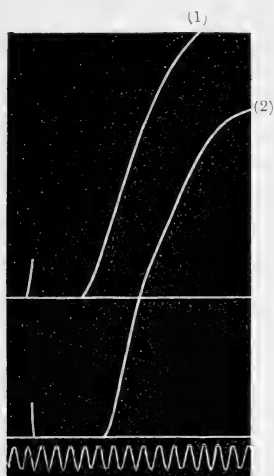


FIG. 3.

(1) Simple reflex contraction, 0·033  
 (2) Crossed reflex contraction, 0·046  
 Difference, 0·008

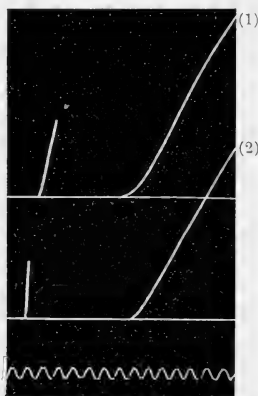


FIG. 4.

(1) Simple reflex contraction (leg), 0·060  
 (2) Transmitted reflex contraction (arm), 0·068  
 Difference, 0·008

There is, of course, no actual incompatibility between these two sets of figures taken in light and in deep strychninisation respectively. It appears to me, however, that the latter as well as the former require to be taken into account before we can accept Miss Buchanan's conclusion that in the same-side reflex a single synapse has to be passed, while in the crossed-limb reflex two such synapses have to be crossed in succession. Given the fact that in deep strychnine intoxication the times of the uncrossed and cross reflex movements are, e.g., 0·04 sec. and 0·05 sec., we have to assume on the

double synapse theory that the first synapse is more profoundly affected than the second. I still prefer, however, not to make this assumption, nor yet that of the double synapse, and to repeat what appears to me to be the most that we may legitimately infer, viz.: that under the influence of strychnine, transmission of a reflex impulse is blocked principally at the junction between afferent nerve fibre and spinal cord cell.

ON THE EFFECT OF STIMULATING THE NERVI ERIGENTES  
IN CASTRATED ANIMALS. By SUTHERLAND SIMPSON and  
FRANCIS H. A. MARSHALL. (From the Physiological Department,  
University of Edinburgh.)

(Received for publication 17th July 1908.)

ECKHARD<sup>1</sup> was the first to show experimentally in the dog that the penis could be caused to erect by the stimulation of certain nerves arising from the sacral part of the spinal cord. These nerves he afterwards designated the *nervi erigentes*. In the dog they are given off from the first and second sacral nerves and sometimes also from the third, but their origin from the cord varies slightly in different species of mammals. Gaskell,<sup>2</sup> and subsequently Morat,<sup>3</sup> found that the *nervi erigentes* leave the cord by the anterior roots only, and these observations have been confirmed by other investigators.

The erection of the penis is brought about partly through the contraction of the ischio-cavernosus (or erector penis) and bulbo-cavernosus muscles, certain of whose fibres pass over the efferent vessels of that organ, and so arrest the outward flow of blood. The result of this contraction is that whereas the blood can freely enter the dilated vascular spaces of the penis, its exit is retarded. This leads to a further distension of the vessels, whose venous outlets become still more compressed. It is clear, however, that whereas the constriction of the outlets assists in causing erection, it is incapable by itself of effecting this result, since erection cannot be induced by ligaturing the efferent veins.<sup>4</sup> The usual view is that the *nervi erigentes* exercise an inhibitory influence upon the tonicity of the walls of the vessels, and so cause them to distend, and that erection is due chiefly to the direct vaso-dilator action of these nerves. According to Langley and Anderson's<sup>5</sup> description, stimulation of the *nervi erigentes* causes inhibition not only of the unstriated muscles, but also of the retractor penis, when that muscle is present.

<sup>1</sup> Eckhard, "Untersuchungen über d. Erektion d. Penis beim Hunde," Beiträge zur Anat. und Phys., vol. iii., Giessen, 1863.

<sup>2</sup> Gaskell, "On the Structure, Distribution, and Function of the Nerves which innervate the Visceral and Vascular Systems," Journ. of Physiol., vol. vii., 1886.

<sup>3</sup> Morat, "Les Nerfs Vaso-dilatateurs et le Loi de Majendie," Arch. de Physiol., 1890.

<sup>4</sup> See Retterer, article "Erection" in Richet's Dictionnaire de Physiologie, vol. v., 1902. This article contains numerous references.

<sup>5</sup> Langley and Anderson, "The Innervation of the Pelvis and Adjoining Viscera," Journ. of Physiol., vol. xix., 1895.

The object of the present inquiry was to ascertain whether the mechanism of erection is present in animals which have been castrated prior to puberty. It is known that erection can take place in castrated adults, at any rate for a considerable time after the removal of the testes, but this may be due to the fact that the accessory generative organs, having once been developed, do not immediately atrophy.

Our experiments were upon cats. Before proceeding to operate on castrated cats, we successfully carried out two experiments upon normal animals. The following is an account of the experiments:—

(1) An adult male cat was anaesthetised, tracheotomised, and fastened face downwards. The spinal cord was exposed in the lumbar region, and the dura mater opened. The cord was then transected at the level of the first lumbar segment. The anterior roots of the sixth and seventh lumbar segments, and of the first and second sacral segments, were exposed on each side. The roots were then ligatured and divided between the ligature and the cord. The posterior roots of the same segments were also divided. The anterior root of the first sacral nerve on the left side was then stimulated by an induced current obtained from an ordinary induction coil without a Helmholtz wire in the primary circuit. The current used was slightly painful on applying the electrodes to one's tongue. The stimulation caused a gradual but distinct erection of the penis, accompanied by a partial ejaculation of semen. Microscopic examination of the latter revealed the presence of living spermatozoa. The erection gradually subsided on shutting off the current, but it could again be induced on renewed stimulation. The same result was produced on stimulating the anterior root of the first sacral nerve on the right side.

(2) This experiment was identical with the first. Erection took place as described above, and was almost immediately followed by ejaculation. On microscopic examination the ejaculated semen was found to contain an enormous number of living spermatozoa.

(3) This experiment, which was upon a castrated cat, was carried out in the same manner, but the result was negative. The animal had been castrated before puberty, and when about half grown; the extirpated testicles being approximately as large as peas. The weight of the cat on the date of castration (3rd December 1907) was 1130 grams. At the time of the stimulation experiment (3rd July 1908) the cat appeared to be fully grown. Stimulation of the anterior roots of the first sacral nerve on either side failed to produce any sign of erection.

(4) This experiment was identical with the preceding, the result being also identical. The cat was castrated on 3rd December 1907, when about half grown, its weight being 1170 grams. The stimulation experiment was on 3rd July, when the cat appeared to be fully grown. There was no indication of any erection.

(5) This experiment was similar in every way to the last two. The cat was castrated on 3rd December, its weight at that date being 1060 grams.



The stimulation experiment was on 3rd July. The result was entirely negative.

(6) In this experiment, which was a control, the first sacral anterior roots of another fully grown normal male cat were stimulated experimentally in identically the same way as in the preceding cases. Erection took place gradually as in the other two control experiments (1 and 2). Ejaculation also occurred, the semen being found to contain numerous spermatozoa, some of which were moving when examined under the microscope.

The experiments show, therefore, that erection cannot be induced experimentally in animals which have been castrated prior to puberty, or, at any rate, that it is far more difficult to cause erection in such animals.

It is well known that in animals castrated in early life the secondary sexual characters as a general rule fail to make their appearance, and that the correlation between the testicles and the accessory generative organs is a still closer one. Thus the prostate and Cowper's glands undergo atrophy after castration, even in cases where the operation of removal is performed after puberty.<sup>1</sup> It is possible, therefore, that in animals which were castrated when young the muscular apparatus of the penis fails to develop sufficiently to admit of erection occurring, but it would seem unlikely that the nervous mechanism is impaired. If erection is due mainly to an inhibition of the vasomotors of the penis, as is ordinarily supposed, there would seem to be no theoretical reason why it should not be possible to bring about that process experimentally (or at any rate to produce a partial erection) in animals which have been castrated. It would appear, therefore, that the process of erection is very possibly a more complex phenomenon than is generally believed, but our experiments throw no further light on the mechanism of that process.

<sup>1</sup> Griffiths, "Observations on the Formation of the Prostate Gland in Man and the Lower Animals," *Journ of Anat. and Phys.*, vol. xxiv., 1890. Wallace, "Prostatic Enlargement," London, 1907.



A CONTRIBUTION TO THE COMPARATIVE PHYSIOLOGY OF THE  
PITUITARY BODY. By P. T. HERRING. (From the Physiology  
Department, University of Edinburgh.) (With one Plate and eight  
figures in the text.)

*(Received for publication 21st July 1908.)*

THE researches of Oliver and Schäfer (7), Howell (4), and others have demonstrated the existence in the mammalian pituitary body of active principles which have a specific effect upon the heart and blood-vessels when injected intravenously. Howell, moreover, pointed out that it is the posterior lobe alone which possesses this property. Magnus and Schäfer (5), and more recently Schäfer and Herring (10), showed that extracts of the posterior lobe have the additional characteristic of producing kidney dilatation and diuresis when injected. Their observations were confined to the pituitary body of certain mammals and of the cod, which was found to have a similar action. Osborne and Vincent (8) had previously shown that extracts of the pituitary body of the cod produce effects upon the heart and blood-vessels similar to those of extracts of the mammalian posterior lobe. The question as to the origin of the active principles found in the posterior lobe of the mammalian pituitary has been discussed in a previous paper (3), and the author has given reasons which appear to him to support the view that they are derived from the cells of the pars intermedia, which in the mammalian pituitary form so close an investment over the nervous tissue of the posterior lobe. It was thought that an examination of the structure of the pituitary body of other classes of vertebrates, combined with an experimental investigation of the action of extracts of the different parts of each, might furnish some interesting facts bearing upon the physiology of the pituitary body, and at the same time throw light upon the mode of origin of the active material.

The difficulty of obtaining sufficient material for extracts has so far prevented an investigation of the pituitary bodies of reptiles, amphibians, and the lower orders of fishes. The present paper is confined to a description of the general structure of the pituitary body, and the physiological action of its extracts, in birds and in bony and cartilaginous fishes.

#### METHODS.

The pituitary bodies of the types examined were fixed for histological examination in Flemming's fluid. Sections were cut by the paraffin method

in the sagittal plane and mounted serially. In all cases a sufficiently large portion of brain was included to display the immediate relationship which exists between the brain and the pituitary body, a precaution which is of importance.

For the experimental part of the research, extracts of the various structures revealed by the histological investigation were prepared, the lobes of the pituitary being carefully separated from one another, finely minced, and boiled in Ringer's fluid. The animals experimented upon were cats, which were anaesthetised with a mixture of chloroform and alcohol. After a tracheal tube had been introduced, anaesthesia was continued by the administration of the same mixture through Brodie's apparatus, with artificial respiration. Injections of the extracts were made through a tube inserted in the external jugular vein. Blood-pressure was recorded by means of a cannula in the carotid artery. The left kidney was placed in a brass oncometer; its movements were registered by a piston recorder. A tube tied into the bladder drained away the urine, which was allowed to fall drop by drop upon a recorder, an electrical signal marking on the paper the moment of the falling of each drop.

## THE PITUITARY BODY OF BIRDS.

### Histological Features.

The type of bird's pituitary investigated has been that of the common fowl, *Gallus domesticus*. The pituitary body of the adult fowl bears certain general resemblances to that of mammals. It has two well-defined lobes—an anterior or glandular, and a posterior or nervous. The epithelial cleft, which is so prominent a feature of certain mammalian pituitaries, e.g. those of the dog and cat, is absent from all the specimens of fowl's pituitary that I have examined. The anterior lobe is a compact cylindrical body with its long axis in an antero-posterior direction, deeply embedded in the sella turcica. Large blood-vessels enter it at its lower posterior margin and are a prominent feature in the initial dissection. The lobe itself is very vascular, and contains large blood-channels running between solid columns of cells. The cells are for the most part small and finely granular; larger cells containing granules which stain more deeply are occasionally met with, but do not resemble the large deeply staining cells which are so characteristic of the anterior lobe of the mammalian pituitary. The cells have a close resemblance to those of the mammalian parathyroid. The lobe is as a rule well defined, but in its upper part strands of cells frequently pass towards the neck of the posterior lobe and are continuous with narrow columns of cells which encircle this and spread over the adjacent brain-tissue. Fig. 1 (of Plate) shows the general relationship of anterior to posterior lobe and adjacent brain. It is taken from a median sagittal section of a fowl's pituitary body.

The posterior lobe is smaller than the anterior, and overlaps it slightly behind. It is hollow, and its cavity is continuous through a narrow neck with the third ventricle of the brain. The lobe is occasionally much convoluted, and its cavity appears at several points in the same section. Its wall is never very thick, and seems to consist chiefly of long ependyma cells, true nerve-cells being absent from it. Colloid bodies are not infrequently present, and the cavity often contains much debris and occasionally rounded clumps of what resemble epithelial cells. In the extension of its cavity by recesses and the convolutions of its wall the posterior lobe suggests a glandular structure opening into the third ventricle. Like the posterior lobe of the mammalian pituitary, that of the fowl possesses an incomplete covering of epithelial cells, which are constantly found in certain positions. They resemble in structure and in their relationship to nervous tissue the cells of the pars intermedia of the mammalian pituitary, and are probably to be regarded as having the same significance. These cells form layers closely investing the nervous substance of the neck of the posterior lobe, and extending forwards between the anterior lamina of the neck and the optic chiasma (fig. 1 of Plate). The layers are few in number, and well supplied by blood-vessels; in fact, the cells often appear to have extended along the sheaths of the blood-vessels. They spread around the neck of the posterior lobe and for a considerable distance backwards over the thin lamella forming the lower wall of the third ventricle. The body of the posterior lobe lies behind, directly upon the anterior lobe, but is readily separated from it. No epithelial cells are seen on its posterior and upper surface, but they are often found laterally, and extend with the blood-vessels into the spaces between the folds of the lobe. In the fowl, therefore, the cells of the pars intermedia come into close contact with the nervous tissue of the posterior lobe, but are aggregated for the most part in the neighbourhood of its neck and on the thin lamina of nervous tissue forming the floor of the third ventricle. It is easy to separate the anterior lobe from the posterior, but impossible to remove the nervous tissue of the posterior lobe without at the same time including epithelial cells of the pars intermedia and their products.

B. Haller (2) studied the pituitary of *Gallus domesticus* and describes two portions in the anterior lobe, one of which, the superior, is closely applied to the infundibulum. The other portion, or anterior lobe proper, Haller believes to be tubular, and to constitute a gland whose secretion is poured into the subdural space by a small mesial opening. Haller noted the diverticula in the posterior lobe, and states that the arrangement met within it gives the lobe a glandular appearance.

Sterzi (11) examined the pituitary of several species of birds, and describes a division of the anterior lobe into two parts, one of which is made up of chromophobe cells and nearly surrounds the posterior lobe, while the other, more massive, consists of chromophil cells.

Gentes (1) also describes two segments in the anterior lobe. One of

these he designates as the "segment juxta-nerveux," consisting of a few layers of cells which have little affinity for stains, and are applied to the posterior lobe in the middle line only. The other segment is formed by the anterior lobe proper, and consists of chromophil cells. Both Sterzi and Gentes deny the tubular character of the anterior lobe assigned to it by Haller. Gentes found a small cleft—the remains of the sac from which the epithelium of the pituitary is developed—in a young duck.

The "segment juxta-nerveux" of Gentes and the chromophobe cells of Sterzi agree in most respects with the cells which are here described as belonging to the *pars intermedia*, but in all the specimens I have examined they have a more extensive disposition than is assigned to them by Gentes. The cells of the anterior lobe may be designated chromophil, but they have not the remarkable affinity for stains which is possessed by the larger cells of the anterior lobe of the mammalian pituitary. They certainly do differ from the cells of the *pars intermedia* in staining property, and occasionally deeply staining granular cells are found among them.

Histological evidence points to the anterior lobe of the fowl's pituitary being, like the mammalian anterior lobe, a gland which secretes directly into the blood-vessels. The posterior lobe has an incomplete covering of cells which are comparable with the cells of the mammalian *pars intermedia* and are chiefly aggregated around its neck, as in some types of mammals. The nervous tissue of the posterior lobe, with its epithelial investment, may be regarded as forming a distinct organ which has probably a similar function to that exercised by the posterior lobe of the mammalian pituitary. It also resembles the mammalian posterior lobe in the occurrence within its nervous substance of colloid or hyaline bodies. The colloid is, however, confined to the nervous tissue of the lobe, and I have not seen it in or between the cells of the *pars intermedia*. No colloid is found in the anterior lobe proper.

#### PHYSIOLOGICAL ACTION OF EXTRACTS OF THE LOBES OF THE FOWL'S PITUITARY.

##### Anterior Lobe.

Extracts of the anterior lobe of the pituitary body of the fowl, when injected intravenously, have little effect upon the blood-pressure, kidney volume, and urine secretion. There is no change in the force and frequency of the heart-beats; the blood-pressure may show a very slight rise, as in fig. 2, but is not much altered. Sometimes the pressure falls slightly and quickly recovers, but I have not seen a marked fall after injection of extracts of the fowl's anterior lobe. The kidney volume increases a little, but not more than it does after the injection of a similar amount of Ringer's fluid alone.

The secretion of urine shows no change. Fig. 1 is a typical tracing of

the effects of the injection of 5 c.c. of an extract of anterior lobe in Ringer's solution into the blood-vessels of a cat. The anterior lobe of the fowl's pituitary does not, therefore, contain any active principles exerting an immediate physiological effect upon blood-pressure, kidney volume, or secretion of urine. In this respect it resembles the anterior lobe of the mammalian pituitary body.

#### Posterior Lobe.

The posterior lobe is readily separated from the anterior, and yields a greyish gelatinous material which dissolves to a certain extent in Ringer's solution. When boiled, filtered, and injected intravenously, such extracts produce immediate and well-marked effects. The blood-pressure begins to rise soon after the injection, the heart beats more rapidly, and

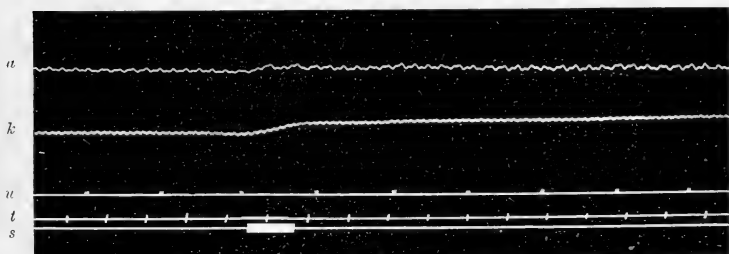


FIG. 1.—Effect of injection into jugular vein of a cat of 5 c.c. of an extract of anterior lobe of the fowl's pituitary in Ringer's fluid.

*a*, blood-pressure; *k*, kidney oncograph; *u*, urine secretion (drops); *t*, time in 5 sec. intervals; *s*, signal. In this and subsequent tracings the line *t* represents the zero of blood-pressure.

the large respiratory waves, when present, are abolished or very much diminished in size. The rise in blood-pressure occurs slowly and attains its maximum about two minutes after the injection. The rise is not a large one, but continues for some time and then gradually falls to normal. The respiratory movements, in spite of the continued supply of air containing the anæsthetic from the air-pump, are sometimes affected. Soon after injection the respiration is increased or inhibited for a short time, and then resumed as before. The kidney volume shows a slight initial increase, followed by a slow and gradual expansion, which, after a 5 c.c. dose (8 glands in 40 c.c. Ringer), attains its maximum in about fifteen minutes, and then falls gradually to what it was before the injection, the whole phase lasting about half an hour. The secretion of the urine increases with the expansion of the kidney, a latent period of one to two minutes usually elapsing before the increase begins.

The increase of urine is very pronounced. In the example of which fig. 2 is a tracing, the increase is from 12 drops in five minutes before the

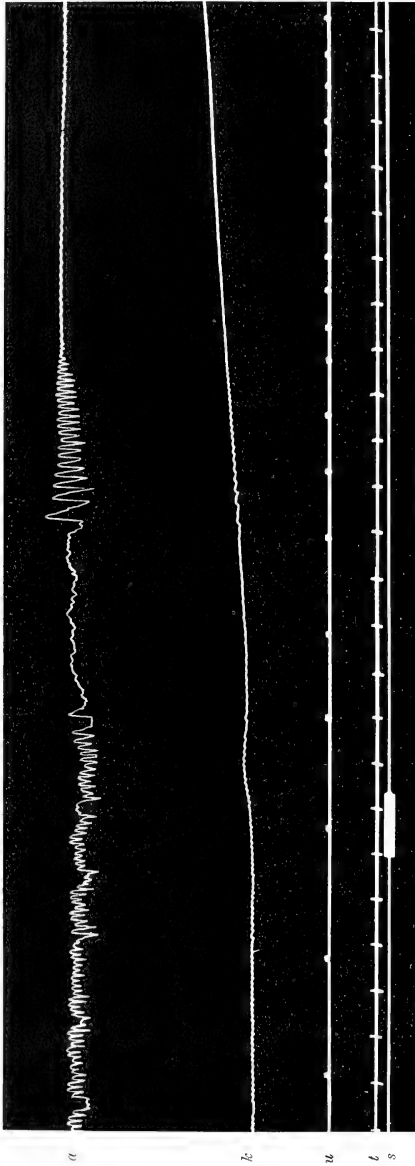


FIG. 2.—Effect of a first injection of 5 c.c. of an extract of posterior lobe of the fowl's pituitary (cat). (8 glands in 40 c.c. Ringer.)  
Letters as in previous figure. Note slight rise of blood-pressure, marked expansion of kidney, and considerable diuresis.



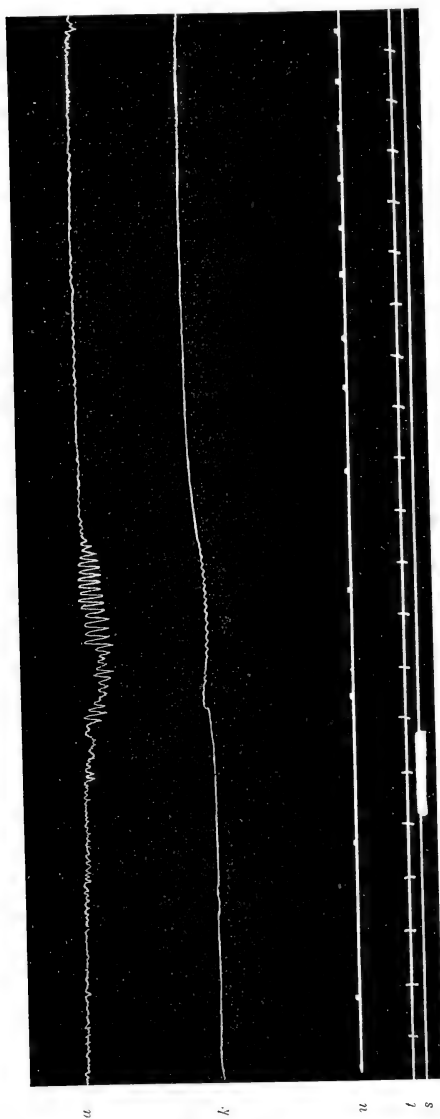


FIG. 3.—Effect of a second injection in the same animal as that used for the last tracing of 5 c.c. of an extract of posterior lobe of fowl's pituitary, administered twenty minutes after the first dose.

Notice the repetition of the above effects.

injection to 42 drops in five minutes afterwards. Where the secretion is very slow to begin with, the subsequent increase may be even more marked. The secretion is independent of the increase of blood-pressure, as was noted by Schäfer and Herring in the case of extracts of the mammalian posterior lobe; it is, however, related to the expansion of the kidney, and decreases when that begins to pass off.

A subsequent dose of the extract in the same animal, if administered after the kidney and urine effect have passed off, is followed by a repetition of the same changes, although there may be an initial fall of blood-pressure, followed by a slow rise (fig. 3).

Extracts of the posterior lobe of the fowl's pituitary have, therefore, an effect on blood-pressure, kidney volume, and urine secretion which is very similar to that produced by extracts of the posterior lobe of the mammalian pituitary. It is impossible to determine whether the active principles in the posterior lobe of the bird's pituitary are products of the epithelial cells of the *pars intermedia* or are formed solely in the nervous substance. The large preponderance of the latter in the bird might be considered as an argument in favour of their nervous derivation; but, on the other hand, if the cells of the *pars intermedia* pour their secretion into the *pars nervosa* of the lobe, it may accumulate there in larger quantities. There is evidence in the mammalian pituitary that the secretion is emptied into the third ventricle of the brain, and is furnished by the cells of the *pars intermedia*. The posterior lobe of the bird's pituitary is so constituted that a similar process may quite well be the normal one in it also.

## THE PITUITARY BODY OF TELEOSTS.

### Histological Features.

The pituitary body of the cod—*Gadus morrhua*—is taken as the type. In this fish the pituitary is a prominent organ lying in front of and below the *lobi inferiores*. The infundibular region is complicated by the presence of a *saccus vasculosus*, which lies immediately behind the pituitary, between the two large *lobi inferiores*. The pituitary body, although forming a single organ, is seen to be composed of two different kinds of tissue, an anterior portion, reddish or white according to the amount of blood in it, and a posterior part, greyish in appearance. The two portions are directly continuous with one another, and the line of division between them can only be recognised by the change of colour in passing from one to the other. On section, the pituitary is found to be a solid organ, and to resemble in general structure the pituitary of mammals and birds; it differs from these, however, in the arrangement of its parts.

In a median sagittal section (fig. 2 of Plate) the general relationship of the different parts is readily appreciated. The pituitary is composed of three varieties of tissue, two of which are epithelial and the third nervous, the

latter being comparatively small in amount. In the anterior part of the pituitary a somewhat quadrilateral or wedge-shaped mass is characterised by the large and deeply staining cells of which it is composed. These cells are almost identical in appearance with the cells found in the anterior lobe of the mammalian pituitary, and the portion containing them is probably the equivalent of the true anterior lobe. The cells are arranged in columns with blood-channels between. There is no trace of tubules, and nothing to support Haller's contention that the anterior lobe of the teleostean pituitary is a tubular gland secreting into the subdural space. This portion of the pituitary of the cod corresponds with that described by Sterzi in other bony fishes as the chromophil segment. Gentes also noted the deeply staining cells met with in certain positions in the teleostean pituitary, and showed that they vary in situation and extent in different species. The chromophil portion is aggregated in the cod's pituitary in the position indicated in fig. 2, *c*, of Plate. It may be regarded as constituting the true anterior lobe, and its similarity to the anterior lobe of the mammalian pituitary suggests that it has a like function.

The other epithelial constituent of the cod's pituitary is widely distributed in the form of small round cells which have little affinity for stains. They surround and invade the nervous tissue of the pituitary, and resemble in this respect the cells of the *pars intermedia* of the mammalian organ. This part of the gland was described by Sterzi as the "chromophobe" portion, and there is little doubt that it corresponds with the *pars intermedia* of mammals and birds. Gentes found it in the types he examined, and states that it surrounds and passes between projections of the nervous substance of the infundibular lobe. The *pars intermedia* in the cod is divided into two main portions, which are continuous with, and separated from one another by, the true anterior lobe. The part which lies in front of the chromophil segment consists of a mass of small cells among which fibres of the nervous substance penetrate. The latter increases in amount towards the junction of the pituitary with the brain substance behind the optic nerves. The thin lamina of nervous tissue connecting the pituitary with the brain in this situation is called by Gentes the *lamina post-optica*. The main mass of the *pars intermedia* lies behind the chromophil portion and makes up the greater part of the lobe. On the surface of the pituitary the epithelial cells form a thick mass and pass deeply inwards among the fibres of the nervous portion.

The *pars nervosa* of the cod's pituitary is small in amount, and appears to be composed of neuroglia and ependyma cells, without any true nerve cells. It is continuous with the brain in front by the *lamina post-optica* or anterior lamina, and at the sides by lateral laminae. A thin layer of nervous tissue separates the chromophil substance from the cavity of the infundibulum. Behind and in the middle line the pituitary is continued into the wall of the *sacculus vasculosus*. The nervous substance of the pituitary closely resembles in structure the *pars nervosa* of the mammalian

pituitary. It is freely invaded by cells of the *pars intermedia*—more so, indeed, than is the case in mammals. It contains, moreover, the colloid or hyaline bodies of mammals and birds, and like them it encloses an infundibular cavity which communicates with the ventricles of the brain. The pituitary of the cod furnishes another example of a brain gland similar in its essential structure and relationships with the brain to the pituitary of mammals and birds. *Pars intermedia*—chromophobe portion of Sterzi—and *pars nervosa* make up a structure strictly comparable to the posterior lobe of mammalian and avine pituitaries. In the cod there is no epithelial cleft, and anterior and posterior lobes are fused together. The fusion is in some cases even more complete than is indicated in fig. 2 of Plate, for it not infrequently happens that some of the chromophil cells of the anterior lobe are found among the cells of the *pars intermedia*, and cells of the latter occur in the true anterior lobe. It is almost impossible, for this reason, to separate one portion from another exactly; but the difference in colour of the two parts is, as a rule, sufficiently obvious to enable one to divide them for the purpose of making extracts.

The *saccus vasculosus* of the cod is single and placed in the middle line. According to Gentes, the *saccus vasculosus* varies considerably in size in different species of teleosts, and may, indeed, be absent altogether, or present only in a rudimentary state. In the cod it is well developed and forms a wide-mouthed sac opening into the infundibulum immediately behind the pituitary recess. It is lined by a single layer of columnar epithelium resting upon a basement membrane. Numerous blood-vessels reach it in the middle line in the interval between the two large *lobi inferiores*. The columnar cells are large, with a nucleus in each situated near the basement membrane, the part of the cell next the lumen of the sac being clear. The epithelium is thrown into numerous folds which are suggestive of an increase of surface for secretory purposes. The arrangement and structure of the *saccus vasculosus* is such as indicates that it is a gland which secretes into the ventricles of the brain. Gentes believes that it is to be looked upon as a ventral choroid plexus, and that its function is to help in the formation of the cerebro-spinal fluid. It was called an infundibular gland by Rabl-Rückhard (9). The *saccus vasculosus* of the cod is attached to the brain behind, and its epithelium is continued for a short distance over the ventricular surface. The brain-tissue above it is remarkable for the large ependyma cells which line its internal surface.

#### PHYSIOLOGICAL ACTION OF EXTRACTS OF THE LOBES OF THE PITUITARY AND OF THE SACCUS VASCULOSUS OF THE COD.

##### Anterior Lobe.

Extracts of the anterior lobe proper—chromophil portion of Sterzi and Gentes—have little immediate physiological effect (fig. 4). The blood-

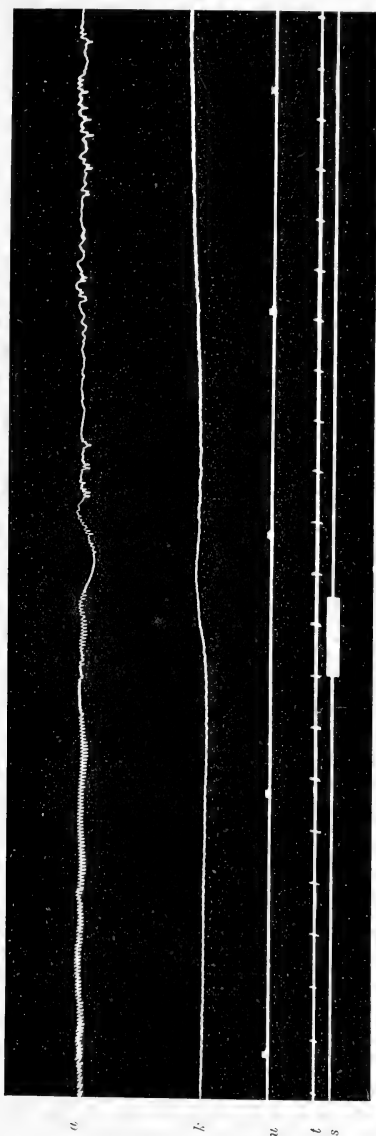


FIG. 4.—Effect of an injection into the jugular of a cat of 5 c.c. of extract of anterior lobe—chromophil cells of St er zi—of the pituitary of the cod. (12 glands in 40 c.c. Ringer.)

This extract probably contained some of the cells of the pars intermedia in addition to those of the anterior lobe.

pressure may show a temporary slight fall or remain unaltered. The frequency and force of the heart-beat are unaffected.

The kidney frequently shows a slight expansion, but not a continued one. The secretion of urine is unaltered or very slightly increased. The difficulty of isolating completely the proper tissue of the anterior lobe from the elements of the posterior make it probable that any effect obtained by the injection of its extracts is brought about by the inclusion of a little of the posterior lobe. If the dissection be so made as to avoid the junction of the two lobes, extracts of the chromophil portion have practically no action. It seems, therefore, that the anterior lobe or chromophil segment agrees in the inactivity of its extracts as well as in its structure with the anterior lobes of the pituitary of mammals and birds.

#### Posterior Lobe.

The general effect of extracts of the posterior lobe is similar to that brought about by extracts of the mammalian and avine posterior lobes.

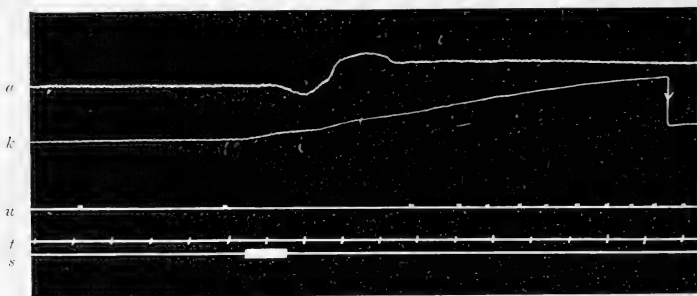


FIG. 5.—Effect of injection into the jugular of a cat of 5 c.c. of an extract of the posterior lobe—pars intermedia and pars nervosa—of the cod's pituitary. (12 glands in 40 c.c. Ringer.)

The arrow on the kidney oncograph indicates an artificial lowering of the writing point. Notice the rise of blood-pressure and rapid expansion of kidney, as well as the well-marked diuresis.

The blood-pressure is almost immediately affected. A considerable rise may take place, preceded sometimes by a slight fall, or the increase of blood-pressure may be only trivial. The same extract frequently produces different results when injected into different cats. In one animal the rise of blood-pressure may be marked, in another very slight, and that notwithstanding that no previous injections of any kind have been made in these animals. Howell pointed out that a repeated dose of pituitary extract does not produce the same results on blood-pressure as are brought about by the first injection, and this observation holds true of extracts of the posterior lobe of the pituitary of avine and teleostean pituitaries as well. The immunity conferred by a first dose does not last very long, and varies with the amount and strength of the injection given; but, in order to obtain

the typical effect, the first injection of extract of posterior lobe can alone be relied upon. Subsequent doses, unless delayed for half an hour, an hour, or longer, according to the amount and strength of the first injection, are followed by a fall of blood-pressure. The same is not the case with regard to the effect upon kidney volume and secretion of urine.

The force and frequency of the heart-beat are scarcely affected, but irregularities in the pressure-tracing due to inhibition of the heart are often abolished for a time.

The kidney expands almost immediately after the injection, and this expansion may be rapid and very considerable, as shown in fig. 5. As was the case after injections of an extract of the posterior lobe of the avine pituitary, the expansion of the kidney may last for twenty minutes or longer and then gradually pass off. The amount of urine secreted begins to increase with the expansion of the kidney, and the increase may be, and usually is, very considerable. In the experiment, of which fig. 5 shows part of the tracing, the increase of urine was from 6 drops in five minutes before the injection to 31 drops in five minutes afterwards, i.e. an increase of five times the amount in a given time. There may be a delay of several minutes after the injection before any increase of urine is detected, and the same extract has different effects in this respect in different cats.

The posterior lobe of the pituitary of the cod has, therefore, an action on blood-pressure, kidney volume, and urinary secretion similar to that of the posterior lobe of the mammalian and avine pituitaries.

Extracts of the posterior lobe of the pituitary of other teleosts, e.g. the ling (*Molva vulgaris*) and the John Dory (*Zeus faber*), have been tried and give the same results. One may conclude, therefore, that the posterior lobe of the teleostean pituitary, corresponding as it does in structure and in the action of its extracts with the posterior lobe of the mammalian and avine pituitary, has a like function. In the case of the teleost the cells of the *pars intermedia* predominate in the posterior lobe and are inseparable from the *pars nervosa*, so that one cannot determine which produces the active material. It seems probable, indeed, that both are concerned; for, wherever cells of *pars intermedia*—chromophobe cells of Sterzi—are bound up with *pars nervosa*, extracts of the resulting tissue produce the effects on blood-pressure, kidney volume, and urine secretion which have been associated with extracts of the posterior lobe of the mammalian pituitary.

#### Saccus Vasculosus.

Extracts of the saccus vasculosus are practically inactive. There is no effect on blood-pressure, and, although there may be some expansion of the kidney, the increase of urine, if any, is very slight (fig. 6). Almost identical effects are produced by rapid injection of a similar amount of Ringer's fluid.

It is of interest to note the observation of Gentes, that in different species of teleosts the saccus vasculosus varies greatly in size and may even

be absent. Gentes further remarks that the presence or absence of the saccus vasculosus brings about no modification of the nervous lobe. It is very probable that the saccus vasculosus has, as Gentes believes, a function similar to that of a choroid plexus.

## THE PITUITARY BODY OF ELASMOBRANCHS.

### Histological Features.

The pituitary body of the skate—*Raja batis*—is taken as the type. In the skate the pituitary is a long, club-shaped body which lies for the most part behind the small *lobi inferiores*. Its anterior extremity is thin, and stretches forward to the optic chiasma. Close to it is the large saccus vasculosus which is bilobed. The lobes of the saccus vasculosus appear to arise just above the anterior part of the pituitary by a common origin with it.

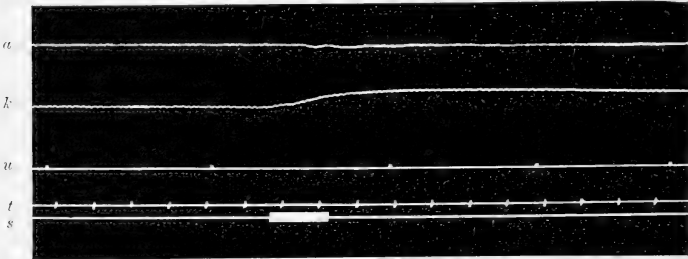


FIG. 6.—Effect of the injection into the jugular of a cat of 5 c.c. of an extract of the saccus vasculosus of the cod. (12 glands in 20 c.c. Ringer.)

The effect upon the kidney is no greater than that produced by rapid injection of 5 c.c. Ringer alone.

Each lobe passes backwards and outwards, and the body of the pituitary lies between them. A fine prolongation of connective tissue passes from the under surface of the pituitary body into the cartilage of the floor of the cranium, binding it closely down to the latter. This is the remnant of the neck of Rathke's pouch, from which the pituitary is developed, and was described by Miclucho-Maclay (6) in the shark. In the skate all connection between buccal mucosa and pituitary body is lost, but a string of connective tissue persists in the cartilage. It is advisable for this reason, in removing the pituitary body, to expose it by cutting away the cartilage of the floor of the cranial cavity.

On making a sagittal section through the pituitary, it is seen to extend for a long distance backwards from the optic chiasma, and to be quite different in structure from the pituitary bodies of mammals, birds, and teleosts. The main body of the organ lies posteriorly, and is the part which Haller designates as the head of the pituitary. It at first sight appears to be composed of tubules lined by large columnar cells, but on careful examination



the tubules are found to consist of columns of cells surrounding blood spaces. The lumen is a blood channel. This feature has been emphasised by Gentes, who states that the elasmobranch pituitary is a typical example of a gland whose secretion is poured directly into the blood-vessels. The epithelial cells surrounding the blood channels are columnar, with nuclei situated at their bases, the part of the cell bordering on the blood-vessel being clear. Outside the columnar cells and separating the vascular tubules from one another is a small amount of what appears to be a very vascular connective tissue. The vascular tubules and this connective tissue make up the body of the lobe. There are no deeply staining granular cells resembling the chromophil cells of the anterior lobe of the pituitary of mammals and teleosts, nor are there any cells exactly resembling those of the *pars intermedia*.

The anterior extremity of the skate's pituitary consists of a comparatively thin prolongation of epithelium enclosing a cavity (fig. 3, *b*, of Plate) which is stated by Gentes to be the remains of the cavity of the original sac from which the pituitary develops. It is lined by columnar epithelium very similar in appearance to that surrounding the blood-vessels in the body of the lobe. The cavity appears to be completely closed, and is much sacculated by convolutions of its wall. Outside this sac are numerous blood-vessels.

There is no differentiation of the pituitary gland of the skate into anterior and posterior lobes. An infundibular cavity is present which runs backwards and downwards to the body of the pituitary. It does not penetrate into the pituitary, but ends in the middle line, as shown in the figure. When followed laterally, however, the infundibular cavity is found to pass on either side into the *saccus vasculosus*. The nearest approach to anything resembling a posterior lobe is seen in the thin lamina of nervous tissue which bounds the infundibular cavity and passes into the tissue of the pituitary. Whether the fine vascular tissue that lies between the epithelial tubules in the body of the pituitary is of nervous origin or not is uncertain, but it is continuous with the lamina of nerve tissue that forms the lower wall of the infundibular cavity. If this tissue really belongs to the posterior lobe, then we have in the skate a very complete intermixture of elements derived from the brain and from buccal epithelium. Gentes states that the posterior lobe is completely absent in elasmobranchs. It is probable, however, that some representative of the *processus infundibuli* exists even in adult life, and that the general plan of development of the pituitary in elasmobranchs does not differ from that of other vertebrates. The nerve tissue in the anterior wall of the infundibulum must be regarded as a representative, in part at least, of the posterior lobe. But its constitution is altered by the large development of the *saccus vasculosus*, and the extension of the epithelium of the *saccus* over the lining wall of the infundibulum.

The *saccus vasculosus* is extremely well developed in the skate, and is a

prominent bilobed organ with a deep red colour due to the amount of blood contained in its vessels. Its wall is thin and convoluted, and consists of one or more layers of epithelial cells outside which are numerous and large thin-walled blood-vessels. The epithelium is continued forwards into the infundibulum, and in median sagittal section the common opening of the two sacs is seen lying above and in front of the body of the pituitary.

The pituitary body of the skate is, then, an example of a type which is entirely different from those of mammals, birds, and bony fishes. There is no differentiation into anterior and posterior lobes, and the characteristics of the cell elements of these are missing. The pituitary body itself furnishes, as Gentes says, a schematic type of gland secreting into blood-vessels. The posterior lobe is not distinct, but is represented to some extent; its infundibular surface appears to be largely devoted to the same purposes as the saccus vasculosus. There are no colloid bodies present in the thin layer of nervous substance, and no cells clearly resembling those of the *pars intermedia*.

#### PHYSIOLOGICAL ACTION OF EXTRACTS OF THE PITUITARY AND OF THE SACCUS VASCULOSUS OF THE SKATE.

##### The Pituitary Body.

Extracts of the whole pituitary body of the skate have little effect upon blood-pressure, kidney volume, or secretion of urine. Strong extracts produce a temporary fall of blood-pressure, but not a marked one (fig. 7). Kidney volume is slightly increased, but there is no continuous expansion, and the effect is merely that of the injection of Ringer's fluid.

Kidney secretion is unaltered or very slightly increased. It is doubtful if this increase is a specific one: it may be solely caused by the rapid injection of so much fluid. The pituitary body of the skate apparently contains none of the active principles which are found in the *pars nervosa* and *pars intermedia* of mammals, birds, and teleosts. If any of these are present, it is only in very small amount; there is no clear evidence of a histological character for the presence of these active principles, and it is probable that they do not exist in the elasmobranch pituitary.

##### The Saccus Vasculosus.

Extracts of the saccus vasculosus, even when very concentrated, have little effect. There may be, as in fig. 8, a temporary fall of blood-pressure, but with weaker solutions there is no change.

Kidney volume and urinary secretion may show a slight temporary increase, but, as was the case with extracts of the saccus vasculosus of the cod, it is not a specific effect, but merely the result of the rapid injection of so much Ringer's fluid.

Extracts of portions of the brain adjacent to the pituitary body and

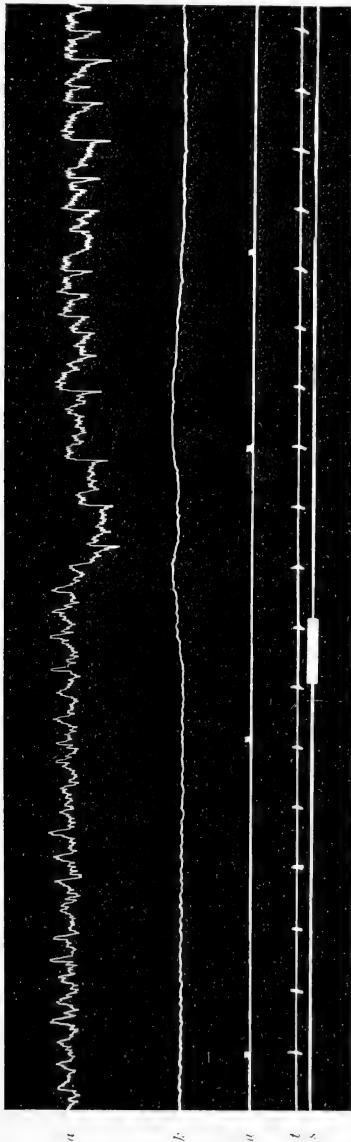


FIG. 7. — Effect of the injection into the jugular of a cat of 5 c.c. of an extract of the pituitary body of the skate. (5 glands in 20 c.c. Ringer.)  
 There is a transient fall of blood-pressure, accompanied by expansion of kidney, but no diuresis.

saccus vasculosus, and extracts of the lobi inferiores, bring about a large fall of blood-pressure; the effects are similar to those seen after injection of extracts of central nervous system in general. There seems to be nothing in the infundibular region of the brain of the skate which is comparable in the action of its extract with the posterior lobe of the pituitary body of mammals, birds, and bony fishes.

### Conclusions and Summary.

In mammals, birds, and bony fishes the pituitary body consists of two lobes, an anterior or epithelial which has the structure of a gland secreting into blood-vessels, and a posterior composed of nervous tissue more or less surrounded and invaded by epithelial cells of the pars intermedia. The posterior lobe may also furnish secretion into blood-vessels, but its arrange-

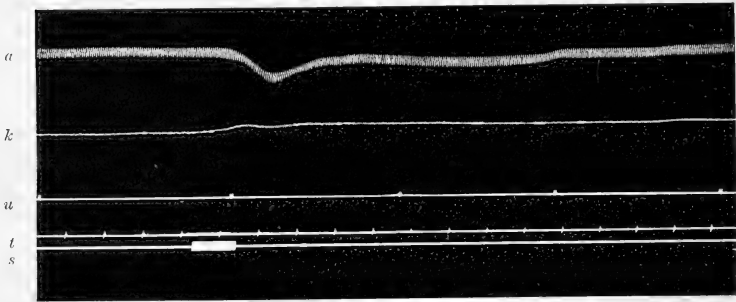


FIG. 8.—Effect of the injection into the jugular of a cat of 5 c.c. of an extract of the saccus vasculosus of the skate. (5 glands in 20 c.c. Ringer.)

There is a transient fall of blood-pressure and slight expansion of kidney, but no diuresis.

ment and histological features suggest a gland which pours its products into the infundibulum, and so into the ventricles of the brain. It may, therefore, be regarded, in part at least, as a special brain gland.

Extracts of the anterior lobe have no immediate physiological action when injected into the blood-vessels.

Extracts of the posterior lobe of birds and bony fishes have an action similar to extracts of the mammalian posterior lobe, bringing about a rise of blood-pressure, expansion of the kidney, and an increase in the secretion of urine. The tissue in which the active principles giving this result are found, contains, when examined histologically, bodies of a colloid nature such as have already been described in mammals in a previous paper. Whether this colloid contains the above-mentioned active principles or not, is undecided; it may possibly be the expression of some other function. The close relationship which exists between pars nervosa and pars intermedia of the posterior lobe renders it probable that the active principles,

and especially the colloid bodies, are furnished by the epithelial cells, but it is possible that the ependyma cells have also a secretory function.

The pituitary body of elasmobranchs differs widely in structure from that of the other classes considered. It is a gland which apparently secretes directly into the blood-vessels, but it contains none of the deeply staining (chromophil) cells which are characteristic of the anterior lobe of mammals and teleosts. Its posterior lobe is absent or merely rudimentary.

Extracts of the pituitary body of elasmobranchs have no immediate physiological activity.

The saccus vasculosus secretes its products into the ventricles of the brain. Its extracts are inactive, and it is probably an auxiliary to the choroid plexus, aiding in the production of the large amount of cerebrospinal fluid which is found in fishes.

I have to thank Mr Richard Muir for the care with which he has executed the accompanying illustrations. The expenses incurred have been assisted by a grant from the Carnegie fund for research-work.

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## DESCRIPTION OF PLATE.

Fig. 1. Median sagittal section through the pituitary body of the fowl—*Gallus domesticus*. *a*, optic chiasma; *b*, anterior lobe of the pituitary—pars glandularis; *c*, cells of the pars intermedia; *d*, neck of the posterior lobe; *e*, cells of the pars intermedia lying behind the neck; *f*, third ventricle; *g*, posterior lobe of the pituitary.

Fig. 2. Median sagittal section through the pituitary body and saccus vasculosus of the cod—*Gadus morrhua*. *a*, optic nerve; *b*, anterior part of pars intermedia—chromophobe cells of Sterzi; *c*, pars glandularis or anterior lobe—chromophil cells of Sterzi; *d*, pars nervosa surrounded by cells of pars intermedia—posterior lobe; *e*, infundibulum; *f*, large ependyma cells; *g*, saccus vasculosus; *h*, space between lobi inferiores occupied by blood-vessels and connective tissue.

Fig. 3. Median sagittal section through pituitary body of skate—*Raja batis*. *a*, optic chiasma; *b*, anterior part of pituitary enclosing cavity; *c*, infundibulum; *d*, opening of saccus vasculosus on either side into infundibulum; *e*, body of pituitary.

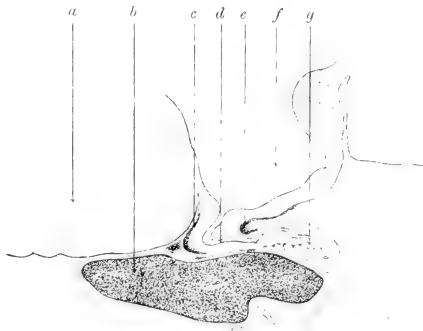


FIG. 1.

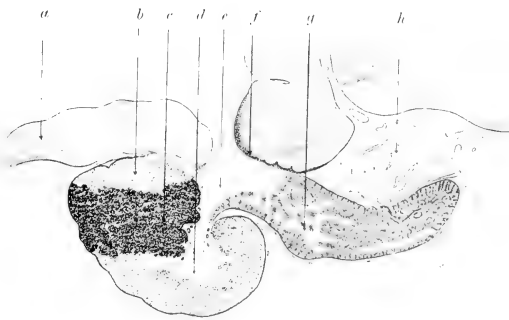


FIG. 2.

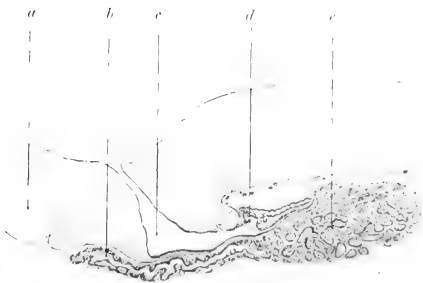


FIG. 3.





THE EFFECTS OF THYROIDECTOMY UPON THE MAMMALIAN  
PITUITARY. PRELIMINARY NOTE. By P. T. HERRING. (From  
the Physiology Department, University of Edinburgh.) (With Two  
Plates.)

(Received for publication 25th July 1908.)

THE occurrence in the pituitary body of a substance resembling the colloid of the thyroid has long been known. The removal of the thyroids is followed in some animals by certain well-marked symptoms ending sooner or later in death; in other animals it has no apparent effect. Rogowitsch (7) went so far as to state that the pituitary acts vicariously for the thyroid, and that in rabbits and other animals which can survive thyroidectomy the function of the thyroid is taken over and maintained by increased activity of the pituitary. Where the pituitary is relatively small, as in the dog, it fails to do this. Rogowitsch found alterations in the pituitaries of thyroidectomised dogs and rabbits of the nature of an increase of certain elements—"Kernhaufen"—in the glandular or anterior portion. He also described the formation of colloid by the chromophil cells of the anterior lobe, and its entry from them directly into the blood-vessels or into small cysts in the "Markschicht." A further change was seen in an increase of the number of vacuoles both in the chromophil cells and in the "Kernhaufen." H. Stieda (9) described hypertrophy of the anterior lobe brought about by an increase in the number of "Hauptzellen" with the formation of vacuoles in them. He saw no change in the chromophil cells and no formation of colloid.

Various other observers, Hofmeister (4), Gley (2), Piseni and Viola (6), Schönemann (8), have found changes in the pituitary body consequent upon removal or disease of the thyroid. Attention has been confined chiefly to alterations in the epithelial constituents of the pituitary body, little mention having been made of the condition of the nervous portion. Klebs (5) states that he has seen hyaline globules in the blood-vessels of the nervous part of the pituitary of strumiprивous dogs, and believes that in this organ is to be sought the "Ausgangspunkt" of the disturbance. Boyce and Beadles (1) found, in cases of myxœdema, enlargement of the pituitary body with increase of colloid in the posterior part of the anterior lobe. Large cysts containing colloid were present in this situation which they termed the medullary layer. The posterior lobe was atrophied but

occupied in its upper portion by a "colloid-like (? plasmatic) material which is not bounded by a cell membrane."

In a previous paper (3), histological evidence has been brought forward to prove that the posterior lobe of the pituitary furnishes a secretion which passes through the nervous portion to enter the infundibulum and ventricles of the brain. The secretion has normally a colloid appearance, and is probably a product of the cells of the pars intermedia. The results which follow thyroidectomy furnish additional and strong evidence in favour of this view.

My observations have up to the present been confined to rabbits, cats, and one dog. Three rabbits, of which the thyroids had been completely removed, were placed at my disposal by Professor Schäfer. These animals showed no symptoms, and were apparently healthy when killed three months after thyroidectomy. The pituitary bodies were removed along with part of the brain, fixed in Flemming's fluid, and cut in serial sections. Unfortunately, during the process of removal the posterior lobe of one animal was badly injured, and was not available for examination. In the other two there is no apparent change in the anterior lobe. Clear cells, deeply granular cells, and transitional forms are present, and there is no evidence of colloid production by them.

The cells of the pars intermedia are distributed as usual, but are somewhat increased in amount, and stain more deeply. Remarkable changes are at once apparent in the posterior lobe, and especially in that portion of it which lies next to the pars intermedia. Masses of a colloid nature lie among the cells and fibres of the pars nervosa (Plate I, fig. 1), and extend forwards and upwards into the neck of the lobe right up to the floor of the third ventricle. Some of this material is irregular in outline, and appears to lie in lymph spaces, but much of it is cellular. The presence of a swollen nucleus occupying the centre of a mass is frequently seen, and in some cases instead of the hyaline or colloid appearance distinct granules are evident. The granular cells and hyaline masses can be traced into the pars intermedia, from the cells of which they apparently take origin.

In the neck of the posterior lobe the colloid is even more plentiful; approaching the floor of the third ventricle it becomes more cellular in appearance. Many of the cells in this situation are extremely vacuolated, others are granular or filled with an amorphous material. In the infundibular recess at the floor of the third ventricle are found numerous cells of varying size and masses of granular and amorphous material. The cells lie free in the cavity of the third ventricle (Plate I, fig. 2), having found their way out of the neck of the posterior lobe by passing between the ependyma cells. After their escape into the cerebro-spinal fluid they become swollen and disintegrate with the production of a granular and amorphous debris.

In addition to this increased production of colloid, there is a distinct budding of the ependyma cells which line the ventricle, and a proliferation of the adjacent neuroglia. The colloid bodies are not only present in the neck

of the lobe, but occupy the thin anterior or post-optic lamina, with the under surface of which the cells of the tongue-like process of *pars intermedia* are closely associated. Changes in the ependyma and neuroglia cells are most noticeable in this region.

In the cats operated upon nervous symptoms followed within twenty-four hours after removal of the thyroids (and parathyroids), and the animals were killed from four to six days after the operation. In the pituitaries of these animals there is comparatively little change. Colloid is still present in the spaces in the *pars intermedia* in which it is normally found. Variations in the amount of colloid in the pituitary of the healthy animal are so great that no change can be definitely asserted, but the amount of colloid in the pituitaries of these recently thyroidectomised animals is not unusually large. The granular bodies and hyaline material in the posterior lobe are more plentiful than is normally the case. Accumulations of colloid cells are seen in numerous places immediately beneath the ependyma cells both in the body and neck of the posterior lobe. They form papular projections of the ependymal surface, and the contents are frequently seen escaping into the infundibular cavity. Localised proliferations of ependyma or neuroglia cells are also found. The changes, as in the rabbit's pituitary, are not confined to the body and neck of the posterior lobe, but extend into the anterior and posterior laminae. They correspond in extent and distribution with the presence of cells of the *pars intermedia* lying outside the nervous part. There is no accumulation of colloid inside the epithelial cleft.

In the only dog as yet operated upon the thyroids (and parathyroids) were completely removed, and no symptoms occurred until five days afterwards, when the animal had attacks of tetany. It recovered, but tetany again developed and extreme weakness came on. The dog was killed nineteen days after the operation. The anterior lobe of the pituitary shows no change. There are no cystic accumulations of colloid in the *pars intermedia*, but a great formation of colloid is taking place at the junction between *pars intermedia* and *pars nervosa*. The nervous substance of the posterior lobe is granular in appearance, and contains masses of colloid accumulated in certain situations. It is most abundant in the neck of the lobe and at the lower end of the infundibular recess. In places the ependyma lining is distended by colloid, some of which bursts through into the infundibular cavity. Here again most of the colloid is cellular, and when it mixes with the cerebro-spinal fluid it takes the form of large nucleated cells full of granular or amorphous material.

In the neck of the lobe appearances are seen of which fig. 3, Plate II., presents an illustration. Cells of the *pars intermedia* pass inwards invading the nervous substance and frequently accompanying blood-vessels. As they approach the infundibular cavity the cells become swollen and look like colloid masses. They accumulate below the ependyma and finally pass into the cavity either in a cellular form or as a hyaline debris.

In this dog there are also marked changes in the ependyma cells, consisting chiefly of a budding off of small round globules into the infundibulum and third ventricle. Fig. 4, Plate II., is a photograph of part of the internal surface of the posterior lamina, and shows the formation and liberation of these small globular bodies. The ependyma cells of the infundibular region are not ciliated, and probably have a secretory power. The combined products of the cells of the *pars intermedia* and of the ependyma cells are mixed, and form a considerable accumulation in the infundibular recess of the third ventricle. The nature and significance of this material is as yet undetermined; its formation in thyroïdectomised animals appears to be an exaggerated condition of a normal process.

#### SUMMARY.

Thyroidectomy is followed by definite histological changes in the pituitary body. The anterior lobe is apparently unaffected; it shows no immediate sign of increased activity as far as can be judged from the animals examined. Whether or not it undergoes an alteration in animals which survive for a long time remains to be determined.

There is increased activity of the cells of the *pars intermedia*, and probably an increase in the number of these cells in animals which live for some time after the operation. In this respect the work of Rogowitsch and others is confirmed.

The most striking changes are manifested in the nervous part of the posterior lobe and in the laminae forming the floor of the third ventricle. In these situations granular, hyaline, or colloid bodies become very numerous. They appear to be, in part at least, of a cellular nature, and to find their way between the ependyma cells into the infundibular recess and ventricles of the brain.

There are also alterations in the ependyma and neuroglia cells in the same regions. The former appear to have a secretory function and give off small clear globular bodies into the infundibular recess and third ventricle. There are also localised proliferations of neuroglia.

The significance of these changes is as yet undetermined. The colloid bodies appear to arise from the epithelial cells of the *pars intermedia*, and their extensive production to be an exaggeration of a normal process. Further work is being carried on to determine the nature of the colloid of the pituitary, its relations to the active principles found in the posterior lobe, and its influence in the production or amelioration of the symptoms, which, in many animals, follow removal or disease of the thyroid.

The expenses incurred in this work are assisted by a grant from the Carnegie endowment for research purposes.

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DESCRIPTION OF PLATES.

PLATE I.

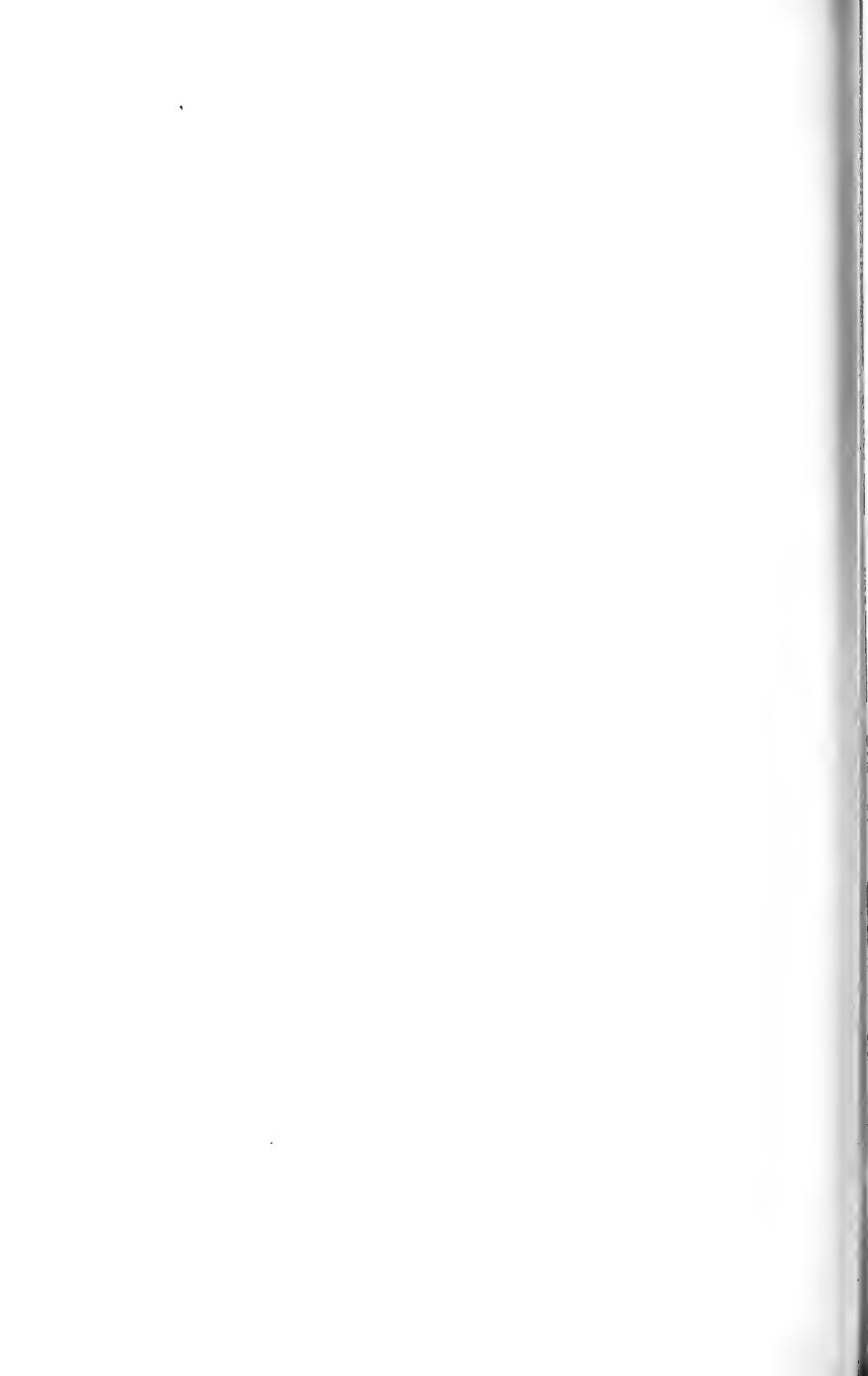
Fig. 1. Median sagittal section through part of posterior lobe of the pituitary of a rabbit three months after removal of both thyroids. (Photograph,  $\times 300$ .) Granular and colloid bodies are seen in the nervous portion of the lobe; some of the cells of the pars intermedia appear on the left-hand side.

Fig. 2. Median sagittal section through infundibular recess and neck of the posterior lobe of a rabbit three months after removal of both thyroids. (Photograph,  $\times 200$ .) Granular and colloid bodies in neck of the posterior lobe. Some of these are definite cells which pass into the infundibular recess. Budding of ependyma cells is seen on the inner surface of anterior lamina on the left-hand side of the photograph.

PLATE II.

Fig. 3. Median sagittal section through lower part of the neck of the posterior lobe of the pituitary of a dog nineteen days after thyroidectomy. (Photograph,  $\times 160$ .) Cells of the pars intermedia seen below are invading the nervous tissue of the neck of the posterior lobe. Colloid bodies are streaming inwards and accumulating beneath the ependyma cells lining the infundibular recess.

Fig. 4. Median sagittal section through part of the nervous substance of the upper part of the neck of the posterior lobe of the pituitary of the same dog. (Photograph,  $\times 300$ .) The active budding of the ependyma cells, and the escape of their products into the infundibular recess of the third ventricle, are shown.



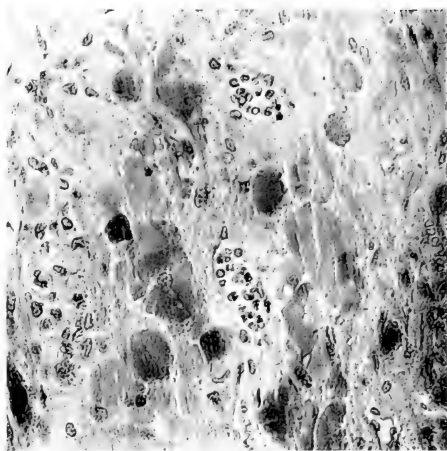


FIG. 1.

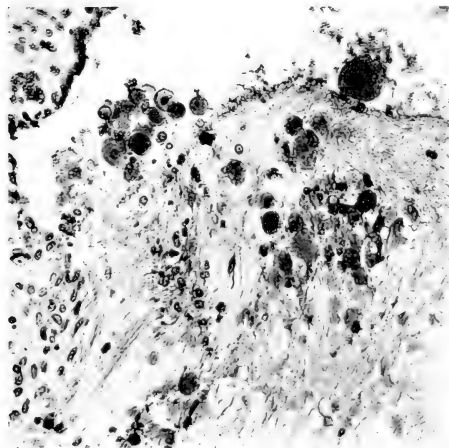


FIG. 2.





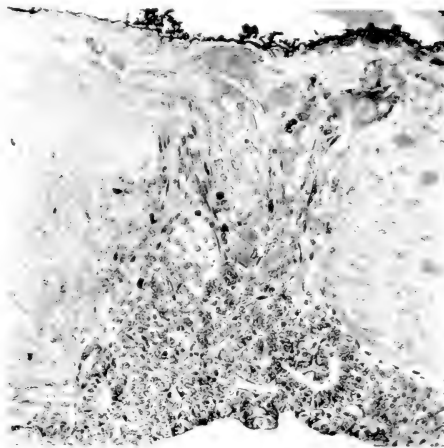
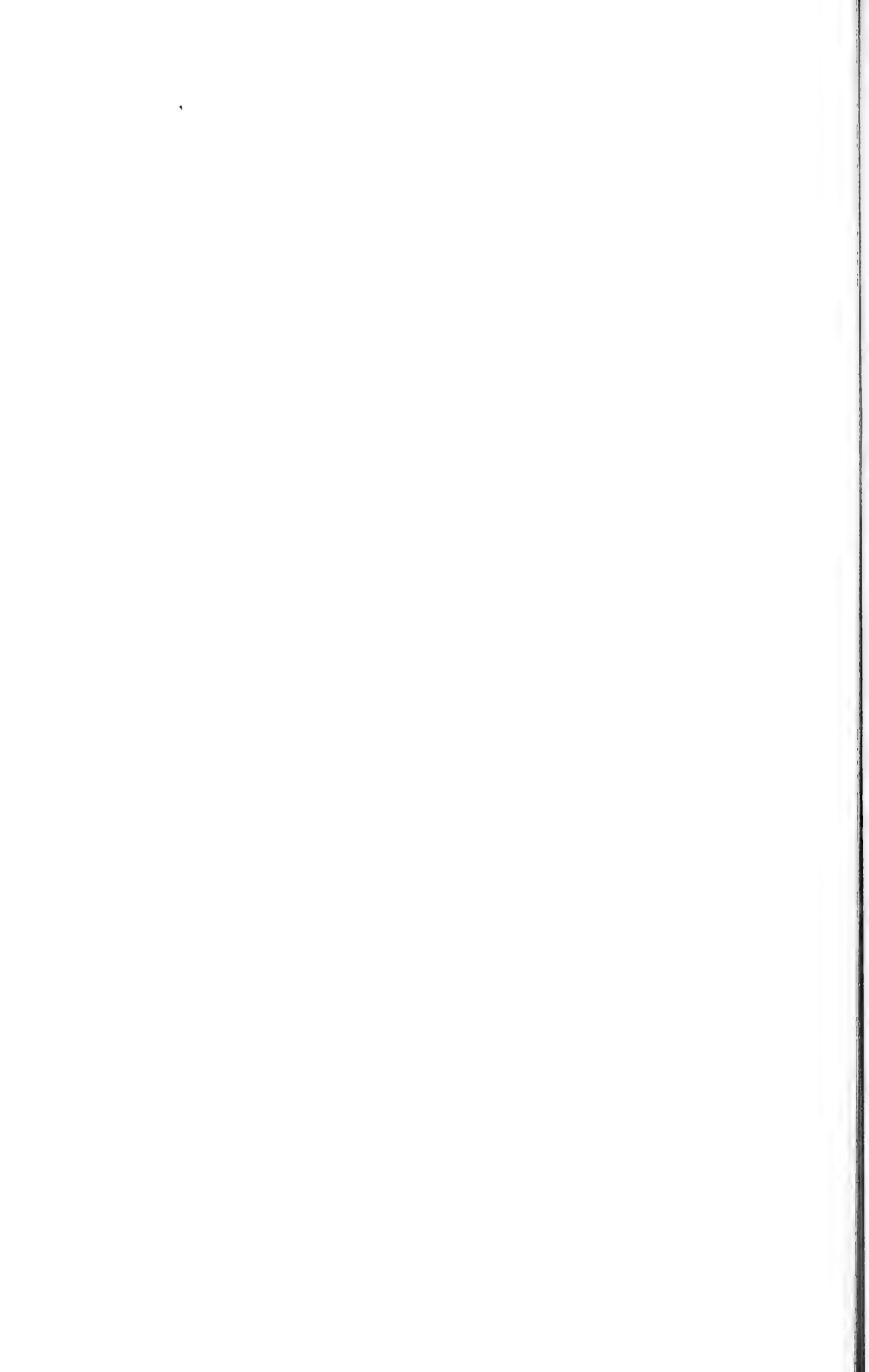


FIG. 3.



FIG. 4.



OBSERVATIONS ON THE NUCLEOLI IN THE CELLS OF HYDRA  
FUSCA. By C. E. WALKER and ALICE L. EMBLETON. (From the  
Laboratory of Cytology, University of Liverpool.) (With One Plate.)

(Received for publication 25th July 1908.)

METHODS AND MATERIAL.

HYDRA FUSCA was used throughout in the observations described here. The hydræ were fixed with Flemming's fluid (strong formula). Dehydration, embedding in paraffin, and the various other processes, were carried out according to the strictest cytological methods.

The following two processes of staining were chiefly employed, and only the results obtained by these are described here.

A. Saturated solution of basic fuchsin in 80 per cent. alcohol, plus a few drops of liquor ammon. fort., for about two minutes. Rinse in water and 30 per cent. alcohol, and water again, getting rid of part of the red stain so as to leave the sections pink. Methylene blue (aqueous solution) for about a minute; rinse in water. Unna's orange tannin until no more blue clouds are to be seen. Dehydrate, clear in xylol, mount in Canada balsam.

B. Saturated solution of saffranin in 80 per cent. alcohol, with a few drops of liquor ammon. fort., for a quarter of an hour, followed by methylene blue and Unna's orange tannin, as in first method.

OBSERVATIONS.

The nucleolus dealt with here is the "true nucleolus." It is a body generally spherical in shape, occasionally oval, bounded by a definite membrane, the contents being usually homogeneous, or finely granular in structure.<sup>1</sup>

The nucleoli in the cells of hydra, both in the ectoderm and in the endoderm, are very striking and definite. Generally only one nucleolus is to be seen in the nucleus, but in some cells we have found two, or even more, of different sizes. While within the nucleus, the nucleoli stain dark

<sup>1</sup> See Wilson, "The Cell in Development and Inheritance," p. 34; Macmillan, London and New York, 1904. Walker, "The Essentials of Cytology," pp. 12 and 13; Constable, London, 1907.

purple with method A, and bright red, sometimes with a blue area in the centre, with method B. They may frequently be seen to be budding in a manner very like the budding of the nucleoli in nerve-ganglion cells described elsewhere.<sup>1</sup>

In both endoderm and ectoderm cells the budding often takes the form of a small excrescence which gradually separates from the nucleolus, remaining joined to it, however, for a considerable period by a membranous process (see figs. 1, 2, and 3). Sometimes, however, particularly in the cells of the endoderm, the nucleoli seem to divide more or less equally from the beginning, and it is impossible to describe this form of division as budding. Very often there is one large nucleolus to be seen and several small ones (fig. 2), but we also find some nuclei containing two or more large nucleoli nearly equal in size (fig. 4). Nucleoli may also frequently be seen in process of passing through the nuclear membrane. As the nucleolus passes through, the nuclear membrane appears to form an encircling lip round it, the membrane being re-formed underneath it very rapidly (figs. 5 and 6). Thus, when the nuclear membrane has re-formed behind the extruded nucleolus, the latter lies in a depression not unlike a crater. Almost as soon as the nucleolus has passed through the membrane, the dark purple colour it exhibits when stained by method A is lost at its periphery, leaving this pink. We thus have a spherical nucleolus with a dark purple area in the centre, surrounded by an area stained pink (figs. 1, 4, 7, 8, and 9). The purple centre appears to decrease rapidly, leaving the nucleolus pale pink throughout. With method B the blue or violet area seen in some nucleoli while within the nucleus is lost as the former passes into the cytoplasm. In every case the nucleolus becomes more and more orange in colour as it leaves the neighbourhood of the nucleus. In the cells of the endoderm, nucleoli may often be seen in process of division, after they have been extruded from the nucleus. It seems that after this, in the endoderm cells, the nucleoli are pushed towards the periphery of the cytoplasm. On approaching the periphery they lose the pink colour (obtained with method A) and take the orange stain, becoming at the same time much less defined, and appear eventually to disintegrate altogether.

We have been unable hitherto to trace the destiny of the nucleoli in the cells of the ectoderm after their extrusion. Occasionally the nucleolus lies pressed upon the surface of the nuclear membrane after its extrusion (fig. 9). Also we have observed the contents of the nucleoli to become granular in a few instances.

The depression, or crater, in the surface of the nuclear membrane appears to last for a considerable time, and gives the appearance of pseudopodial processes when seen in optical section (figs. 4, 7, 8, and 9).

<sup>1</sup> "On the Multiplication and Migration of Nucleoli in Nerve Cells of Mammals," by W. Page May and C. E. Walker, this Journal, vol. i., No. 2, 1908.

The cytoplasm of the endoderm cells appears to be almost structureless, while there is generally some structure to be seen in the ectoderm cells.

#### CONCLUSIONS.

The phenomenon here recorded seems to be something quite apart from anything connected with cell division, whether mitotic or amitotic: indeed, it is only to be observed in cells that are in the vegetative condition. The probability that it is intimately connected with, and dependent upon, metabolism taking place in the nucleus, is very strong. While it is readily observed in almost all the cells of the endoderm, it can be followed only to a very limited extent in the cells of the ectoderm. The processes connected with digestion are carried out by the cells of the endoderm. It would therefore appear probable that the kind of metabolism in the nucleus that is connected with digestion produces this phenomenon in a more striking manner, and more frequently, than is the case with the nuclear metabolism taking place in the cells of the ectoderm.

The rapid and very marked change in the staining reaction of the nucleolus as it passes into the cytoplasm, suggests that some important chemical or physical change takes place in the contents at this time. Similar changes have been observed in the nucleoli of nerve-ganglion cells.<sup>1</sup>

It seems probable that the bodies here described as extruded nucleoli have been frequently described as food particles. But we would point out that the structures here dealt with are apparently in process of disintegration at the periphery of the cell, become more defined in the neighbourhood of the nucleus, and are found in their most definite form and in process of active multiplication within the nucleus itself.

<sup>1</sup> Page May and Walker, loc. cit.

#### DESCRIPTION OF PLATE.

(In these drawings the cytoplasm of the endoderm cells is, for the sake of convenience, considerably diminished in size in proportion to the nuclei.)

Fig. 1. Two endoderm cells showing the nucleoli budding. Some nucleoli are to be seen in the cytoplasm.

Fig. 2. Two endoderm cells showing the nucleoli budding. Several nucleoli may be seen in the same cell.

Fig. 3. Four ectoderm cells showing nucleoli budding.

Fig. 4. An endoderm cell showing two large nucleoli and pseudopodial processes in the nucleus, as well as several nucleoli in the cytoplasm.

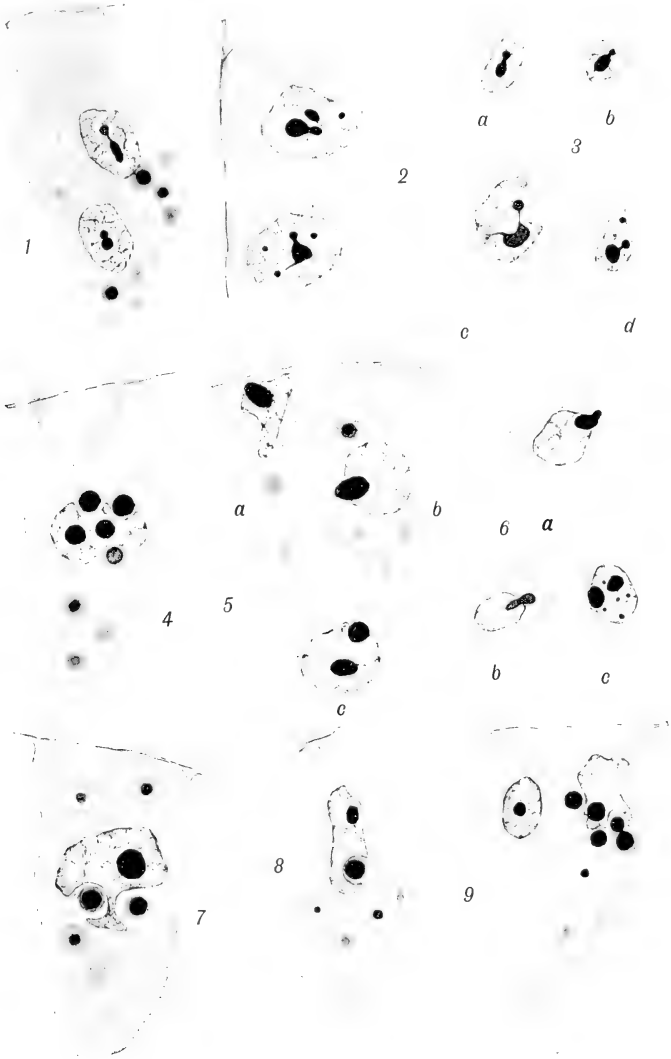
Fig. 5. Three endoderm cells showing nucleoli passing out of the nucleus. In *c* a nucleolus which remains within the nucleus is seen to be as large as that which is passing out.

Fig. 6. Three ectoderm cells showing nucleoli passing into the cytoplasm.

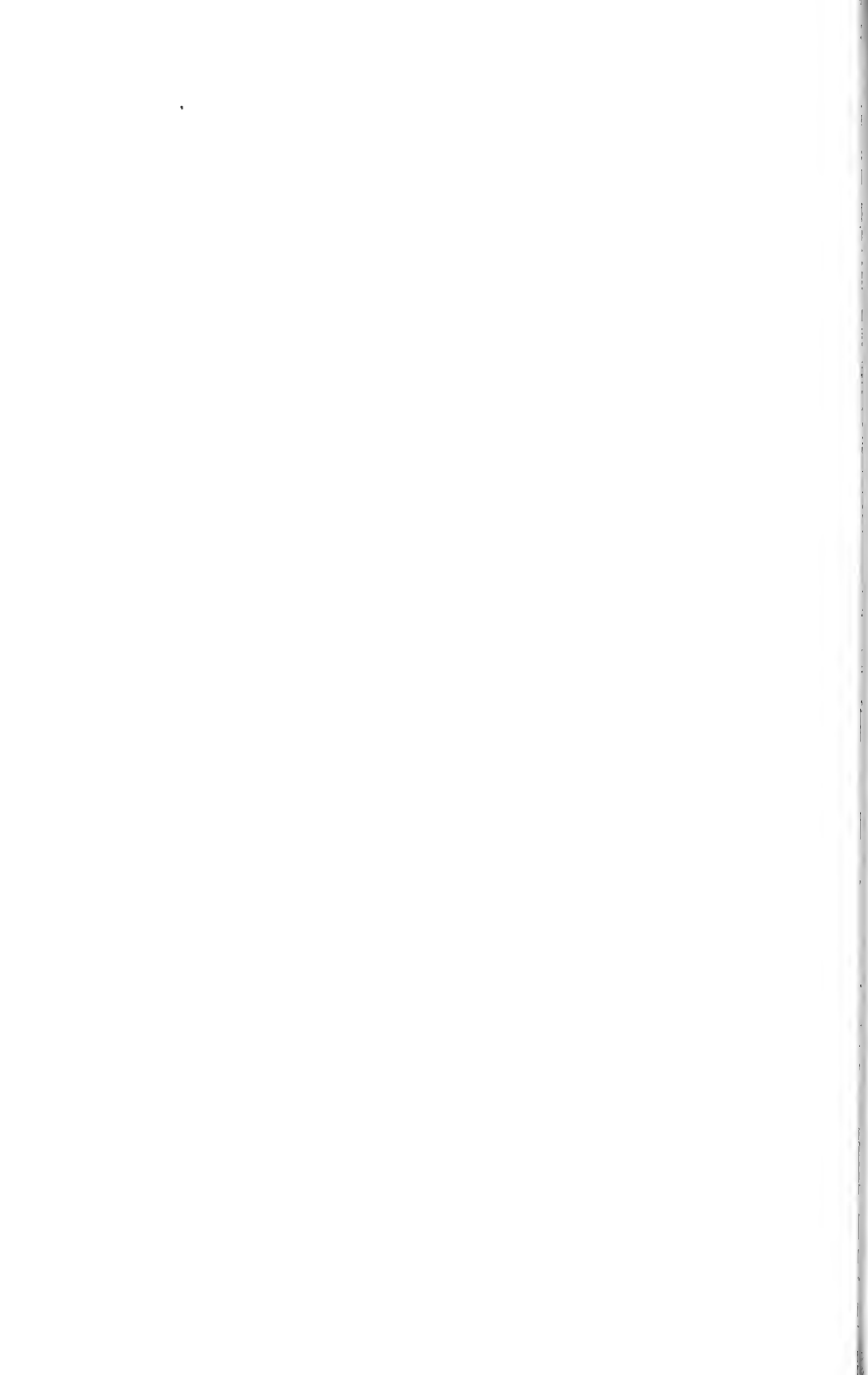
Fig. 7. An endoderm cell showing nucleoli lying in the cytoplasm. Some have a dark centre ; in others the dark centre has disappeared. The nucleus also exhibits pseudopodial processes.

Fig. 8. An endoderm cell in which a nucleolus is seen pressed against the outside of the nuclear membrane.

Fig. 9. Two endoderm cells, one of them containing a number of nucleoli in the cytoplasm. One of these nucleoli is seen to be dividing.



C. E. WALKER and ALICE L. EMBLETON, "Observations on the Nucleoli of Hydra Fusca."





IS CHOLINE PRESENT IN THE CEREBRO-SPINAL FLUID OF  
EPILEPTICS? By S. KAJIURA, Imperial Japanese Navy. (From  
the Physiological Laboratory, King's College, London.) (With  
one Plate.)

(Received for publication 30th July 1908.)

IN their investigations on the presence of choline in cerebro-spinal fluid and blood, Halliburton and Mott pointed out that it was only present in organic diseases of the nervous system, and that this was a fact of diagnostic value in distinguishing diseases where there is an actual breakdown of nervous material from those which are merely functional.<sup>1</sup> Although these observers, and those who immediately followed them, employed as their principal chemical test the formation of crystals of the choline platino-chloride—a test which it has since been shown is not absolutely conclusive by itself—nevertheless the absence of choline can be inferred when this test gives negative results.

On the other hand, it has been contended by Donath<sup>2</sup> that choline is generally found in the cerebro-spinal fluid in cases of epilepsy; and he relies for the detection of choline upon the fact that the crystals of the platinum compound of choline are doubly refracting, and can thus easily be distinguished from platinum compounds of potassium and ammonium chloride, which under the ordinary microscope are liable to be mistaken for those of the choline salt.

In his Oliver-Sharpey lectures,<sup>3</sup> Professor Halliburton said: "This requires confirmation. Granting that the diagnosis of epilepsy in Donath's cases was correct, it appears necessary to remove epilepsy from the list of functional diseases, if the existence of choline in the fluids of such cases is a fact."

The research of which this paper is the outcome was undertaken with the view of ascertaining whether or not Donath is correct in his statements. The difficulty of demonstrating the presence of choline when mixed with potassium and ammonium salts, as always happens when one is dealing with physiological fluids, has been overcome by the introduction of the periodide test by O. Rosenheim,<sup>4</sup> and it appeared necessary to investigate the question by means of this characteristic and trustworthy

<sup>1</sup> See fully Halliburton, *Ergebn. d. Physiologie*, iv., pp. 72-74, 1905.

<sup>2</sup> *Journ. of Physiol.*, xxxiii., p. 211, 1905-6.

<sup>3</sup> *British Medical Journal*, May 4, 1907.

<sup>4</sup> *Journ. of Physiol.*, xxxiii., p. 221, 1906; xxxv., p. 445, 1907.

reaction. Dr Donath had sent to Dr Rosenheim seven samples of cerebro-spinal fluid from cases of genuine epilepsy, and the latter kindly placed them in my hands for the purpose of this research. I have to thank Dr Rosenheim for his care in supervising my work. I am also deeply indebted to Dr F. E. Batten, Dr A. Connel, and Dr J. Bernstein for a number of other specimens which have enabled me to extend the series of observations. In all cases the fluid was removed by lumbar puncture during life.

In order to make myself acquainted with the method, and at the same time to test its reliability and sensitiveness, I carried out a series of tests by adding pure choline to an artificial cerebro-spinal fluid. The latter was prepared by mixing egg-albumin and dextrose in the necessary proportions with a solution of potassium and sodium chloride of a strength corresponding to that found in cerebro-spinal fluid. The choline hydrochloride employed was prepared from lecithin and purified by repeated crystallisation of its mercury double salt.<sup>1</sup> Using Rosenheim's simplified iodine test,<sup>2</sup> I have been able to detect choline easily and with certainty when it was mixed with the artificial cerebro-spinal fluid in the proportions 1:1500, 1:5500, and 1:10,500. In the last two instances, only 15 c.c. of the mixture were used. Dilutions greater than 1:10,500 I should regard as of no practical importance; but Rosenheim obtained positive results when the proportion was 1:20,000.

In the examination of the material sent by Dr Donath, my general plan of testing for choline was (1) to try the periodide test with the purified alcoholic extract; (2) to employ Donath's micro-polariscopic method, all his directions being carefully observed; and (3) to use Rosenheim's original periodide test as applied to the platinum salt.

The results are given in Table I, p. 293.

In all cases Donath's test gave a positive result; a few doubly refracting crystals could always be distinguished under the crossed nicols of the polarising microscope (see fig. 2). The yield of the platinum precipitate was in all cases extremely small, amounting usually to not more than a slight haze; so that it was necessary to wait many hours for it to settle before collecting it on a filter. In no instance could the periodide reaction be obtained either with the alcoholic extract direct, or from the platinum precipitate.

<sup>1</sup> In his first communication on the periodide reaction, Rosenheim applied the iodine solution to the crystals of choline platino-chloride, and found that they dissolved and were replaced by crystals of choline periodide. Dr Rosenheim informs me that the mercury salt may be used instead of the platinum salt for this purpose. I have further examined the lead, zinc, and cadmium salts in the same way, and found that they all show the reaction. The cadmium salt, owing to its insolubility in alcohol, seems to be the most suitable to replace the platinum salt, if that should be necessary (see also F. W. Schmidt, *Zeits. f. physiol. Chem.*, liii., p. 428, 1907). Still, as Rosenheim pointed out in his second paper, it is not really necessary to prepare a metallic salt first, for the iodine reaction can be obtained straight from the alcoholic extract of any fluid which contains choline.

<sup>2</sup> *Journ. of Physiol.*, xxxv., p. 465, 1907.

It follows from these results either that choline is present, but in amounts too small for the periodide test to reveal it, or that Donath's test is untrustworthy and choline is absent. If the first alternative is correct, Donath's test is much more sensitive than the periodide reaction.

TABLE I.

Donath's cases.	Quantity of cerebro-spinal fluid.	Periodide test.	Platinum compound prepared.	
			Donath's polariscopic test.	Periodide reaction.
1	33 c.c.	-	+	-
2	36.5 "	-	+	-
3	24 "	-	+	-
4	28 "	-	+	-
5	15 "	-	+	-
6	36 "	-	+	-
7	11 "	-	+	-

The formation of anisotropic crystals on the addition of platinum chloride is really not so characteristic of choline as Donath appears to consider. It does not exclude the possibility that other bases are present in cerebro-spinal fluid which form anisotropic crystals, and which do not give the periodide reaction for choline. Rosenheim has already pointed this out in one of his papers,<sup>1</sup> and I have been able to confirm his observations.

But, before coming to a final conclusion, it seemed advisable to examine a few more cases of epilepsy, and the results are given in Table II.:-

TABLE II.

Number of case.	Disease.	Quantity of cerebro-spinal fluid.	Periodide test.	Platinum precipitate.	
				Donath's test.	Periodide test.
8	Epilepsy	5 c.c.	-	+	-
9	"	3 "	+	+	
10	"	10 "	+	+	+
11	"	2.5 "	-	+	

The results of the examination of these four specimens are very interesting. When sent to the laboratory, they were simply labelled epilepsy; and it will be seen that in two of them choline was found by the periodide test, notwithstanding that in one of them (case 9) the quantity of fluid at my

<sup>1</sup> Journ. of Physiol., xxxv., p. 469, 1907.

disposal was very small. The quantity, however, was too small to enable me to complete the series of tests by the application of the periodide reaction to the platinum compound. This was also the case in No. 11.

In view of these two positive results, a closer investigation of them appeared necessary, for it seemed improbable that they were cases of simple epilepsy; the notes which Dr Batten kindly forwarded confirmed this view.

Case 9 was a child (F. L. R.) one year old, with congenital mental defect, with chorea and epilepsy culminating in status epilepticus. Her symptoms while in the hospital appear to have been very obscure, and the diagnosis of meningitis and later of encephalitis was made. The fluid removed by lumbar puncture contained excess of lymphocytes. She was still in the hospital when I last heard of her.

Case 10 was also a child (L. H.) ten years of age, who had had frequent fits since she was eighteen months old. It did not appear, however, to be a simple case of epilepsy, for there was also considerable mental defect, though the cause of the latter is uncertain.

In case 9 the clinical evidence is quite clear that something more than a functional disorder was present. In case 10 the clinical evidence is not so clear, although it was evidently recognised as something more than a simple case of epilepsy. The finding of choline in both cases is therefore not unexpected, and these are just the cases where the choline test should come to the assistance of the clinical observer, in pointing to the probability that an organic lesion is at the root of the malady.

It now remains to explain how it is that Donath's method gave always a positive result, although choline could not be detected by the periodide test.

In the first place, Donath's method always gives positive results, not only in cases of epilepsy, but also in normal cerebro-spinal fluid, or in the cerebro-spinal fluid of every disease in which I have had the opportunity of examining it.

These results are shown in Table III., p. 295.

In cases 12 and 13 the fluid was normal, and in case 16 the fluid was also approximately normal.

Negative results (by the periodide test) in cases of meningitis were previously noted by Rosenheim;<sup>1</sup> inflammation of the meninges does not necessarily cause any noteworthy change in the underlying cerebral matter. Yet, as the table shows, all cases gave a positive result when subjected to Donath's test.

As it seemed possible, as mentioned before, that the obtaining of anisotropic crystals may indicate the presence of other organic bases, I collected the platinum precipitates of several cases (which gave a positive

<sup>1</sup> Journ. of Physiol., xxxv., p. 467, 1907.

Donath's test, and a negative periodide reaction), ignited them in a platinum dish, and extracted the residue with dilute hydrochloric acid. Donath's test was again performed with this extract. Although all the organic matter had been destroyed, nevertheless doubly-refracting crystals, although less numerous than before, were obtained. It is clear, therefore, that part at least of the anisotropic crystals represent inorganic substances.

TABLE III.

Number of case.	Disease.	Amount of fluid examined.	Periodide test.	Platinum precipitate.	
				Donath's test.	Periodide test.
12*	Cancer of stomach	33 c.c.	-	+	-
13	Nephritis	28 "	-	+	-
14	Post-basic meningitis	10 "	-	+	-
15		5 "	-	+	-
16	Congenital hydrocephalus				
(a)		5 "	-	+	-
(b)		15 "	-	+	-
(c)		5 "	-	+	-
(d)		5 "	-	+	-
(e)		60 "	-	+	-
(f)		45 "	-	+	-
17	Epidemic cerebro-spinal meningitis	13 "	-	+	-
18		?	-	+	-
19	No record given	10 "	-	+	-
20	"	5 "	-	+	-

\* In this case the fluid was removed twelve hours after death.

This result led me to carry out a control experiment, using distilled water instead of cerebro-spinal fluid. The method was carried through in the usual way, and the inorganic salts employed (potassium carbonate, potassium chloride, and platinum chloride) were carefully purified specimens. Finally, under the polarising microscope a crop of doubly-refracting crystals, indistinguishable in quantity and form from those obtained in cases of epilepsy, were obtained. I have repeated this several times with the same result. This is illustrated by the accompanying photographs. Fig. 1 shows the appearance of the doubly-refracting crystals obtained in a blank experiment, using 20 c.c. of distilled water instead of cerebro-spinal fluid. Fig. 2 shows the same from a case of genuine epilepsy. If these are compared with those published by Donath,<sup>1</sup> they will be seen to be practically identical.

It is clear, therefore, that such crystals are no proof of the presence of choline, but owe their origin (at least in part) to minute impurities in the

<sup>1</sup> Loc. cit., p. 215.

reagents employed. It must be remembered that the delicacy of the polarisation microscope is so great that impurities which cannot be detected by ordinary chemical methods are revealed. Whilst it may of course be possible to purify the salts used in the test to such a degree that they are free from these impurities, I have been unable up to the present to obtain any specimens which do not show some anisotropic crystals under the conditions of Donath's method. Kahlbaum's purest potassium chloride and potassium carbonate give the reaction easily.

Whilst this investigation was in progress a paper appeared by A. Ziveri<sup>1</sup> on the presence of choline in cerebro-spinal fluid in mental diseases. By applying Rosenheim's periodide test, he found choline absent in cases of epilepsy, only one positive result being obtained in twenty-six examinations.

The conclusions arrived at by M. Kaufmann,<sup>2</sup> so far as they refer to the absence of choline in epilepsy, confirm my results; but his further statements that the base found by him in cerebro-spinal fluid in other cases is not choline do not seem to be borne out by his own experiments, and are not in agreement with the results of previous observers and my own.

The following are the general conclusions to be drawn from the investigations:—

1. Rosenheim's periodide test for choline is both trustworthy and sensitive.

2. Relying upon this test, choline is found to be absent from the cerebro-spinal fluid in cases of genuine epilepsy.

3. The detection of a few fragments of anisotropic crystals by Donath's micro-polariscopic test is not in itself sufficient evidence of the presence of choline in the cerebro-spinal fluid of epileptics; for the same result may be obtained with normal cerebro-spinal fluid, or even with distilled water.

<sup>1</sup> Riv. di Neuropatologia, etc., vol. i. p. 1, 1908.

<sup>2</sup> Neurolog. Zentralbl., No. 6, March 1908, p. 260. The errors in Kaufmann's experiments have been fully demonstrated by J. Donath in a paper which will be published in the forthcoming number of the same Centralblatt, an advance proof of which has reached me through the kindness of Dr Donath.



FIG. 1.



FIG. 2.





ON SO-CALLED "PROTAGON" By OTTO ROSENHEIM and M. CHRISTINE  
TEBB. (From the Physiological Laboratory, King's College, London.)  
(With one Plate.)

(Received for publication 31st July 1908.)

IN the first number of this Journal<sup>1</sup> Wilson and Cramer made an attempt to rehabilitate "protagon" as a substance of definite chemical composition and of constant physical properties. For this purpose the old assumption of Liebreich, previously abandoned by Cramer, that "protagon" is decomposed by warm or boiling alcohol, is revived. The decomposition is assumed to be a hydrolytic one, and explains, according to Wilson and Cramer, the discrepancies in the analytical figures obtained for "protagon" by other investigators. By means of a method which is essentially a modification of Couerbe's (1834) original method for the preparation of what he called *cérébrote*<sup>2</sup> (= "protagon"), and by limiting the time of extraction to one and a half minutes, they prepared a new standard "protagon" and maintain that it can be recrystallised from alcohol without decomposition, the only condition being to limit the action of the solvent to a short period of time. As an index of the decomposition they rely upon a change in the supposed physical constants. The specific rotation of "protagon" in pyridine is stated by Wilson and Cramer to be  $[\alpha]_D^{30} = +6.8^\circ$ , whilst "decomposed protagon" according to them shows  $[\alpha]_D^{30} = (+?)13.3^\circ$  (13.08 to 13.43). By a strange oversight the sign of the rotation in the latter case is not stated.

In the course of an investigation on the behaviour of "protagon" solutions in polarised light, which has led us to the discovery of a new phenomenon (Spherorotation), we have found that any "protagon" (provided that its constituents are present in such proportions as to make the amount of phosphorus about 1 per cent.) possesses an initial dextrorotation of  $[\alpha]_D^{30} = +6.8^\circ$  and a final levorotation of  $[\alpha]_D^{30} = -13.3^\circ$ .<sup>3</sup> This change from dextrorotation to levorotation, the nature of which has been

<sup>1</sup> Journ. of Exp. Physiol., vol. i., 1908, p. 97.

<sup>2</sup> It seems hardly necessary, in view of the results to be discussed in the present paper, to enter into the charge of inconsistency made by Wilson and Cramer against our identification of "*cérébrote*" and "acetone-protagon" with "protagon." It can be easily shown by the methods to be described that these two products represent the same heterogeneous mixtures as, and are identical with, "protagon." With regard to the different solubility of "protagon" in acetone before and after extraction from brain, Wilson and Cramer seem to have forgotten that exactly the same behaviour of "protagon" towards ether was described in Liebreich's original paper. This is, indeed, now generally recognised as characteristic of the behaviour of mixtures of lipoids.

<sup>3</sup> Journ. of Physiol., vol. xxxvii., 1908, p. 341 and p. 348.

explained fully in the other papers just referred to, has not been mentioned by Wilson and Cramer. It is a property of any "protagon," whether the same has been subjected to the supposed decomposing influence of boiling alcohol or not. The value  $[\alpha]_D^{20} = (+ \text{ or } -) ? 13.3'$  of Wilson and Cramer is therefore in no way characteristic for "decomposed protagon," and this fact alone deprives the whole decomposition theory of its foundation.

During these investigations we have also obtained further chemical evidence, if such should be still wanted, which proves clearly that our previous conclusions with regard to the composite nature of "protagon" also apply to the new standard "protagon" of Wilson and Cramer.

#### THE COMPOSITION OF "PROTAGON" IS COMPLETELY CHANGED BY RECRYSTALLISATION FROM ALCOHOL

The main chemical fact on which Wilson and Cramer rely for their view that "protagon" is of a definite composition is the possibility (which has never been denied) of its recrystallisation from alcohol without change in its composition. The one and only condition which must be fulfilled to attain this end consists, according to them, in limiting the exposure to the hot solvent to a short period of time.

It is clear, however, that this fact, even if the above condition was the correct one for its achievement, would in no way prove the definite composition of "protagon"; for a mixture of substances which possess approximately the same solubility in a given solvent, may also retain its relative composition under these conditions. This is well illustrated by the case of phytosterin, which since its discovery by Hesse<sup>1</sup> has also been assumed to be a substance of definite chemical and physical properties, a product of constant composition, melting point, and optical activity being always obtained by recrystallisation from alcohol. Windaus and Hauth<sup>2</sup> recognised, however, that Hesse's product was a mixture of two substances.

In the recrystallisation of "protagon" there is, however, another factor which has been completely neglected by Wilson and Cramer. We have looked in vain to find in their paper any statement as to the proportion of "protagon" to alcohol employed for the recrystallisation. This proportion is evidently of no importance if one is dealing with a definite chemical substance such as cholesterol. Whatever the amount of solvent used, the recrystallised product will always be cholesterol. But in the case of a mixture of substances the amount of solvent used for recrystallisation is obviously of the greatest importance.

We found that, by simply varying the proportion of "protagon" to alcohol, we obtained variations in the phosphorus percentage of "protagon" of over 50 per cent., whilst taking care at the same time to avoid the

<sup>1</sup> Ann. d. Chem., 192, 1878, p. 175.

<sup>2</sup> Ber. d. d. chem. Ges., 39, 1906, p. 4378.

supposed decomposing influence of alcohol by limiting the time of heating during recrystallisation to one minute.

We give the following experiment in detail; it is perfectly typical of several we have performed.

"Protagon" was prepared from ox-brain, following scrupulously Wilson and Cramer's directions. The greatest care was taken to limit the time of extraction with hot alcohol to one and a half minutes, and the time of heating during recrystallisation to one minute. The extracts and solutions were cooled at once on ice. An insoluble residue remained in the first and second recrystallisation, very little in the third and fourth. The following analytical figures were obtained:—

	Proportion "protagon" to alcohol.	P %
Original "protagon" . . . . .	3 extractions; 1 part brain : 2 parts alcohol	1.33
1st recryst. . . . .	1 : 18	0.83
2nd " . . . . .	1 : 30	0.54
3rd " . . . . .	1 : 20	0.44
4th " . . . . .	1 : 20	0.39

It will be seen from these results that the phosphorus percentage of "protagon" falls during four recrystallisations from 1.33 per cent. to 0.39 per cent., notwithstanding the strictest adherence to Wilson and Cramer's conditions.

From the final phosphorus-poor product we obtained easily by recrystallisation from glacial acetic acid (Koch), or by a slight modification of Thudichum's method, a substance which agrees in all its properties with Thudichum's phrenosin (= Gamgee's "pseudocerebrin" and Thierfelder's "cerebron") and which is free from phosphorus and sulphur.

We give below the results of a complete analysis of this substance in order to show the striking differences from the composition of the original "protagon":—

	C %	H %	N %	P %	S %
Phrenosin (our analysis) . . . . .	68.96	10.32	1.82	none	none
Protagon (Wilson and Cramer's analysis, sample D)	66.40	10.71	2.55	1.02	0.68

The results obtained in the recrystallisation of protagon from alcohol, which agree with those recently published by Gies and Cohen,<sup>1</sup> are in direct contradiction to those of Wilson and Cramer, who maintain that the phosphorus percentage of "protagon" remains constant, although they obtain the relatively low figure of 0.92 per cent. after the fourth recrystallisation. (The phosphorus percentage of their original "protagon" is not given.)

<sup>1</sup> Proc. Soc. Exper. Biol. and Med., vol. v., 1908, p. 97.

We were, however, able to find the correct explanation for this discrepancy, which by itself proves the indefinite composition of "protagon." By carrying out a tedious series of systematic recrystallisations, constantly controlled by analysis, we have found out the exact conditions under which the phosphorus percentage of "protagon" remains approximately constant. Wilson and Cramer seem to have arrived at this result empirically without, however, recognising or stating the true conditions. These depend in no way on the time of heating and the supposed decomposition, but simply on the proportion of "protagon" to alcohol employed. Wilson and Cramer favoured evidently a very small proportion, as is indicated in the paper by Loehhead and Cramer.<sup>1</sup> We found that, by limiting the amount of alcohol used for recrystallisation to a minimum ("protagon": alcohol = 1:5 for the first and 1:2 or less for the subsequent recrystallisations), we were able to keep the phosphorus percentage at the figure held by Wilson and Cramer to be characteristic for "protagon."

It is obvious that these are not the conditions which favour the intended purification. When using reasonable amounts of solvent for this purpose, as in the preceding series of recrystallisations, it will be seen that "protagon" undergoes a complete change of composition.

We again quote only one typical experiment, in which another sample of Wilson and Cramer's "protagon" was employed. The same precautions were taken during its preparation and recrystallisation as indicated above, the only altered factor being the proportion of "protagon" to alcohol. The results were as follows:—

	Proportion of "protagon": alcohol.	P %
Original "protagon" .	3 extractions ; 1 part brain : 2 parts alcohol	1.18
1st recryst. . . . .	1 : 5	0.99
2nd „ . . . . .	1 : 2	0.96

Even here a distinct drop in the phosphorus percentage is noticeable, but owing to the whole of the dissolved substance being precipitated again by cooling on ice, no efficient separation of the constituents can possibly take place.<sup>2</sup>

The above results, which agree with those of Wilson and Cramer,

<sup>1</sup> Biochem. Journ., ii., 1907, p. 350. See also Cramer's letter to Posner and Gies, published by the latter in Journ. Biol. Chem., i., 1905-6, p. 79.

<sup>2</sup> We should like to point out that the term "recrystallisation" is hardly justified in this case, as the product precipitated from its hot solution by cooling on ice is amorphous. "Protagon" can only be obtained in crystalline form, according to Liebreich and Gamgee, if the temperature is allowed to fall very gradually. Under these conditions "protagon" would, however, be decomposed in Wilson and Cramer's sense. According to Gamgee and Blankenhorn (Zeitschr. f. physiol. Chem., iii., 1879, p. 277), their best result was obtained when the temperature fell in seventeen hours from 41.25° to 27.5°.

show that, whilst it is possible to retain approximately the composition of "protagon" during recrystallisation, this achievement is in no way due to the prevention of a hypothetical decomposition. The great variations in the analytical figures usually obtained for "protagon" are explained in the simplest manner. There is, therefore, no need to take refuge in the far-fetched "decomposition" theory which, besides not being confirmed by our critical re-examination of Wilson and Cramer's statements as to the physical constants of "protagon," is also a priori most improbable in view of the fact that alcohol is not a hydrolytic agent.

THE ISOLATION OF THE CONSTITUENTS OF "PROTAGON"  
BY MEANS OF PYRIDINE.

A generally recognised method of showing the uniformity of any substance consists in subjecting the same to fractional crystallisation or precipitation.<sup>1</sup> Wilson and Cramer considered the results of our previous fractionations as being produced by "decomposition." Although this criticism has now been shown to be unfounded, we thought it nevertheless advisable to repeat our fractionation experiments under conditions which make a decomposition in Wilson and Cramer's sense impossible. This can be easily done, as already stated in a previous communication,<sup>2</sup> by using a mixture of inert solvents.

It is remarkable that Wilson and Cramer refrain from employing fractional crystallisation as a test for the uniformity of their new "protagon," especially as the solvent (pyridine) chosen by them for the determination of the supposed physical constants of "protagon" lends itself admirably to this purpose. A decomposing influence at the low temperature in question is not to be assumed, and pyridine would not have been employed by them as their standard solvent if such an influence had been suspected.

"Protagon" is fairly soluble in pyridine at 30° to 45° C., and a precipitate is formed on cooling its solution. Evidently, if "protagon" is of a uniform composition, the precipitate must again consist of "protagon" with 1 per cent. phosphorus. Although we hardly expected this from our previous experience with this substance, we were nevertheless surprised to find on analysis that the precipitate contained more than double the amount of phosphorus, namely, 2.5 per cent. This result led us to a systematic examination of the fractions into which "protagon" can evidently be divided by means of pyridine.

For this purpose a 3 per cent. solution in pyridine of a recrystallised "protagon," prepared by Wilson and Cramer's modification of Gamgee's method, was employed. The solution was effected at 45° C., and the temperature kept at 45° for only one minute. The perfectly clear solution

<sup>1</sup> See H. Meyer, *Analyse, etc., organ. Verbindungen*, p. 13.

<sup>2</sup> *Proc. Physiol. Soc.*, xxxvii., 1908, p. 1.

was at once cooled on ice to room temperature (15° C.) and the precipitate filtered either after a few minutes or in some cases after half an hour. The filtrate was poured into two volumes of acetone, which produced a small quantity of a flocculent precipitate. After the removal of the latter by filtration, the acetone-pyridine solution was cooled on ice, by which process the bulk of the dissolved product was brought down.

The results of the analysis are given below:—

	P %	N %
Original "protagon" . . . . .	1·07	2·46
Fraction I. (insoluble in pyridine at +15°) . . . . .	2·51	3·03
Fraction II. (precipitated by acetone at ±0°) . . . . .	0·09	1·73

These results furnish the most striking proof for the composite nature of "protagon." Under the conditions of the experiment a decomposition in Wilson and Cramer's sense is impossible, and nevertheless "protagon" is divided by one fractionation into a practically phosphorus-free part and one with two and a half times as much phosphorus as that of the original "protagon." The nitrogen figures also undergo characteristic changes.

(a) The phosphorus-rich constituents.—The quantity of this fraction averages one-third of the "protagon" employed. We possess, therefore, in pyridine a solvent by means of which the phosphorus-rich moiety of "protagon" can be easily isolated. Its phosphorus percentage is not appreciably raised by repeated (three times) recrystallisation from pyridine, a fact which would, according to Wilson and Cramer, speak for its definite composition. We have, however, succeeded in isolating its main constituent, the di-amino-phosphatide, sphingomyelin, by a fractionation method with alcohol-chloroform and acetone, which we shall communicate later in detail. For the purpose of comparison with "protagon" we give below a complete analysis of the purest preparation which we succeeded in obtaining so far. We have, however, reasons for believing that this substance, which agrees in its properties with Thudichum's sphingomyelin, is not yet perfectly uniform

	C%	H%	N%	P%	S%
Sphingomyelin (our analysis)	62·90	11·54	3·33	3·46	...
"Protagon" (Wilson and Cramer's analysis, sample D)	66·40	10·71	2·55	1·02	0·68

(b) The phosphorus-free constituents.—Fraction II. as obtained above may be easily rendered perfectly phosphorus-free by further recrystallisation from glacial acetic acid (Koch) or by the method indicated previously.

## MICRO-CHEMICAL PROOF OF THE COMPOSITE NATURE OF "PROTAGON."

We have examined the physical properties of the substances isolated from "protagon" somewhat more closely, and found characteristic differences in their optical activity, melting point, solubility, etc. They possess further the remarkable property of crystallising from pyridine under certain conditions in fluid spherocrystals,<sup>1</sup> and we had indications that they also exist in a liquid-crystalline state between the solid and completely fused condition. During the latter observations we noticed a striking difference between them by the help of the polarising microscope, which furnishes a further proof of the heterogeneous composition of "protagon."

If a small quantity of the phosphorus-rich material mentioned above (Fraction I.) is carefully fused on a slide under a cover-glass, the clear fused liquid is seen to be isotropic between the crossed nicols of a polarising microscope. On allowing the slide to cool a shower of separate bright spherocrystals, showing dark crosses, appears on the black background. The spherocrystals grow rapidly on cooling until they touch each other, forming finally a complete mosaic on solidifying. The same property is shown still better by the specimen of sphingomyelin described above (see fig. 1).

Quite different is the behaviour of the phosphorus-poor fraction (Fraction II.) and of pure phrenosin. An isotropic fluid is also produced on complete fusion, but instead of spherocrystals it will be observed that on cooling bright anisotropic needles shoot out on the dark background (see fig. 2). In ordinary white light both the needles of phrenosin and the spherocrystals of sphingomyelin can only be faintly distinguished by their outlines.

It seemed to be of interest to examine in the same way samples of "protagon," and this method also proved it to be a mixture. As will be seen from the illustration (see fig. 3), "protagon" when fused and allowed to cool slowly gives rise to an indefinite crystalline mosaic, in which the needles of phrenosin seem to predominate. The identical result is obtained from "artificial protagon," i.e. a mixture of the phosphorus-rich and phosphorus-free constituents which we described previously (*loc. cit.*) and found to be identical in chemical and physical properties with "protagon."

Summary.—It will be remembered that the "protagon" idea was originally conceived by Liebreich (1865). In his opinion all the constituents of nervous tissue (known at this time as phosphorised fats, lecithin, cerebrin, etc.) do not exist preformed, but are derived from the decomposition of the one and only mother-substance, which was therefore called "protagon." This simple idea had no doubt a certain attraction for the earlier physiological chemists, especially as it was for a time at least adopted by Hoppe-Seyler. Several additional theories, none of which were supported by facts or stood the test of experimental criticism, had to

<sup>1</sup> Journ. of Physiol., vol. xxxvii., 1908, p. 348.

be made in order to keep the original theory alive. There is now no doubt that lecithin, to quote only one of the supposed derivatives of "protagon," occurs independently in brain, and besides in no way enters into the composition of "protagon." If we consider the diversity of the mixtures of lipoids occurring in other organs (as demonstrated by the recent researches of Erlandsen on the lipoids of the heart, of Bang and others on those of the blood, of Thierfelder and Stern and of Fränkel on those of the egg), there seems to be no justification for the assumption that the lipoids of the brain, the most complex organ, should be derived from one uniform substance, "protagon." In view of the clear evidence against it, we think, therefore, that the time has arrived to dismiss finally the primitive "protagon" idea as unfounded and contrary to modern conceptions and knowledge. It has for the last forty years unfortunately retarded progress in the investigation of the chemistry of the nervous tissues, instead of stimulating it. We are forced by the results of our prolonged study of the question to repeat our previous conclusion:

"Protagon" is a heterogeneous mixture, and the term "protagon" has only a historical justification.

The expenses of this research have been in part defrayed from a grant from the Government Grant Committee of the Royal Society.

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#### DESCRIPTION OF PLATE.

- Fig. 1. Crystals of sphingomyelin.  $\times 70$ . Polarised light: crossed nicols.  
Fig. 2. Crystals of phrenosin.  $\times 88$ . Polarised light: crossed nicols.  
Fig. 3. Crystals obtained from "protagon" after fusion.  $\times 130$ . Polarised light: crossed nicols.



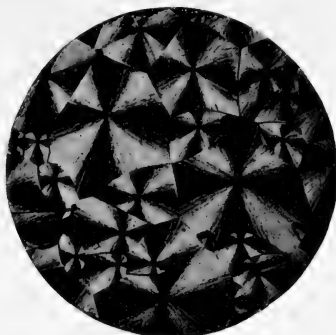


FIG. 1.



FIG. 2.

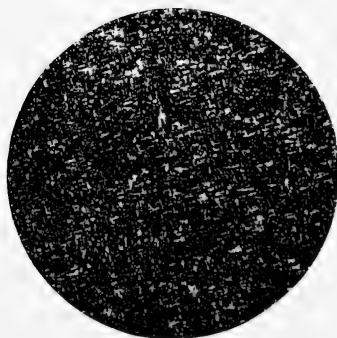


FIG. 3.



THE COAGULATION TIME OF THE BLOOD IN MAN. By T. ADDIS.  
 (From the Physiology Laboratory, University of Edinburgh.) (With  
 five figures in the text and two Plates.)

(Received for publication 1st August 1908.)

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I. A NEW METHOD OF ESTIMATING THE COAGULATION TIME.

WHEN a current of oil streams against the edge of a drop of blood suspended in oil, a continuous smooth flow of the corpuscles is induced, although the drop as a whole does not rotate. Under the microscope this flow will be seen to cease quite suddenly after a certain time has elapsed. This is due to the occurrence of coagulation in the drop.

The method is a modification of Brodie and Russell's method (1). Instead of intermittent jets of air at an unknown and variable temperature, a continuous stream of oil at a known and constant temperature is used. The end point also is entirely different.

The whole apparatus is placed on a small table to which an upright has been attached (see Plates at end of article).

A reservoir (P) of filtered mineral oil such as is used for burning in lamps is hung from the upright by a cord passing through a pulley so that its height can be varied at will. Any vessel will do for a reservoir so long as it contains a sufficiently large surface of oil, in order that the amount which runs out during an observation may not materially affect the pressure of the flow of oil by altering its level in the reservoir.

The oil is conducted from the reservoir through six feet of flexible metal asbestos-lined quarter-inch tubing (*p*). The usual flexible metal tubing should not be used, because it is packed with rubber which soon rots under the influence of the oil.

The last  $4\frac{1}{2}$  feet of the tubing are coiled spirally within a tank of water (V), and the lower end emerges through the side of the tank at the level of the stage of a microscope and terminates in a stop-cock (fig. 1).

The next part of the apparatus is an adaptation of Bogg's modification of Brodie and Russell's method (2). Bogg's apparatus consists of a small circular metal box, the floor of which is of glass. The box is closed above by an inverted truncated glass cone. It is pierced on one side by a small metal tube which ends in a nozzle (figs. 1 and 2).

The metal tube is screwed on to the stop-cock on the side of the tank.

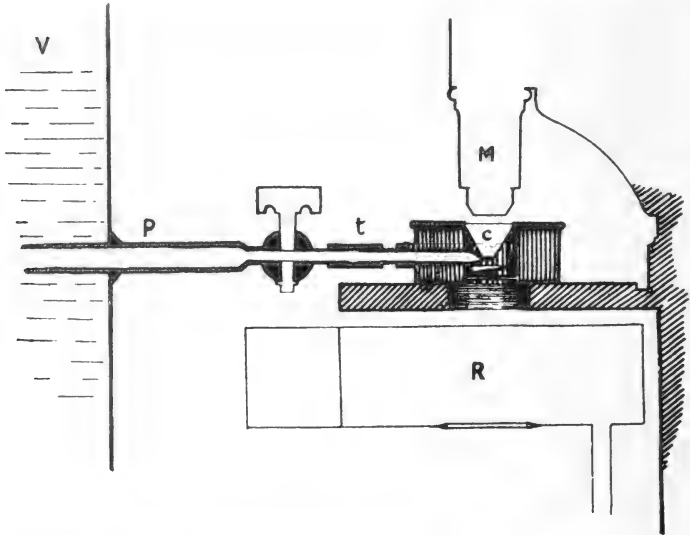


FIG. 1.—Diagram showing the stage apparatus in section.

V, tank of water; P, flexible metal tubing; t, metal tube screwing into tap; c, truncated glass cone in position; M, low-power lens of microscope; R, box hung below the stage to catch the oil which flows out of the coagulometer.

The box is then fitted on to it and locked by a small brass collar which fits into pegs driven into the tube and the side of the box, so that any rotation of the tube in its socket is rendered impossible. A special nozzle must be fitted on to the end of the tube. It is made of soft brass, and is about half a millimetre in diameter and 4 mm. long. It is essential that the nozzle should point in exactly the right direction. It should be placed so that when the stop-cock is turned and a jet of oil issues from it, the stream travels tangentially against the edge of the drop of blood which hangs from the end of the glass cone. This direction has to be determined experimentally by making slight alterations in the direction of the tube with

a pair of fine pliers, until it is found that a smooth and continuous flow is produced by a low pressure of oil.

The nozzle should then be plastered round with solder to preserve it, for the slightest knock may alter it a little, and unless it is exactly right the flow of the corpuscles is rendered jerky and inconstant.

When the stop-cock is open the box is of course filled with oil, and the jet from the nozzle produces a current which streams across the edge of the drop of blood which is surrounded by oil on all sides except where it is in contact with the end of the cone. When everything is in position the box lies on the stage of a microscope, and the flow of the corpuscles can be observed with a low-power lens. The oil runs out of the box through a hole in the metal fitting of the cone and forms a pool on the top in which the lens lies when it is in focus.

The oil is allowed to run over the stage of the microscope and falls into a vessel hung beneath it, from which it is collected by a pipe which conveys it over the edge of the table into a receptacle on the floor.

An arrangement is also necessary for keeping the oil which surrounds the blood at a constant temperature. The water in the tank is warmed by a small gas-jet which is regulated by Schäfer's thermostat.

This tank is round, is 7 inches high, and has a diameter of 7 inches. It has a dome-shaped bottom so as to raise it some distance above the gas-flame, and is placed on a metal ring 3 inches high which is pierced by holes for the inlet of air to the flame.

The oil in the 4½ feet of metal tubing which is immersed in the water soon acquires the same constant temperature.

A finely graduated thermometer (fig. 2, T) pierces the box into which the cone fits, so that though the shaft is outside, the bulb lies in the interior very close to the suspended drop of blood. In this way, the temperature of the oil immediately surrounding the blood is accurately known. By means of this arrangement the temperature of the oil in which the blood lies can be kept constant at any desired temperature for any length of time.

The pressure of the flow depends on the height of the level of the oil in the reservoir, and on the calibre of the nozzle.

With the calibre of nozzle which is at present used by me, the surface of oil must be 10 cm. above the blood.

The pressure may vary from the obstruction of the nozzle by dust, etc. To obviate this, the oil is twice filtered, before it is introduced into the reservoir.

It is necessary to have a standard by which the pressure can be gauged. This is most simply attained by measuring the length of the jet of oil when the box is removed.

When the receiving vessel, which is hung below the stage of the microscope, is 3.9 cm. from the end of the nozzle, the jet of oil just falls into it. If it does not do this, there must be something wrong. The nozzle can then

be screwed off and the length of the jet, as it issues from the stop-cock, is measured in the same way: it should be 6.5 cm. In this way the location of the obstruction can be ascertained.

This does not need to be done before every estimation, but it should be done every now and then, especially if the apparatus is not being constantly used. Of course, any considerable variation in pressure reveals itself, by its different action on the blood.

A temperature of 18.5° C. is a convenient one to work with. It is easily maintained by regulating the temperature of the water in the tank. The correct temperature having been attained, it is advisable not to stop the flow of oil by turning the stop-cock between successive

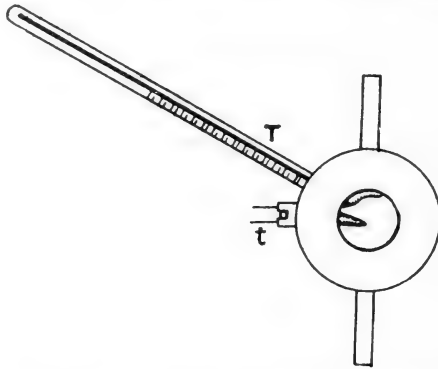


FIG. 2.—Diagram of the metal box with the truncated glass cone removed.

The thermometer (T) and the tube (t) are shown piercing the side of the box; the bulb of the thermometer and the nozzle of the tube being inside the chamber.

estimations, because it takes a little time for the box to be warmed up again.

The parts of the apparatus which come in direct contact with the blood, i.e. the end of the glass cone and the instrument used for puncturing the skin, have to be freed from any possible contamination with fibrin ferment.

The only way to do this with certainty is to expose them for some time to a temperature above 65° C. At temperatures above that point, fibrin ferment is destroyed.

The puncturing instrument and the glass cone are placed in a vessel full of oil, the lid of which has three holes cut in it. Into one of these a thermometer is fitted, and through the other two the cone and lancet can be hung, so that they dip below the level of the oil. The vessel is placed in a tin of water, which is then heated until the thermometer indicates that the

temperature of the oil is 70° C. or more. After they have been left in for a few minutes they are fitted into the necks of small bottles containing ether. The bottles are then well shaken, and the ether dissolves off all the oil.

This is sufficient as regards the puncturing instrument, but it is of the utmost importance that the end of the cone shall be not only free from fibrin ferment, but that it shall also be free from any dust, or anything else which might impede the flow of the blood.

In the first place, to clean the drop of coagulated blood from it, it is put under a strong jet of water. Then it is washed in absolute alcohol, and put into the hot oil. After rinsing in ether, a perfectly clean handkerchief, made of fine silk, is dipped in ether, and drawn once or twice gently across the end of the cone. After this treatment, the cone is fitted into the neck of a small bottle, and thus preserved from contamination with dust until it is required.

The following is the method which I find best fitted to obtain at once a drop of blood of the right size.

A slip-knot is placed round the finger, and the arm is swung round ten or twelve times. In this way the finger is filled with blood, and becomes bright red. When the swinging is stopped the slip-knot is tightened up, so that the condition of vascular engorgement is maintained. A superficial puncture is made, and a spherical drop of blood about 4 mm. in diameter at once appears. The time is then noted.

The glass cone is gradually approached to the drop. Before it has quite touched it, the blood seems to leap up, and flows smoothly right up to the edge. It is put at once into the apparatus. The whole procedure, from the pricking of the finger to the fitting of the cone into its position, should not take more than 10 seconds.

Jenner's vaccinostyles are well adapted for pricking the finger, but it is necessary to fit them with a guard of some description, to prevent too deep a puncture being made.

If the wound is too deep, the drop of blood is apt to spill over, and a stream runs from the puncture, from which it is impossible to obtain the proper quantity of blood. The only way to get a constant amount of blood on the end of the cone is to produce a drop of the right size. This is a difficulty in dealing with people who have not been pricked before, for there is a considerable variation in the rate of flow in different individuals.

That the size of drop taken up by the cone should be approximately constant, is of considerable importance. Variable results are obtained if this point is neglected.

As soon as the cone has been introduced into the box, the reservoir must be raised to a little more than twice its original height. This is necessary because a higher initial pressure is required, partly to overcome the inertia of the corpuscles, but mainly, I think, to break up the blood from the

state of slight agglutination into which it passes whenever it leaves the vessels.

That the inertia of the corpuscles is not the only factor is shown by the fact that the initial pressure required varies according to the time the blood is left exposed to the action of the air. If this time is 2 or 3 minutes, it is often found that a pressure of four or five times the standard pressure is not sufficient to start the flow.

In different individuals, also, there seem to be slight variations in the rate at which the blood agglutinates.

If the technique as regards the taking of the blood is strictly observed, it will be found that a height of slightly more than twice the standard pressure will act uniformly. Thus, with my present apparatus, in which the standard pressure is represented by a height of reservoir above the blood of 10 cm., an initial height of 20 to 22 cm. is required.

The outline of the drop, as the reservoir is being raised, should be watched. When the agglutination has been overcome, a tongue of blood is seen to stream out from the drop. The reservoir is, then, at once lowered; but it should be done slowly, so as gradually to lessen the rate at which the corpuscles are revolving.

When the low power is brought into its focal position, the lens lies only a few millimetres above the top of the glass cone, and is immersed in the oil which flows out of the chamber. If the necessary conditions have been observed, the corpuscles are seen streaming round fairly rapidly, each one separate from the other. The part where the flow is most rapid is the point at which the oil stream impinges on the blood. This is the part which should be watched. After about 7 minutes have elapsed with out any observable change, one or two stationary streaks appear a little way from the edge of the drop. These rapidly increase in number and length, and with this there is an appreciable diminution in the rate of flow.

Within a half to one minute more, the streakiness will have extended right up to the edge, and there will now form a laminated clot, within the meshes of which more and more corpuscles become entangled. Only a small part of the total number of corpuscles continue to flow slowly and interruptedly round.

The signs of a clot associated with the cessation of flow of the great body of the corpuscles, is the end-point adopted. When this is attained the time is again taken and the estimation is complete.

To a greater or a less extent all the conditions necessary for accurate results are realised in this method. There is one essential which is not fully carried out in any other method, but which is perfectly complied with in this, i.e. the maintenance of a constant temperature in all comparative observations.

The temperature of the blood, as it comes from the capillaries is about



37° C. In the 10 seconds or so which are required to place it in the oil, the temperature falls; and it continues to fall until it reaches 18·5° C., the temperature of the oil, after which it remains constant.

As variability of temperature is the most important source of fallacy in other methods, so its constancy is the chief advantage in this one. This is the one reason why the results are so much more constant than can be obtained by other methods. Another point which may be looked upon as a special advantage is that coagulation occurs under conditions not very dissimilar to those which obtain in an injured vessel, or one into which a foreign body has been introduced. In both cases the blood is flowing, and thrombokinase from the tissues has been added to it. In the one case it is surrounded by the vascular endothelium, and in the other case by oil which in its neutrality as regards coagulation is strictly comparable to the lining of the vessels.<sup>1</sup>

Coagulation may, therefore, be said to occur under conditions much more nearly allied to those under which blood sometimes coagulates within the body than when observed by any method hitherto employed. Nevertheless the method here described is far from being a perfect one. This is shown by the fact that irregular variations in the time of coagulation still occur even with a constant temperature. These variations are not due to alterations in the actual coagulability of the blood, but must, I think, be attributed to experimental error.

They appear to be due to two causes—first, slight errors in technique, and second, a want of absolute definiteness in the end-point.

The errors in technique most likely to arise are those connected with the picking up of the drop of blood by the cone.

When the margin of the drop of blood on the end of the cone is examined under the microscope, it will be seen that it does not always come absolutely up to the edge of the glass surface—a thin margin is sometimes left. The blood being then a little further away, the stream of oil will not affect it in quite the same way. Again, the drop is then not always quite circular in outline, and the flow of the corpuscles is liable thereby to be slightly obstructed.

With regard to the errors arising from want of definiteness in the end-point, it may be observed that there are three possible stages which might be adopted as indicating coagulation:—

- (1) The first appearance of a streak of clot.
- (2) The stoppage of the main flow of blood and the clear appearance of a laminated clot; and
- (3) The complete cessation of flow.

On an average 60 seconds passes between (1) and (2), and 50 seconds between (2) and (3).

<sup>1</sup> In order to explain why the blood does not coagulate in the vessels, some have assumed that the vascular endothelium secretes an anti-body (anticoagulin). Loe b (3) has shown experimentally that this theory is not tenable.

The first appearance of a streak of clot has been found too variable to use as an end-point.

In the following columns the times given by the second stage, and by the third, are compared :—

STAGE II. Stoppage of main flow of blood and appearance of a laminated clot.		STAGE III. Practically complete stoppage.	
Min. Sec.	Variation from mean.	Min. Sec.	Variation from mean.
7 25	- 25 sec.	8 0	- 41 sec.
7 10	- 40 "	8 4	- 37 "
7 55	+ 5 "	9 10	+ 29 "
7 45	- 5 "	8 30	- 11 "
8 45	+ 55 "	9 0	+ 19 "
7 55	+ 5 "	8 10	- 31 "
7 55	+ 5 "	9 25	+ 44 "
7 45	- 5 "	9 5	+ 24 "
8 35	+ 45 "	9 5	+ 24 "
8 5	+ 15 "	8 45	+ 4 "
7 5	- 45 "	8 0	- 41 "
7 30	- 20 "	9 0	+ 19 "

The average time taken to arrive at stage II. was 7 minutes 49 seconds, and to arrive at stage III. was 8 minutes 41 seconds.

When the second stage was taken as the end-point, the average variation from the mean was 20 seconds; when the third stage was adopted, it was 27 seconds.

The second stage has, therefore, always been observed as the end-point. It is better than the third also because it is difficult to say when the stoppage is to be considered "practically complete." The positively complete cessation of flow is very variable, since a few clumps of corpuscles sometimes wander slowly round for a considerable time.

But the second stage also is not always quite definite, though it much more often is so. The main mass of the blood usually stops moving at a definite moment, and in the next second or two a clear laminated clot stands out. But sometimes this develops slowly, and in these cases it is impossible to be quite accurate, for judgment is necessary to decide when the clotting is distinct enough to be considered as the end-point. The amount of possible error is, however, strictly limited, because the flow always stops completely within, at most, 90 seconds after the commencement of the second stage.

Now and again the main body of the blood ceases to flow without the clear appearance of any clot.

This, I think, is usually due to the agglutination of the blood not

having been properly overcome at the beginning. The time obtained in these cases is, as it happens, usually approximate to the mean time.

Variations in the agglutination of the red blood corpuscles of healthy people, as shown by differences in the pressure of oil needed to set the corpuscles flowing each one separately from the other, are slight, and have never given rise to serious error or difficulty.

I found, however, when I came to apply the method to pathological cases, that this agglutination of corpuscles was greatly increased in disease.

In one instance this was so marked that no flow could be started in the blood even after the pressure had been raised high enough to drive part of the drop off the end of the cone. I cannot at present say in what class of cases this condition is most marked, for I have made but few observations on pathological conditions; it was, however, present in a varying degree even in some convalescent patients.

In one or two instances no accurate estimation of the coagulability could be made, because even the highest pressures failed to produce a smooth and even flow, the corpuscles remained sticking together in clumps, and flowed slowly and with jerks.

It is interesting to note that Fleming (4) has found, in connection with work on the opsonic method, that in 90 per cent. of patients at St Mary's Hospital the red blood corpuscles are agglutinated by their own serum. This condition is very rare in health. Hektoen (5) says that auto-agglutinin is very seldom if ever demonstrable *in vitro* in the blood of healthy people. In disease the agglutination is possibly due to the action of bacterial hæm-agglutinins, for it has been shown by Pearce and Winne (6) that certain bacteria produce a substance which agglutinates red blood corpuscles *in vitro*, and which when injected into animals leads to the formation of thrombi composed of agglutinated corpuscles. In 1873 Hueter (8) showed that certain thrombi in infectious diseases were due not to coagulation but to agglutination. Flexner (9) confirmed this, and reproduced it experimentally by the injection of bacterial filtrates. Boxmeyer (10), Kraus and Ludwig (11), Volk and Lipschütz (12), Kayser (13), and Eisenberg (14) have also demonstrated the action of bacterial hæm-agglutinins.

In cases in which this increased tendency to agglutination has developed, the flow of the corpuscles is hindered and the estimation of the coagulation time may be impossible.

In many pathological conditions, therefore, the method is inapplicable, though in these cases it may be of value in showing the degree of auto-agglutination which is present.

Unless otherwise stated, the coagulation times given in the following sections were taken by this method at a temperature of 18.5° C.

## II. THE CONDITIONS WHICH ARE ESSENTIAL FOR THE ACCURATE ESTIMATION OF THE COAGULATION TIME OF THE BLOOD BY ANY METHOD.

### 1. The Blood must be obtained under the same Conditions in each Experiment.

Pratt (15), using a modification of Brodie and Russell's method, concluded that blood from deep wounds took longer to coagulate than blood from superficial ones. He gives as an example a coagulation time of 7 minutes when the cut was deep, and of 2 minutes when it was superficial. I have found that there is no appreciable difference in the coagulability of blood from deep and from superficial punctures.

In eight comparative observations, the average time from deep punctures was 8 minutes 8 seconds, whereas, when the puncture was superficial, it was 8 minutes 4 seconds.

Several observers have stated that congestion of the part from which the blood is obtained leads to a diminution of the time, the explanation being that a greater quantity of coagulation-accelerating substances from the tissues are added to the blood. I have not been able to confirm this. Very marked congestion was produced in one arm by the application of a Bier's bandage above the elbow. The average of twelve comparative estimations of the coagulation time of blood taken from the congested fingers was 8 minutes 2 seconds, while the average time of blood from the uncongested fingers was 7 minutes 51 seconds. No conclusion can be drawn from so slight a difference as 11 seconds, because it is well within the limits of experimental error.

Pressure near the wound while the blood is issuing has been supposed to act in the same way, but again I have not been able to find that this makes any appreciable difference, the average time with pressure being 8 minutes 5 seconds, and without pressure 8 minutes 4 $\frac{3}{4}$  seconds.

The rate of flow of blood from the wound has proved to be of importance in so far as it affects the time during which the blood is exposed to air before its introduction into the apparatus.

The other factors requisite to cause coagulation being present, air has a marked influence on coagulation: its temperature is variable, and dust particles no doubt attach themselves to the drop to a variable extent during its exposure to air.

The finger is the most convenient part from which to obtain blood, because in it a constant size of drop and rate of outflow can be secured by the employment of temporary congestion.

In continuous hæmorrhage the point of time during the course of the bleeding at which a specimen is taken for examination is of considerable importance. I could not use my method to demonstrate this, as exposure of the blood to air leads to an increase in the agglutinability of the corpuscles. M'Gowan's method (16), modified by the addition of an apparatus

to keep the tubes at a constant temperature, showed that there was a rapid and progressive diminution of the coagulation time as the hæmorrhage went on. Thus the coagulation time (at 18° C.) of the first blood to appear from a wound was 7 minutes 15 seconds. The blood flowing from the wound after 1 minute coagulated in 5 minutes 15 seconds, and after 2 minutes in 3 minutes 40 seconds.

It is important, therefore, to take for examination only the first blood to appear after a puncture.

A comparison of coagulation times, taken when the blood was protected from contact with the skin by smearing the surface with lanoline before the puncture was made, with the time taken in the ordinary way, shows that contact with the skin for the short period which elapses before the blood can be introduced into the apparatus has no appreciable effect.

On the other hand, when the skin of the part into which the puncture is made is covered with recently shed blood, a very marked diminution of the coagulation time results.

Skin free from blood.		Skin covered with a film of recently shed blood.	
min.	sec.	min.	sec.
6	40	4	50
7	50	4	20
7	55	3	40

This diminution no doubt results from the addition to the freshly issuing blood of preformed fibrin ferment.

It is, therefore, necessary to make sure that there are no traces of fibrin ferment left on the skin, on the puncturing instrument, or on the parts of the apparatus with which the blood comes in contact.

The only certain method is by destruction of the ferment by heating to a temperature above 65° C. This can be done for the apparatus. In the case of the skin thorough washing in running water, with subsequent drying by alcohol and ether is, in practice, sufficient to eliminate this fallacy.

In connection with the method of obtaining the blood, therefore, it is necessary that the skin and instruments should be clean, that the rate of outflow should be approximately constant, and that the first blood which appears should be taken for examination.

## 2. All Estimations must be made at the same Temperature.

The value of any method is mainly determined by its success, or want of success, in maintaining a constant temperature.

Where no attention is paid to differences of temperature the method is practically worthless. The influence of even the slight variations which occur in rooms, wards, or laboratories is so great as to make comparative observations valueless.

This is in direct contradiction to the conclusions of the originators of some of the methods, who, while admitting the importance of large variations, believe that slight ones have so little effect that they may, for practical purposes, be neglected. No one of them, however, has brought forward any experimental proof of this assumption.

The extraordinary variations in the coagulation times obtained by the use of their methods are to a great extent due to variations of the temperature. This is illustrated in the following three charts:—

Figure 3 is a chart showing consecutive coagulation times, taken in

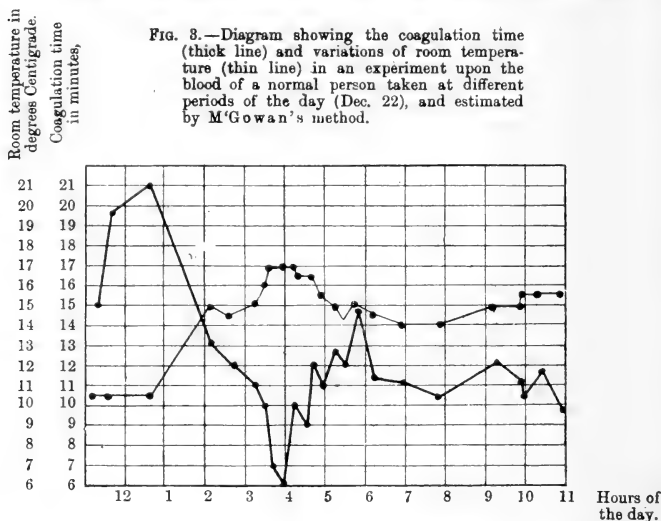


FIG. 3.—Diagram showing the coagulation time (thick line) and variations of room temperature (thin line) in an experiment upon the blood of a normal person taken at different periods of the day (Dec. 22), and estimated by M'Gowan's method.

a room in which the temperature never varied so much as to make the room noticeably cold or hot.

M'Gowan's method (16) was used. The originator of this method does not think that accurate results can be gained by the use of the method without the addition of some means of keeping the temperature constant.

In this case, however, the tubes were simply left to acquire the temperature of their surroundings.

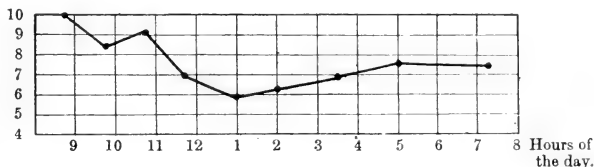
The amount of variation in the time is very great, and the curve of coagulation, though complicated by experimental error due to other causes, is seen roughly to run in the opposite direction to the curve of temperature.

In the next chart (fig. 4) the times were taken by the same method, modified by keeping the tubes in an apparatus I had constructed in order

to keep them at a constant temperature. This gave an almost constant temperature, and the variations are seen to be smaller.

Coagulation time  
in minutes.

FIG. 4.—Diagram showing the coagulation time of the blood of a normal person taken at different periods of the day. M'Gowan's method, modified by the addition of an apparatus for maintaining a constant temperature. The temperature was approximately 20° C. throughout.

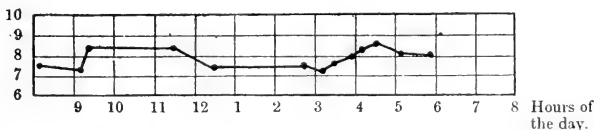


The coagulation times in the next chart (fig. 5) were taken by my method, in which the temperature is kept absolutely constant.

Here the variation is still less, and is due entirely to other experimental errors (see Daily Variations in the Coagulation Time, Section IV.).

Coagulation time  
in minutes.

FIG. 5.—Diagram showing the coagulation time of the blood of a normal person taken at different periods of the day (Addis' method). The temperature of the oil was maintained absolutely constant at 18.5° C.



The following is a list of coagulation times taken by my method at temperatures between 10° C. and 20° C., which may be looked on as the extremes of ordinary room temperature:—

Temperature.	Coagulation time.
°C.	min. sec.
10.25	21 30
12.25	16 30
13.5	14 32
14.5	12 58
15.5	11 46
16.5	10 10
17.5	8 27
18.5	7 34
19.5	6 2
20.5	5 22

It is thus clear that variations of room temperature have a very great effect on the coagulation time, and it follows that methods in which the

temperature is not kept constant cannot yield comparative results which may be relied upon.

### 3. The Contact of the Blood with the same Amount and Kind of Foreign Body in each Observation.

A foreign body, as regards blood-coagulation, may be defined as anything which hastens or retards the coagulation time.

Besides the intact endothelial lining of the vessels, there is only one class of substances which may be said not to act as foreign bodies, i.e. the oils. I do not think that the ordinary commercial mineral oils can, strictly speaking, be entirely excluded from classification as foreign bodies, for I found that the coagulation time of blood surrounded from the moment it issued from the wound by "motor spirit" was less than the time taken when ordinary paraffin oil was used. Paraffin oil gave a time of from 70 to 80 minutes, while with Pratt's Motor Spirit it was 50 to 60 minutes. Nevertheless, their action as foreign bodies is so slight, in comparison with that of other substances, that they may be considered as having practically no effect at all.

This fact may be utilised to estimate the effect of foreign bodies on the coagulation time. For, by placing a drop of blood in partial contact with a foreign body of constant nature and surrounding it elsewhere by oil, the complicating effect of other substances is excluded, and the resulting coagulation time gives an indication of the influence of that particular foreign body.

In the following experiments coagulation was said to have occurred when a visible mass of fibrin was left after drawing off the fluid portion of the drop with filter paper. I found that, when blood was drawn under paraffin oil in a vessel lined with paraffin-wax, it took 70 to 80 minutes to coagulate. This, then, may be taken as representing the coagulation time when the process is allowed to occur without the intervention of any foreign body. In this case coagulation is due simply to that amount of injury which the blood receives in its passage from the wound, and to the thrombokinase added to it from the tissues of the wound.

Drops of blood which were placed on clean glass slides and then immersed, blood downwards, in oil, took from 20 to 26 minutes to coagulate. Here the only foreign body was glass.

Other slides were coated with a smooth film of paraffin-wax and drops of blood placed on them. In this case the foreign body was air, and the coagulation time was 10 to 16 minutes.

When the blood was exposed to both glass and air it coagulated in 5 minutes. These results are of course rough, for no attempt was made to keep the temperature constant; but they were all done within 3 hours on the same afternoon, and they serve to indicate that different foreign bodies have different effects on the coagulation time. They also make it clear that the



effect of the wound on the time taken is slight in comparison with the effect of the environment of the blood after it has left the wound. Any method, therefore, in which the blood is not in contact with exactly the same amount and kind of foreign body is likely to give erroneous results.

4. The End-point must be Clear and Definite and must always Indicate the same Degree of Coagulation.

The various methods may be divided into two classes, those in which the end-point adopted is the first appearance of fibrin, and those in which the evidence that coagulation has occurred is an indirect one deduced from some change in the behaviour of the blood to its surroundings.

Thus in Wright's (17), Bürker's (21), Sabrazès' (22), and M'Gowan's (16) methods the coagulation time is the time elapsing from the issue of the blood from the wound to the first appearance of a thread of fibrin.

In the second class various phenomena are taken as indicating coagulation. Thus in Hayem's (23), Milian's (24), and Brodie and Russell's (1) methods change in the contour of the drop of blood is the end-point. Biffi (25) and Hingston Fox (26) take the non-diffusibility of the blood when introduced into water. Vierordt's (27) method depends on the fact that when the blood has attained a certain degree of coagulation it no longer adheres to a horse-hair. The cessation of movement of the corpuscles in a film of blood under the action of gravity is the indication in Buckmaster's (28) method, and in the coagulometer which I have described there is a combination of the methods of both classes, for a fibrinous clot is seen, and the flow of the corpuscles is stopped.

It might be thought that the recognition of a thread of fibrin was an absolutely certain proof that at least a certain amount of coagulation had occurred, but even this is not sure. Buckmaster (29) has noted that fibrin threads several millimetres in length may be drawn by a needle from a drop of blood within 10 seconds after it has issued from the wound, He takes this as an indication that coagulation is a gradual process which begins whenever the blood leaves the vessels. I have very often noticed the same thing when withdrawing the glass cone used in Brodie and Russell's method from a drop of blood which had just appeared, and yet the same drop will be found to have a normal coagulation time. It may be true that coagulation begins at once, but I do not think that it gradually increases in amount up to entire coagulation, for it is a matter of common experience when large quantities of blood are used that it remains perfectly fluid for a time and then suddenly develops all the signs of coagulation.

If, therefore, the fine fibrin filament which can be demonstrated so early indicates a certain amount of coagulation, it must remain very limited in extent until there is a sudden crisis of coagulation throughout the whole body of the blood.

On the other hand there is some reason to believe that the appearance of such a thread does not necessarily imply coagulation at all, but may be a purely physical phenomenon. Mann, in his "Chemistry of the Proteids," 1906, p. 382, says: "Ramsden, in a paper not yet published, states that fibrinogen solutions, free from fibrin ferment, can be made to yield mechanical surface aggregates" indistinguishable from typical fibrin, and that "fibrinogen, mechanically produced fibrin, and ferment-produced fibrin have the same heat-coagulation temperature, 53°-58°."

There is, therefore, the possibility that this may be an instance of the mechanical production of fibrin.

In the four methods which adopt the fibrin thread as their end-point the method of demonstration is by the slow withdrawal of glass from blood, just the circumstances under which Buckmaster has found this very early appearance of fibrin to occur.

To adopt this as the end-point would appear, therefore, to be very fallacious, and to a certain extent it no doubt is so. In practice, however, there is very often an appearance not of a thread but of a considerable mass of fibrin, and there is no good reason to suppose that this is due to anything but fibrin-ferment coagulation.

Nevertheless, even when a fibrin thread is taken to indicate the occurrence of coagulation, it is probable that it will not be very constant in the time of its appearance, for fibrin is primarily deposited in an amorphous and invisible condition, and it is only under the influence of mechanical stress that it acquires the appearance of threads or of a visible mass. These physical factors are impossible to control or keep constant. Possibly also there may be conditions of the blood which favour or retard the appearance of fibrin in a recognisable form.

In the case of the indirect method of determining coagulation, various alterations in the behaviour of the blood to its surroundings have been adopted as end-points. All these are based on one change in the physical character of the blood, i.e. a loss of fluidity.

When clear signs of this are seen, it is assumed that coagulation has occurred. This, however, is not always the case, for it is sometimes due simply to agglutination of the corpuscles.

As has already been mentioned, the agglutinability of the blood is increased in disease, and for this reason methods which depend on an indirect method of determining coagulation will probably give untrustworthy results in pathological conditions.

Agglutination commences whenever the blood is drawn, and the longer it is left exposed to air the more marked does it become. This is well seen in using Brodie and Russell's method. If the blood after its introduction into the apparatus is not put in motion for a minute or two, agglutination may be so strong as to give all the signs which are considered to be conclusive of coagulation; although, if movement of the corpuscles had been induced at once and repeated at short intervals, a coagulation time of 7 to 12 minutes

would have been given. Agglutination is, then, a process which is entirely distinct from coagulation, which it may nevertheless closely simulate.

No end-point which has yet been suggested can be considered free from fallacy. With the direct methods fibrin may be inconstant in the time of its appearance or may possibly be sometimes due to purely physical causes, and with the indirect method agglutination may yield appearances usually considered characteristic of coagulation.

### III. OTHER METHODS OF ESTIMATING THE COAGULATION TIME.

#### Wright's Method.

Wright (17) published his method in 1893. Since then it has undergone several slight changes.

In the latest modification (1905) capillary glass tubes are calibrated by an ingenious method. They are filled with blood and placed in water at 37° C. At intervals the blood is expressed from one after the other until fibrin is found.

The method has probably been more widely used than any other. Murphy and Gould (30) compared Wright's (17) and Brodie and Russell's (1) methods. In 15 per cent. of estimations no result was arrived at with Wright's method. Ross (31) gives fourteen cases in which the coagulation time as shown by this method was diminished after calcium. He appears to think that the method is an accurate one. Coleman (32) preferred Brodie and Russell's (1) method. Solis-Cohen (33) made sixty-five observations. He says: "The results obtained were all practically negative, and were, moreover, unsatisfactory." He attributes this to fallacies in connection with the method. Douglass (34) estimated the coagulation time of normal, pregnant, and eclamptic women, but does not express any opinion as to the method. Nias (35) used it to show that strontium as well as calcium diminishes the coagulation time. He says: "In spite of criticisms which have appeared as to the sufficiency of this method, it has proved itself amply adequate for the purpose in hand, very consistent results having been obtained." Hinman and Sladen (36) think that "the pathological differences, and those dependent on technique, in this method, are of about the same relative value, which must confuse the results." Turner (37) made over one thousand observations on normal and epileptic people. His average coagulation time was 2 minutes 40 seconds, and he says that he often found differences of two minutes in the results of estimations of blood taken from the same individual by successive punctures. He attributes this to rapid variations in the coagulability of the blood and not to any deficiency in the method.

I made forty-three estimations with the method as described in 1897, with the exception that each tube was filled from a separate puncture as is recommended in 1902. In nearly 50 per cent. of them only an approximate

time was arrived at, because coagulation occurred in one tube although it was not present in others until later. Considerable variations occurred in the coagulation time. These were, I think, largely due to differences of temperature. It is practically impossible to keep a small tin of water at a constant temperature simply by adding warm water at intervals in the manner recommended, especially since the observer's attention is fully occupied in filling and emptying the capillary tubes.

The 1905 method is essentially the same, except that the tubes are kept at a temperature of  $37^{\circ}$  C. instead of  $18.5^{\circ}$  C. I do not think that this can be regarded as an improvement.

With this apparatus Wright (17) sometimes obtained coagulation times as low as 30 seconds. I found that it took me nearly as long to fill the tube and introduce it into the water. With times so short any variation in the period during which the tubes are exposed to the room temperature must lead to considerable error. It is in connection with the end-point, however, that the most serious fallacy may be introduced. When the blood is expressed on to filter paper it is difficult to see the fibrin unless it is in large amount. In practice, therefore, one is apt to spread out the drop or to move the pipette over the filter paper while expressing the blood. It is here that the fallacy of the mechanical production of fibrin comes in, for in removing the fine tube from the blood the fibrin thread which is seen may not be due to coagulation at all. I think that the very low times recorded by Wright (17) after the administration of calcium are to be explained in this way. If the blood were not touched after it had been blown out, there would be no opportunity for this error.

#### Bürker's Method.

A drop of blood from the finger is allowed to fall into a drop of distilled water on a glass slide let into the lid of a box within which water at any desired temperature is kept.

Fine rods of glass are passed through the mixture of blood and water every half minute until a thread or mass of fibrin is picked up.

Bürker (21) states that the mixture of blood and water on the lid of the box very quickly arrives at the same temperature as the water beneath, and that variations in the temperature of the room do not materially affect the temperature of the blood mixture. Even accepting this, there is still the difficulty of keeping the water at a constant temperature. I found that it was impossible to keep it from varying considerably. A thermostat would certainly be necessary for accurate work. Bürker (21) constructed a curve showing the effect of temperature on coagulation. This was compiled from only half-a-dozen observations, and as he later on described considerable daily variations in the coagulation time, it is difficult to see how his observations can be accepted as an accurate guide by which observations conducted at different temperatures may be made comparable.

Bürker found that his results were untrustworthy unless he diluted the blood with water. The reason for this may be that the mechanical production of fibrin is prevented. Further, the end-point is not a good one to select, for sometimes a thread and sometimes a large mass of fibrin is demonstrated. In the latter case an earlier stage of coagulation has been missed.

#### Sabrazès' Method.

This method depends on the fact that when blood coagulates in capillary glass tubes a fine thread of fibrin can be demonstrated when the tube is broken and the ends drawn slowly apart. The rest of the apparatus is intended to keep the tubes at a constant temperature. It consists of two superimposed glass boxes. The lower box contains warm water in winter and ice in summer. In the upper box the tubes are placed in close proximity to a thermometer.

The end-point, as will be shown in speaking of the next method, is a good one, but the method is not accurate, because it is impossible to maintain the temperature of the tubes constant throughout the experiment. A tube is taken out and broken across every half minute. To do so it is necessary to remove the lid, and the temperature inside at once tends to rise or fall according to the temperature of the air in the room. For this reason I found it was quite impossible to maintain anything approaching a constant temperature, and as a consequence the results obtained were very variable. Geneuil (38) has used this method, and has obtained very variable results in different pathological conditions.

#### M'Gowan's Method.

Glass tubes 1.5 mm. in diameter and 7 inches long are partially filled with blood. Portions are broken off every half minute until a thread of fibrin is seen.

The author believes that for clinical work variations of temperature between 15° C. and 20° C. may be neglected, though he admits that to obtain accurate results some method of keeping the temperature constant would be necessary. He also suggests that in clinical work the coagulation time of the patient should be compared with the coagulation time of the observer's blood, which might be assumed to represent the normal.

In testing this method it was at once apparent that it was useless even for rough clinical work unless some means were employed of keeping the temperature of the tubes constant. Thus when two tubes, both filled from the same drop of blood at the same moment, were kept at 15.5° C. and 19° C. respectively, the coagulation times were 9 minutes 30 seconds and 5 minutes 45 seconds. I have had an apparatus constructed which is fairly successful in maintaining a constant temperature, and with this addition I regard the method as more trustworthy than any of those hitherto used.

In order to find whether the end-point adopted was to be depended on,

I determined the coagulation time in tubes filled simultaneously and kept under the same conditions.

Two hundred and fifty-six estimations were made, and the average difference in the coagulation time of each couple of comparable estimations was found to be 30 seconds. The slight variations which exist in the calibre of the tubes do not make any appreciable difference in the times, so that half a minute represents the average error due to the deficiency in the delicacy of the end-point. The average coagulation time was about 8 minutes. This amount of error is, I think, very much less than obtains with the end-points adopted in other methods.

#### Brodie and Russell's Method.

The apparatus consists of a small circular chamber, floored with glass and roofed in above by a metal ring, into which an inverted truncated glass cone is fitted. A few drops of water are kept in the box, to lessen evaporation. Surrounding the sides of the air chamber is a water-jacket with inlet and outlet tubes for the circulation of water at a constant temperature.

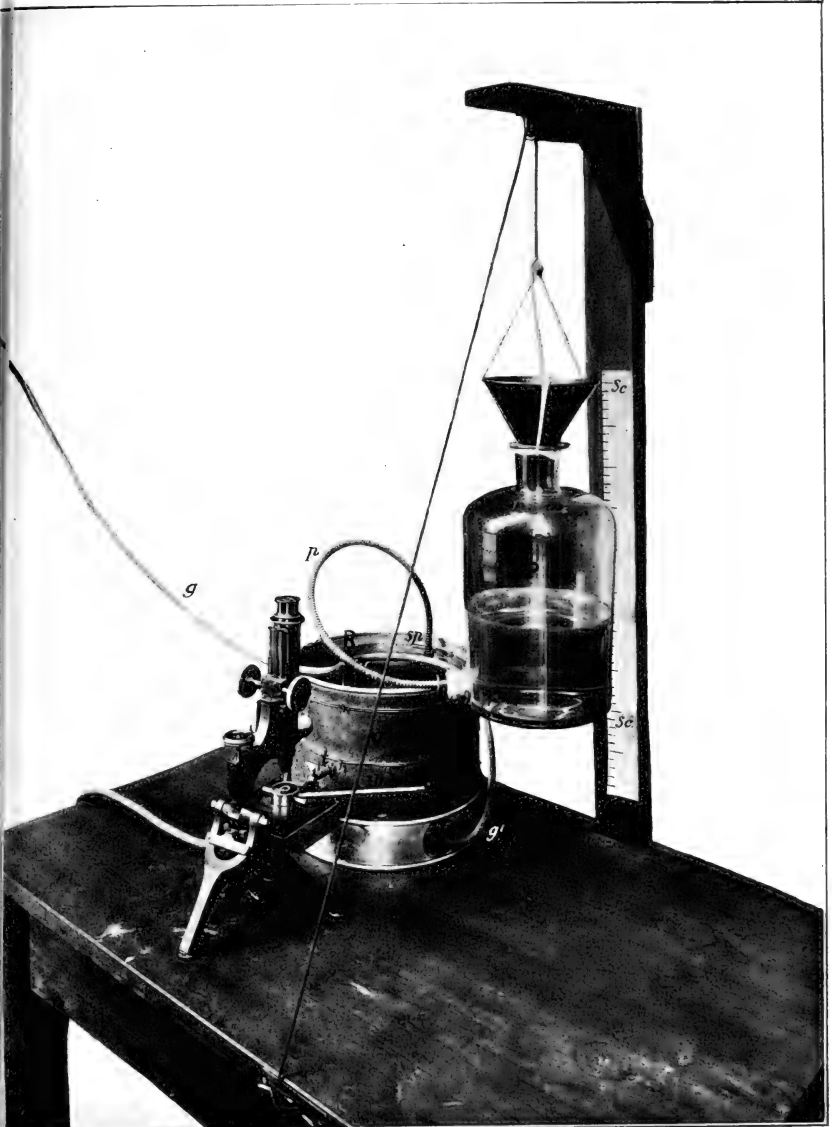
A small glass tube runs through the water-jacket and enters the air chamber. It is so directed that when air is blown through the draught impinges on the edge of the drop and causes the corpuscles to stream round. This is repeated at short intervals, and the movement watched under the microscope. Coagulation is considered to be present "as soon as a rim at the periphery is solid, and blowing simply indents this rim, without causing rotation."

. In Bogg's (2) modification the water-jacket is discarded as unnecessary and a more convenient form of box is adopted. Pratt (15) did not use either the water-jacket or the glass cone, but simply directed a stream of air on to a drop hanging from a glass slide.

Brodie and Russell (1) themselves give only a few coagulation times illustrating the effects of temperature. Pratt (15) found great variability in the coagulation time, but was unable to discover the factors which led to this. Murphy and Gould conclude that it is a more accurate method than Wright's (17). They say that in 5 per cent. of the cases no result could be arrived at, because the blood would not flow at all. The times which they obtained were very variable. Coleman (32) obtained very consistent results by this method, working for the most part with rabbit's blood. Hinman and Sladen (36), after using Vierordt's, Hayem's, Wright's and Milian's methods, came to the conclusion that Brodie and Russell's was the best "as having the greatest accuracy, combined with simplicity of technique." In 251 observations in health and disease, the limits were from 33 minutes to less than 3 minutes.

Widely variable times were recorded even in consecutive observations on healthy people.

I have made more than two hundred estimations with this method or



T. ADDIS, "The Coagulation Time of the Blood in Man." PLATE I.



T. ADDIS, "The Coagulation Time of the Blood in Mar." PLATE II.



with Bogg's (2) modification of it. I found that with its use the coagulation time varied considerably in consecutive observations on the same person. Thus a time of 4 minutes might be followed by one of 12 minutes, and so on, without any regularity. Moreover, simultaneous observations with two similar coagulometers gave contradictory results. These discrepancies appear to have been due either to temperature variations or to the want of definiteness of the end-point, or to actual fallacies in connection with the end-point.

The arrangement for the circulation round the air chamber of water at a constant temperature is inadequate to keep the temperature of the air which surrounds the blood constant. Not only is the cone taken off at the end of each observation, leaving the chamber in direct communication with the air of the room, but fresh air of unknown temperature is blown in every half minute or so when the cone is in position. The uselessness of the water-jacket is so apparent that, so far as I am aware, no one has used it except Brodie and Russell (1), who recorded the effect of running water at different temperatures through it.

Another cause of error is the indefiniteness of the end-point. Hinman and Sladen (36) point out that different observers have used three different stages as end-points. They believe that a radial elastic movement is the most definite occurrence to fix on as indicating coagulation. I find, however, that it is very difficult to be sure of the exact time of occurrence of this condition. Often it is found to be present quite early, but a blast of air stronger than usual causes it to disappear entirely. In these cases it was due simply to agglutination of the corpuscles. In cases where agglutination is allowed to become advanced, as when the blood is not set in motion immediately after it is introduced into the apparatus, no amount of blowing is sufficient to break it up. The blood appears to be coagulated, yet on removing the cone and examining it directly no coagulum can be found.

I believe that this end-point is due partly to agglutination and partly to coagulation. If the blowing is done frequently and strongly, the amount of agglutination is slight or absent and the coagulation time is relatively long. If, on the other hand, the blowing is done at longer intervals and lightly, the end-point is really due to agglutination, and the resulting time is short.

#### Hayem's Method.

Blood is received in a test-tube which is then tilted at intervals until the blood becomes so solid that the level no longer changes with each movement of the tube. Dastre and Floresco (39) and Brat (40) have worked with this method. Floresco thinks that it gives more accurate results than either Wright's or Vierordt's methods. Bezançon and Labbe (41) have used a slightly modified form of it. A great disadvantage is the considerable quantity of blood which is required. Moreover, Hayem appears to have made no attempt to exclude temperature variations.

### Milian's Method.

Drops of blood are allowed to fall on numbered glass slides. From time to time the slides are tilted and the contour of the drop watched. When the drop remains convex instead of sagging down, coagulation is assumed to have occurred.

Hinman and Sladen (36) have modified this method by only including drops of uniform size. They recommend it as a "quick, convenient, and practical means of determining the coagulability of the blood," and state that it is thoroughly reliable. They hold, however, that "it is only marked differences in temperature which affect the time, which, as a rule, are not met with in the wards or in the laboratory." As I have already shown, this is far from being true, and the great variations in the coagulation time as determined by this method are principally due to slight changes in temperature.

### Biffi's Method.

Five loops such as are used for bacteriological work are made on a platinum wire which is fused into a glass rod. The rod passes through the cork of a jar half full of water. The wire is drawn through a drop of blood so that each loop takes up a film of blood. At short intervals of time the films are successively pushed down into the water until one is reached which does not diffuse into the water but remains unaffected. This is taken as indicating coagulation.

I found, in using this apparatus, that whenever the wire touches the water the blood in all the films becomes diluted. I was not able to find a way of obviating this difficulty. I have not seen any reports of work in which this method was used.

### Hingston Fox's Method.

A series of calibrated capillary tubes are filled at intervals of half a minute with blood from the same wound. After some time a rubber nipple is applied and the blood expressed into water. The end-point is the time when the blood no longer diffuses in the water, but remains as a definite worm-like clot, "or until the contents have become so dense that they are with difficulty expressed. This occurs, in some cases, apart from the formation of a worm-like clot, the mass being partially diffusible in the water."

Coagulation times taken at five different temperatures are given in order that corrections to the standard temperature of 60° F. may be made. No formula is given for this reduction, which "must therefore be made from the diagram graphically."

### Vierordt's Method.

This was the first clinical method for determining the coagulation time. A graduated glass tube with a bore of 1 mm. is filled with blood up to the

5 cm. mark. A carefully prepared white horse-hair is then introduced into the tube, so that a segment of it is surrounded by blood. At intervals of time the hair is pulled a short distance out of the tube. As coagulation advances small masses of fibrin and red blood corpuscles will be found sticking to the hair, but later still the blood becomes so solid that none adheres to it. This is taken as the end-point.

Vierordt (27) fully recognised the importance of a uniform method of obtaining the blood, and the necessity of always having the same amount of contact with foreign bodies. He also mentions that the temperature should, as far as possible, be constant.

He made a large number of estimations, principally on himself, and he gave the first list of coagulation times in disease.

His results were very variable. The limits in the case of his own blood were from  $3\frac{1}{2}$  to  $17\frac{1}{2}$  minutes, and he was unable to establish any regularity in the fluctuations. These must have been due mainly to changes of temperature and to want of reliability in the end-point.

#### Buckmaster's Method.

The method is based on the fact that, when a loop of wire is drawn through a drop of blood so that a film of blood is taken up by it, the corpuscles can be observed by the aid of a lens to flow in response to changes in the position of the loop. Thus when it is held vertically they can be seen to fall slowly downwards until a clear circle of plasma is left at the upper pole, while in the horizontal position they spread themselves evenly over the film. As time goes on the flow of the corpuscles becomes slower and more impeded until a moment arrives at which no movement can be seen. The blood is then considered to have coagulated.

The apparatus is devised with the object of keeping the film of blood at a constant temperature. It consists of a box, the lower part of which is filled with water. The film is in an inner chamber. Panes of glass are fitted in the sides so that it is possible to see the blood when it is in position.

When the wire with its film of blood has been introduced all openings are closed, and the film can be rotated from outside the box. The water is warmed from below, and a thermometer whose bulb is in the inner chamber records the temperature of the air surrounding the blood.

This is an extremely original and ingenious method. No work has as yet, so far as I know, been done with it. Buckmaster (28) himself gives only a few times taken at slightly different temperatures.

In attempting to make a series of observations by it at the same temperature, I found that temperature variations were apt to occur even during the course of an experiment, while it was altogether impracticable, without tedious waiting and manipulation, to get a series of times taken at a constant temperature. This is not remarkable when it is remembered that whenever the wire loop is removed for cleaning and filling with a fresh

film, a large communication exists between the inner chamber and the air of the room. The temperatures at which Buckmaster (28) worked varied between 30° and 40° C. This is so much removed from room temperature that there must necessarily be a rapid fall whenever the loop is removed. When the opening is closed again by the reposition of the wire the temperature rises again.

I further found it impossible to heat the water with a spirit-flame or a gas-jet so as to keep the temperature constant, and the effect of an access of heat to the water is not at once apparent in the inner chamber where the thermometer is placed, so that one is constantly at fault in attempts at regulation.

By making a considerable number of observations and picking out those which chanced to have been taken at the same temperature, some idea of the amount of variation due to other causes than temperature was obtained.

Temperature.	Coagulation times in minutes and seconds.					
27° C.	7 30	5 30	7 15	7 30	9 15	6 15
29° C.	4 15	5 15	6 0	5 5	...	...
31° C.	3 30	4 0	3 10	3 5	...	...
35° C.	4 15	4 45	4 0	4 10	3 15	4 15
36° C.	4 0	3 25	4 10	3 15	3 0	...
37° C.	5 0	5 15	5 30	4 30	2 30	...
38° C.	5 0	3 30	4 35	4 15	3 40	...
39° C.	3 15	5 0	3 50	4 25	...	...

There are thus considerable variations even when the temperature is constant.

I think that this inconstancy in my results was due to differences of thickness of film. After the loop is filled with blood a shake is given to it in order to detach part of the blood. Unless this is done the film is so opaque that it is difficult to see the streaming of the corpuscles. I did not pay particular attention to the amount I shook off, and some of the films were much thicker than others.

When very thick and very thin films are compared at low temperatures so as to give long coagulation times, there was found to be an enormous difference in the time.

Temperature.	Thin film.	Thick film.
°C.	min. sec.	min. sec.
11.5	7 30	21 20
12.5	7 0	17 0
13	5 30	17 0
14	3 15	13 45

At higher temperatures the effect is less marked but still distinct.

Temperature.	Thin film.	Thick film.
°C.	min. sec.	min. sec.
29	4 15	6 0
32	3 35	4 20
34	2 5	3 30
37	5 15	4 30
38	4 15	5 0

If the temperature of the water were kept constant by a gas regulator and some means adopted for preventing the inflow of air at room temperature when the wire is removed, the method would be a good one, for no doubt with practice it would be possible to shake off the extra quantity of blood so as to get films of approximately equal thickness.

#### IV. THE EFFECT OF LOW AND HIGH TEMPERATURES ON THE COAGULATION TIME OF THE BLOOD.

The low temperatures were obtained by adding ice to the water in the tank. The following is a list of coagulation times taken at temperatures from 3.25 to 51.5° C. :—

Temperature.	Coagulation times.
	min. sec.
3.25 C.	63 20
7.25	32 45
10.25	21 30
12.25	16 30
13.5	14 32
14.5	12 58
15.5	11 46
16.5	10 10
17.5	8 27
18.5	7 34
19.5	6 2
20.5	5 22
24.0	5 0
26.0	3 40
28.0	2 55
30.0	2 35
32.0	2 15
34.0	1 40
36.0	1 25
38.0	1 30
40.5	1 30
42.5	1 40
44.5	2 25
45.5	2 55
46.3	3 30
47.25	3 10
48.25	3 30
49.5	3 30
51.5	5 15
53.5	?

When the results are plotted out so as to form a curve it can be seen that the effect of variation of temperature is much more marked at the lower temperatures. Thus from 3.25 to 7.25° C. there is a diminution of the time of over 30 minutes, while from 7.25 to 12.25° C. there is a difference of only 16½ minutes. From 12.25° C. there is at first a fall of about 2 minutes for every degree of temperature, but this diminishes until at about 28° C. it is only half a minute. From this point the periods become very slowly shorter until 36° C. is reached, when the blood coagulates in 1 minute 25 seconds. Above 36° C. they begin to increase slowly up to 51.5° C., at which temperature the blood takes 5¼ minutes to coagulate.

At temperatures below 10° C. a tendency for the red blood corpuscles to become agglutinated became apparent, so that twice the usual pressure of oil was necessary to keep the corpuscles flowing separately from each other.

Above 40° C. the same phenomenon occurred, and became progressively more marked as the temperature rose. Above 51.5 a regular flow could not be induced. At 53.5° C. there was no evidence of any clot after 6 minutes, but the movement was very slow and irregular. At 56° C., at which temperature fibrinogen would be heat-coagulated, the blood was not moved even by the highest pressures, but collected in whorls and clumps of agglutinated corpuscles. No change was apparent in their shape or size.

#### V. THE ABSENCE OF DIURNAL VARIATION IN THE COAGULATION TIME.

Vierordt (27) tried to determine whether there were any regular diurnal fluctuations in the coagulation-time but was unable to show directly that these existed, although he found differences in the averages of the observations taken at different periods of the day.

Bürker (21) has stated, as a result of two-hourly observations for three days, that the coagulation time is longest in the morning and diminishes until it reaches its lowest point about 2 o'clock p.m., after which it rises again. This statement is not, however, quite borne out by his figures.

Coleman (32) says that the time is shortest in the morning and longest in the early hours of the afternoon.

Hinman and Sladen (36) do not come to any definite conclusion, but are inclined to think that the time is longest in the morning.

The fact that diurnal variations exist has never been called in question, simply because it was often found that different coagulation times were obtained at different periods of the day. This was attributed to true differences in the coagulability of the blood instead of deficiencies in the technique of the method which was used.

When working with M'Gowan's (16) method I noticed that the more temperature variations were excluded the smaller was the amount of difference in the coagulation times, and that fluctuations in the coagulation curves, which I had formerly considered to be indicative of intrinsic

changes in coagulability, were really due to variations in the surrounding temperature.

With the method described in Section I., in which temperature variations are entirely excluded, the coagulation time was found to be remarkably constant at all times of the day and night. The slight variations which occur are irregular and are due to experimental error.

For twenty days the coagulation time of my own blood and that of certain other persons was taken every hour or two, and in no one of the charts prepared from these observations do any variations occur which are outside the limit of experimental error.

The following figures are the averages of more than three hundred observations taken at different hours of the day, at a temperature of 18.5° C. :—

Time of day.	Average coagulation time.	
	min.	sec.
8-9 a.m.	7	36
9-10 "	7	50
10-11 "	7	24
11-12 noon	7	37
12-1 p.m.	7	46
1-2 "	7	28
2-3 "	7	49
3-4 "	7	41
4-5 "	7	50
5-6 "	7	54

The assimilation of food has been described as a cause of variation in the coagulation time by Bürker (21), Coleman (32), and others.

The coagulation time was estimated on ten different occasions both before and after breakfast. The average time before food was 7 minutes 47 seconds, and the average after was 7 minutes 46 seconds.

## VI THE EFFECT OF THE ADMINISTRATION OF CALCIUM AND CITRIC ACID BY THE MOUTH ON THE COAGULATION TIME.

The details of work done in this connection will be published elsewhere (42). The general conclusion arrived at is that calcium and citric acid, when given by the mouth, have no influence on the coagulation time. This is not due to entire non-absorption, but to the fact that the change they produce on the calcium content of the blood is so slight that no appreciable effect is produced.

## VII. CONCLUSIONS.

1. There are four conditions which must be fulfilled by any method which is to yield results which can be relied upon. (a) There must be a uniform method of obtaining the blood. (b) The temperature must be the same during each experiment. (c) The amount of contact with foreign bodies must be always the same. (d) The end-point must be

clear and definite and such as always to indicate the same degree of coagulation.

2. A method is described which conforms to these conditions.

3. Other methods which have been employed are reviewed, and, where necessary, the result of work undertaken to test their accuracy is given.

4. The general conclusion of this testing is that no one of these methods fulfils all the conditions mentioned above, and that in particular all fail to comply with the essential condition that in comparative observations exactly the same temperature must be maintained throughout.

5. The effect of variations of temperature is described, and it is shown that at about the normal temperature of the body the coagulation time is shortest, becoming gradually longer at temperatures above 40° C. and below 36° C.

6. The coagulation time is constant for the same individual at different times of the day, and even on different days. The daily variations which have been described do not exist, but are due to fallacies in connection with the methods which have been employed.

7. The conclusion that calcium and citric acid, when administered by the mouth, have no effect on the coagulation time, is referred to.

I wish to express my thanks to Professor Schäfer and to Dr Cramer for advice and criticism during the progress of these investigations.

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## DESCRIPTION OF PLATES.

## PLATE I.

P, large glass bottle containing paraffin oil suspended from an upright, so that it can be raised or lowered; *Sc*, scale on upright; *p*, flexible metal tubing leading the oil from the glass bottle to the spiral (*sp*) contained within the water vessel (*V*); *t*, metal tube continuous with the spiral, and emerging from the water tank at the level of the microscope stage; *ch*, Bogg's coagulometer chamber, with thermometer (*th*) inserted into it; *r*, reservoir into which the overflow of oil from the stage is

received; *w*, waste tube by which the oil from *r* is removed; R, Schäfer's thermostat (gas regulator); *g*, rubber tube from gas-tap; *g'*, rubber tube from thermostat to gas-burner under the water vessel.

## PLATE II.

This gives a closer view of the stage apparatus. The lid of the Bogg's coagulumeter (*ch'*) has been taken off, and the glass cone with its truncated apex, from which the drop of blood hangs, is shown.

THE ACTION OF TOBACCO SMOKE, WITH SPECIAL REFERENCE  
TO ARTERIAL PRESSURE AND DEGENERATION By W.  
EMERSON LEE. (From the Pharmacological Laboratory, Cambridge.)  
(With fourteen figures in the text and one Plate.)

(Received for publication 28th August 1908.)

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I. PREVIOUS HISTORY.

THE following investigations were conducted with a view to determine, first, the action of tobacco smoke apart from its various constituents, and second, whether smoking may cause arterial degeneration.

It is not necessary to refer to papers dealing with the physiological action of nicotine; a complete bibliography is attached to Langley's paper (1). The toxic effects of nicotine on man also require little notice; most of the important work on this subject is found in Allbutt's "System of Medicine." The effect of tobacco smoke has received little or no attention from the experimental standpoint.

II. COMPOSITION OF TOBACCOS AND TOBACCO SMOKE.

The composition of tobacco smoke, obtained by an aspirator from the slow combustion of 100 grams of tobacco, was as follows :—

Nicotine, 1.165 grams. This represented 50 per cent. of the total nicotine present before combustion.

Pyridine bases, 0.146 g. Chiefly pyridine and collidine, the former

being produced during the destruction of some of the nicotine, the latter from the combustion of the fibres in the tobacco.

Hydrocyanic acid, 0.08 g.

Ammonia, 0.36 g.

Carbon monoxide, 410 c.c.

These amounts vary with many factors. Thus the length of the tube through which the smoke passes—by allowing the deposition of the solid matter and the condensation of vapour—materially affects the composition of the smoke, the principle of this is illustrated in the “churchwarden” pipe. Again, the quality of the tobacco varies within the widest limits; evidence will be produced on this point later. For the purposes of experiment some standard tobacco must be adopted and retained throughout. The tobacco which was chosen for these experiments was of two varieties:—1. A sample of Virginian tobacco from the “untreated” leaf, prepared for smoking in cigarette form; this was kindly provided by Mr. Player. 2. A very strong variety of Manilla cigar.

In order to determine the amount of nicotine or blood-pressure-raising substances present in these two tobaccos, equal weights were taken and macerated, each in the same quantity of normal saline solution. The solutions were then filtered, and the amount of nicotine or blood-pressure-raising substances estimated physiologically by their power of raising blood-pressure. For this purpose cats were pithed or anæsthetised with urethane, and the blood-pressure recorded by a mercurial manometer. The following figures show the result of these experiments:—

One gram of cigarette tobacco and 1 gram of Manilla tobacco were macerated each in 100 c.c. of saline and allowed to stand for two days. The infusion resulting was filtered, and a fluid suitable for intravenous injection was obtained.

Equal quantities of the two solutions were injected into the jugular vein of a pithed cat, and the relative rise of blood-pressure when the amount injected was not excessive was as follows:—

Manilla tobacco, 25.6 mm. Hg.

Cigarette „ 42.4 „

A solution of the crude leaf from which this sample of cigarette tobacco was prepared (1 gram to 100 c.c.) produced a rise of 56 mm. Hg. The last result shows that during the course of preparation a considerable quantity of nicotine or pressor substance is destroyed. This is not surprising, as it is well known, that fermentation reduces the amount of nicotine in tobacco. From these experiments it is obvious that the Manilla tobacco contains much less nicotine than the Virginian. It does not, however, necessarily follow that because one tobacco contains less nicotine than another, it will yield less nicotine when it is smoked. For this reason a second series of experiments was conducted by drawing the fumes from the

combustion of the tobaccos through saline solution by means of a suction pump, 1 gram of each variety of tobacco being used with 100 c.c. of saline. The stroke of the pump was so arranged that the smoke was drawn through the saline about twelve times a minute. The procedures with the two tobaccos were conducted under identical conditions, and so arranged that the combustion of the two tobaccos was effected in equal times. The relative amounts of nicotine were determined as before, by comparing the rise in blood-pressure produced when the solutions were injected into a pithed cat. The results showed that when the smoke solution from the Manilla tobacco caused a rise of 2 mm. Hg, the smoke solution from the cigarette tobacco caused a rise of 1 mm. Hg.

From these experiments the remarkable fact comes out that, whilst the Virginian tobacco contains a much greater percentage of nicotine than the Manilla, yet, after combustion, the smoke from the Manilla contains considerably the larger percentage. This circumstance is explained as follows:—During the slow combustion of a cigar, as in ordinary smoking, immediately behind the point of combustion is an area in which the water and other volatile substances in the tobacco condense; during the act of smoking the greater portion of the nicotine at the seat of combustion is destroyed (50 per cent.), and the nicotine which finds its way into the mouth of the smoker is probably derived from the hot gases passing through the moist area and volatilising certain of the more volatile principles of the tobacco, of which nicotine is certainly one. So that the smaller the moist area behind the point of combustion, the less likely is the smoke to contain volatile toxic bodies. It will be immediately suggested that a thin cigar or a cigarette will yield fewer of these products than a thick cigar, for the thin cigar or cigarette obviously permits a relatively greater evaporation to take place. Moreover, if a thick cigar be unrolled and made up in a thinner body, the percentage of nicotine destroyed during combustion is increased. The experience of many smokers also agrees with this hypothesis, for there are those who will always avoid a thick cigar because, whatever be the strength of the leaf from which it is made, unpleasant symptoms are invariably experienced.

### III. THE RELATIVE ACTION OF THE CONSTITUENTS OF TOBACCO SMOKE.

It has been pointed out already that the important constituents of tobacco smoke are nicotine and certain pyridine bases, including especially pyridine itself and collidine. The following experiments were conducted with a view to determine the relative effects of these alkaloids.

i. Plain Muscle.—The action on plain muscle was determined first by means of "ring" preparations of the frog's stomach. These were suspended in Ringer's solution and arranged to record on a slowly moving drum by means of suitably weighted levers.

A solution of collidine, 1 in 1000 in saline, was applied to such a preparation; the movements were promptly inhibited and the tonus diminished. Nicotine, 1 in 1000, was then applied; the muscle entered into tonic contraction, the waves for the time ceasing, but it soon regained its normal activity. Pyridine, 1 in 1000, was administered and produced hardly any result; collidine was applied once more, when it again produced its typical effect.

It is clear from this that nicotine and collidine act in opposite directions: the former causes the muscle to increase in tonus, the latter inhibits movements and causes it to relax; pyridine, in these doses, is almost without action.

ii. The Heart.—It has been pointed out that experiments made by dropping drugs on the frog's heart are not likely to lead to valuable results, since in many cases the action differs from that obtained when the drug

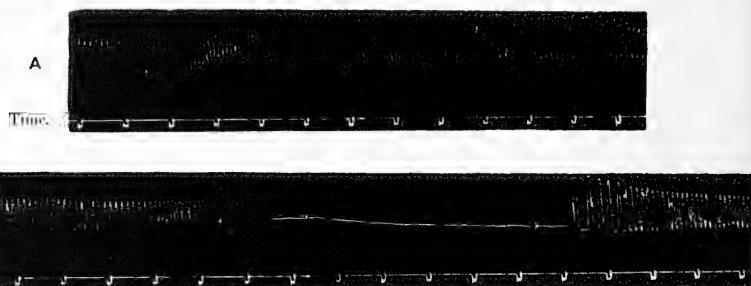


FIG. 1.—Record of movements of an isolated frog's heart perfused through the hepatic vein. A shows the effect of 0.1 per cent. nicotine. B shows the effect of 0.1 per cent. collidine. Time, 30 seconds.

passes through the circulation of the heart. The following experiments on the isolated frog's heart were therefore performed by perfusing Ringer's solution through one hepatic vein and allowing it to escape by the aorta, the heart-beat being recorded by the suspension method. A solution of 1 in 10,000 nicotine raised the tonus of the heart muscle and slightly quickened the beat. The same strength of pyridine produced no effect, while the same strength of collidine produced some slowing and a fall in tonus. A more concentrated solution of nicotine (1 in 1000) always causes the heart to enter into very marked tonus. The effect of pyridine (1 in 1000) slightly weakens the beat of the heart; it never produces a rise of tonus.

From such experiments it is shown that nicotine has much the most toxic effect on the heart, and that pyridine has the least. The effect of collidine compared with nicotine is shown in fig. 1. It will be noticed in these experiments, which were performed under identical conditions, that the nicotine (A) quickens the heart at first, but later causes some slowing:

these effects of nicotine, we know, are due to excitation of the ganglion-cells. The collidine effect bears a superficial resemblance to that of nicotine, except that the inhibition is more pronounced in the case of collidine (B); but there is one important difference in the complete absence of rise of tonus.

The action on the isolated mammal's heart was determined by perfusing the hearts of rabbits with Ringer's solution, by means of a modified Langendorff apparatus.

At A, in fig. 2, 2 c.c. of a 1 per cent. solution of pyridine were injected by means of the lateral tube, but on its reaching the heart only a very small effect was noticed—there was no alteration in tonus, but there was evidence of some quickening of the beats. After a period of 20 minutes' perfusion with Ringer's solution, during which time the heart's action had become quite normal, 2 c.c. of 1 per cent. solution of collidine were injected at B, at the same rate as before, into the perfusing fluid. The heart was immediately inhibited in diastole; but, after the drug had passed through, recovery gradually ensued until the normal rhythm was regained, no permanent depression resulted. After a further period of 20 minutes' rest, 2 c.c. of a 1 per cent. solution of nicotine were injected (at C); the strength of the heart was immediately increased, the heart-beat was accelerated, and the tonus was gradually raised; indeed, this gradual increase of tonus with nicotine is a most characteristic effect, and affords a marked distinction between the action of nicotine and the other constituents of tobacco smoke.

The periods of inhibition found in the tracing, following injection of nicotine, are due to excitation of the intracardiac ganglion-cells; they are not seen in the atropinised animal.

I now propose to compare the action of a solution of nicotine with a solution through which tobacco smoke has been drawn. For this purpose the smoke from 1 gram of tobacco, slowly burnt, was drawn slowly through 100 c.c. of saline solution. The isolated rabbit's heart was again used; fig. 3 illustrates the two effects, at A the effect of injecting the smoke solution being shown, and at B the effect of nicotine. The first effect (A) shows initial inhibition followed by acceleration of the heart and some increase in strength of the beat: there is no rise in tonus. B shows an almost identical result, but with the difference that the heart does not relax properly in diastole, so that the diastolic tone gradually rises. We know that the pyridine bases, especially collidine, reduce tonus in muscle, and it seems possible that while the inhibition and subsequent acceleration shown at A may be due to nicotine, the absence of increased tonus may result from the antagonistic action of the pyridine bases to nicotine.

iii. Some Effects of Perfusion.—The blood-vessels of the frog were perfused through the right innominate artery, and the outflow from the veins determined by placing the frog in a funnel and allowing the drops to fall upon a lever that recorded on a slowly moving drum.

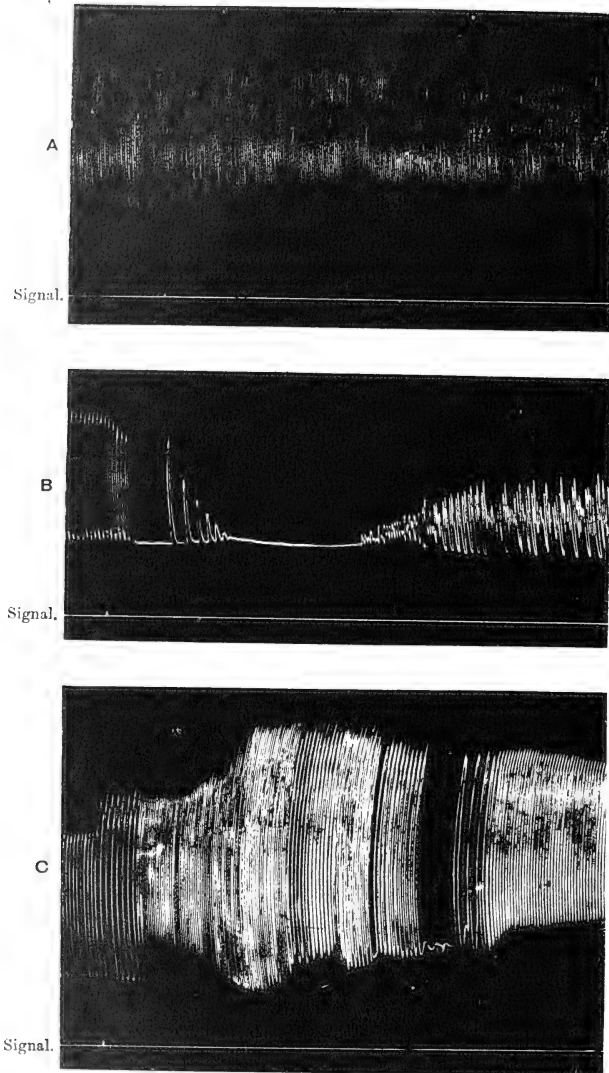


FIG. 2.—Isolated rabbit's heart perfused with Ringer's solution by the method of Langendorff. A shows the effect of injecting into the lateral tube 2 c.c. 1 per cent. pyridine; B, injection of 2 c.c. 1 per cent. collidine; C, injection of 2 c.c. 1 per cent. nicotine. Time, 1 cm. = 10 seconds.



Ringer's solution was employed as the perfusing fluid. All three drugs reduce the flow through the vessels if in sufficient concentration, but this result is not obtained with dilute solutions such as 1 in 10,000. It is known that nicotine solutions of this dilution constrict blood-vessels in an intact animal to a very decided degree: all that these experiments therefore show is that nicotine in dilute solutions does not act directly on the vessel wall.

Collidine and pyridine, in doses which one may regard as possible in man, have practically no action upon the blood-vessels.

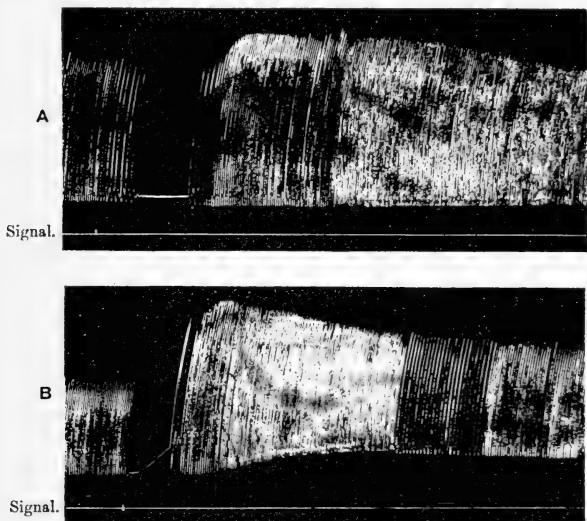


FIG. 3.—Isolated rabbit's heart perfused by Langendorff's method.

A shows the effect of injecting into the lateral tube 3 c.c. of the smoke solution, and B, 2 c.c. of 1 per cent. solution of nicotine. Time, 1 cm. = 10 seconds.

iv. The Effect of Intravenous Injection of the Constituents of Tobacco Smoke. (a) On the Spinal Cord.—Small doses of these drugs injected directly into the circulation of rabbits excite the spinal cord, and if the doses be sufficiently large, convulsions are produced. These convulsive movements differ from those produced by strychnine in that there is no antecedent tonic stage; the animal passes into a condition of anaesthesia associated with clonic contractions of all the muscles supplied by the spinal cord. This effect is much the most marked with nicotine; it is very small in the cases of collidine and pyridine. That the convulsions are spinal in origin may be shown by painting the spinal cord of a decapitated frog with the drug, when the muscles supplied by that part will be observed to

twitch. This has been already shown and commented on by Dixon (3). Moreover, these drugs differ in their action from strychnine in that, whilst this drug affects the sensory cells so that the convulsions can only be produced by an appropriate afferent stimulus, the drugs under consideration act on the motor cells. This is proved, first, because on being applied to the cord they produce immediate twitchings of the muscles which are supplied by that section of the cord; second, because they are not reflex in origin; and third, because the twitchings are limited to the cells affected. That is to say, strychnine applied to one small portion of the cord produces convulsions over the whole body; but nicotine applied in the same way causes twitchings only in the muscles supplied by the corresponding part of the affected cord.

(b) On the Circulatory System.—Pyridine produces remarkably little effect; in fig 4, A, 5 c.c. of a 1 per cent. solution were slowly injected and produced practically no alteration in the blood-pressure, nor in the general condition of the circulation. Collidine, however, in small doses causes considerable dilatation of the blood-vessels, and a corresponding fall in blood-pressure. This is shown in fig. 4, B, in which the upper tracing represents intestinal volume, and the lower the blood-pressure. It will be noticed that as the vessels dilate the pressure falls. In this case, however, there can be no doubt that some of the fall of pressure is due to cardiac depression. Larger doses of collidine weaken the heart, and consequently lower the blood-pressure to such an extent that the intestinal vessels, instead of filling with blood, shrink, secondarily to the fall of blood-pressure.

The effect of nicotine is shown in fig. 4, C, for the sake of comparison. It also lowers the blood-pressure, as shown in the second (detached) part of the tracing, but only after an initial rise. I shall have occasion later, in dealing with the action of tobacco smoke on man, to refer again to this well-known rise followed by a fall in blood-pressure.

#### IV. TOXICITY AS ESTIMATED BY INJECTION INTO INTACT ANIMALS.

The relative toxicity of pyridine, collidine, and nicotine was estimated by determining the minimal lethal doses in frogs.

Three frogs, each within a fraction of 23 grams in weight, were injected with solutions containing a quarter of a grain of each drug respectively. The injections were made into the dorsal lymph sac.

During the two hours after injecting pyridine the animal was very active, the pupils were dilated, and respiratory efforts were increased; there was no paralysis.

The second frog, after collidine, became paralysed in two minutes, respiration almost ceased, the pupils were widely dilated, and reflexes were entirely absent. After a lapse of twenty minutes feeble respiratory movements became evident, and the animal gradually recovered.

Nicotine was much the most toxic. The frog died about two minutes

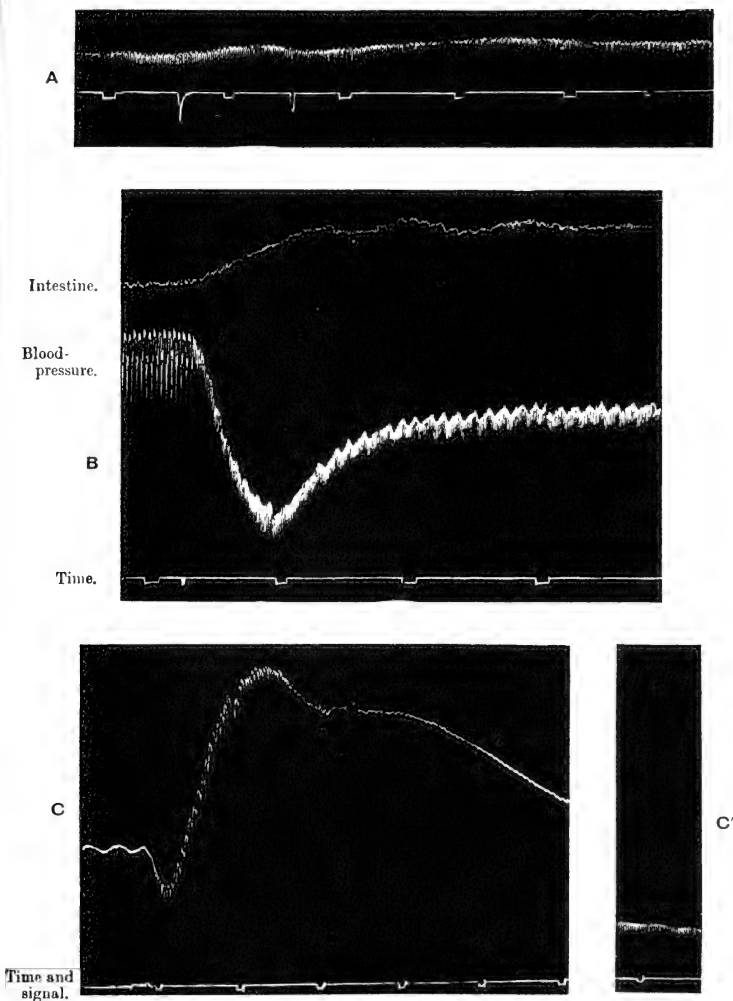


FIG. 4.—Cat; A-C-E mixture: urethane. Shows the comparative effect of pyridine, collidine, and nicotine on blood-pressure.

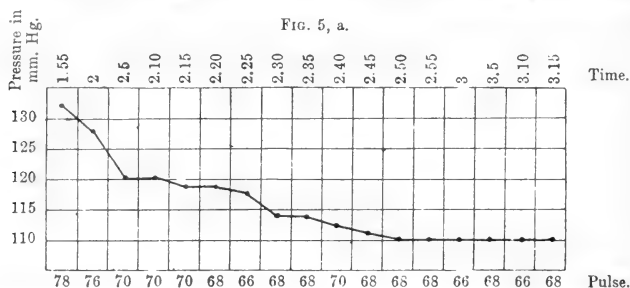
A, the effect of injecting into a vein 5 c.c. of 1 per cent. pyridine; B (in which the intestinal volume is also recorded), the effect of injecting 5 c.c. of 1 per cent. collidine; C shows the effect of 1 c.c. of 1 per cent. nicotine. These tracings were obtained from different animals, but may be taken for comparison. Time, 30 seconds.

after injection, from complete paralysis of the central nervous system. After systemic death it was found that the motor nerve endings were almost paralysed, but that the heart was still beating.

After a series of similar experiments upon frogs whose weights approximated closely to those given in the previous experiment, it was decided that the following were the minimal lethal doses of the drugs for a 20-gram frog:—

Pyridine	.	.	$\frac{3}{10}$ grain = '039 gram.
Collidine	.	.	$\frac{1}{4}$ grain = '016 gram.
Nicotine	.	.	$\frac{1}{10}$ grain = '006 gram.

Therefore, if the toxicity of pyridine be represented by 1·0, that of collidine will be 2·4, and that of nicotine 6·0. From this it will be seen that the toxicity of these three drugs varies in the same way as their effect



on isolated tissues. That is to say, their effects on the heart, plain muscle, and central nervous system all run a parallel course, nicotine in each case being much the most active and pyridine much the least.

#### V. THE EFFECT OF SMOKING ON MAN.

A series of experiments were conducted upon men whose habit varied from that of the novice to that of the seasoned smoker. I have described below a series of experiments on the blood-pressure of men.

The blood-pressures were taken with Martin's modification of the Riva Rocci instrument, the pressure band being fixed in each case to the bared left arm. The normal blood-pressure was always taken a number of times before a final figure was fixed upon. I invariably found that merely fixing the instrument upon the arm was sufficient to raise blood-pressure several mm. Hg, and the final figure was never determined until the man was accustomed to all his surroundings and his blood-pressure was quite constant. The habitual smokers were required to observe abstinence from tobacco smoke for some six hours before being subjected to an experiment.

Experiment 1 shows the necessity of obtaining a correct normal reading of the blood-pressure before determining the effect of any drug.

EXP. 1. A youth aged 18 was placed in a chair in as comfortable a position as possible, and a normal chart prepared for comparison with those in which smoke was inhaled (see fig. 5, a).

Time.	B.P.	Pulse.
1.55 P.M.	134	78
2	128	76
2.5	120	70
2.10	120	70
2.15	118	70
2.20	118	68
2.25	116	66
2.30	114	68
2.35	114	68
2.40	113	70
2.45	112	68
2.50	110	68
2.55	110	68
3	110	68
3.5	110	68
3.10	110	66
3.15	100	68

The following experiments show blood-pressure changes and pulse-rates during smoking.

EXP. 2. Youth aged 17½, an occasional smoker of cigarettes; normal blood-pressure, from a series of observations, found to equal 117 mm. Hg (systolic reading); pulse-rate 72. (See fig. 5, b.)

Time.	Blood-pressure.	Pulse-rate.	Observations.
5 P.M.	114-116	72	Started to smoke and inhale the standard Manilla cigar.
5.15	128	...	A distinct pallor of face, and sensation of weakness in legs.
5.20	128	...	Eyes "sleepy"; appears shaky.
5.25	128	...	Feeling faint, cigar half finished.
5.30	78	...	Intense pallor of the skin, cold sweat on forehead.
5.35	...	60	Feeling very faint and weak, colicky pains in abdomen, cigar three-quarters finished. Stopped smoking.
5.40	114	66	Feels less faint.
5.44	108	72	Lips regaining their colour.
5.50	95	64	Feels better, muscles stronger but is still incapable of physical exertion.
6.10	104	54	Better, but feels weak all over.
6.25	110	54	Slight indisposition.
Next morning	121	72	Normal state.



EXP. 3. Boy aged 15, moderate smoker of cigarettes. Shows effect of smoking one and a half Manilla cigars; occasionally inhaled. Normal blood-pressure and pulse-rate were found to be 120 and 84 respectively. (See fig. 5, c.)

Time.	Blood-pressure.	Pulse-rate.	Observations.
12 P.M.	120	84	Normal sensations.
12.30	122	88	
2.50	120	80	Started smoking.
2.55	120	84	
3.4	120	84	
3.15	124	90	
3.20	130	88	Heavy feeling in head.
3.30	128	88	Feels shaky.
3.35	128	88	Slight dizziness.
3.40	130	92	Feels weak; forehead shows beads of perspiration.
3.50	120	106	Felt suddenly faint—nausea and misty vision. Smoking stopped.
4	120	104	No change from 3.50.
4.5	120	102	Nausea—stiff feeling about the back of neck. Great lassitude.
4.30	104	88	Feels slightly better.
5	120	80	Feels well.

EXP. 4. Boy aged 17, smoker of cigarettes only. Smoking two Manilla cigars, with occasional inhalation. (See fig. 5, d.)

Time.	Blood-pressure.	Pulse-rate.	Observations.
2.50 P.M.	134	54	
2.55	132	60	
3	132	60	
Normal	blood-pressure therefore	132-134	Pulse 60.
3.5	135	68	Started smoking.
3.10	138	68	
3.15	140	70	Pulse irregular.
3.20	142	74	
3.25	145	68	Pulse intermittent.
3.40	142	76	
3.45	146	82	Pulse irregular and intermittent.
3.55	150	70	Sensations of vertigo; hands show distinct tremors.
4	145	72	Smoking stopped.
4.5	142	78	
4.10	136	78	Considerable salivation—a feeling of nausea.
4.20	134	66	Feels better.
4.30	132	62	
4.40	132	62	
4.50	133	64	

EXP. 5. Man aged 30, moderate smoker, but not an inhaler. Smoked one Manila cigar.

Time.	Blood-pressure.	Pulse-rate.	Observations.
11.30 A.M.	122	52	<p>Started smoking.</p> <p>No change in sensations. No alteration in colour.</p> <p>Stopped smoking.</p>
11.40	122	56	
11.45	120	56	
11.50	123	60	
11.55	120	58	
12.0	120	58	
12.10	128	60	
12.15	130	58	
12.20	130	60	
12.25	130	60	
12.30	128	58	
12.35	126	56	
12.40	125	56	
12.45	124	56	
12.50	123	56	

EXP. 6. Man aged 31½, habitual smoker, but non-inhaler. Smoked one Manila cigar, inhaling all the time.

Time.	Blood-pressure.	Pulse-rate.	Observations.
11.20 A.M.	124	60	<p>Started smoking.</p> <p>Stopped smoking.</p> <p>No subjective or objective changes noted throughout the experiment.</p>
11.25	120	60	
11.30	120	60	
11.40	120	62	
11.45	122	64	
11.50	124	66	
11.55	128	66	
12	128	66	
12.5	129	69	
12.10	128	66	
12.15	128	64	
12.20	126	64	
12.25	126	66	
12.30	128	63	
12.35	126	68	
12.40	122	64	
12.45	122	60	
12.50	122	64	
12.55	122	64	
1 o'clock	122	66	
1.5	120	64	
1.10	120	64	



EXP. 7. Man aged 24, a smoker of seven years' standing. Smoked one cigar, not inhaling.

Time.	Blood-pressure.	Pulse-rate.	Observations.	
10.20 A.M.	120	72	Average reading for 9.55-10.20. Started to smoke.	
10.30	120	72		
10.35	120	56		
10.40	121	60		
10.45	124	56		
10.50	125	56		
10.55	127	60		
11	128	64		
11.10	130	60		
11.15	130	62		
11.20	131	60		
11.25	130	62		
11.30	128	60		Stopped smoking.
11.35	126	60		There were no subjective or objective changes noticed.
11.40	126	60		
11.45	126	60		
11.50	124	60		
11.55	122	64		
1 o'clock	120	46		

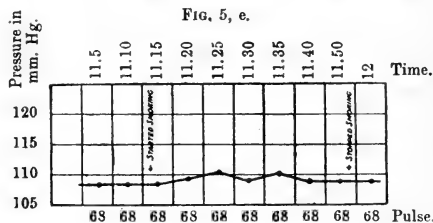
EXP. 8. Man aged 29, habitual smoker, frequent inhaler of cigarettes. Smoked one Manilla cigar, inhaling all the time. (See fig. 5, e.)

Time.	Blood-pressure.	Pulse-rate.	Observations.	
11.5 A.M.	108	68	Started smoking.	
11.10	108	68		
11.15	108	68		
11.20	110	68		
11.25	108	68		
11.30	110	68		
11.35	108	68		
11.40	108	68		
11.50	108	68		
12	108	68		
				No alteration in sensation.
				Stopped smoking.

One last experiment in the series was made by smoking leaves which are known not to contain nicotine. Dried lavender leaves were used for this purpose, these being sold in certain parts of this country as "boys' tobacco." The result of smoking such leaves is to cause a sensation of stinging or scalding in the throat and mouth and some slight rise in blood-pressure, the latter, however, not being comparable to that of tobacco. Fig. 5, f, shows the effect of smoking such leaves. This chart was obtained from the same subject as fig. 5, a and b.

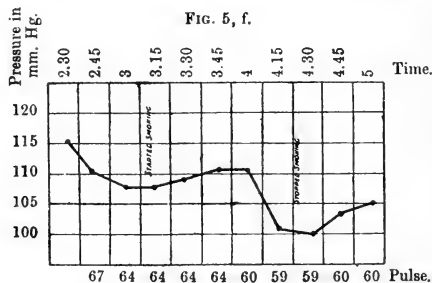
These protocols are typical of the effect of tobacco smoke on man. They may be divided into three groups: the first including those in which the smoker was a novice; the second, the group of moderate smokers; and the third, the group containing the "excessive smokers."

In the case of the novice there is always an initial rise of blood-pressure very shortly after the inhalation has well started, and lasting half an hour or perhaps even a shorter time. The height to which the blood-pressure



rises above the normal varies, but is usually from 10 to 20 mm. Hg. This effect is associated with some quickening of the pulse; for example, in protocol 2 the increase is from 72 to 80, and in 3 from 84 to 106.

At first the smoker has no unpleasant symptoms, but rather a feeling of well-being and exhilaration. As the smoking continues, however, a sudden change occurs in the blood-pressure, which begins to fall rapidly,



so that, as in the case shown in protocol 2, there may be a fall of 50 mm. Hg. within five minutes.

When the smoker, though a novice, is less affected by the inhalation, as in protocol 3, the fall, though still rapid in onset, does not so closely resemble a crisis as in experiment 2. This fall in blood-pressure is associated with all the symptoms characteristic of shock or collapse. The face becomes pale, the skin is covered with a clammy sweat, there is general weakness of all the muscles, faintness, shallow respirations, and a slow and feeble pulse; sometimes nausea or vomiting may be present, and

sometimes colicky pains are felt in the abdomen, suggesting increased peristalsis.

These experiments strongly suggest that fall in blood-pressure is the essential factor in the production of collapse, for all the symptoms of collapse are such as are obtained from a sudden fall in blood-pressure. I believe that these simple experiments amply confirm the hypothesis of Crile (4), that shock and collapse are conditions resulting from a severe fall in blood-pressure.

In tobacco smoke there is only one constituent (nicotine) which has the power of increasing blood-pressure appreciably, but there are many substances, such as the pyridine bases, which lower the pressure. During the inhalation of tobacco smoke, the action of nicotine overshadows that of the other constituents; the nicotine stimulates nerve cells, and for a time exercises unchallenged its vaso-constrictor influence, with the accompanying rise of blood-pressure. But a stage in smoking is reached when the stimulation of nerve cells by the nicotine gives place under the same influence to their depression, with resulting vaso-dilatation and a fall in blood-pressure. This condition will be exaggerated by the other constituents of the smoke, such as pyridine and collidine, which throughout have been tending to lower blood-pressure.

The action, then, of tobacco smoke on man is exactly what might be anticipated from a knowledge of the action of nicotine: while the stimulation stage lasts the pressure is raised; then, as the nerve cells are depressed, the blood-pressure falls. But I have obtained plenty of evidence of variations in the degree of idiosyncrasy for nicotine. Thus, in comparing experiments 2 and 3, although the subjects were very similar as regards their smoking habits—in fact, they were both almost complete novices—yet the elder was much more affected than the younger, twice the quantity of smoke being required to produce a similar effect in 3 as in 2.

The second group is composed of moderate smokers, and this group probably includes the majority of those who smoke regularly. Experiments 5, 6, and 7 are typical examples. In these the blood-pressure rises slowly, unlike those in the first group, where the rise is rapid; the height to which the pressure rises, however, is about 10 mm. Hg, and the tendency is for the blood-pressure to continue rising slightly, or at least to maintain the higher level, whilst the smoking lasts. With the novice, on the other hand, blood-pressure falls, and collapse ensues whilst smoking is actually in progress. In these temperate smokers, after smoking has ceased, the blood-pressure falls gradually to the normal, but shows no tendency to move below that level. The whole effect, then, consists of a small and gradual rise of blood-pressure lasting until the smoking ceases. The rise in pressure is usually associated with some acceleration of the pulse.

Of course these experiments only show the effects of moderate smoking

on the moderate smoker. If the moderate smoker smokes to excess, he assumes the position of a novice, and a climax would be reached in which the nicotine and other constituents of tobacco smoke would accumulate in the blood to an extent which would paralyse the nerve cells, and produce the sudden fall of blood-pressure characteristic of collapse. Why the moderate smoker is able to withstand the action of smoking so much better than the novice, is a question with which I propose to deal elsewhere.

The third group—the excessive smokers—an example of which is shown in protocol 8, is merely an exaggeration of group 2. The pressure rises only 2 mm. or 4 mm. Hg, and such as it is, is maintained in the same way as in group 2 until smoking ceases, when it returns again to the normal. The pulse in these cases is not affected.

## VI. THE EFFECT OF SMOKING ON ANIMALS.

(a) Immediate Effects.—The effect of inhalation of tobacco smoke on anaesthetised animals was determined by connecting a tracheal tube, which has in it a suitable lateral opening, capable of being regulated in size, with a lighted cigarette. The animal will then inhale the smoke with a variable admixture of air according to the size of the aperture in the lateral tube: the inhalation will be on much the same principle as when man inhales. The objection to this mode of procedure is the effect of the smoke on respiration: the animal ceases to breathe for a time, or the respiration becomes feeble and irregular, and this results in a variety of secondary circulatory disturbances from the spasmodic breathing, or from partial asphyxia, so that it is almost impossible to say which circulatory effects are due directly to smoking and which are due to irritation of the respiratory tracts.

To obviate this difficulty an alternative mode of administering the smoke was adopted. A normal animal was first killed by the destruction of the brain and medulla oblongata by pithing, without the use of any anaesthetic, artificial respiration being started. In the course of the air tube was inserted a special apparatus, devised for these experiments, which is shown in fig. 6. The current of air was divided by a Y-tube, the one limb carrying the air directly to the trachea tube, the other through a chamber in which, by allowing a regulated quantity of air to pass through it, tobacco in the form of a cigar or cigarette could be burnt at any required rate. The two streams of air were brought together again by a second Y-tube, the single limb of which was connected to the trachea tube.

A glance at fig. 6 shows that when a lighted cigarette or cigar is placed in the tube A, if the tube B is closed during the down-thrust of the pump all the air will pass through the tube A and through the cigarette or cigar, and thus the animal will receive the smoke. By regulating the

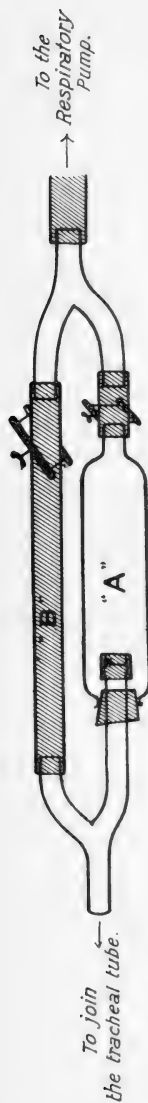


FIG. 6.—Apparatus used for administering tobacco smoke to animals.



FIG. 7.—Cat. Brain destroyed by pithing. Artificial respiration. Blood-pressure. Shows the effect of inhalation of smoke from Manilla cigar. Time, 30 seconds.

Smoking,  
On.

Oil.

amount of air passing through B, it is possible to obtain a condition in which the smoke is comparable in amount to that which is inhaled by man: this condition is gauged by the rate at which the cigarette or cigar burns.

The effect of such smoking on a cat is shown in fig. 7.

In this cat the blood-pressure rose 20 or 30 mm. Hg during the first five minutes of smoking, and then began to fall, in spite of the fact that the smoking was still going on. This condition may be regarded as typical, and bears a close analogy to that which occurs in man. Occasionally, when the blood-pressure began to fall, the animal showed convulsive movements. The ultimate effect, after smoking two or three cigarettes, was a considerable fall in blood-pressure.

In one experiment, during the inhalation, a quantity of moisture from

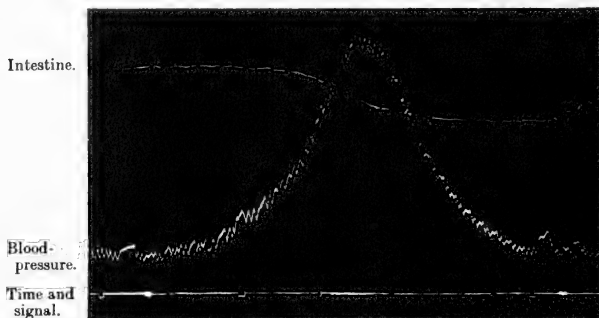


FIG. 8.—Cat. Brain destroyed by pithing. Artificial respiration. Intestinal volume. Blood-pressure.

Shows the effect of accidental inhalation of tobacco juice. Time, 30 seconds.

the cigar condensed on the glass tube, just in front of the tracheal tube. This during one inspiration was blown into the trachea, and produced an immediate rise of blood-pressure with marked constriction of the blood-vessels. This is shown in fig. 8, in which the upper tracing represents the intestinal volume, the lower the blood-pressure. It is just such sudden increases of blood-pressure as these which stretch and rupture the elastic fibres in the vessels, as described later. The experiment illustrates the possible dangers attendant on the use of foul pipes and the latter end of a cigar, although they may be exaggerated in this instance, since the fluid passed directly into the trachea and not into the mouth, as would be the case in man.

The ultimate effect on blood-pressure is shown in fig. 9. In this case a rabbit was used, anaesthetised with urethane. The tracing A shows the height of the blood-pressure before the smoking commenced, while B

represents the height after the inhalation of three cigarettes, smoked during thirty minutes.

The first effect of the inhalation of tobacco smoke on the heart is shown in fig. 10. In this experiment the outflow of blood from the heart was

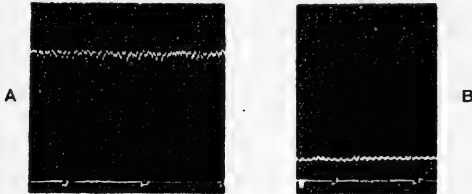


FIG. 9.—Rabbit. Urethane.

A shows normal blood-pressure, and B shows the fall after smoking three standard cigarettes. Time, 30 seconds.

measured by the cardiometer. It will be noticed that the heart filled with blood, but that its systole was a little incomplete; that is to say, it did not empty itself quite as completely as it normally does: nevertheless, the total output from the heart was increased.

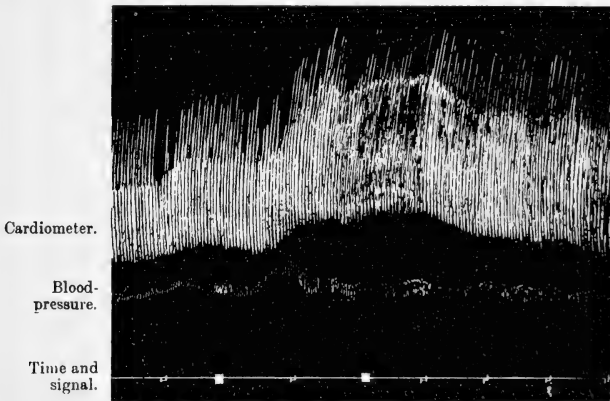


FIG. 10.—Cat, A-C-E: urethane. Artificial respiration. Cardiometer and blood-pressure. Shows effect of inhaling smoke from a Manilla cigar with a plentiful supply of air. Time, 30 seconds.

From half to three-quarters of an hour after smoking, the blood-pressure does not rise on the administration of large doses of nicotine, and the vagi are found paralysed, although adrenaline causes its normal effect. This must mean that an excess of smoking produces paralysis of the autonomic nerve cells in exactly the same way that nicotine does.

(b) Remote Effects.—Rabbits were used for this experiment, because they are known to be very susceptible to changes in the arterial system. Two were made to inhale tobacco smoke regularly for about fifteen to twenty minutes on alternate days throughout a period of five months. Two methods were used. In the one a mask was fitted over the mouth and nose of the animal and, with the apparatus already described, through which the supply of both air and smoke could be regulated, the smoke from mild cigarette tobacco was directed into the mask. A very dilute mixture was at first given, but as the animal became accustomed to the treatment the percentage of smoke was increased. The second method consisted in placing the animals in a small chamber with inlet and outlet valves; one side of the chamber was of glass, to enable observations to be made, and through this chamber smoke in varying quantities was passed continuously. The latter method was chiefly employed. Whilst the animals were inhaling the smoke, it was noticed that salivation was present, but no other changes from their normal condition appeared. On being removed from the chamber, for the ensuing three or four minutes they appeared lethargic, although their reflex sensibility was somewhat heightened. At the end of five months each animal had received about seventy inhalations of tobacco smoke; during this period they had increased in weight, as did the control animal, and, as far as one could see, had suffered in no way, for they took their food and behaved as normal rabbits, and during the later inhalations showed no objection to the process.

We now know that any substance which has the power of raising blood-pressure suddenly and to any considerable extent, say 30 or 40 mm. Hg, tends to injure the aorta. This has been shown by Harvey (5) in this laboratory. Harvey raised the blood-pressure by compressing the abdominal aorta, thus excluding all the hypotheses and speculations which have been made suggesting that the "irritation" of the drug causes the arterio-sclerosis observed. Moreover, irritant drugs which do not raise blood-pressure do not cause arterial disease.

We may, therefore, assume that if the smoking to which these animals were subjected was capable of raising the blood-pressure to any considerable degree, signs of arterial degeneration may be expected.

Rabbit B was killed. A few miliary patches were visible in the ascending aorta, and also a small plaque in that sinus of Valsalva which does not give off a coronary artery. The lungs showed some congestion, but no consolidation, and the bronchial glands were normal. In the spleen was an old infarct. The kidneys were normal.

The aorta was prepared for microscopical examination; portions of it were hardened in formalin and dehydrated in a series of alcohols of increasing strength up to 100 per cent.; they were cleared with chloroform, and embedded in paraffin. Sections were cut and stained with:—(a) acid hæmatoxylin (Ehrlich) and aqueous eosin, or with picric acid fuchsin (Weigert's modification of Van Giesen); (b) acid orceïn (Unna-Taenzer



elastic tissue stain); and (c) silver nitrate, 5 per cent. aqueous solution (v. Kossa's calcium test). The sections were mounted in Canada balsam. Photomicrographs of the three specimens are shown in the accompanying Plate 1 shows extensive fibrosis of the tunica media, invading to a slight extent the tunica intima. It also shows, in parts, the antecedent stage of fibrosis as an inflammation, with plentiful cell-proliferation. 2 shows marked erosion and rupture of the elastic fibres; some of these fibres are encased in calcium salts. 3 shows considerable deposits of calcium salts—which are stained black in this specimen.

Rabbit A was killed. The animal was found to have tuberculosis of the lungs. The heart and aorta of this animal showed hardly any naked-eye change. The trachea was acutely inflamed, and with the exception of the tuberculous lesions, there were no other abnormalities.

Sections from the aorta of this rabbit (A) show changes similar to those exhibited by section 1 of rabbit B.

From these experiments I conclude that it is possible to obtain arteriosclerotic changes by the inhalation of tobacco smoke.

It gives me much pleasure to record my thanks to Dr W. E. Dixon for his advice, and to Dr W. H. Harvey for preparing the photomicrographs.

## VII. GENERAL CONCLUSIONS.

1. Nicotine is the most important poison in tobacco smoke.
2. Pyridine bases, in the quantities in which they are present in tobacco smoke, are not injurious to the smoker.
3. Smoking raises the blood-pressure by vaso-constriction, accelerates the heart and respiration, and increases intestinal movements. In excess, cerebral depression may occur, and, with the coexisting depression of the vaso-motor centre, may lower the blood-pressure to such an extent that collapse may be induced.
4. The amount of nicotine inhaled during smoking depends not so much on the tobacco smoked, as upon the form in which it is smoked. The greater the condensation area between the point of combustion and the entrance into the mouth, the more nicotine will be inhaled.
5. Arterial disease may result from prolonged tobacco smoking.

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- (3) CRILE, *Experimental Research into Surgical Shock* (1897).

(4) ZEBROWSKI, Centralbl. f. allgem. Path. u. path. Anat., 1907, vol. xviii, p. 337.

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#### DESCRIPTION OF PLATE.

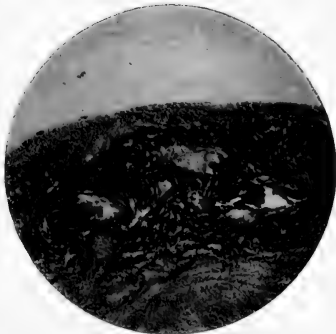
The figures represent photographs taken under a moderately high power of the microscope of part of the wall of the aorta of a rabbit which had been caused to inhale tobacco smoke at intervals during five months before being killed. For further description see text, pp. 356, 357.



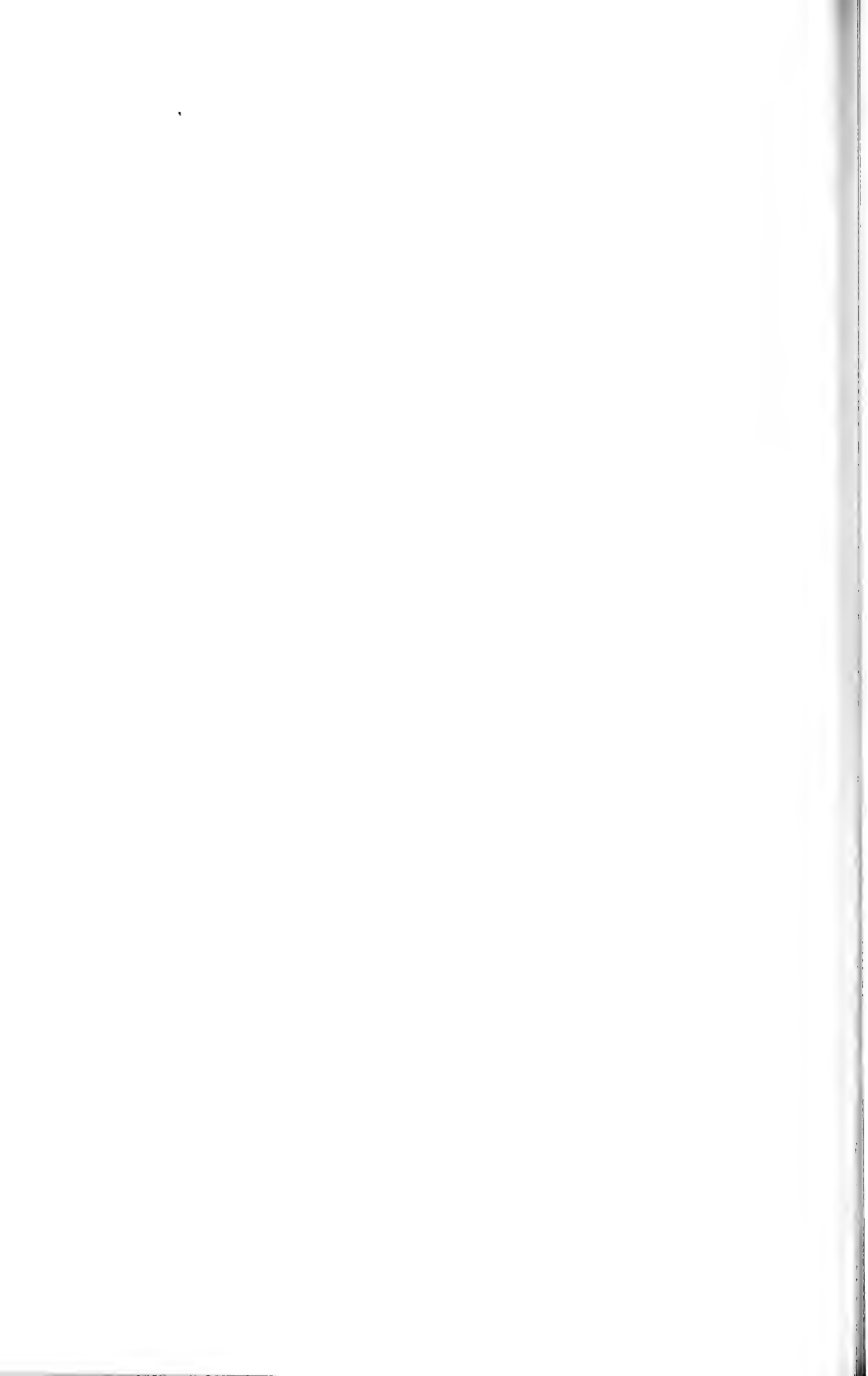
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# CONTRIBUTIONS TO PHYSIOLOGICAL TECHNIQUE.

By F. S. LOCKE.

(Received for publication 28th August 1908.)

## I. SIGNALLING MORE THAN ONE KIND OF EVENT WITH ONLY ONE WRITING-LEVER.

WHEN events of more than one kind have to be graphically recorded electrically, it is usual to employ a separate electromagnetic signal for each kind of event. The proper arrangement of more than one electric signal in relation to the other recording apparatus is often a matter of irritating difficulty, when they do not form a permanent fixture on the kymograph employed (Hering-Rothe, Brodie-Palmer), and even here the limited adjustability of the signals in height renders the application of another system of signals frequently desirable. Combinations of two or more signals on one supporting rod suitable for use on any recording surface have been designed (Carl Ludwig, Langendorff, and others), but at best the multiplication of writing-points is not a thing to be wished for, and in many cases it is of distinct advantage to be able to make one writing-point record distinguishably in addition to time-intervals at least one other kind of event. I have devised two ways whereby this end is attained, one of which demands an electromagnetic signal of special construction, while the other, although necessitating more complicated electrical connections, can be used with any signal possessing an adjustable armature-spring and iron cores not exhibiting too much hysteresis.

### A Double Electromagnetic Signal with a Single Writing-Lever.

The signal in question consists of two electrically separate electromagnets, the armature of each of which, instead of being connected with a separate recording lever, is connected with one common recording lever by means of a flexure joint which serves either as point of application to the lever of the force of attraction of its own electromagnet, or as relatively fixed axis of rotation of the lever when acted on by the force of attraction of the other electromagnet, the movement of the writing-point attached to one end of the lever being in opposite directions in the two cases. The actual mode of construction is shown in fig. 1, and is so obvious that a detailed description is unnecessary. The special points worthy of mention are:—

(1) The armature-springs, by which the armatures are connected with the solid brass frame of the apparatus, are of sheet phosphor-bronze, of

triangular outline, with the armatures fastened across their apices. The triangular shape not only produces on flexion a rapidly increasing resistance to further flexion, which prevents an armature being brought in contact with a pole, but also makes its movement very much more dead-beat, owing to interference in their tendency to vibration of the longitudinal elements of different lengths.

(2) The screw adjustment of the strength of the armature-springs and the distances of the armatures from the poles. The screws act indirectly on the armature-springs through two accessory springs of sheet phosphor-bronze, the mode of action of which is obvious in the figure. These, besides producing a very smooth and gradual adjustment, increase further the dead-beatness of the armatures in the case of downward movements.

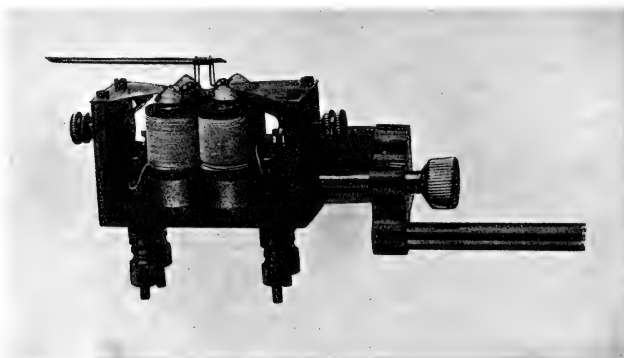


FIG. 1.—Double signal.  $\frac{2}{3}$  real size. The "legs" of the suspension of the writing-lever are a little longer than usual for the sake of greater clearness.

(3) The armatures and pole-pieces are of special construction, the latter being conical in shape, and the armatures stamped out conically to fit over them. Magnetic attraction is thus made to take place through a longer range, with a strength less rapidly increasing with approximation of the armature than with the usual arrangement.

(4) The suspension of the writing-lever between the two armatures consists of two spirals, one right- and the other left-handed, of thin German-silver, brass, or platinoid wire, which fit into one another with their coils alternating to form a tubular socket 1.2–1.4 mm. in diameter and 4 mm. long, for the writing-lever. The ends of the two spirals make four legs and feet, so to speak, a pair of feet being attached to each armature by the same screws which attach it to its spring, and form a flexure joint permitting sufficient freedom of movement without the complicated construction and other disadvantages of friction-joints.

(5) In the double-spiral socket a writing-lever about 5 cm. long of

any suitable material can be fixed. Fine straws serve admirably, but I prefer myself to use a strip of thin sheet-magnalium,<sup>1</sup> 1.5–2 mm. wide sharpened and bent to form a writing-point, and stiffened by having a line drawn down its middle by means of a blunt metal point and straight-edge. Such a magnalium lever can also be stiffened by making its transverse section curved in outline by drawing it through a short piece of glass tubing constricted at one end in the blowpipe-flame. If the fixed end be suitably tapered no special aid to fixation is necessary for ordinary work, but if desired a tiny wood or magnalium wedge can be employed.

The bilateral symmetry of the apparatus both in the transverse and longitudinal directions permits the writing-lever being fixed so as to point backwards if required on either side. This conveniently avoids the necessity of ever having the direction of the writing-lever against that of the motion of the recording surface. The necessity of the writing-lever projecting somewhat towards one side of the apparatus for application to the recording surface, is better met by a torsion to the required degree of the by no means fragile suspension by means of the fingers, than by too much flexion of the writing-lever. Any flexion it may be desired to give to it is better done by a sharp angle than a gradual curve.

(6) Adjustability of the degree of pressure of the writing-point on the recording surface is given by means of a strong flat spring suspension, the tension of which is regulated by a screw. The latter is, however, placed longitudinally instead of, as usual, transversely. Its head remains therefore always conveniently accessible whatever the position of the apparatus.

As would be anticipated from its mode of construction, the mobility of the signal is very considerable. It will respond easily to 100 interruptions per second. Beyond this I have not yet tested it. The latency of the signal to the rupture of an electric circuit is of the same order as that of the Pfeil and Deprez signals (0.5–1 $\sigma$ ), and is very constant under identical conditions.

Specimens of records by the double-signal are given in fig. 2. One electromagnet was connected up with the Brodie-Palmer clock writing seconds, and the other with the primary circuit of a Du Bois coil. The speed of the recording surface was different in each tracing. Records of make and break and of "tetanisation" are shown. The rate of interruption during the latter is well shown in the lowest tracing (52 per second).

The double signal was shown, in a form differing only in its details, at the meeting of the Physiological Society on 1st March 1902. It is made by Mr John Sinclair, Physiological Laboratory, University of London. I may add here that I have applied the same principle of the electromagnetic shifting of the

<sup>1</sup> Recording levers of magnalium were employed by Dr O. Rosenheim and myself for heart-work in 1903. The much greater rigidity of this alloy of magnesium and aluminium, with its actually less density, makes it much more suitable for the purpose than aluminium.

axis of rotation of the lever recording intervals of time to the Jaquet Time Marker, which thus becomes an electric signal as well. A description of this will be published later.

### The Polygraphic Use of a Single Simple Electromagnetic Signal.

In order to get the most polygraphically out of an ordinary electromagnetic signal, it is essential that it be used to record time-intervals of not much less than one second, by closures of the chronograph-circuit of markedly unequal length to that of the openings alternating with them. Absolute continuity of the time-record must also be not all important, a condition very rarely unfulfilled in the present perfection of recording apparatus. Given a signal writing seconds by short makes of the battery-

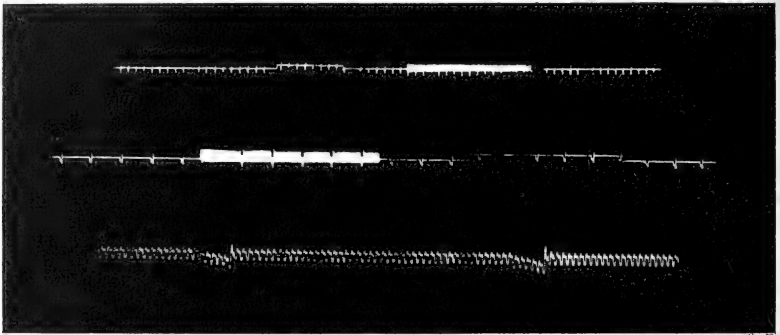


FIG. 2.—Records of time in seconds (every tenth second missed); of makes and breaks of a constant current, and of "tetanisation" by means of the interruptor of an ordinary Du Bois coil. The speed of the drum was increased between each tracing from above downwards. In the lowest tracing the interruptor was continuously in action, and two clock-contacts a second apart are shown.

current through its electromagnet (e.g. by means of the Brodie-Palmer clock), there are three distinct ways (cf. fig. 3) in which it can be made to notify the occurrence of something other than the closures of the seconds-clock: (1) The rendering of these closures ineffective by opening the circuit at some other point, an unbroken abscissa being thus produced. This will be called Signal 1. (2) The continuous closure of the chronograph-circuit by means of a key "in parallel" with the clock-contact, the abscissa being thus shifted in height, while seconds cease to be recorded. This will be called Signal 2. (3) Arranging that the clock-closures, instead of magnetising the signal, demagnetise it, this being attained by continuous closure of the chronograph-circuit as in (2), while at the same time the clock-contact is made to act as short-circuit to the signal, the seconds-record being thus inverted. This will be called Signal 3.

The sharpness of Signal 1 is obviously less than that of Signal 2 in the



case of fig. 3, the possible uncertainty of the beginning and end of the former approximating more or less to the time-interval of the clock.<sup>1</sup> Signal 1 is therefore best used for such an event as an injection. If, however, the closure of the clock-contact be longer in duration than its opening, as is the case with the Leipzig clock, the case is reversed, and Signal 1 becomes sharper than Signal 2.

All three signals can be made by a single movement of the hand, by means of a very simple switchboard consisting essentially of two spring two-contact keys fixed parallel and close to one another on the same board; so that one or the other or both together can be depressed at will.

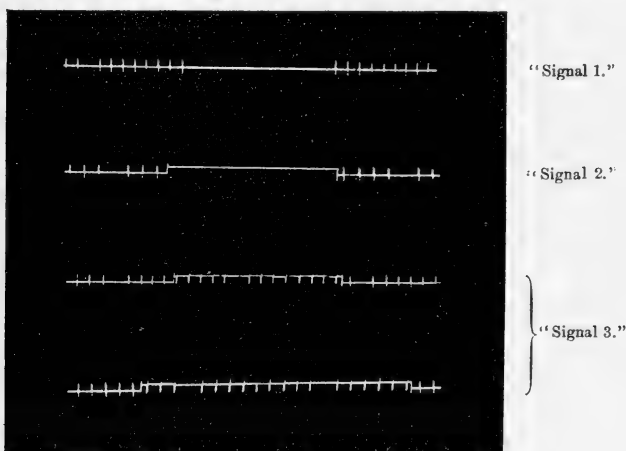


FIG. 3.—For description see text.

One of them acts as a two-way switch, the other as a make and break key. A complete scheme of connections is shown in fig. 4, A, and the connections actually functioning in each case in fig. 4, B, C, D, and E. Fig 4, B, shows the actual connections when time alone is being recorded. Depressing the left-hand key (switch) produces Signal 1 by creating the connections shown in fig. 4, C. Depressing the right-hand key (key) produces Signal 2 by the connection shown in fig. 4, D<sup>2</sup> De-

<sup>1</sup> The Brodie-Palmer clock is usually made to drop every tenth second, as in the tracings accompanying this paper. The lack of sharpness of Signal 1 may therefore rise with it to two seconds, but this is of course not necessary when the time of occurrence of the event to be signalled can be deliberately chosen.

<sup>2</sup> If Signal 2 be rapidly repeated by a succession of short taps on the right-hand key, an additional easily distinguishable signal is produced more or less resembling on a slowly moving drum the upper records of "tetanisations" in fig. 2, p. 362. This variation can, of course, be also made use of with any ordinary signal not recording time with simply a make and break key in its circuit.

pressing both keys together produces Signal 3 by the connections shown in fig. 4, E.

As is indicated in fig. 4, A, by dotted lines, the right-hand key

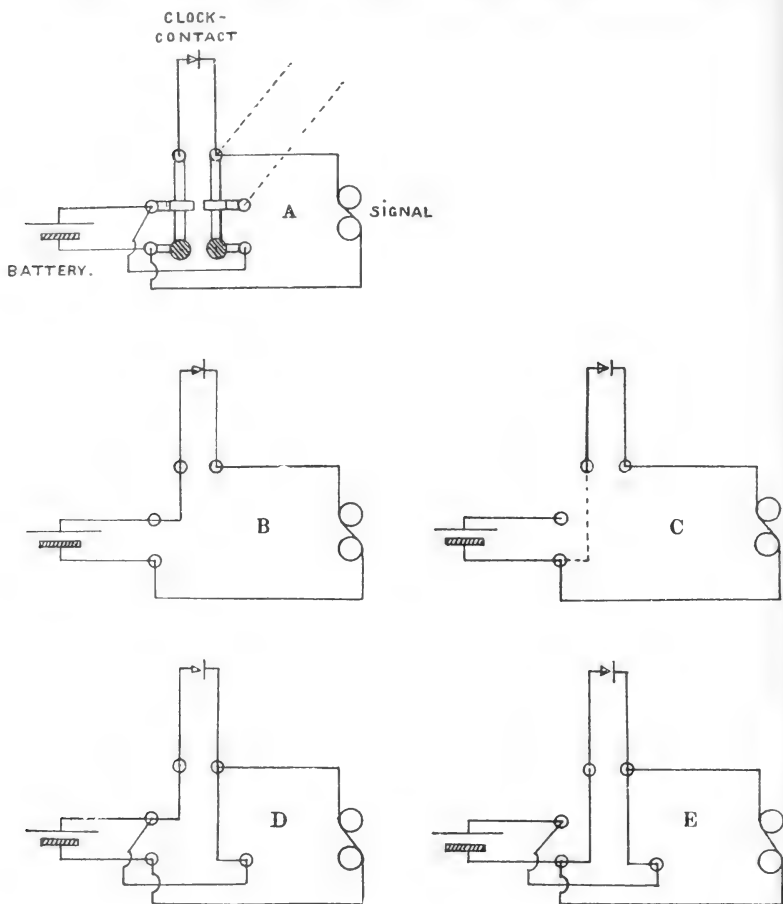


FIG. 4.—For description see text.

thanks to its spare-contact, can also if required serve as short-circuiting key to the stimulating electrodes in the secondary circuit of an inductorium, in order to signal automatically the duration of a tetani-

sation.<sup>1</sup> Of course it is impossible to give Signals 1, 2, and 3 simultaneously, but if it be necessary to signal the simultaneous occurrence of two events, this can be indicated by Signal 3, the separate events being indicated by Signals 1 and 2.

The connections of fig. 4, A, are not the only ones possible to attain the end in view, and in fact are not applicable to the Brodie-Palmer clock on account of the permanent connection in that apparatus of one battery-pole with one side of the clock-contact. Altogether there are four modes of arranging the connections possible according to whether the continuous closure of the circuit independently of the clock in Signal 2 is effected (a) by the "switch" or (b) by the "key"; and in each of these cases whether the clock-contact is put in permanent connection (*ac*, *bc*) with the battery or (*ad*, *bd*) with the signal. These four different possible arrangements will now be referred to as *ac*, *bc*, *ad*, and *bd* respectively. It is unnecessary to describe them in detail systematically; they may be easily worked out by those interested.

The arrangement of connections in fig. 4, A, corresponds with *ad*. This is theoretically the most preferable of the four, because short-circuiting of the battery is only made use of in Signal 3. With the arrangement *bd* short-circuiting is continuous during Signal 1. It is therefore best avoided. With the arrangement *ac* short-circuiting of the battery tends to be, and with the arrangement *bc* is certainly, produced during Signal 1, but only during the clock-contact closures, and is therefore practically negligible. Both of these arrangements can therefore be used with the Brodie-Palmer clock, and the way of connecting it up in the arrangement *ac* is shown specially in fig. 5.

In order to ensure that the short-circuiting of the electromagnet of the chronograph effectively demagnetises it for the production of Signal 3, sufficient strength must be given to the armature-spring, and actual contact of the armature with the poles must in some cases be prevented, best by a screw adjustment, but extemporaneously by paper or other material intervening. An absence of inertial overthrow of the writing-lever is also desirable for Signal 3, which is not given in fig. 3 as well as it can be got with a more suitable form of signal than that actually used.

If it be only desired to give Signal 3—the advantage of which is, of course, that there is no temporary loss of time-record—there can be

Any risk of unipolar excitation by the extra-current of the chronographic electromagnet may be avoided by connecting up in parallel with the clock-contact an electrolytic "spark-trap" consisting of aluminium electrodes in a solution of sodium sulphate (cf. Ostwald-Luther, *Physiko-chemische Messungen*, 1902, p. 397). This should, in fact, be in permanent use with the clock if only to prolong the life of the contact.

used, instead of the switch-board above described, the double switch frequently used as commutator on small spark-coils, consisting of two parallel bars playing over three contacts,<sup>1</sup> or a Pohl's rocking switch with only one cross-wire. In the case of both of these also the spare contact can be used for the short-circuiting of stimulating electrodes in a secondary circuit.

After devising the methods just described for connecting at will two current-paths in series or parallel (in the special case in question clock-contact and signal), I found that Ewald<sup>2</sup> has described and figured the special arrangement *bc* for Pohl's rocker with one cross-wire with a view to quite other practical applications than the one made here. I find too that Kronecker,<sup>3</sup> in his report on chronographic methods, mentions the possibility of using temporary omission of the time-record as a signal

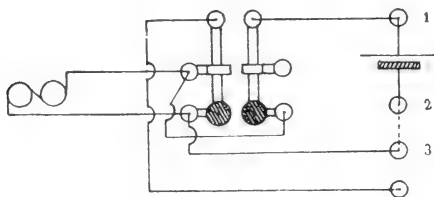


FIG. 5.—The terminals numbered 1, 2, 3, and 4 are those of the Brodie-Palmer Clock from right to left. (Cf. the figure showing the clock-connections in Proc. of the Physiol. Soc., Dec. 8, 1900, Journal of Physiology, xxvi., p. xii.)

("Signal 1"). He also states that Judin<sup>4</sup> used as a signal the diminution of the amplitude of the excursions of the chronograph produced by the passage of a weak constant current through its electromagnet. I believe, although I cannot now recover the reference, that Kronecker has stated in a much earlier publication that Dew-Smith long ago employed on a continuous paper-kymograph a chronograph with polarised armature, so that currents in reverse directions produced reversed excursions.

The methods of signalling described in this section were shown to the Physiological Society on 1st March 1902.

<sup>1</sup> In this case the usual permanent connection of the outer two of the three contacts must be broken. Since the above was written, this form of switch has been applied to general electrophysiological work by H. G. Roaf and W. G. Smith (Proc. of the Physiol. Soc., Nov. 11, 1905; Journal of Physiology, xxxiii., p. xiv.), who have made the connection in question breakable at will.

<sup>2</sup> J. R. Ewald, Arch. f. d. ges. Physiol., xlii., p. 478, 1888.

<sup>3</sup> H. Kronecker, Arch. Ital. de Biol., xxxvi., p. 135, 1901.

<sup>4</sup> A. Judin, La Physiologiste Russe, v., p. 67, 1898, cited after Kronecker.

## II. A NEW FORM OF BELLOWS-RECORDER.

The bellows-recorder introduced by Brodie,<sup>1</sup> while capable of yielding the most admirable results, has in ordinary practice disadvantages well known to those who have worked with it, in particular the difficulty of making it air-tight in the first place and maintaining it so afterwards without stiffening its joints. The volume-recorder here described is of

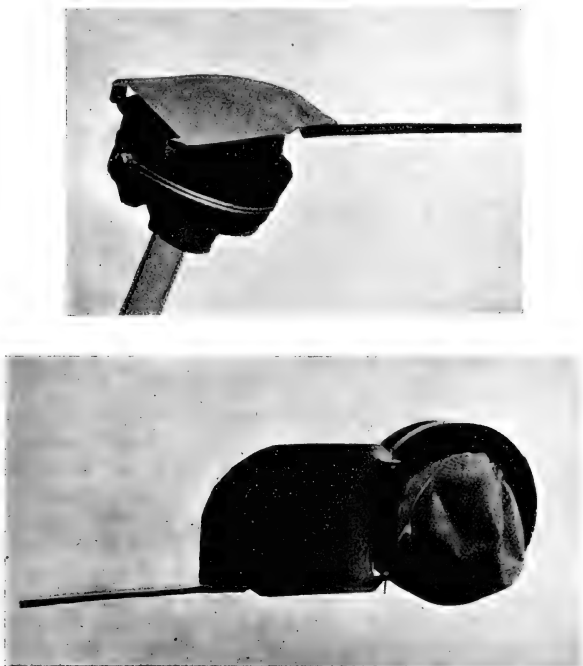


FIG. 6.—Two views of the recorder. In the lower one it is seen from above with the cover and recording-lever thrown back. In the upper one the loose open end of the condom has not been cut close off.

very easy construction in a range of sizes suitable for a number of purposes, and does not tend to leak. Its air-chamber is readily renewed when required, consisting merely of a few centimetres of the closed end of a readily procurable tube of thin rubber (condom).<sup>2</sup>

The apparatus is shown in fig. 6.

<sup>1</sup> T. G. Brodie, *Journal of Physiology*, xxvii., p. 473, 1902.

<sup>2</sup> Renewal is, however, not often necessary. Made with condoms of good red rubber, I have had recorders in good condition for as long as two years.

A glass tube 7-8 cm. long, and of large enough bore (up to 1 cm.) is fixed in a rubber stopper of a diameter of 4-6 cm. flush with its broader upper end. It is convenient to divide an ordinary rubber stopper of suitable size transversely on the lathe, thus getting two of suitable diameter and thickness (15-20 mm.). Over the broader upper end a condom of good quality is drawn till only 2.5-5 cm. of the closed end remain clear above it. The condom should be of such diameter that it requires to be somewhat stretched to pass over the stopper, so that no creases are formed on the stopper while for a few millimetres it remains in light contact with the outer part of the upper surface of the latter. This constitutes the air-chamber, and it remains to make it air-tight, and furnish it with an index capable of recording its volume.

A short length (about 2.5 cm.) of wide (3-4 cm. diam.), rather thin-walled rubber tubing,<sup>1</sup> is stretched over three fingers of each hand and passed over the end of the condom and on to the rubber stopper, this being firmly held by means of the glass tube by an assistant. A little of the wide tubing is left above the edge of the stopper, and by virtue of its elasticity



FIG. 7.—*a* indicates how the rubber stopper is cut.

and less diameter than that of the stopper lies flat upon its upper surface, forming an orifice through which the end of the condom suddenly pouches out. By varying the diameter of the wide rubber-tubing selected orifices of varying diameters are produced, and a corresponding variation in the capacity of the air-chamber obtained. The rubber-tubing around the stopper forms an elastic collar fixing the condom upon it. To render the joint absolutely air-tight, however, it is necessary to add a couple of turns of well-tied string.

Upon the air-chamber rests a hinged cover consisting of a plate of thin sheet magnalium prolonged into a writing-lever stiffened by a longitudinal indentation. The axis of rotation of the cover is obtained in the following way:—

Before the condom is applied over the rubber stopper, a transverse notch about 2 cm. long and 3 mm. deep is made in the latter at the edge of its upper surface by cutting out a slip of rubber in the way shown in profile in fig. 7. In the seat thus formed, but outside the condom and under the rubber collar, a short piece of small glass-tubing, somewhat longer than the

<sup>1</sup> I use for this purpose old bicycle tyre inner tubes. The short bits of tube should be turned inside out before being fitted, to get the seam outside.

seat, is made to lie. The ends of this have been previously fused in the flame, so that they only form apertures nicely admitting a sewing-needle of No. 9 size. Such a needle, preferably one longer than ordinary and known technically as a "Straw," is now thrust through the rubber collar on one side, through the length of the glass tube and out through the rubber collar on the other side, taking care not to injure the condom, the projection outwards of the rubber collar by the ends of the glass tube making this easy. Its sharp end is now cut off so that only a few millimetres project on either side.

The magnalium-plate, shaped as shown in fig. 6, the hinges and writing-lever attached to it being formed by cutting and bending, is now fitted to the needle-axis, two beads on each side, or a bead and glass spangles as in fig. 6, minimising side-lash and friction.

If preferred the plate can now be fixed to the summit of the condom by a touch of rubber solution. I have not myself found this to be any advantage. The merely resting plate immediately forms the condom into the folds natural to it, which if not disturbed remain permanently the same. If the condom has been fitted carefully and symmetrically, preferably with its summit projecting a little forward and its seam, if it have one, in the middle line, the folds assumed by it are surprisingly regular and symmetrical.

For actual use the recorder is best fixed by means of its glass tube in a clamp in the somewhat oblique position shown in fig. 6.

Many variations in the details of construction can of course be made. Instead of rubber, cork or suberit shives can be used to build the recorders on. But both of these substances are so leaky that they must be completely and fairly thickly covered externally with Prout's glue or some such substance, the edges of the condom being embedded in this. The cover and its hinge can be made in different ways of different materials. Cardboard can be used instead of metal, hinged by a strip of soft silk to another piece of cardboard slipped under the rubber collar, the rubber stopper being cut flat on one side to receive this. The needle-axis, if used, can be simply made by thrusting the needle through the thickened seam of the rubber collar when this is made of "inner tube" turned inside out, but this method is by no means so lasting as the one described above.

It might be thought that the use of a thin distensible rubber air-chamber was not permissible in a volume-recorder, but if care be taken that the cover moves easily on its axis and that there be no excessive friction of the writing-point, no rise of pressure occurs in the air-chamber sufficient to introduce error in ordinary volumetric work. The absence of any real elasticity of form in the limp air-chamber wall prevents, of course, any suction applied to the air-chamber acting directly upon the recording

lever, which has to follow negative changes of volume merely by virtue of its own weight and that of the air-chamber cover to which it is attached. The recorder is therefore, unlike Brodie's bellows, incapable of following very rapid changes in volume such as those producible by a tuning-fork acting on a tambour. Its rapidity of action is, however, sufficient for all ordinary physiological experimental work. It gives, for instance, excellent results as an oncograph.

The calibration curve of the recorder resembles that of Brodie's. Starting from zero, i.e. with the air-chamber emptied by the weight of the cover and writing-lever, successive uniform increases of volume (of 0.5 c.c. or 1 c.c.) produce successively greater movements of the lever for the first 10°-15° of its upward rotation, after which equal increases of volume produce sensibly equal movements of the writing-lever till distension is approached. For most purposes it may be assumed that only the middle portion of its range would be employed, in which no special reduction of the excursions obtained to correct relative values is necessary.

The volume-recorder here described was shown to the Physiological Society on 20th January 1906.

### III. A PERFUSION STOPCOCK.

A stopcock was described by me four years ago,<sup>1</sup> which among other possibilities permitted practically as rapid a change from one perfusion-fluid to another as is possible with an ordinary three-way tap, whilst giving at will a bye-pass to the fluid not being perfused, so that any ill-effect when turned on of its previous stagnation may be avoided. To accomplish this two separate three-way "keys" in one barrel were employed. The tap here described effects it with one key only, which is a considerable simplification.

The construction, as may be seen from fig. 8, is based on the well-known Greiner and Friedrichs three-way tap, both the barrel and key of which are modified.

To the middle of the barrel at right angles to the other three tubes is attached a fourth tube D, to act as a bye-pass to either A or B, conveying the two perfusion-fluids employed. The tube C, connected with the perfused organ, is functionally prolonged upwards and forwards for a quarter of the circumference of the barrel by a channel blown out from it and bulging out its front.

Two transverse channels, each occupying a little more than half the circumference of the key, are ground out of its surface in the planes of A and B on opposite sides of it, so that the outer ends of the two parallel oblique key-borings open at the middle points of these channels, which form functional continuations of them.

<sup>1</sup> F. S. Locke, Proc. of the Physiol. Soc., March 19, 1904; Journal of Physiology, xxxi., p. xii.



When, therefore, the key is in the position shown in fig. 8, A is shut off, while B is connected to C. If the key be now turned clockwise through  $90^\circ$ , B will still be connected with C by virtue of half of the one transverse channel, and the prolongation of C upwards and forwards, but A will be put in connection with the bye-pass D, the hitherto unemployed perfusion-fluid now running to waste. Another turn clockwise through  $90^\circ$  shuts off B from C, but connects A with it instead of with the bye-pass, a change from one perfusion-fluid to the other being now effected. Another turn clock-

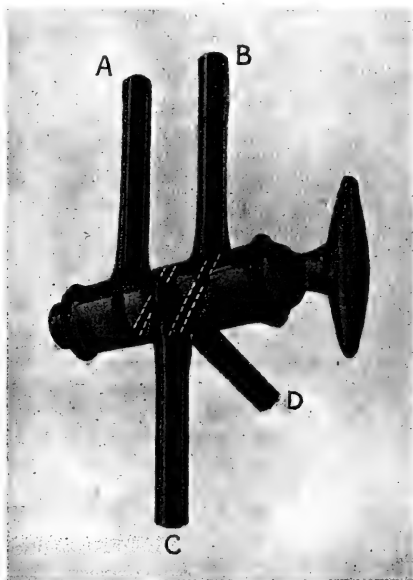


FIG. 8.—For description see text. The Greiner and Friedrichs oblique key-borings are indicated by light dotted lines. The special additional passages are shown dark. The stopcock was filled with dark fluid before being photographed.

wise through  $90^\circ$  leaves A still in connection with C, but connects B with D, the perfusion-fluid first employed now running to waste. Another turn clockwise through  $90^\circ$  restores the state of affairs shown in fig. 8, changing the perfusion-fluid to the one in use at the start.

Midway between the second and third, and also between the third and fourth (first), of the positions of the key just described, A and B are completely shut off from C and D, all perfusion being cut off, with no perfusion-fluid running to waste.

Spots of different coloured enamel are fused on the ends of the key-

handle, and corresponding ones on the tubes A and B near the barrel, so that the spot on the end of the key handle pointing downwards in the direction of C is of the colour of the spot on the tube A, or, as the case may be, B, at the time in connection with it. Confusion as to the effect of movements of the key on the perfusion is thus avoided.

The form of stopcock described was shown at the meeting of the Physiological Society on 20th January 1906. It is made by Greiner and Friedrichs, and is supplied by Messrs Baird & Tatlock, London. This also now applies to my earlier form of perfusion-stopcock (*loc. cit.*), which is still of special use for certain purposes.

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The apparatus and methods described in this paper have been mainly worked out in Dr Halliburton's laboratory, King's College, London. I have, however, to acknowledge my indebtedness to Dr Brodie and Dr Waller for the facilities put at my disposal in their laboratories also. Grants in aid of the expenses incurred have been received from the Government Grant Committee of the Royal Society.

THE FORM AND MAGNITUDE OF THE ELECTRICAL RESPONSE OF THE EYE TO STIMULATION BY LIGHT AT VARIOUS INTENSITIES. By W. EINTHOVEN and W. A. JOLLY.<sup>1</sup> (From the Physiological Laboratory of the University of Leyden.) (With twenty-five figures in the text.)

(Received for publication 29th September 1908.)

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I. HISTORICAL INTRODUCTION.

THE following paper gives an account of our study of the electrical response of the frog's eye to light by means of a sensitive and rapidly moving galvanometer.

Before discussing our own results, it is desirable shortly to pass in review the principal steps by which our knowledge of the retinal currents in the frog has been built up upon the foundation laid by Holmgren.

Holmgren<sup>2</sup> found that when light is allowed to fall upon the eye of

<sup>1</sup> The research was performed during the tenure by one of us (W. A. J.) of a Carnegie Research Fellowship of the University of Edinburgh.

<sup>2</sup> "Method at objektivera effekten af ljusintyck po retina," Upsala Lakareforenings Förhandlingar, vol. i. p. 177, 1866. *Physiol. Untersuch.*, Heidelberg, Bd. ii. p. 81, and Bd. iii. p. 308.

a frog that has been kept in the dark and again when the light is removed, there is an increase, in the positive direction, of the current present during darkness. A similar result is obtained when, during continued illumination, the light varies rapidly in intensity. The strength of the current is, within certain limits, proportional to the intensity of the light. The onset and removal of light is attended with the same electrical changes when the posterior half of the eyeball alone is employed.

In the case of the viper, rabbit, dog, and cat, a negative variation accompanies lighting, followed on continued illumination by a slow positive variation, and darkening produces a positive variation.

Dewar and M'Kendrick,<sup>1</sup> who rediscovered the electrical changes caused in the retina by light, found that "when diffuse light is allowed to impinge on the eye of the frog, after it has arrived at a tolerably stable condition, the natural electromotive power is in the first place increased, then diminished; during the continuance of light it is still slowly diminished to a point where it remains tolerably constant; and on the withdrawal of light there is a sudden increase of the electromotive power nearly up to its original position."

The effect of moonlight upon the eye was found to be about equal to that of a candle distant a few feet. The eye is more sensitive to variations in light of weak intensity than to variations in light of great intensity.

Certain of the colours of the spectrum were arranged by Dewar and M'Kendrick, with reference to their power of altering the electromotive force, in the following order—yellow, green, red, blue.

It was found that the anterior segment of the eyeball, including cornea, iris, and lens, yielded a current which was not affected by light.

The interesting fact was discovered that in the crustacean eye the retinal currents are reversed in direction, in accordance with the inverted arrangement of the sensory epithelium. In investigating the eye of the cat, the onset of light was found to be attended by a diminution of the electromotive force; during illumination the electromotive force gradually rose to a point where it became steady, and on darkening a rise was well marked.

Kühne and Steiner<sup>2</sup> occupied themselves chiefly with the investigation of the reactions of the isolated retina. They found that the electrical change on lighting and darkening is a complex one, the variation being first positive, then negative, and finally again positive. The reaction is divisible, according to these observers, into two parts: the first, due to the onset and continuance of illumination, consists of a negative variation preceded by a positive; and the second, caused by disappearance of the light, consists of a simple positive variation. Kühne and Steiner discuss the question whether the former of these parts can be divided further into a positive variation caused by the onset and a negative due to continuance of illumina-

<sup>1</sup> Trans. of the Roy. Soc. Edin., vol. xxvii. p. 141, 1872-73.

<sup>2</sup> Physiol. Untersuch., Heidelberg, Bd. iii., S. 327, 1880.

tion, but regard such a division as indefensible. In a later research<sup>1</sup> it was observed that, in some cases, instead of a negative variation following the positive, the latter merely suffered diminution. This, since it occurred in the freshest preparations, was regarded as the normal reaction of the retina.

No current was obtained from the posterior half of the eyeball from which the retina had been removed, the pigment layer being left behind. The observers concluded from this that the pigment epithelium possesses no electromotive power, and no ability to give rise to electrical changes on stimulation by light.

The latent period of the lighting effect was investigated by Fuchs<sup>2</sup> by means of the rheotome. He obtained much smaller figures than have been observed by other workers. The method employed by Fuchs, however, is not applicable to the changes of long duration which constitute the reaction of a dark-adapted eye.

Waller<sup>3</sup> studied the phenomena presented by the frog's eyeball with the help of photographic records of the galvanometer variations. He found that the effects obtained in the posterior half of the eyeball or in the isolated retina were precisely those manifested by the injured eyeball, and therefore regarded the reaction of the intact eyeball as typical. Waller found that the deflection of the galvanometer with illumination is positive at the commencement and at the end, and is also positive during the continuance of the illumination. He regarded the final deflection as a subordinate feature of the main change, and described the reaction simply as a positive (upward) current during illumination and no such current during darkness.

Some of the curves figured by Waller indicate that the positive deflection evoked by lighting is not simple, but of a double nature. In his figure 15 it is evident that the curve, after a steep ascent, is interrupted in a step-like manner, after which it progresses by a more gradual rise and fall during the course of the illumination, and upon this slow deflection the positive effect due to darkening is superposed. The interruption of the rise is noted by Waller and referred to a negative restraint setting in at that point.

The period of latency between onset of light and positive deflection were found by this observer to be very long amounting in some cases to several seconds. This delay he explains as due to a period of hesitation during which two opposed currents are developed from the retina at nearly equal rates, and finds that a short negative swing frequently precedes the main positive effect.

De Haas,<sup>4</sup> working in this laboratory, investigated in detail the strength

<sup>1</sup> *Physiol. Untersuch.*, Bd. iv., S. 64, 1881.

<sup>2</sup> *Pflüger's Arch.*, Bd. 56, S. 408, 1894. *Ibid.*, Bd. 84, S. 425, 1901.

<sup>3</sup> *Phil. Trans.* vol. 193, B, p. 123, 1900.

<sup>4</sup> *Lichtprikkels en retinastroomen in hun quantitatief verband*, Dissertation, Leiden, 1903; and *Onderzoekingen Physiol. Lab.*, Leiden, 2nd ser., vol. 6.

of the electrical response to light stimuli differing in intensity and duration, and the relation which the response to light at different intensities bears to Fechner's law. He employed a slow-moving Deprez-d'Arsonval galvanometer for his research. This instrument, although fairly sensitive, is not well adapted for following the rapid alternations of current present in the response of the eye. A more suitable instrument for that purpose is found in the capillary electrometer which was used by Gotch.<sup>1</sup> The electrical reaction of the eye to light was divided by this observer into three portions: (1) the rise due to the sudden illumination, termed by him the "on-effect"; (2) the continuous change occurring during the continuance of illumination; and (3) a second rise due to the sudden change from light to darkness, termed by him the "off-effect." In addition to these changes, careful examination of the records with a lens showed the presence in some cases of a small negative deflection of short duration immediately preceding the on-effect. The curve deduced from the electrometer records shows that the on-effect having reached its maximum, subsides; this subsidence is checked, and a continuous effect is present during the illumination. The continuous effect is not necessarily steady, but in some cases gradually increases until it exceeds the value of the on-effect, and the off-effect is superposed upon it. The off-effect depends for its production upon previous illumination. When the illumination is of short duration—half a second—it does not appear; it is just visible with slightly longer illumination, and as the period increases the off-effect becomes more pronounced. The latent period of the on-effect varies with the temperature and with the nature of the light. The delay was found to be shortest in the case of white light, longest in the case of red light, and intermediate in duration with blue-violet light. It was not, however, possible under the conditions of the research—the light being passed through filters of coloured fluid—to determine the absolute intensities of the rays of different wave-length used as stimuli. The latent period of the off-effect is shorter than that of the on-effect, nor does it vary with the nature of the illumination as does the latter.

Having regard to the facts that the on- and off-effects are both positive, and that they differ in time relations, Gotch concluded that they cannot be regarded as merely two different aspects of one chemical change, but that there must be two distinct substances, one reacting to light, the other to darkness.

The results obtained by Piper<sup>2</sup> from the eye of the frog agree generally with those of Gotch. The latent periods at onset of light, according to this observer, range from 0.133 sec. to 0.164 sec. The lighting effect is a sudden increase of the electromotive force, which endures for 0.4 sec. to 0.5 sec. and does not exceed 1 millivolt; upon this follows typically a slight diminution of the light effect. During illumination the curve remains

<sup>1</sup> Jour. of Physiol., vol. xxix. p. 388, 1903, and vol. xxxi. p. 1, 1904.

<sup>2</sup> Arch. f. Anat. u. Physiol., Suppl.-Bd., S. 133, 1905.

almost completely constant. The reaction on darkening has a latent period of about 1287 sec. and consists of a renewed increase of electromotive force more gradual than the lighting effect and less in amount. Immediately thereafter the electromotive force sinks at first rapidly, then slowly, to the original amount. Piper did not find in the case of the frog's eye any gradual continual increase after the first increase and diminution. In the course of his study of the vertebrate eye, however, he found such a variation. The reaction of the eye of a cat, which had not been treated with atropin, after a first deflection presenting the typical appearance of the lighting effect, showed a second slow positive deflection. This was not observed in eyes treated with atropin, and Piper attributes its presence to electromotive changes accompanying contraction of the iris muscles. The second deflection was considerably greater in amount than the initial positive deflection which followed the onset of light.

We have seen that a positive deflection during the continuance of illumination has been figured by several observers as an occasional feature of the reaction of the frog's eye, but it is to Ishihara<sup>1</sup> that we owe the recognition of this deflection as a typical constituent of the curve, independent of the primary positive deflection on lighting. This worker followed out the suggestion yielded by Waller's curves, and recognised clearly the double nature of the positive deflection. According to his description the positive deflection on lighting is at first rapid. It then continues more slowly until it reaches a maximum, after which it gradually diminishes. A close investigation shows that the ascending limb of the curve contains a step or notch which occurs about the same time after lighting as the maximum of the off-effect after the moment of darkening. Ishihara concludes that at lighting there is a rapid positive deflection similar to that which occurs at darkening, although the former, owing to the slow movement of the galvanometer used by him, was less clearly visible than the latter, tending to be fused with the succeeding slower deflection which he names the "Helligkeitsschwankung." On one of the curves figured by this observer may be seen the positive deflection on lighting, which, after a slight diminution, is followed by a slow rise above the value of the first, upon which the darkening reaction is superposed.

Brücke and Garten<sup>2</sup> have recently made valuable contributions to our knowledge of the retinal currents by the employment of the capillary electrometer and string galvanometer. By the aid of the latter instrument they have demonstrated clearly the preliminary negative variation visible to Gotch on examining his curves by a magnifying glass. This deflection occurs in the majority of cases, and its latency is 0.078 sec. to 0.099 sec. Its value is greatest in the freshest eyes. The continuous effect during illumination of the isolated eyeball consists of a slow rise independent of, and two or three times greater than, the first positive variation. Its return

<sup>1</sup> Pflüger's Arch., Bd. 114, S. 569, 1906.

<sup>2</sup> Ibid., Bd. 120, S. 290, 1907.

to the original level during lighting lasts two or three times as long as the ascent. The positive darkening reaction does not alter the course of the continuous effect. When the eye is submitted to short repeated illuminations the successive on-effects are superposed upon the continuous effect. The state of adaptation of the eye has an important influence upon the continuous effect. When the eye is light-adapted no second rise is exhibited on the curve, but after the on-effect the current sinks gradually during illumination without, however, reaching the zero line. During a series of experiments on the dark-adapted eye the continuous effect is evoked more strongly at first and becomes progressively weaker as illumination is repeated. The continuous effect is exhibited not only by the whole eye but also by the posterior half. This is inconsistent with Piper's view that it is derived from the iris muscles.

The continuous effect differs from that just described when the experiments are performed not upon the isolated eye but upon the eye, remaining in situ, of a curarised frog. Here the continuous effect does not exhibit subsidence during illumination but remains constant at a high level, the illumination being continued for half an hour.

The latent period of the on-effect is on an average 0.2 sec. The very short latent periods obtained by Fuchs cannot be attributed to the short duration of the light stimuli used by him, as similar short stimuli were made use of by Brücke and Garten and yielded latencies of 0.108 sec. to 0.111 sec. Fatigue may be observed in the on-effect which diminishes in strength after repeated stimulation of the eye. The on-effect differs from the continuous effect in that it is but little affected by the state of adaptation of the eye. The off-effect is found, as a rule, with dark-adapted eyes, especially after long illumination, to be stronger than the on-effect, while in light-adapted eyes the latter is usually as strong as or occasionally stronger than the off-effect. The off-effect is, as a rule, somewhat steeper than the on-effect.

Brücke and Garten have extended their researches over a large number of vertebrates and find a marked similarity of reaction in the different classes when the eyes are quite fresh and investigated under the most favourable conditions. From their results it appears probable that the electromotive changes caused in the eye by the stimulation of light are essentially of the same nature throughout the vertebrate series, and when allowance is made for the reversed direction of the currents, also in crustaceans.

Although the electrical response of the eye to stimulation by light has been studied by numerous observers, there has not, so far, been undertaken a systematic investigation of the electromotive changes which are caused by stimuli of very varying strength. Such an investigation, however, can, as we hope to show, contribute to our comprehension of the retinal processes.



## II. METHOD OF INVESTIGATION.

### 1. General Remarks.

We have in our work employed exclusively isolated frogs' eyes. We have been enabled on the one hand, by means of the string galvanometer<sup>1</sup> which, for the retinal currents, may be regarded as the most sensitive instrument available, to record and measure very weak electromotive forces, such as are evoked by light of extremely low intensity; on the other hand, we have tried by a suitable system of lenses to concentrate light of as great intensity as possible upon the retina of the eye under observation.

### 2. The Arc Light.

The weakening of any light may be continued indefinitely. On the other hand, the increase of the intensity of light radiating upon a given area is restricted within limits, theoretically as well as practically.

We have chosen as the source of illumination the crater of the arc light, which of all terrestrial sources of light possesses the greatest intrinsic intensity. In making this choice it has been necessary to consider, in the first place, whether the radiation from the crater is sufficiently constant for use in a series of observations an essential part of which consists in the use of measurable quantities of light.

With regard to this we may observe that we have never employed the entire crater. If we desired to avail ourselves of the maximum of white light we made use of a relatively large part of the crater, while in all other cases only a small area of its central depression was employed. Thus in all our experiments the question as to the constancy of the crater light reduces itself to the question of the constancy of the light which is radiated per square millimetre from the crater.

We may assume that this constancy is sufficiently guaranteed. Thus Violle<sup>2</sup> demonstrated that through variations in current intensity of 10 to 400 amp., and of energy from 500 to 34,000 watt, the brightness of the crater does not change. It has even been suggested from several sides to take the light derived from 1 mm.<sup>2</sup> of the crater as a light unit and thus raise the crater to the position of a light standard. In all probability the temperature of the crater is that of vaporising carbon.

On the other hand, some difficulties arise; thus, for example, Petavel<sup>3</sup> says: "Even when the most favourable conditions are selected and the intensity of current and the length of the arc are maintained constant,

<sup>1</sup> Cf. "Ein neues Galvanometer," *Annalen der Physik*, 4 Folge, Bd. 12, S. 1059, 1903. "Ueber einige Anwendungen des Saitengalvanometers," *ibid.*, Bd. 14, S. 182, 1904. "Weitere Mitteilungen über das Saitengalvanometer," *ibid.*, Bd. 21, S. 483, 1906.

<sup>2</sup> Cf. Waidner and Burgess, *Bulletin of the Bureau of Standards*, Washington, vol. i. p. 109, 1904.

<sup>3</sup> *Proc. Roy. Soc. London*, vol. lxx. p. 469, 1900.

it is difficult to obtain consistent results, variations of over 5 per cent. being by no means unfrequent." Petavel made use of a lamp regulated by hand with carbons placed at right angles with one another. To what extent the inconstancy of his results is to be attributed to this circumstance we do not venture to judge.

We have ourselves made use of a differential lamp of Siemens and Schuckert of 20 ampères, which burns quietly when fed either by a battery of accumulators at 65 volts or by a dynamo at 110 volts with appropriate resistances. At the same time the distance between positive and negative carbon is great enough to permit of our utilising the radiation from the centre of the crater depression. The lamp has occasionally during the course of our work shown some irregularity, but this does not often happen, and when it occurs the observation is repeated. The

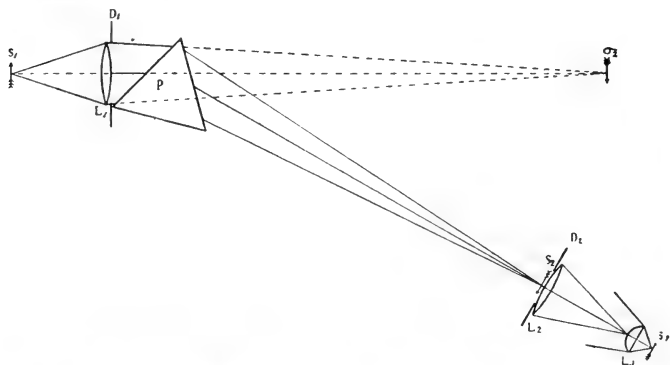


FIG. 1.—Spectroscopic arrangement and system of lenses designed to transfer a maximum of spectroscopically isolated rays from the crater of the arc lamp to the pupil of the frog's eye.

photographic records made of the movements of the string of the galvanometer, where in the same way only the centre of the crater depression was used, give us no reason to assume that variations of 5 per cent. in the intrinsic brilliancy of the crater light occur with a steady lamp.

### 3. System of Lenses.

As we chiefly desired to make use of light between definite wave-lengths, we employed a spectroscopic arrangement. In constructing this we had to solve the question as to how a maximum of spectroscopically isolated rays might be obtained from the crater and transferred to the pupil of the eye to be investigated.

The solution was found to be as follows:—From the slit  $S_1$  (fig. 1) a spectrum is formed by the aid of lens  $L_1$  and prism  $P$  in  $S_2$ . From this spectrum there is cut off by the octangular diaphragm  $D_2$  a part lying between definite wave-lengths.

Close to  $D_2$  there is placed a second lens  $L_2$  which forms a sharp image of  $L_1$  upon a third lens  $L_3$ . By this last lens there is formed a diminished image of the spectrum which passes within the pupil of the eye and which is denoted in the figure by  $S_3$ .

The light rays which pass out from  $S_1$  and are refracted by the lenses and prism arrive weakened in  $S_3$ . Through reflection from the refracting planes and absorption in the refracting media, a part of the light is lost. But if we neglect this loss, then we determine as the basis of our construction that all the light rays of a definite wave-length which are collected by the lens  $L_1$  from the slit must again be united in  $S_3$ .

Changes in the breadth of the slit  $S_1$  and in the horizontal dimension of the rectangular diaphragm  $D_2$  bring about some variations in the nature of the light in use. If it is desired without changing the quality to vary the intensity of the radiation, then one must especially take into account the height of the slit and diaphragm. We name the height of the slit and of the two spectra respectively  $s_1$ ,  $s_2$  and  $s_3$ . The angle of aperture of the cone of light falling upon lens  $L_1$  may be named  $\omega_1$ , that of the cone of light passing from this lens  $\omega_1$ . The analogous angles of aperture for the lens  $L_3$  may be named  $\omega_3$  and  $\omega_3$ .

In the space between the lenses, the prism, and the slit there is air with the refractive index 1. On the other hand, we leave the possibility open of filling the space between  $L_3$  and the eye with any medium, whose refractive index may be termed  $n$ .

According to known laws we may write:—

$$\frac{s_3}{s_1} = \frac{\sin \frac{1}{2}\omega_1}{n \sin \frac{1}{2}\omega_3} \quad (1)$$

If all the rays of light which are refracted by the lens  $L_1$  pass through  $L_3$ , the numerical aperture of  $L_3$  must be equal to or larger than  $n \sin \frac{1}{2}\omega_3$ .

We therefore write

$$n \sin \frac{1}{2}\omega_3 \geq N_3 \quad (2)$$

where  $N_3$  is the numerical aperture of the lens  $L_3$ .

In the system of lenses  $L_2$  cannot be dispensed with. If all parts are exactly centred and if the requirement of formula (2) is satisfied, the light rays which are directed upon the point of intersection of  $S_2$  and the optical axis, will all pass through the lens  $L_3$ . Here  $L_2$  does not function, but this lens is required to bring about for all other points of  $S_2$  what holds good without the lens for the point of intersection above mentioned. To attain that object the lens  $L_2$  must form a focussed image of the second principal plane of  $L_1$  upon the first principal plane of  $L_3$ .

From formulæ (1) and (2) it follows that

$$\frac{s_1 \sin \frac{1}{2}\omega_1}{N_3} \leq s_3 \quad (3)$$

In order to be certain that all the light which falls upon the eye can always pass through the pupil, we have given to  $s_3$  a fixed value which is

less than the pupil diameter of any of the frogs' eyes which we have investigated. The question above stated as to the maximum of light which can be radiated from the crater upon the pupil now reduces itself to the question as to the most favourable values for  $s_1$ ,  $\sin \frac{1}{2}o_1$ , and  $N_3$ .

The basis of our construction was, as we mentioned, that all the light which falls upon  $L_1$  shall enter into the pupil. If we were to leave  $N_3$  out of account, then a maximum of light would fall upon the pupil when a maximum of light radiates upon  $L_1$ . For that purpose one must give a maximum value both to  $s_1$  and  $o_1$ ; in other words, the slit must be large, and at the same time the collimator lens  $L_1$  must have a large diameter and a short focal distance.

But proportionally to the increase of  $s_1 \sin \frac{1}{2}o_1$ ,  $N_3$  must also be increased, and in this we soon practically reach an unsurmountable limit. It is among microscopic objectives that one can find lenses with the greatest numerical aperture. We selected from Carl Zeiss' catalogue the water-immersion lens D\* as the most suitable lens for our purpose. The numerical aperture of the lens is 0.75, while the free object distance amounts to 1.5 mm. The lenses with greater aperture which are mentioned in the catalogue have all a much smaller object distance which renders them unsuitable for our purpose of forming the light image in the pupil plane of the intact frog's eyeball.

Formula (3) can be written in the form

$$\frac{s_1 \sin \frac{1}{2}o_1}{s_3} \leq N_3 \quad (4)$$

In our experiments  $s_1 = 1.9$  mm.,  $\sin \frac{1}{2}o_1 = 0.118$  mm., and  $s_3$  about 0.33 mm. From this it follows that

$$\frac{s_1 \sin \frac{1}{2}o_1}{s_3} = 0.68.$$

$N_3$  having as mentioned the value of 0.75, the requirements of formulæ (3) and (4) are satisfied, and by an appropriate arrangement we can ensure that in fact all the light falling upon the collimator lens  $L_1$  enters through the pupil into the eye.

The slit  $S_1$  is only a few millimetres distant from the crater. We could, of course, easily have placed a higher slit behind a larger crater, and much stronger collimator lenses with greater diameter are available, but by making use of such means we could not in any case, as we mentioned above, have increased the illumination of the image in the pupil.

The intensity of illumination which we obtained by means of our construction with a lamp of 20 amp. and a simple collimator lens was already very great. If one imagines in place of the slit of 1.9 mm. in height a square diaphragm with side  $s_1 = 1.9$  mm., and if the rays are not dispersed by a prism, so that in place of a spectrum of height 0.33 mm. in  $S_3$  a square white spot of light is formed of side  $s_3 = 0.33$  mm., then the illumination of this spot can be expressed in metre-candles or Lux.

According to Blondel<sup>1</sup> the light intensity of 1 mm.<sup>2</sup> of the crater surface amounts to 158 bougies, according to Petavel<sup>2</sup> to 147 candle-power. To make the two values comparable one to the other it is necessary to express them in the same units. We choose for this purpose the Hefner candle (HK). Regarding 1 bougie as equal to 1.075 (HK) and 1 candle-power equal to 1.095 (HK)<sup>3</sup>, and if the mean of the figures found by Blondel and Petavel be taken, a value of 165 (HK) per mm.<sup>2</sup> crater surface is obtained.

If we assume the intrinsic brilliancy of the crater light to be  $i$ (HK) per mm.<sup>2</sup>, then the intensity of radiation of our square spot in a direction at right angles to its surface equals that of a light source of

$$I = s_1^2 i(\text{HK}) \quad . \quad . \quad . \quad . \quad (5)$$

The total flux of light passing out from this spot is the light flux which is directed upon the inner surface of a hemisphere. The centre of this hemisphere coincides with the centre of the diaphragm, while the plane upon which the hemisphere rests is the plane of the diaphragm.

Taking Lambert's law into account we calculate the total flux of light as amounting to

$$\phi = \pi I \text{ Lumen}^4 \quad . \quad . \quad . \quad . \quad (6)$$

The flux  $\phi_1$  which forms the image in the pupil is but a part of  $\phi$ , and indeed is

$$\phi_1 = \frac{\phi}{p} \sin^2 \frac{1}{2} \sigma_1 \text{ Lumen} \quad . \quad . \quad . \quad . \quad (7)$$

where  $\frac{1}{p}$  is that part of the light which remains after the loss by absorption in the refracting media and reflection from the refracting surfaces.

From  $\phi_1$  the strength of illumination of the image in the pupil can easily be calculated. If the flux of light were distributed equally on an area of 1 M<sup>2</sup>, the illumination intensity of the surface would equal  $\phi_1$  metre-candles or Lux. But the flux here being concentrated on the area of the image =  $s_3^2$  square millimetres, the illumination intensity amounts to

$$E = 10^6 \times \frac{\phi_1}{s_3^2} \text{ Lux} \quad . \quad . \quad . \quad . \quad (8)$$

To calculate the value of E numerically we remember that  $i = 165$  HK,  $s_1 = 1.9$  mm.,  $s_3 = 0.33$  mm.,  $\sin \frac{1}{2} \sigma_1 = 0.118$ .  $p$  alone remains to be determined. Referring to Section IV., regarding the energy of stimulation in absolute measurement, we put  $p = 2$ , and then find for E the value  $120 \times 10^6$  metre-candles.

The illumination of a plane whereon the direct rays of the sun in zenith fall vertically through a clear atmosphere is given as 288000

<sup>1</sup> Cf. Liebenthal, *Praktische Photometrie*, Braunschweig, 1907, p. 139.

<sup>2</sup> *Loc. cit.*, p. 475.

<sup>3</sup> Cf. Liebenthal, *loc. cit.*, p. 434.

<sup>4</sup> One Lumen is the flux of light that radiates from a punctiform light source of 1 (HK) to an area = 1 of the surface of a sphere described round the light point with a radius = 1.

metre-candles.<sup>1</sup> Our image in the pupil of the frog's eye is therefore rather more than 400 times more strongly illuminated than this:

As fig. 1 is only a diagram it does not show the dimensions of the system of lenses which we have used. We therefore give here some of the actual dimensions:—

Height of slit . . . . .	1.9 mm.
Breadth . . . . .	1.1 "
Distance of $S_1$ from the anterior surface of $L_1$ . . . . .	165.0 "
Diameter of $L_1$ . . . . .	39.2 "
Distance of the posterior surface of $L_1$ from $\sigma_2$ . . . . .	825.0 "
Distance of $D_2$ from $L_3$ about . . . . .	100.0 "

#### 4. Weakening the Light.

Our original plan was to weaken the light by means of smoked glasses in order to leave unchanged the form, magnitude, and colour of the retinal image, and to vary solely the intensity of the light. But it soon became apparent that even the best of the so-called neutral glasses, if they absorb a great part of the light, do not allow all colours to pass through equally. Before they could be used it was therefore necessary to measure the transmitting power of these glasses for each colour separately.

As we had not an opportunity of carrying out these measurements in a sufficiently accurate way, we have contented ourselves with the use of diaphragms. In the first place the opening of lens  $L_1$  can be diminished by diaphragm  $D_1$ , and in the second place the opening of lens  $L_2$  by diaphragm  $D_2$ .

The first diaphragm, which varies the illumination intensity of the image  $S_3$  in the pupil, leaves, it is true, the form and magnitude of this image unchanged, but diminishes the spot of light on the retina as it considerably diminishes the size of the diffusion circles which contribute not a little to the formation of the retinal spot.

The second diaphragm  $D_2$  diminishes the image  $S_3$  in the pupil, whereby a further diminution of the retinal image is brought about. Moreover,  $D_2$  intercepts the rays of light at the margin of  $S_2$ , which have another wave-length than the rays at the centre, but the colour of the retinal image is thereby very little altered.

By measurements which have been made to determine the sensitiveness of the human eye to very weak light, it is found that if the spot of light on the retina does not exceed certain limits, the illumination of the spot that is necessary to give rise to a sensation is inversely proportional to its area. In these circumstances the quantity of energy required for a sensation is therefore independent of the size of the retinal area illuminated.<sup>2</sup>

If the spot of light is not small enough the rule above mentioned does

<sup>1</sup> Arrhenius, Lehrbuch der kosmischen Physik., Leipzig, p. 93, 1903.

<sup>2</sup> Piper and Asher; cf. v. Kries, Zeitschr. f. Psychol. u. Physiol. d. Sinnesorg., 2 Abt., Bd. 41, 1907, pp. 376 and 377; also cf. Henius, Zentralbl. f. Physiol., Bd. 22, p. 229, 1908.

not hold good, and with larger retinal areas the amounts of energy required increase proportionately with the square root of the area.

We may expect that analogous rules exist for the photo-electric reaction of the isolated frog's eye. In our experiments the flux of light on the retina was often diminished by the use of diaphragms from 1 to  $10^{-9}$ . When once it has been diminished to  $10^{-3}$  or  $10^{-4}$  the spot of light on the retina has in all probability become so small that the quantity of light required for a photo-electric reaction has become independent of the area of the spot.

Four groups of diaphragms were prepared. Those of the first group could be placed close to the collimator lens at  $D_1$ , while at  $D_2$  the diaphragms from the three other groups served respectively for the three parts of the spectrum used by us. It need scarcely be mentioned that all diaphragms were applied centrically round the optical axis. The diameters of the diaphragms, of which the size was exactly controlled by aid of the microscope, were so chosen that the successive members of a series each weakened the light by ten times.

The following are the dimensions of the diaphragms used:—

TABLE I.  
Circular Diaphragm  $D_1$ .

Diameter.	Flux of Light.
39.2 mm.	I.
12.39 "	$10^{-1}$ "
3.92 "	$10^{-2}$ "
1.239 "	$10^{-3}$ "
0.392 "	$10^{-4}$ "
(0.124 " )	$10^{-5}$ "

TABLE II.  
Diaphragm  $D_2$ .

Red from $\lambda=0.670$ to $\lambda=0.590$ .		Green from $\lambda=0.590$ to $\lambda=0.497$ .		Blue from $\lambda=0.497$ to $\lambda=0.460$ .	
Dimensions.	Flux of light.	Dimensions.	Flux of light.	Dimensions.	Flux of light.
9.5 × 14.5 mm. <sup>2</sup> (rectangular).	$I_r$	9.5 × 27.5 mm. <sup>2</sup> (rectangular).	$I_g$	9.5 × 18.2 mm. <sup>2</sup> (rectangular).	$I_b$
Diameter of circular diaphragm.		Diameter of circular diaphragm.		Diameter of circular diaphragm.	
4.19 mm.	$10^{-1}$ "	5.77 mm.	$10^{-1}$ "	4.69 mm.	$10^{-1}$ "
1.324 "	$10^{-2}$ "	1.823 "	$10^{-2}$ "	1.483 "	$10^{-2}$ "
0.419 "	$10^{-3}$ "	0.577 "	$10^{-3}$ "	0.469 "	$10^{-3}$ "
(0.132 " )	$10^{-4}$ "	(0.182 " )	$10^{-4}$ "	(0.148 " )	$10^{-4}$ "

The largest of the diaphragms used by us at  $D_2$  has, as mentioned in Table II., a length of 27.5 mm. An image of it is formed in its full length by the water-immersion lens  $D^*$  in the pupil of the frog's eye. The image so formed measures only 0.955 mm., while the objective is able to form a circular image of 1.3 mm. diameter, although this image is very much distorted at the margin.

The diameter of the pupil of the eyes investigated by us has always been greater than 0.955 mm.

The smallest diaphragm of each group in both tables is placed within brackets, as it produces only on one condition the calculated weakening of the light. The smallest diaphragm at  $D_1$  is only used when at the same time the light is diminished 100 times at  $D_2$ , and the smallest diaphragms of the three groups at  $D_2$  are only used when at the same time the light is 10 times weakened at  $D_1$ .

If these conditions are not satisfied the diminution of intensity becomes greater than that calculated on account of the diffraction of the light. It can easily be shown that when the conditions are satisfied, the influence of the diffraction upon the image formation at all three places  $S_2$ ,  $S_3$ , and  $L_3$  may be neglected.

### 5. The Spectrum.

The collimator consisting of the slit  $S_1$ , the lens  $L_1$ , and the tube connecting these two parts, is fixed upon a horizontal plank which may be rotated around a vertical axis. The continuation of this axis runs in the plane which bisects the refracting angle of the prism  $P$ , and in order to be able always to obtain the minimum of deviation the prism may also be rotated separately around this axis.

The prism, which has walls of mirror glass, is filled with carbon disulphide and is 115 mm. in height. Its base is an equilateral triangle whose side measures 105 mm.

The lamp is placed on the same horizontal plank as the collimator, and always therefore rotates together with this.

The whole is placed upon tables which are nailed to the floor, so that the axis on which the lamp, the collimator, and the prism rotate holds an unchangeable position with regard to the system of lenses and the eye.

There are, as already mentioned, for illuminating by the three parts of the spectrum, three rectangular diaphragms in use which can successively be placed at  $D_2$ . In order to be able to judge if the spectrum forms here a sharp image, and if in fact only light of the desired wave-length passes through the diaphragm, the pasteboard tube which lies between the prism and  $D_2$  for the purpose of excluding the daylight, is taken away and the spectrum is directly viewed. The self-regulating lamp is replaced by an arc lamp with hand regulation which can easily be done without displacing in the slightest the collimator or other parts of the installation. Between the carbons of the hand-regulated lamp, a salt, either of sodium or of



lithium, is then placed and the light of the arc exhibits a sharp spectrum of lines in  $D_2$ .

The dimensions of the rectangular diaphragms placed here, which are given in Table I., are so chosen that the spectrum in its vertical dimension radiates exactly through the opening of the diaphragm, while each rectangle is so long that two previously determined easily recognisable spectral lines fall upon its lateral margins.

For illumination with red the lithium line  $\lambda = 0.67$  and the sodium line  $\lambda = 0.59$  are thrown upon the left and right margins respectively of the rectangle  $D_{2r}$ . For illumination with green the sodium line and the lithium line  $\lambda = 0.497$  are thrown upon the left and right margins of the rectangle  $D_{2g}$ , and for illumination with blue the lithium lines  $\lambda = 0.497$  and  $\lambda = 0.460$  are thrown upon the left and right margins of  $D_{2b}$ .

When the spectrum is focussed upon  $D_2$ , in order to illuminate with one of these three parts, the exact position of the collimator and the prism, which can be easily controlled, is read from a scale. After both of these have been firmly screwed in their places, the hand-regulated lamp is replaced by the self-regulating lamp of Siemens and Schuckert.

## 6. The Movable Screens.

We must now describe the arrangement which enabled us to cause the light to enter the eye at the desired moment and to radiate during any desired time. This arrangement, which agrees in principle with that used by de Haas,<sup>1</sup> is placed between the prism and  $D_2$  and consists of two parts. The first part is composed of a system of two black, equal-sized, vertically placed discs which may be rotated upon a common axis at right angles to their centre. This axis is parallel to the course of light rays  $PS_2$ .

The discs are pressed one against the other and cover one another completely, but a portion is cut out from the circumference of each, and by rotating one disc over the other, an orifice is left of any desired breadth through which the light can pass to the eye.

With the aid of an electromotor the pair of discs can be rotated as one with exactly determinable velocity. The speed of rotation and the breadth of the opening at the circumference determine together the duration of illumination.

In order to ensure that the light does not pass with every rotation, the second part of the apparatus is constructed. In this second part a small screen, formed like a semicircular disc, is present. By the aid of a strong spring the disc is rotated when a catch is withdrawn, until after half a rotation it is stopped by a second catch. In one position of the screen the light rays may pass, in the other they are intercepted.

The catches are brought into action by an electromagnet which is fed by a battery of accumulators. The circuit which carries the current

<sup>1</sup> Loc. cit.

from the battery to the electromagnet is interrupted in two places. The first place lies near the galvanometer, where the circuit can be opened and closed by the observer by means of a key. The second place lies near the rotating discs, where the circuit is closed automatically for a short time after every five revolutions of the pair of discs.

During the time when the circuit is thus automatically closed, both the discs and the screen are in a position to permit of the rays passing.

In this way the observer is easily able to illuminate the eye only once during a definite short period. He may close at any moment the circuit of the electromagnet, and then merely wait until the electromagnet comes automatically into action. So soon as this has done its work, which is accompanied by a clicking sound, easily audible, the observer opens the circuit again.

If it is desired to radiate the light during a longer period, the pair of discs are placed at rest, while the light is allowed to pass freely by the opening in the circumference which is made large. The action of the magnet is then dependent alone upon the closing of the circuit by the observer.

An electrical contact arrangement is connected with the screen, by means of which a current is made at the exact moment at which the light passes, and is broken at the exact moment of interception of the light. This current sets in motion a signal recording these times photographically.

The signal resembles a small string galvanometer, and consists of a silver strip 120 mm. long,  $143 \mu$  broad, and  $6.4 \mu$  thick, stretched between the poles of a permanent magnet. It registers the make and break of the current with a latency which has been found by previous measurements not to exceed  $.0001$  sec.

## 7. The Moist Chamber and the Connection of the Eye with the Galvanometer.

In a large, well-illuminated room, where the galvanometer is arranged, there is constructed a small dark room. This consists of a frame of wood covered with linen and paper. Its base is rather more than one square metre, and its height rather more than two metres. Within this dark room, resting upon a stone pillar, is the moist chamber containing the eye. The moist chamber, as is usual, consists for the most part of glass, and has an opening in front through which the tube of the microscope is inserted.

This tube reaches the wall of the dark room, and is here closed by the lens  $L_2$  and the diaphragm  $D_2$  (fig. 1). Everything in front<sup>1</sup> of  $D_2$ , and thus outside the dark room, is covered with pasteboard tubes and black cloth, so that no light can enter the dark room through  $D_2$ , other than what is sent from the crater through the slit of the collimator.

<sup>1</sup> Following Helmholtz, we designate the direction towards the light source, forward, away from the light source, backward.

The microscopical objective, Zeiss' water-immersion D\* moistened with Ringer's solution, is placed almost in contact with the cornea of the frog's eye. The latter is connected with a pair of du Bois-Reymond's non-polarisable electrodes in such a way that one takes the potential of the cornea and the other of the fundus oculi (fig. 2).

The moist chamber is so arranged that the air within can be saturated with water vapour, while the eye and the electrodes which are attached to separate glass tubes projecting freely through openings in the floor of the chamber, and which are nowhere in contact with its moist walls, remain completely insulated.

The electrodes are, by means of insulated wires, led to the galvanometer in a manner similar to that which has been employed in this laboratory for recording electrocardiograms.<sup>1</sup> One has in this way an opportunity of compensating the current of the resting retina, of measuring in a simple and rapid manner the resistance of the preparation, and of regulating the sensitiveness of the galvanometer as desired.

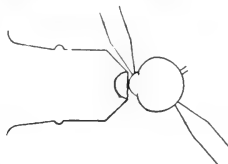


FIG. 2.—Arrangement of the eye in relation to water-immersion lens and non-polarisable electrodes.

We need scarcely mention that we have repeatedly controlled the exactitude of our adjustments. The insulation resistance lies as a rule between  $10^{10}$  and  $10^{11}$  ohm. The crater is so large that the cone of light radiating through the slit extends all round beyond the margin of the collimator lens. All lenses are accurately centred, and we experimentally determined that all the light passing through the outer margins of  $L_1$  contributes to the formation of  $S_3$ .

In conclusion, we did not omit to ascertain that all enclosing arrangements were light tight. If  $L_1$  is covered, all diaphragms and the movable screens may be withdrawn without the slightest photo-electric response being obtained.

### III. RESULTS.

#### 1. The Form of the Photo-electric Reaction to Light of Moderate Intensity.

If the isolated eye, which has not shortly before been exposed to strong light, be illuminated by rays of intermediate strength, a form of curve is obtained similar to that recorded by previous observers.

<sup>1</sup> Cf. "Weiteres über das Elektrokardiogramm," Pflüger's Archiv, Bd. 122, p. 517, 1908.

Fig. 3 may serve as an example. The curve shown in this figure must, like all the curves here reproduced, be read from left to right, while the connections of the eye with the galvanometer are made in such a way that a current passing from the cornea through the instrument to the posterior surface of the eye deflects the image of the string in an upward direction. An action current in this direction may be termed positive, and in the reverse direction negative.

Fig. 3 gives the reaction following on a momentary illumination. In the rectangular system of co-ordinates 1 mm. of an abscissa represents 0.5 sec., 1 mm. of an ordinate 100 microvolts. The illumination is by means of green light, which is reduced to 0.01 of its original intensity, and which, in accordance with the two tables given in the previous chapter, we may name  $10^{-2} I_g$ . The duration of the illumination is 0.1 sec.

One observes, after a latent period, a small preliminary negative deflection A which is immediately followed by an upward movement of the string. After the curve has reached a somewhat acute peak B, it sinks first rapidly, then more gradually, but while still distant from the zero line it mounts again. This latter ascent begins 2.5 to 3 sec. after the beginning of the illumination, while much later the curve reaches its second maximum C, which lies about 1 mm. higher than the first positive peak B. Finally, the curve gradually regains the zero line.

The potential differences which have given rise to these three summits amount to—

For the preliminary negative deflection A . . . . .	-70 microvolts.
For the first positive summit B . . . . .	1050 "
For the second positive summit C . . . . .	1150 "

The form of the photo-electric reaction when evoked under similar circumstances to fig. 3 is always essentially the same, but the absolute size of the deflections as well as their proportional size may differ. When a number of curves are compared one with another, which all agree in having summit B of the same height, the deflection A as well as the slow succeeding wave C may show very different heights.

In the case of illuminations of short duration, the energy of the light is, as may be expected, the measure of the stimulation.<sup>1</sup> A stronger light must shine a shorter time than a weaker in order to produce the same effect.

In fig. 4 is seen a curve which is obtained by illuminating another eye during 0.01 sec. with the full amount of  $I_g$ . In the system of co-ordinates 1 mm. abscissa is again 0.5 sec., but the sensitiveness of the galvanometer is now ten times greater, so that 1 mm. ordinate amounts to 10 microvolts.

Notwithstanding that the energy of the light stimulation is greater,

<sup>1</sup> Cf. de Haas, loc. cit.

the amount of the potential differences brought about is here less than in

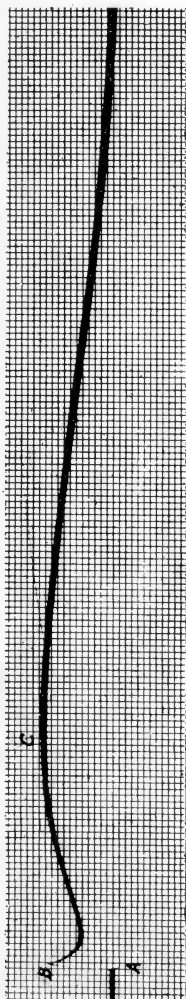


FIG. 3.—The combined reaction of the three substances, Dark eye. Absc. 1 mm. = 0.5 sec. Ordin. 1 mm. = 100 microvolts, Flash 0.1 sec. Green light. Intensity of illumination =  $10^{-2} I_k$

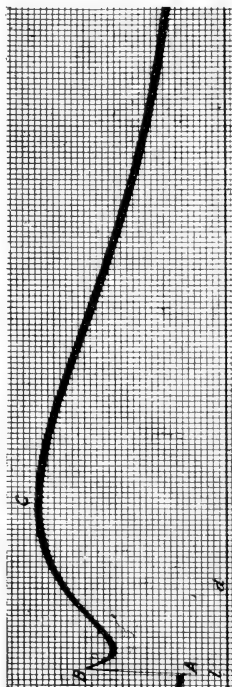


FIG. 4.—The combined reaction of the three substances, Dark eye. Absc. 1 mm. = 0.5 sec. Ordin. 1 mm. = 10 microvolts, Flash 0.01 sec. Green light. Intensity of illumination =  $I_k$

fig. 3. This need not surprise us, as the curve in fig. 4 is obtained from another eye, and considerable differences exist in the reaction intensities of different preparations.

The absolute amounts are—

For summit A . . . . .	- 15 microvolts.
"    B . . . . .	150       "
"    C . . . . .	230       "

In proportion to B, both A and C are greater than in fig. 3.

If the illumination is weaker and continued for some time, then one observes at the moment when darkening begins after a latent period, a new elevation of the curve, an off-effect  $A_1$ .

In fig. 5 there is given an example. In photographing the movements of the string, the sensitive plate is here moving more quickly, so that in the system of co-ordinates 1 mm. abscissa is now equal to 0.2 sec. The sensitiveness of the galvanometer is regulated in such a way that 1 mm. ordinate amounts to 20 microvolts. This is visible in the control curve, which is obtained at the end of the curve by suddenly introducing into the circuit a potential difference of 200 microvolts. The intensity of the illumination is  $10^{-4} I_g$ , while the duration of illumination as indicated by the signal amounts to 4.58 sec.

The latent period of the preliminary deflection A is 0.1 sec., that of the off-effect  $A_1$  is 1.8 sec.

The absolute amounts of the potential differences are—

For A . . . . .	- 20 microvolts.
"    B . . . . .	384       "
"    C . . . . .	670       "

The potential difference of  $A_1$  cannot easily be given. It may be estimated at 60 microvolts, and is measured after connecting the beginning and end of the curve  $A_1$  by a line running in the course of the main curve. We may remark here that the off-effect  $A_1$  is in general higher the longer the illumination has been continued.

Further, we draw attention to the fact that the curves in figs. 3, 4, and 5, although they may differ in details, are yet formed essentially in precisely the same way. It may also be remarked that fig. 15, which we shall discuss later, shows no essential difference from figs. 3, 4, and 5.

## 2. The Three Substances.

The complicated structure of the curves above described and the striking fact that a deflection in the same direction takes place both on illumination and on darkening, suggest that there are in the eye two or more different processes occurring partly simultaneously, partly successively, whose fusion determines the form of the electrical reaction.

Further investigation confirms this suggestion, and if recourse is had to very weak or very strong light, it seems even to be possible to bring about a separation of the supposed processes. The phenomena are explained in

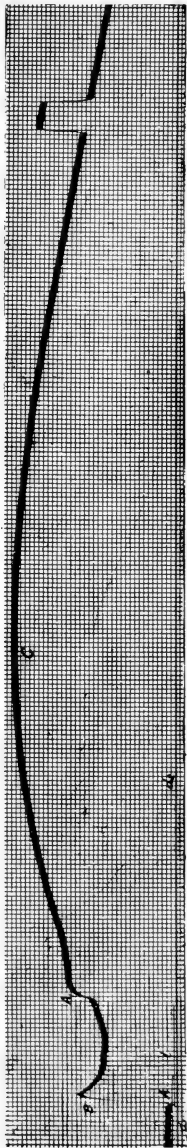


FIG. 5.—The combined reaction of the three substances. Dark eye. Absc. 1 mm. = 0.2 sec. Ordin. 1 mm. = 20 microvolts. The deflection at the end of the curve is caused by the introduction of a known difference of potential into the galvanometer circuit. Green light. Intensity of illumination =  $10^{-4} I_g$ . *l*, light; *d*, darkness.

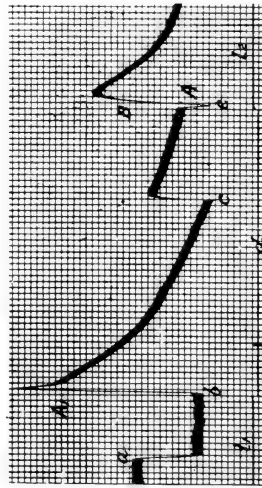


FIG. 6.—The reaction of the first substance. Light eye. Absc. 1 mm. = 0.2 sec. Ordin. 1 mm. = 20 microvolts. White light. Intensity of illumination =  $I_w$ . *l*, light; *d*, darkness. At *a* and *c* are control curves.

the simplest manner by the assumption that the processes are three in number, whether they are together dependent upon the same substance or each upon a separate one. For the sake of convenience we shall speak of three substances, and as we do not intend in the meantime to attempt to define them anatomically in the eye, we shall try to describe their characteristics and to mention the conditions under which their effects appear as pure as possible.

### The First Substance.

The substance which we have termed the first reacts more quickly than the other two. On lighting it displaces the image of the string downwards, on darkening upwards. Its effect, which can be obtained pure only during a short period, is very marked in a light-adapted eye—which for the sake of brevity we may call a light eye<sup>1</sup>—and the more so the stronger the illumination has been.

From the nature of the case the darkening stimulation can be employed very strong in a light eye, and accordingly an eye which has been illuminated strongly develops on darkening a huge positive potential difference. The upward deflection so evoked cannot, however, be of long duration, because by the darkening the light eye is beginning to be changed into a dark eye, and therefore the effect of our first substance is no longer so clearly indicated.

An example of these phenomena is reproduced in fig. 6. Here 1 mm. abscissa is 0.2 sec., 1 mm. ordinate is 20 microvolts. The eye has been illuminated for a fairly long time with almost the strongest light at our disposal; that is to say, practically the full amount<sup>2</sup> of  $I_w$ . At *a* a constant potential difference of 200 microvolts is introduced into the circuit whereby the image of the string is displaced 10 mm. downwards. At *b* the eye is suddenly darkened. It is seen that after a latent period which, according to a rough estimate, has a duration of about 0.04 sec., a high positive off-effect  $A_1$  occurs, whose top is elevated 29.5 mm. above the original position of the string. If the indicating speed of the galvanometer left nothing to be desired, this height should correspond to a potential difference of 590 microvolts, but the galvanometer was in this case given too great sensitiveness to be able to follow exactly the extremely rapid current variation. The amount of the latter must be calculated from the form of the curve.<sup>3</sup> We have not made this calculation in detail but it is easy to ascertain that the actual potential difference attained is considerably higher than the amount above mentioned. We may estimate it at about 1000 microvolts (1 millivolt).

<sup>1</sup> An eye which is dark-adapted may be termed a dark eye. The terms are analogous to "Lichtfrosch" and "Dunkelfrosch," which are commonly used.

<sup>2</sup> There was placed in the path of the light rays a very weak smoked glass, whereby the intensity was a little lessened.

<sup>3</sup> For a method of calculation cf. "Weitere Mitteilungen über das Saitengalvanometer," *Annalen der Physik*, Bd. xxi., S. 483, 1906.



This enormous off-effect is thus about sixteen times higher than that shown in fig. 5. It is, as we have already remarked, of but short duration. By the darkening the light eye is beginning to be changed into a dark eye. The image of the string is seen to descend at first rapidly, then more slowly.

Although in the light eye the conditions are less favourable for the lighting than for the darkening stimulus, it is nevertheless possible to apply the former in either of two ways. In the first place, we may suddenly increase the intensity of the light that is radiating on the eye, and secondly, we may darken the light eye for a short period, so that it has not yet become a dark eye, and then suddenly illuminate it.

The second method gives better results than the first, and we possess numerous curves where, after a short darkening of a light eye, a strong light stimulus was applied. An example of this is seen at the end of fig. 6. The potential difference of 200 microvolts which was introduced at *a* is cut out at *c*, and the strong white light  $I_w$  is suddenly allowed to radiate upon the eye at *e*. The reaction A of the first substance attains here as a downward directed deflection a value of  $-90$  microvolts. The positive deflection B following thereon belongs, as we shall explain later, to the action of the second substance. It has nothing to do with the reaction of the first substance, and becomes smaller the more the reaction of the first substance appears unmixed. The positive deflection E (lighting reaction of the second substance) must be reckoned as beginning at the lowest point of the negative deflection A. The height of B must thus be measured from this lowest point to the peak. It amounts at *e* in fig. 6 to 370 microvolts, and is therefore more than four times greater than the lighting reaction A of the first substance.

But it is not difficult to increase the lighting effect of the first substance and at the same time to diminish that of the second substance, which is, in other words, to produce the lighting effect of the first substance more purely. For that purpose one requires to darken the light eye during a shorter time so that it preserves better the attributes of a light eye. In an eye that is darkened during a very short time the lighting effect of the first substance can even surpass that of the second. The negative deflection A becomes then larger than the immediately following positive wave B. Fig. 7 may illustrate this.

Here 1 mm. abscissa is equal to 0.2 sec., 1 mm. ordinate to 21 microvolts. The eye is illuminated with white light of the full strength  $I_w$ . The periods of lighting are, as in all our other figures, denoted by *l*, those of darkening by *d*.

If  $l_{12}$  of fig. 7 be compared with  $l_2$  of fig. 6, it is clear that the reaction of the first substance at  $l_{12}$  has become greater, that of the second substance has become smaller than at  $l_2$ . The reaction on lighting at  $l_{12}$  has followed upon a shorter period of darkness. We may remark, however, that at  $l_{12}$  B is still twice as great as A (see diag., fig. 8, a).

On further shortening the period of darkness the proportion becomes continuously changed in favour of the first substance, so that on lighting at  $l_2, l_3, l_4, l_5,$  and  $l_{11}$  the negative wave of the first substance is about as great as the positive wave of the second (see diag., fig. 8, b), and finally,



FIG. 7.—The reaction of the first substance. Light eye. Absc. 1 mm. = 0.2 sec. Ordin. 1 mm. = 21 microvolts. White light. Intensity of illumination =  $I_w$ .  $l$ , light;  $d$ , darkness.

after very short periods of darkening, the positive wave of the second substance at  $l_6, l_7, l_8, l_9,$  and  $l_{10}$  becomes smaller than the negative of the first (see diag., fig. 8, c), especially at  $l_7, l_8,$  and  $l_{10}$  the lighting effect of the first substance appears almost pure.

To understand fully the significance of fig. 7, we must further pay special attention to the darkening deflections. As already mentioned, the

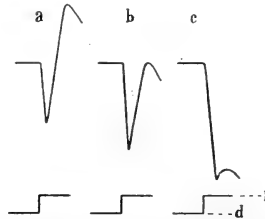


FIG. 8.—Diagram of the reaction to strong light of a light eye which has previously been darkened for a short time. The preceding period of darkness has been longest in  $a$ , shorter in  $b$ , and shortest in  $c$ , and the upward deflection, due to the action of the second substance, becomes progressively less, while the downward deflection due to the first substance becomes greater.  $l$ , light;  $d$ , darkness.

first substance reacts in a completely light eye on stimulation by darkness with the development of a huge positive potential difference, which, however, cannot have a long duration, since by the darkening the light eye begins to change into a dark eye. The descent of the image of the string caused thereby, a descent which is produced by the action of the second substance, is relatively slow. It can easily be distinguished from the very

rapid descent with which the first substance reacts to a strong light stimulation.

In the case of darkening of moderate duration, as for example at  $d_2$  and  $d_3$ , there therefore appears a curve which begins with a rapid ascent, continues in the middle with a slow descent, and concludes with a rapid descent (see diag., fig. 9, a).

The rapid ascent at the beginning and the rapid descent at the end are the reactions of the first substance; the slow descent in the middle is produced by the action of the second substance.

As the duration of the darkening is shortened the middle part of the curve diminishes more and more until, with very short darkening (a flash of darkness), it entirely disappears. In these circumstances, therefore, the action of the second substance is totally cut out and the curve shows the pure reaction of the first substance. These phenomena are fully depicted in fig. 7. The effects of darkening at  $d_5$  and  $d_{10}$  are reproduced diagram-

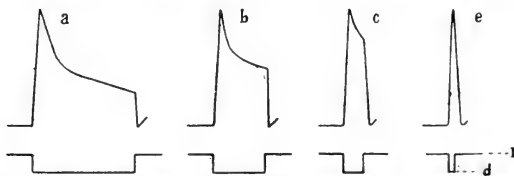


FIG. 9.—Diagram of the reaction of a light eye to darkness. The period of darkening is progressively shortened from  $a$  to  $e$ . The initial upward and terminal downward deflections are due to the first substance, and the intermediate slow descent to the second substance. In  $e$ , where the darkening is very short (a flash of darkness), the reaction of the first substance is seen pure.  $l$ , light;  $d$ , darkness.

matically by fig. 9, b, at  $d_3$  by fig. 9, c, and the effects of the very short periods of darkening at  $d_6$ ,  $d_7$ , and  $d_9$ , where the reaction of the first substance appears practically pure, are shown by fig. 9, e.

The absolute amount of the darkening reaction is in  $d_1$  very great. The deflection being 31 mm. in height, should represent a potential difference of 650 microvolts, if the galvanometer had been able, with the sensitiveness here employed, to follow the current variations exactly, but the actual potential difference is, for reasons explained above, much greater, and must be estimated at more than 1200 microvolts.

The absolute amounts of the lighting reactions are not so great, but the potential differences here developed may nevertheless be termed considerable. At  $l_{10}$  and  $l_8$  downward directed deflections occur of 17 and 18 mm. which, if the galvanometer were rapid enough, would represent potential differences of 357 and 378 microvolts, but must in reality be estimated at more than 600 microvolts.

In cases where the darkening is of short duration and the darkening reaction is greater than the lighting reaction, curves are recorded as at  $d_1$  and  $d_4$ , where the string does not return to its original position (see diag., fig. 10, a).

In ng. 10, b, on the contrary, is diagrammatically reproduced a curve with a long period of darkness as at  $d_{11}$ , where the string descends below its original position.

### The Second Substance.

The second substance reacts less quickly than the first. On lighting it moves the string with moderate velocity upwards, and on darkening slowly downwards; thus on applying stimuli of the same kind, it develops potential differences which are opposed to those of the first substance. Its effect appears almost unmixed in a dark eye which is illuminated for a short time by weak light.

If when illuminating with light of very low intensity, the darkening follows rapidly upon the lighting, in a similar way as in a momentary illumination, there is recorded a curve of simple form with a steeper

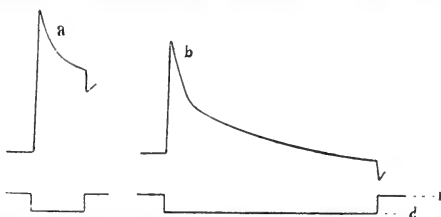
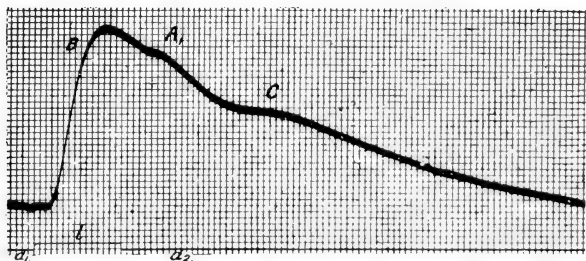
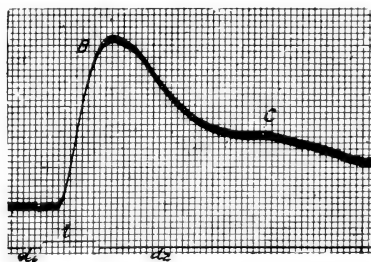
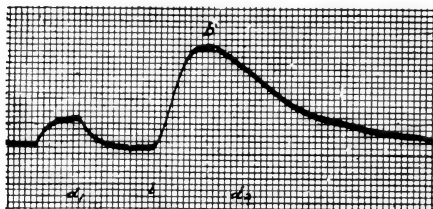
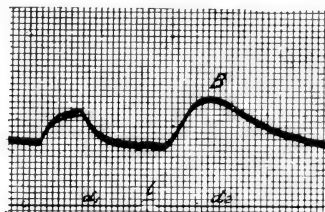


FIG. 10.—Diagram of the reaction of a light eye to darkness. In *a*, where the darkness is of short duration and the darkening reaction is greater than the lighting, the curve does not return to its original position. In *b*, where the darkening is of long duration, the curve descends below its original position. *l*, light; *d*, darkness.

ascending part which is evoked by the lighting and a less steep descending part evoked by the darkening. The top of the curve lies, within certain limits, higher the more the energy of the illumination is increased either by using greater intensity or longer duration of the light. These limits are determined by the functioning of the other two substances, which, when their effects become perceptible, influence the form of the curve and considerably complicate it.

The series of four photographs which are taken from one and the same eye and are reproduced in figs. 11, 12, 13, and 14, may serve as examples of illumination of this description. Here 1 mm. abscissa is equal to 0.2 sec., and 1 mm. ordinate equals 4 microvolts. The eye is illuminated each time by green light, which, by making use of diaphragms, is reduced to one ten millionth of its original intensity, and which we thus name  $10^{-7} I_0$ . The duration alone of the illumination is varied. From fig. 11, where this duration amounts to 0.48 sec., it increases gradually: in fig. 12 it is 1.12 sec.; in fig. 13, 1.9 sec.; and in fig. 14, 3 sec.

The heights of the summits increase regularly with the increasing



FIGS. 11, 12, 13 and 14.—The reaction of the second substance. Dark eye. Absc. 1 mm. = 0.2 sec. Ordin. 1 mm. = 4 microvolts. Green light. Intensity of illumination =  $10^{-7}$  Ig. *l*, light; *d*, darkness. A control curve precedes reactions 11 and 12. The duration of lighting increases from fig. 11 to fig. 14. In figs. 11 and 12 the second substance is acting alone. In fig. 13 the action of the third substance is seen at C, and in fig. 14 also that of the first at A<sub>1</sub>.

duration of the lighting times. In the four figures the successive amounts are 31.2, 66.8, 110 and 116 microvolts. The first and second members of the series—figs. 11 and 12—exhibit the reaction of the second substance practically unmixed. In the third member of the series—fig. 13—a complication begins to be visible. There is formed another summit at C which, as we shall later explain, must be considered as the effect of the action of the third substance.

In the last member of the series—fig. 14—the complication has considerably increased. Not only does summit C of the third substance appear more clearly, but the action of the first substance also becomes apparent; thus at A<sub>1</sub> we perceive the darkening effect of this substance.

The lighting effect of the first substance is the negative deflection A. This does not yet appear in fig. 14, which need not surprise us, since the first substance acts specially strongly in a light eye, and therefore from the nature of the case the darkening reaction must appear sooner than the lighting reaction. Nevertheless the lighting effect makes itself appreciable to some extent during the record of the curve; for if we regard the duration of lighting and the proportional heights of the summits of the four figures in the series (figs. 11, 12, 13, and 14), it is evident that the summit height in fig. 14 is only little greater than that in fig. 13. The increase is only 6 on 110 microvolts, while the duration of lighting is increased from 1.9 to 3 sec. It is the lighting effect of the first substance which here hinders the development of a higher summit B.

### The Third Substance.

The third substance reacts in the same direction as the second, but more slowly. On lighting it displaces the image of the string slowly upwards, and on darkening still more slowly downwards. So much slower is the third substance than the other two that its effect in a recorded curve appears, as a rule, almost entirely isolated, and can thus be easily followed.

The summit of the wave which is evoked by the action of the third substance is denoted by the letter C in figs. 3, 4, and 5. In the case of the momentary but very strong illumination of fig. 4, this summit occurs 16 sec. after the beginning of lighting. In the case of the less strong illumination of figs. 3 and 5, it occurs 20 sec. after such beginning.

The effect of the third substance falls out under two conditions: (1) in a dark eye exposed to very faint light for a short time, and (2) in a completely light eye. The first condition is realised in figs. 11 and 12, while on the contrary, in the figs. 13 and 14 of the same series, where the energy of the light stimulation exceeds certain limits, the effect of the third substance appears again.

The second condition, a completely light eye, is practically realised in fig. 7. After the short lighting at  $t_{11}$  there is seen in the curve no slow deflection which could be the analogue of the deflection C in figs. 3, 4, and 5.

In the case of an eye which has been exposed for a long time to strong light and thereafter maintained in darkness for a few minutes, so that it does not yet differ much from a completely light eye, we see the third substance acting very weakly.

Fig. 15 may serve as an example of this. The summit C of the third substance is here elevated only 1 mm. or 18 microvolts above its base. It will clearly appear that this amount is both absolutely and relatively very low when the summits B,  $A_1$  and C of figs. 5 and 15 are compared one with another.

### Composite Curves.

Having thus considered the effects of the three substances separately, it is not difficult to analyse the composite curves of figs. 3 and 4 into their

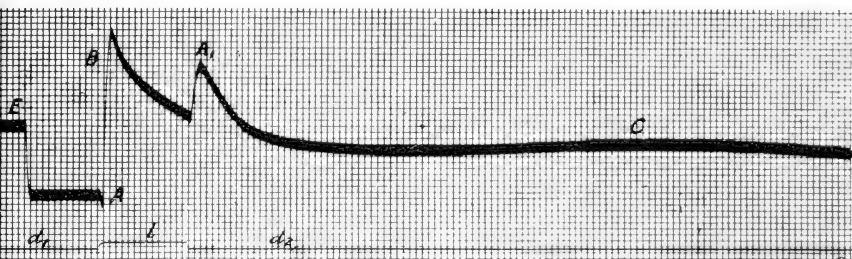


FIG. 15.—The reaction to light of an eye which has previously been exposed for a long time to strong light and thereafter darkened for some minutes. The third substance is seen acting feebly at C. Absc. 1 mm. = 0.2 sec. Ordin. 1 mm. = 18 microvolts. E, control curve. *l*, light; *d*, darkness.

component parts. The negative deflection A is evoked by the action of the first substance, the summit B by that of the second substance, and the summit C by the action of the third substance.

In fig. 16 those three reactions are diagrammatically represented as three separate curves. By superposition of these curves we obtain a figure similar to what is shown in figs. 3 and 4.

In fig. 5, where the lighting duration is 4.58 sec., there is seen in addition to the deflections of figs. 3 and 4 still another summit  $A_1$ . This is produced by the darkening reaction of the first substance. As already mentioned, the upward movement of the string which is produced by the darkening reaction of this substance is only of short duration. In agreement therewith the summit  $A_1$  is superposed in such a way upon the slow wave C that the form of the waves may easily be recognised separately (see diag., fig. 17).

What has been noted with regard to fig. 5 is also true of fig. 15. The two figures, which differ considerably in outward appearance, nevertheless

exhibit complete agreement in essentials. The analogous summits are denoted in both figures by the same letters A, B, A<sub>1</sub>, and C.

Specially remarkable are the curves obtained if the duration of lighting of a dark eye is systematically changed, and we wish to direct attention more particularly to the off-effect in such cases. If the duration of the lighting is very short and the light weak, then, as already mentioned, the effects of the second substance appear unmixed. The off-effect here consists in the descent of the curve to the zero line.

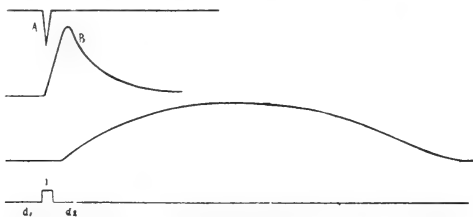


FIG. 16.—Diagrammatic representation as three separate curves of the reactions to light of the three substances. A, first; B, second; and C, third substance. *l*, light; *d*, darkness.

If the duration of the lighting is a little longer and the effects of the other two substances begin to become perceptible, the off-effect is determined by the resultant of three forces. The first substance tends to displace the image of the string upwards. It is at first acting weakly, but its strength increases regularly during illumination so that it soon surmounts the effect of the other substances. In the case of longer lighting the off-effect



FIG. 17.—Diagram of the combined action of the three substances. A is the lighting, and A<sub>1</sub> the darkening reaction of the first substance; B is the action of the second, and C of the third substance.

therefore is always an upward movement which increases with the duration of the lighting.

The second substance tends to depress the image of the string, acts first with moderate strength, but decreases gradually during lighting. As the second substance in particular is acting in a dark eye, the conditions for its functioning grow during the illumination more unfavourable. A strong darkening effect cannot be expected in a dark eye.

The third substance is so slow that the darkening effects of the first and second take place usually at a moment when the third substance is still tending to displace the string upwards. The darkening effect of the



third substance itself, consisting in a slow descent of the string, appears much later and fairly isolated.

The general result is that we can observe in a series of curves obtained from a dark eye, where the light has gradually been lengthened in duration, that the darkening effect, in the first curves a negative deflection, becomes in the later ones a positive deflection. The latter, on further lengthening the duration of lighting, gradually increases in size. In the conflict between negative and positive deflections there is sometimes seen an upward movement, which is immediately preceded by a small downward one.

To illustrate reactions of this description we reproduce in the first place fig. 18, where 1 mm. abscissa = 0.2 sec., and 1 mm. ordinate = 2 microvolts. The intensity of the stimulation cannot be given exactly, as the weakening of the light has been brought about by the aid of a

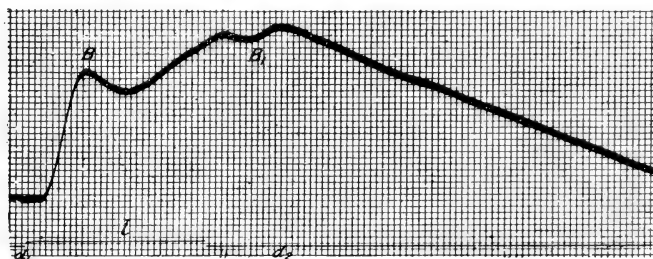


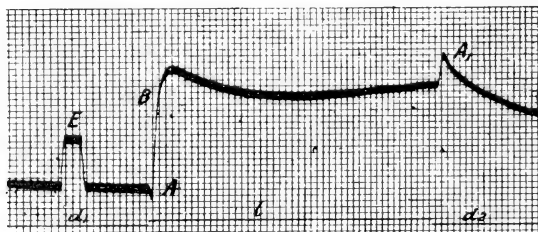
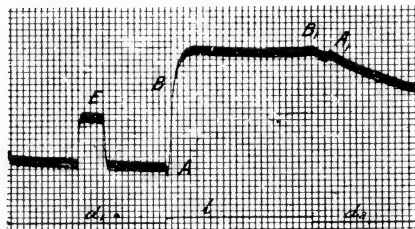
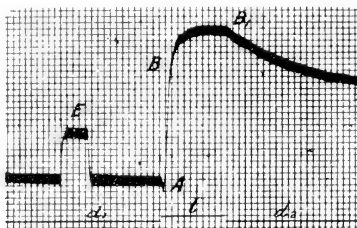
FIG. 18.—Conflict between the reactions of the three substances. Dark eye. Absc. 1 mm. = 0.2 sec. Ordin. 1 mm. = 2 microvolts. Green light. Intensity of illumination =  $10^{-6} I_g$ . *l*, light; *d*<sub>2</sub>, darkness. Instead of the usual upward deflection on darkening, due to the first substance, there is seen a downward deflection *B*<sub>1</sub> caused by the action of the second substance.

coloured light screen in addition to the usual diaphragms. The light intensity may be estimated at  $10^{-5}$  or  $10^{-6} I_g$ .

One observes that in these circumstances the negative deflection *A* is not present, while the summit *B* is developed in the ordinary way. As a result of the darkening there is seen in place of the usual upward directed summit *A*<sub>1</sub> a descent of the curve which is denoted by *B*<sub>1</sub>. So deep a depression at *B*<sub>1</sub> as occurs here is not found again in our whole collection of photographs.

We desire, in the second place, to draw attention to figs. 19, 20, and 21. which were obtained successively from the same eye. In all three figures 1 mm. abscissa equals 0.2 sec., 1 mm. ordinate equals 26 microvolts. In each figure there is reproduced in front of the photo-electric response a control curve *E* which is obtained by introducing a potential difference of 200 microvolts, which remains constant for a short time. The summits *A* and *B* are present in the three figures. In the first of the series, fig. 19, the darkening reaction at *B*<sub>1</sub> is seen as a sudden descent of the

curve after a short period of illumination. In fig. 20, where the illumination has lasted somewhat longer, there is evidence of the conflict between the actions of substances 1 and 2. At  $B_1$  is seen a negative deflection which is immediately followed by a positive deflection at  $A_1$ .



FIGS. 19, 20, and 21.—Conflict between the reactions of the three substances. Dark eye. Absc. 1 mm. = 0.2 sec. Ordin. 1 mm. = 26 microvolts. White light. Intensity of illumination = 0.2  $I_w$ .  $l$ , light;  $d$ , darkness. E, control curve. The three curves are from the same eye. The duration of lighting increases from fig. 19 to fig. 21. The darkening reaction is in fig. 19 a descent of the curve  $B_1$ ; in fig. 20 a descent  $B_1$  followed by an ascent  $A_1$ , and in fig. 21 an ascent  $A_1$ .

In the last figure of the series (fig. 21), where the illumination has lasted longer still, the characteristics of the organ as a light eye, to which condition the eye must attain more and more, appear in the foreground. The darkening effect at  $A_1$  here consists of a pronounced positive deflection.

Finally, we draw attention to the difference which exists between figs. 6 and 7 on the one hand, and the series of figs. 19, 20, and 21 on the other. In both cases very strong light was used, but in the first-mentioned figures we had the organ as completely as possible a light eye. In the last-mentioned figures we endeavoured to keep it as completely as possible a dark eye.

### 3. Light of Different Colours.

We shall now mention some further results of our investigations, and in the first place, those obtained by illuminating with rays of different wave-lengths. Our expectation that we should find marked differences in the form of the photo-electric reaction when light of different wave-lengths is employed for stimulation has not been realised. When experiments are performed with light of a single colour there appear very different forms of photo-electric reactions according as we have to do with a light eye or with a dark eye, and according as we have illuminated the eye with weaker or stronger light, and during shorter or longer periods. This is sufficiently proved by the figures of our plates. To study the influence which the variation of colour exerts upon the development of these numerous forms would require a long and detailed investigation which we have not had an opportunity of carrying out. We have only been able in our investigations to confirm what is already known, viz. that for the same energy of stimulation the reaction to green rays is stronger than that to red and blue.<sup>1</sup>

### 4. Rhythmical Reaction on Continuous Stimulation.

We take the opportunity of referring in a word to the possibility of a rhythmical reaction to constant illumination. As a rule the eye reacts to constant illumination with an electrical current which increases and decreases very gradually, but in some cases it is open to question if this rule holds good. In fig. 22 an example of this is reproduced. We have here to do with a light eye which is illuminated by strong white light  $0.2 I_w$ . Here 1 mm. abscissa = 0.2 sec., 1 mm. ordinate = 15 microvolts. The string shows, during each period of illumination  $l_1$ ,  $l_2$ , and  $l_3$ , rhythmical oscillations which fall during the periods of darkness  $d_1$  and  $d_2$ .

After making the record shown in fig. 22, the eye was maintained for a quarter of an hour in the dark and thereafter exposed again to light of the same strength, with the result given in fig. 15. The value of 1 mm. abscissa has remained unchanged and is equal to 0.2 sec., while the value of 1 mm. ordinate is increased to 18 microvolts. At E a control curve is recorded, while the reaction evoked by a short exposure to strong light shows the usual summits A, B, A<sub>1</sub>, and C. We draw attention to the

<sup>1</sup> Himstedt and Nagel, *Berichte der Naturforsch. Ges. zu Freiburg*, Bd. xi, p. 153, 1901.

pure line which appears during the lighting period  $l$ , and in which every trace of rhythmical oscillation fails.<sup>1</sup> It is to be noted that the illumination employed here, where the slit of the collimator is used, is derived from the central part of the crater, but we cannot altogether exclude the possibility that the arc lamp has burnt irregularly during the recording of fig. 22. In that case the oscillations of potential difference in the eye during illumination would have their natural origin in the rhythmically varying intensity of the light stimulation. But we did not observe an irregular condition of the lamp during the experiment, and must not, therefore, overlook the other possibility that it has been burning quietly. The oscillations in potential difference of fig. 22 would then have their origin in the rhythmical reaction of the eye itself.

Their failure in fig. 15 may, if the latter explanation is adopted, be

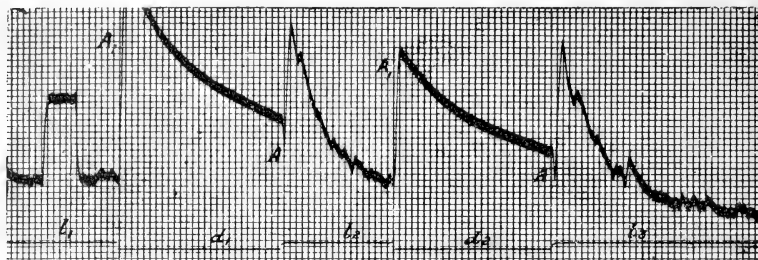


FIG. 22.—Rhythmical reaction to a continuous stimulus. Light eye. Absc. 1 mm. = 0.2 sec. Ordin. 1 mm. = 15 microvolts. White light. Intensity of illumination = 0.2  $I_w$ .  $l$ , light;  $d$ , darkness.

attributed to the rest of a quarter of an hour in darkness which has been given to the eye, during which it may be supposed to have recovered.

### 5. The Latent Period.

As we have mentioned when describing our method of investigation, the screen which intercepts the light rays is automatically moved by a strong spring set in action by means of an electromagnet. The movement of the hand required in closing the circuit of the electromagnet and the movement of the armature of the magnet itself give rise to slight jerks whereby the signal line and the string itself are sometimes set slightly in movement. One or two tenths of a second later, at the exact moment at which the light enters the eye or at which it is intercepted, a circuit is made or broken, by means of which the signalling instrument receives its current. This instrument itself has, as we have already mentioned, a

<sup>1</sup> If we study the curve with a magnifying glass we observe very fine rhythmical oscillations of about 25 or 30 periods per second. These are caused by the technical deficiency of the recording apparatus, and have no bearing upon changes in potential difference which might occur in the eye.

latency of only 0.0001 sec., so we may assume that the light stimulation begins or ceases simultaneously with the interruption of the signal line.

The breadth of the image of the slit in front of the sensitive plate in our photographic apparatus is 0.05 mm., and as the majority of our photographs were made on a sensitive plate moving at the rate of 5 mm. per second, time differences of 0.01 sec. may be observed.

So it is just possible to deduce from figs. 6, 7, 15, 19, 20, 21, and 22 that the latent period of the negative deflection A, as it occurs on very strong illumination, may be as short as 0.02 sec. or even 0.01 sec.

In order to be able to measure this duration exactly, we would require to give greater velocity to our sensitive plate. In the meantime we must content ourselves with the round figures given above.

The duration of the latent periods of the different deflections of the photo-electric reaction is in a high degree dependent upon the intensity of the stimulation.<sup>1</sup> Thus we see that the latent period of the negative deflection A in fig. 5, where the light stimulation amounts to  $10^{-4} I_g$ , is increased to about 0.1 sec.

In other photographs not reproduced here we have even been able to measure a latent period of this deflection amounting to 0.14 sec., but much larger periods are difficult to obtain, since, on employing weaker light, the negative deflection diminishes, and soon fails entirely.

Our observations, which are in agreement with those of Brücke and Garten, extend over a wider range. These investigators have made exact measurements, but their intensity of stimulation has not been greatly varied. They find, as we have mentioned, for the shortest latent period of the negative deflection A 0.078 sec. and for the longest 0.099 sec.

Fuchs<sup>2</sup> gives much shorter latent periods than we do, but the method employed by Fuchs is, as shown by Gotch,<sup>3</sup> open to serious criticism.

The latent period of the darkening deflection  $A_1$  shows greater variations than that of the negative deflection A. With very strong illuminations the latent period of  $A_1$  is also very short, 0.04 sec. and less, even diminishing to 0.01 sec. With weaker illuminations it becomes longer, and since we can still obtain a summit  $A_1$  with very weak illuminations, there occur also very long latent periods of this summit.

In fig. 5 the latent period of  $A_1$  is about 0.2 sec., with the intensity of illumination of  $10^{-4} I_g$ . In fig. 14 the latent period of  $A_1$  has the value of 0.8 sec., with an intensity of illumination of  $10^{-7} I_g$ ; while in fig. 24, where an intensity of illumination of  $10^{-9} I_g$  is employed, the latent period attains the enormous value of 2.2 sec.

The latent period of the summit B, the reaction of the second substance, cannot be measured when illuminating with strong light, since the beginning of the wave is here masked by the reaction of the first substance. Where

<sup>1</sup> This relation has already been mentioned by Brücke and Garten, loc. cit.

<sup>2</sup> Loc. cit.

<sup>3</sup> Loc. cit.

a negative deflection A is present, the commencement of B cannot be pointed out.

On the other hand, when employing weaker light, where the deflection A fails the latent period of B can easily be determined. We have found, for

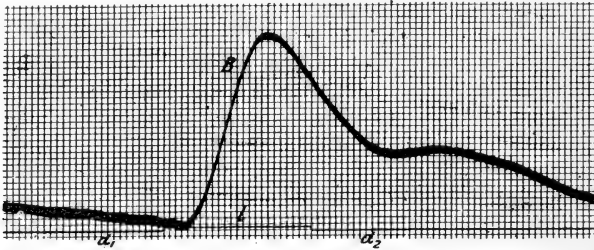


FIG. 23.—A strong reaction to a weak stimulus. Dark eye. Absc. 1 mm. = 0.2 sec. Ordin. 1 mm. = 2 microvolts. Green light. Intensity of illumination =  $10^{-9}$   $I_g$ .  $l$ , light;  $a$ , darkness.

instance, with a light intensity of  $10^{-5}$   $I_g$ , in a photograph not reproduced here, a value of 0.24 sec.

With weaker illumination this amount increases considerably. With  $10^{-7}$   $I_g$  (figs. 11, 12, 13, and 14) it is on an average 0.6 sec. In fig. 23, with  $10^{-9}$   $I_g$ , it is 0.8 sec.; and in fig. 24, where the lighting is also  $10^{-9}$   $I_g$ , it even reaches 2.1 sec.

Brücke and Garten<sup>1</sup> give for the analogous values amounts which lie

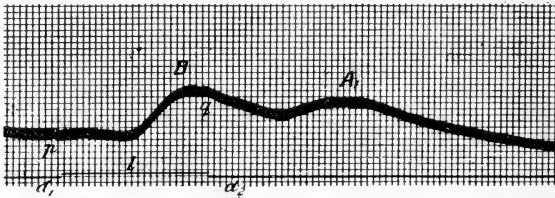


FIG. 24.—A long latent period. Dark eye. Absc. 1 mm. = 0.2 sec. Ordin. 1 mm. = 2 microvolts. Green light. Intensity of illumination =  $10^{-9}$   $I_g$ .  $l$ , light;  $a$ , darkness;  $p$ , slight jerk caused by movement of hand in closing circuit of electromagnet and of armature of magnet.

between 0.108 sec. and 0.244 sec. Gotch<sup>2</sup> mentions a minimum of 0.16 sec., and a maximum of 0.30 sec. When we consider that the duration of the latent periods is dependent in so high a degree upon the intensity of the light stimulation, it may not seem too rash to suppose, in accordance with the data supplied by these investigators, that the intensities of the light stimuli employed by them ranged between  $10^{-3}$   $I_g$  and  $10^{-5}$   $I_g$ .

<sup>1</sup> Loc. cit., pp. 312 and 315. See also Piper, loc. cit.

<sup>2</sup> Loc. cit.

The latent period of the wave C, the reaction of the third substance, is difficult to measure, since the commencement of the wave is always masked by the reaction of one or both of the other substances.

Mention must be made of the measurements of Waller, who, in opposition to Gotch and Brücke and Garten, observed latent periods which are as great or even greater than ours. Speaking of latent periods of 3, 5, and even 7 seconds, Waller says: <sup>1</sup> "Such an interval is altogether in excess of any possible physiological lost time, and highly suggestive of a period of hesitation during which two opposed currents were developed from the retina at nearly equal rates."

As Waller made use of a slowly acting Thomson galvanometer, he was entirely right in supposing that opposite forces were in question which compensated one another at first, while later one of them obtained the mastery. The forces assumed by Waller are realised in our first and second substances.

Waller's explanation, however, excellent though it may be when a slow galvanometer is used, does not hold good for the curves recorded by us, for, where these show great latent periods, the actions of the various substances are practically completely isolated, while our measuring instrument reacts almost instantaneously.

From figs. 11, 12, 13, 14, 18, 23, and 24, we are, therefore, compelled to conclude that, in fact, on weak stimulation a considerable latency occurs in the action of those parts of the retina which give rise to the development of electricity.

The latency found in the photo-electric reaction of the frog's eye is in complete agreement with the latency of light-perception in the human eye. As evidence for this we may quote the work of two astronomers which has not, so far as we know, been referred to up to the present in physiological literature.

Van de Sande Bakhuyzen <sup>2</sup> uses as lighting point a small opening in a copper plate, placed behind the flame of a petroleum lamp. Behind this opening there is a metal screen which can be quickly withdrawn by means of a strong spring. At the moment at which the margin of the screen passes the light point, a metal projection from the screen dips in mercury. In this way an electrical circuit is closed, by the aid of which a mark is written upon the moving strip of paper of a recording apparatus.

The observer at a distance of 25 metres from the point of light views the appearing of the light through a telescope. At the moment at which he perceives it he closes a second electrical circuit whereby another mark is recorded on the same strip of paper. The distance between the two marks measures the reaction time of the observer.<sup>3</sup>

In order to diminish the brightness of the light point, coloured glass is

<sup>1</sup> Loc. cit., p. 143.

<sup>2</sup> Arch Néerlandaises des sc. exactes et naturelles, sér. 2, t. 6, p. 727.

For a critical review of the literature relating to measurements of reaction time see Nyman, Skand. Arch. f. Physiol., Bd. 19, S. 365, 1907.

used, and also a pair of Nicol's prisms which can be rotated upon each other. The brightnesses used are expressed in stars of known magnitude, as these are seen by a telescope of definite dimensions. We reproduce a table which gives for different star magnitudes the reaction times of the observer H. B. in thousandths of a second.

Observer H. B.

	m.	m.	n.	m.	m.	m.	m.	m.	m.	m.	m.	m.
Star-magnitude	3·7	4·5	5·2	5·9	6·0	6·6	7·4	8·2	8·3	9·0	9·4	9·7
Reaction time	253	253	271	285	276	288	332	375	351	450	514	605

From these figures it is seen that for the observer H. B. the reaction time in the case of weak light of the star magnitude 9·7 m. is considerably greater than that of strong light of the star magnitude 3·7 m. The difference amounts to  $0·605 - 0·253 = 0·352$  sec.

If we assume that the light perception on stimulation by strong light takes place instantaneously, the latent period for the perception of the weak light here used amounts to 0·352 sec. As however the perception of strong light must also take some time, we must conclude that the latent period for the perception of the weak light here used is greater than 0·352 sec.

Bakhuyzen also mentions the results which have been obtained by some other observers in an analogous way. We would draw attention in particular to E. B. II. where, for a star magnitude 5·0 m., a reaction time of 0·279 sec. was found, and for a star magnitude 9·8 m. a reaction time of 0·815 sec., and who therefore shows a latent period for the perception of weak light which is greater than  $0·815 - 0·279 = 0·536$  sec.

Particular interest attaches to the observations of Pihl,<sup>1</sup> who has calculated the latency of light perception from direct observations of stars. For the methods employed by him we refer to his detailed communication, and need only mention here that Pihl finds, for the latent period of the perception of weak light, amounts which exceed a full second.

Considering what we have quoted it need not surprise us that we have been able to determine latent periods for the photo-electric reaction of the isolated frog's eye amounting to 2 sec.

#### IV. THE ENERGY OF THE STIMULATION IN ABSOLUTE MEASUREMENT.

It may not be devoid of interest to know the amount of energy of the radiation employed by us in absolute measurement. Our original intention to determine this amount by direct bolometric observations required to be given up for several reasons, but nevertheless we can form a fairly good idea of it if we attempt to calculate it in accordance with the known laws of radiation.

We can identify the crater of an arc light as regards its radiation

<sup>1</sup> The stellar cluster  $\gamma$  Persei micrometrically surveyed, Christiania, 1891.





Had our absolute radiations been exactly determined by the bolometer, it would have been worth our while also to measure  $p$  accurately. Since, however, we have based our calculations of the absolute radiation on the temperature of the crater, we may content ourselves with an estimation of  $p$ . Further, this estimation will suffice, because  $p$  is very small in comparison with the enormous ratios of the radiating energies employed by us, which have ranged from 1 to more than  $10^{10}$ .

Our estimation of  $p$  is based on a measurement which has recently been made by von Kries,<sup>1</sup> who gives the analogous loss in his spectroscopic arrangement as  $\frac{1}{0.548} = 1.825$ .

According to the above data the amount of the radiation entering the eye is calculated to be as follows:—

$$H_1(\lambda = 0.460) = 229$$

$$H_1(\lambda = 0.497) = 277$$

$$H_1(\lambda = 0.590) = 379$$

$$H_1(\lambda = 0.670) = 418$$

These results are represented diagrammatically in fig. 25. Here the wave-lengths of the normal spectrum are plotted in a system of rectangular co-ordinates as abscissæ, and the values of  $H_1$  as ordinates.

The radiation  $h$  of our three parts of the spectrum expressed in g. cal. per sec. is represented by the area of the three parts of the diagram:—

For blue, from  $\lambda = 0.460$  to  $\lambda = 0.497$  .  $h_b = 9.36 \times 10^{-4}$  g. cal. per sec.

„ green, „  $\lambda = 0.497$  to  $\lambda = 0.590$  .  $h_g = 30.5 \times 10^{-4}$  „

„ red, „  $\lambda = 0.590$  to  $\lambda = 0.670$  .  $h_r = 31.9 \times 10^{-4}$  „

To obtain a maximum of radiation in the eye the largest diaphragm is placed at  $D_2$ , viz. the diaphragm for green of  $9.5 \times 27.5$  mm.<sup>2</sup> The slit of the collimator and the prism are removed from the spectroscopic apparatus and the uncoloured image of the crater thrown directly on the diaphragm  $D_2$ . The crater image, which is about 20 mm. high and 32 mm. long, has an elliptic form and overlaps all margins of the diaphragm.

In order to calculate the radiation energy so obtained, we have to determine the part of the crater to which the rectangular diaphragm at  $D_2$  corresponds. If this is calculated for the image magnification, a rectangle of 1.9 mm. high and 5.5 mm. long is found as the piece cut out from the crater. It is by this rectangle, when we employ our maximum amount of white light, that the slit of the collimator is replaced. Its area is exactly five times larger than the area of the slit.

It is useless to calculate the radiation energy of the crater light for all wave-lengths together. First we must bear in mind that a considerable part of the ultra-red and ultra-violet rays is absorbed by the glass lenses

<sup>1</sup> Loc. cit., p. 391.

of the spectroscopic apparatus and the refracting media of the eye; and secondly, we must remember that these rays, although they reach the retina, do not practically exert here a photo-electric action.

We therefore may limit our calculation to rays of wave-lengths between  $\lambda = 0.460$  and  $\lambda = 0.670$ . The entire active radiating energy is then easily calculated to be 5 times the area of diagram (fig. 25), making an amount of  $h_w = 0.0359$  g. cal. per sec.

The photographs in our plate where the weakest radiations are employed are figs. 23 and 24. The strongest radiations have been employed in the case of figs. 6 and 7. The ratio between the weakest and the strongest radiation is  $10^{-9} h_g : h_w$ ; that is,  $1 : 1.18 \times 10^{10}$ .

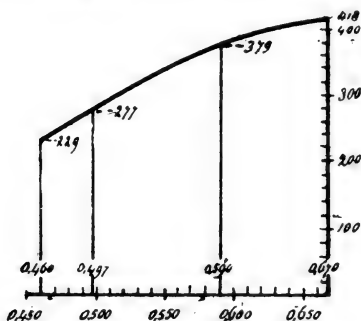


FIG. 25.—Diagram in which the wave-lengths of the normal spectrum are plotted in a system of rectangular co-ordinates as abscissæ, and the values of  $H_1$ —the radiation entering the pupil—as ordinates. The areas of the three parts of the diagram represent the radiation of the three parts of the spectrum used for illuminating in g. cal. per sec.

Previous investigators have already repeatedly demonstrated that the eye acts as a very sensitive reagent to light.

Waller<sup>1</sup> mentions that radiation by moonlight during not more than 0.01 sec. is able to evoke a strong action current, and that a distinct galvanometric deflection is caused when the eye is illuminated by a white card 10 feet distant from the eye which itself receives illumination from a standard candle 10 feet distant.

With regard to the sensitiveness of light perception in the human eye, we refer to the measurements of Langley, Grijns and Noyons and von Kries.

Langley<sup>2</sup> gives as the least energy required to cause a light perception when stimulating with green light

$$6.7 \times 10^{-17} \text{ g. cal.} = 2.8 \times 10^{-9} \text{ erg.}$$

<sup>1</sup> Loc. cit., p. 124.

<sup>2</sup> American Jour. of Science, p. 376, 1888.

Grijns and Noyons' <sup>1</sup> minimum is

$$0.95 \text{ to } 2.6 \times 10^{-18} \text{ g. cal.} = 0.4 \text{ to } 1.1 \times 10^{-10} \text{ erg.}$$

Von Kries <sup>2</sup> gives

$$3.1 \text{ to } 6.2 \times 10^{-18} \text{ g. cal.} = 1.3 \text{ to } 2.6 \times 10^{-10} \text{ erg.}$$

In fig. 23 we see the development of a fairly strong photo-electric reaction to a stimulus which uses  $30.5 \times 10^{-13}$  g. cal. per sec., which lasts for 4.84 sec., and thus possesses a total energy of  $1.47 \times 10^{-11}$  g. cal. In all probability there may be obtained a perceptible galvanometric deflection with a stimulus 10 times, and perhaps even 100 times weaker, but our experiments have not extended further in that direction. Nevertheless, it is sufficiently clear that for the photo-electric reaction of an isolated frog's eye, there is required much more light than for the development of a light perception in the human eye.

On the other hand, a comparison of the isolated frog's eye with the most sensitive bolometer constructed by human skill is greatly to the disadvantage of the latter.

#### V. THE RELATION BETWEEN THE ENERGY OF THE STIMULATION AND THE ENERGY OF THE REACTION.

Generally speaking, the rule holds good that for moderate and strong stimuli the energy of the reaction increases or decreases much less rapidly than the energy of the stimulus. Perhaps it is to be expected that here the Weber-Fechner law is true; that is to say, that when the stimuli increase in geometrical progression the reactions increase in arithmetical progression. This should be investigated for each of the three substances separately, which has not so far been done.

If very weak radiations are employed, the above-mentioned law does not hold good, as is sufficiently evident from the series of four photographs, figs. 11, 12, 13, and 14. Here the energy of reaction is increasing considerably, whereas the increase in the energy of radiation is but small. If the radiations are weakened still more, the reactions decrease relatively quickly in energy, and it is reasonably to be expected that under these conditions all perceptible galvanometric deflections will soon fail. The results of the experiments are in complete agreement with this expectation.

In reference to these problems, we may recall the work of de Haas,<sup>3</sup> who has confirmed the Weber-Fechner law within wide limits; but de Haas used a slowly deflecting galvanometer, so that the separation by him of the action of three substances was impossible. Presumably, his measurements with stronger stimuli relate principally to the action of the third substance, while those with weaker stimuli may have had reference to the combined action of the second and third substances.

<sup>1</sup> Engelmann's Arch. i. Physiol., p. 25, 1905.

<sup>2</sup> Loc. cit.

<sup>3</sup> Loc. cit.

In conclusion, we may institute a comparison between the absolute amount of the energy of the stimulus and that of the response.

We choose as an example fig. 23, where we have already determined the energy of stimulus as amounting to  $1.47 \times 10^{-11}$  g. cal. The energy of reaction must be calculated from the form of the curve. If the galvanometer is replaced by a wire of a small negligible resistance the current passing through it is  $A = \frac{V}{R}$  amp. Here  $V$  is the electromotive force in volts developed at each moment, while  $R$  is the resistance in ohms of the preparation. For the present we assume that  $V$  remains unchanged, when the resistance of the galvanometer is diminished. The energy of the reaction during the time  $dt$  is expressed by  $VAdt$ , the total energy of the reaction by  $W = \int VAdt$  or  $W = \int \frac{V^2}{R} dt$  Joule.

In the figure 1 mm. abscissa = 0.2 sec., 1 mm. ordinate = 2 microvolts. The resistance of the preparation is  $R = 9000$  ohms.

With the aid of the above data, the amount of  $W$ , as somewhat roughly calculated from the form of the curve, is  $W = 2 \times 10^{-12}$  Joule =  $4.8 \times 10^{-13}$  g. cal., and it is thus evident that in the case of fig. 23 the energy of the reaction is more than 30 times less than the energy of the stimulus.

Following on this result, there are good grounds for stating as a general rule that the absolute energy of the photo-electric reaction is always less than that of the light stimulus. It is true that we have to consider the possibility that in a curve which is recorded under other conditions, the energy ratio might be altered in favour of the photo-electric reaction, but in our collection of photographs, taken in very varying circumstances, we have not found an example of this.

The curve (fig. 23) has been chosen just because its energy ratio is specially favourable to the photo-electric reaction.

In judging of the energy of the photo-electric reaction, we have to take into account that there exists short circuiting in the eye itself, and that the current measured by the galvanometer is presumably only a small part of the current passing through the eye.

This last current is not easily determined, so we shall take, as is usually done, the potential difference or the current, as these are measured by an instrument outside the eye, to be the real photo-electric reaction. In our above calculations we have assumed that the electromotive force developed by the eye remains unchanged when the resistance of the galvanometer is diminished, and we have assumed the amount of this resistance to be equal to zero. Both assumptions were made for the purpose of calculating the possible maximum of the reaction energy for this special case. If we take the resistance of the galvanometer into account, we find for the reaction energy an amount which is 1.75 times less, the resistance of the galvanometer being 6800 ohms.

The photo-electric reaction of the eye is, in regard to the energy ratios,

comparable with the electric reaction of a nerve or a muscle, not with the mechanical reaction of the latter, for the muscle in contracting is able to develop a quantity of energy which far exceeds that of the stimulus.

## VI. SUMMARY OF CONCLUSIONS.

1. The photo-electric reaction of an isolated eye is specially adapted to the study of the effect of stimuli of very different intensities. In our experiments light stimuli have been used whose energy varies from  $3.05 \times 10^{-12}$  to  $3.95 \times 10^{-2}$  g. cal. per sec.

Short illumination with the strongest light has not been found to damage the eye, while the weakest light was capable of producing a fairly considerable photo-electric reaction.

2. The form under which the photo-electric reaction manifests itself under different conditions gives ground for the supposition that there occur in the eye three separate processes, and each of these may be dependent upon a separate substance. We speak of three substances for the sake of convenience.

3. The first substance reacts more rapidly than the other two. On lighting it develops a negative, on darkening a positive potential difference. Its action comes strongly into prominence in a light eye and appears almost unmixed on sudden darkening of short duration (a flash of darkness).

The second substance reacts less rapidly than the first, and in an opposite sense. On lighting it develops a positive, on darkening a negative potential difference. Its action appears almost unmixed in a dark eye which is illuminated for a short time with weak light.

The third substance reacts in the same sense as the second, but much more slowly. Its action fails in a completely light eye, and also in a dark eye which is illuminated very weakly for a short time.

4. The latent period of the photo-electric reaction is in a high degree dependent upon the intensity of the stimulus. With strong stimuli it is of the order of 0.01 sec., while with very weak stimuli it may be lengthened to more than 2 sec. These values are in agreement with the latent periods of light perception in the human eye.

5. For each of the three substances the rule holds good that with moderate and strong light the energy of the stimulus increases much more quickly than the energy of the reaction.

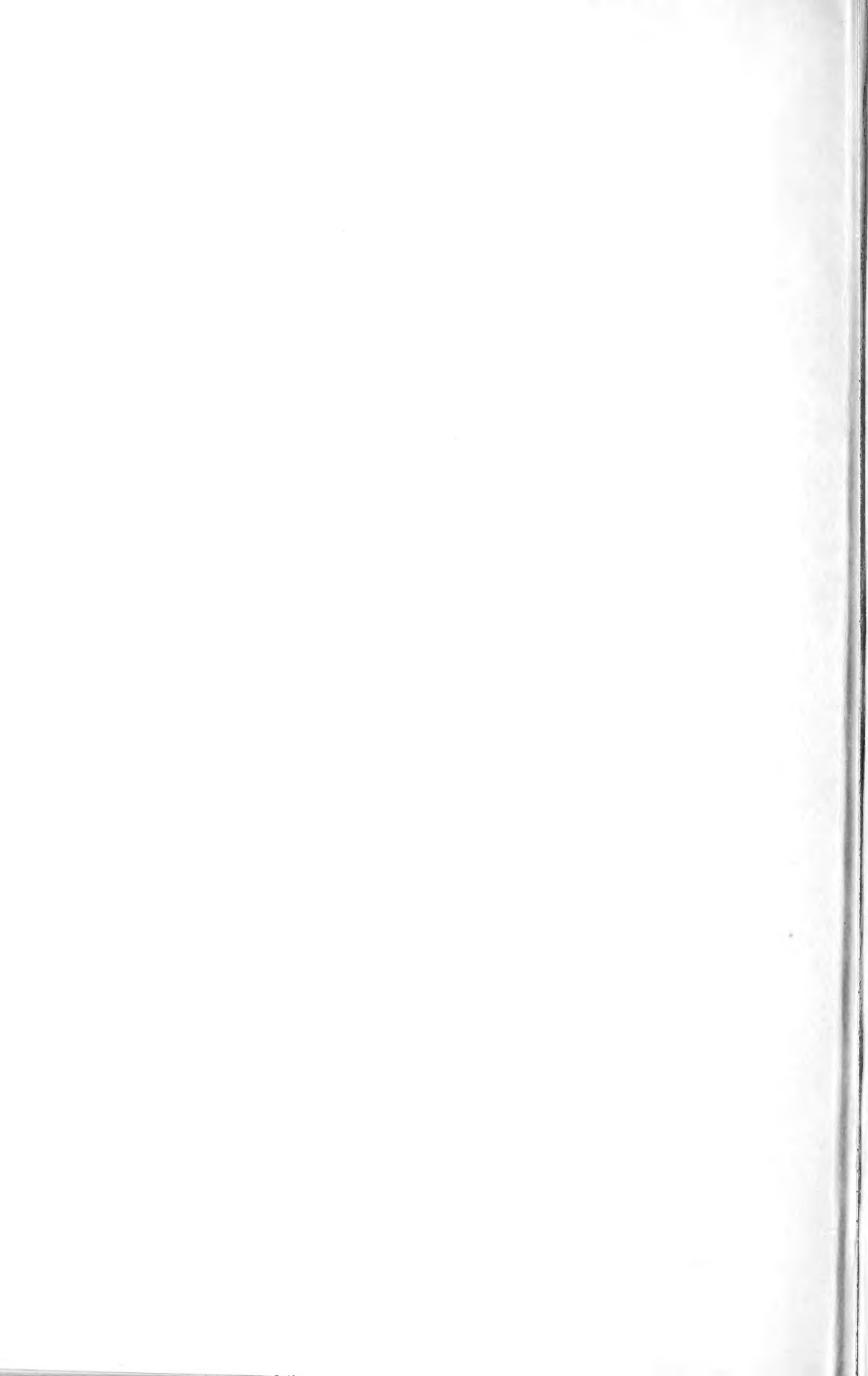
6. Although the eye is far more sensitive than the most sensitive artificial bolometer, the energy of the reaction remains below that of the stimulus even in the most favourable circumstances. The photo-electric reaction of the eye is in this respect comparable with the action current of a muscle or nerve.











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