

STUDIES ON THE COMPARATIVE PHYSIOLOGY
OF THE HEART.

by

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ON THE BASIS OF CARDIAC AUTOMATICITY.

Historical Introduction:

From the time of Harvey until the middle of the nineteenth century practically nothing that was fundamental had been added to our knowledge of the physiology of the heart - a circumstance which is the more remarkable considering the stimulus to investigation in this very field which the publication of Harvey's immortal work (1) should have afforded. The mid-nineteenth century, however, saw the commencement of investigations along several distinct lines, the results of which were to lead to a greater advance in our knowledge of the heart in the succeeding sixty years than previous hundreds had witnessed. This new era, which we may take as having been initiated by the work of Remak, Stannius and the brothers Weber, has continued to the present day, and as it concerns aspects of cardiac physiology with which it is the business of this paper to deal - the question of the cause of the heart beat and of the cause of the effects produced by the extrinsic regulatory nerves - a brief consideration of the historical aspects of these questions may not be amiss.

On the cause of the heart's motion various theories have been propounded at different times, some of which must appear as fantastic to the modern mind as modern/

modern theories will to the physiologist of three hundred years hence. Descartes (2), for instance, applying as mechanistic a view as was possible in his day was of the opinion that the beats of the heart were due to a succession of explosions brought about by the accumulation of steam inside the organ. Sylvius (3), considering the matter more from a chemical point of view, concluded that the motions were due to an effervescence arising from the intermixture of alkaline blood with the acid chyle. Stahl (4), on the other hand, avoided the difficulties inherent in both theories by attributing the cardiac movements to a continual activating and guiding influence of the immortal soul! Haller (5), though probably sufficiently vitalistic in outlook to accept such a view as the latter, held that the heart beat was a direct result of stimulation of the inner surface of the heart by the blood, and this view held practically unchanged until the rise of two opposed schools of thought in the late nineteenth century.

The discovery by Remak (6) of that ganglion in the sinus venosus which now bears his name, was followed by Ludwig's (7) demonstration of a similar collection of cells in the interauricular septum, and by that of Bidder (8) who showed a double mass of ganglion cells at the junction of the auricles and ventricle. These discoveries marked the birth of the neurogenic/

neurogenic theory of the heart beat, a theory which seemed to find its greatest support and indeed its final proof in the classical experiment of Stannius (9).

According to this theory in its early form Remak's ganglion sent rhythmic discharges into the otherwise non-rhythmic muscle of the sinus venosus: from here the impulse was conducted by presumably nervous paths to the ganglion of Ludwig where it suffered a delay before passing to the auricular musculature on the one hand and the ganglion of Bidder on the other, passing finally from the latter ganglion to the ventricular musculature. The proposed delay in the passage of the impulse from the lower ganglia to the muscles subserved by these assured the normal sequence of contraction in each cycle. Suppression of Remak's ganglion caused impulse initiation to be assumed by the less highly automatic Ludwig centre, while suppression of this again led to Bidder's ganglion assuming this function - an interpretation of Stannius's experiment which was as striking as it appeared complete.

Experiments performed by Eckhard (10), and by Foster and Dew Smith (11), who showed that the ganglion free ventricular apex would beat rhythmically if stimulated by a constant current, and experiments of Bowditch (12) and Merunowicz (13), who showed that/

that a similar result was obtained by subjecting the apex to the constant stimulus afforded by the pressure of some nutrient fluid, caused the upholders of the motor ganglion point of view to withdraw the conception of Remak's centre as exerting its effect by a discontinuous or rhythmic action and substitute instead the view that it produced its rhythmic effect by a continuous stimulating action.

The "neurogenists", though they were of course aware that rhythmicity could be conferred by stimulation, made a fundamental error in failing to recognise the importance or indeed the existence of rhythmicity as an inherent function of cardiac muscle, and it is the merit of Engelmann (14) and of Gaskell (15), especially the latter, to have built up a theory of the heart beat which was in opposition to the neurogenic view in that it considered the heart beat to be the expression of an inherent rhythmicity of cardiac tissue rather than a rhythmicity superimposed by a stimulus of nervous origin.

Gaskell's work illustrates how valuable is the broad outlook even for one concerned with what might be considered as narrow and particular problems. From the fact that most muscles may be made to exhibit rhythmic contractions under certain conditions he came to regard rhythmicity as a primitive character of/

of such tissue showing that the more primitive forms of muscle have this property in greater degree than have those forms which are more highly developed in which, presumably, rapidity of contraction has been gained at the expense of rhythmicity. Considering the heart from a phylogenetic and ontogenetic standpoint he was able to correlate the degree of modification of the original cardiac tube with the rhythmical power of the different parts of the heart, showing that rhythmicity was greatest in those parts where the original musculature had been least modified, that is to say in the sinus venosus, the auriculo-ventricular junction and the conus arteriosus. He showed further that the conduction of the impulse from the sinus to the ventricle did not take place along nervous paths as had been supposed, but that nevertheless conductivity like rhythmicity was subserved in different degree by different parts of the heart.

While it is not our purpose to enter into a discussion of the controversy between the upholders of the neurogenic and the myogenic theories*, a conception arose from the work of Gaskell and Engelmann which in point of fruitfulness must rank next to the myogenic view itself - though this will be disputed by/

* A full account will be found in the recent monograph of Fredericq (16), and in the paper of Haberlandt (17).

by some. This is the view that cardiac tissue is possessed of five fundamental properties or functions - excitability, rhythmicity, conductivity, contractility and tonus - and though Engelmann's view that these five attributes are distinct in "time and space" may not, as Lewis (18) observes, prove to be true, nevertheless the most recent work on the physiology of the heart seems in respect at least of the three main functions - rhythmicity, conductivity and contractility - to have justified the hypothesis in a remarkable manner.

The stimulus given to the study of the heart by this work on the lower Vertebrates led to the morphological discoveries of Kent (19) and His (20), and the later and equally remarkable discoveries of Tawara, Keith and Flack in the mammal.

That the bundle of specialised tissue, connecting the auricles with the ventricles, discovered by Stanley Kent and by His, was intimately concerned in the conduction of the cardiac impulse from auricles to ventricles was soon proved (21-23). Following this discovery came the demonstration by Tawara of a collection of special tissue differing from ordinary cardiac muscle lying near the base of the interauricular septum and forming the commencement of the auriculoventricular bundle of Kent and His, and in the/

the same year Keith and Flack (25) discovered a similar collection of tissue lying in the sulcus terminalis between the inferior and superior venae cavae and the right auricle. To these last two tissues were given the names auriculoventricular and sinoauricular node respectively, and at the outset the latter was regarded by its discoverers as a remnant of the sinus venosus and therefore probably of importance from a functional standpoint.

The announcement of these discoveries led a host of investigators to attack the physiological problems presented by these tissues. A complete account of such work is to be found in the admirable review by Eyster and Meek (26) and we will content ourselves by stating the general conclusion which may be drawn from the investigations, which is that the normal seat of impulse initiation in the mammalian heart is the sinoauricular node: that the impulse arising here spreads by way of the auricular musculature to the auriculo-ventricular node where it suffers a delay before passing finally by way of the bundle of His to the ventricular musculature. Suppression of the activity of the sinoauricular node leads to impulse initiation being taken up by the less automatic auriculoventricular node, while suppression of this in turn causes some part of the "special system" in the ventricles to act as centre for the production of the/
the/

the idioventricular rhythm.

This last work is of great interest for not only has it brought the physiology of the mammalian heart into line with that of the frog as regards impulse initiation but it has given morphological support to Engelmann's views on the discreteness of the fundamental attributes of cardiac tissue in so far as it has shown that not only rhythmicity but conductivity also is subserved, mainly at least, by distinct tissues.

We may consider that of the three main functions of cardiac tissue the condition of our knowledge, as a result of the work we have rapidly reviewed, was more complete in respect of contractility and conductivity than as regards automaticity - as far at least as the nature of these functions was concerned. The essentially physical nature of conductivity and even of contractility led to views regarding the nature of these phenomena which we need not discuss, but which, because they were mechanistic were satisfying if only provisional. Although the environmental conditions which determined cardiac automaticity had been determined with some degree of accuracy - thanks to the stimulus afforded by the work of Ringer (37) - and though the myogenic view was generally adopted, no real advancement as regards insight to the causative factor of rhythmicity in the/

the tissue itself, its essential nature or basis, had been obtained; and though there were theories enough and to spare, it seemed that on a last analysis rhythmicity was to be regarded, like irritability itself, as a primitive attribute of living matter - a truly vitalistic phenomenon, a mechanistic interpretation of which was unthinkable!

While it is perhaps true philosophically speaking that we can never know the ultimate nature of any process, to show that a physiological phenomenon has a physical (or chemical) basis and to determine what that basis is, is to advance the problem far indeed towards the goal of the general physiologist, even if it merely, as it usually does, transfers the problem from being one of the mode of action of one tissue on another tissue, to being a problem of the mode of action of some chemical substance or some physical process on that tissue.

The rapid and indeed sensational advance of endocrinology and the consequent demonstration of the comparatively simple chemical basis of some of the most complicated of physiological processes led to the evolution of a new viewpoint and a new technique in physiological research - hormonal, indeed, in more senses than one. Investigations in this new field by this new technique left practically no organ in the/

the body untouched and so the heart, in common with the other organs, came in for its due share of attention.

Soon after the appearance of Loewi's work, which will be reviewed in a later section of this paper, articles appeared almost simultaneously from three different laboratories describing experiments which were interpreted as showing that the rhythmicity of cardiac tissue was due to the action on the otherwise non-rhythmic myocardium of substances produced by, or at least acting through, the special (nodal) system of the heart.

Thus Demoor (39), Professor of Physiology in the University of Brussels, showed that non-rhythmic portions of mammalian cardiac muscle could be made to beat rhythmically if bathed in Locke solution to which had been added an extract of the sinoauricular node, and he concluded from this that the automatic rhythmicity of the heart was dependent on the continuous action on the myocardium of what he called the "substances actives", produced by the sinoauricular node, and, as demonstrated later, found also in the node of Tawara, bundle of His, and the Purkinje tissue of the subendocardium.

Haberlandt (40), of Innsbrück, working independently of Demoor and in ignorance of the latter's work/

work, showed that the sinus venosus of the frog's heart produced a substance - "Herzhormon" - which would initiate rhythmic contractions in the long perfused and quiescent frog ventricle, and to which therefore the normal automaticity of the heart was presumed to be due.

Finally, Zwaardemaker (41), of Utrecht, working mainly with the eel's heart, came to the conclusion as a result of experiments commenced as far back as 1916, that the automaticity of cardiac tissue was due to the presence in the heart of substances which he called "Automatins" which were produced in various parts of the body by the action of the β rays of potassium on an inactive precursor which he called "automatinogen". These automatins were supposed to be concentrated in the special system of the heart, and to work, as it were, from this system as a base.

Thus was initiated what has now generally come to be known as the work on the "heart hormones" - an inaccurate though convenient term by which to designate the various substances described by the different workers.

It is the purpose of this paper to review the work of each of the three schools, to add the author's own observations, to discuss the whole critically, and, if possible, assess the value and significance of this work.

THE SCHOOL OF DEMOOR.The basis of Demoor's work.

Though the fact that some parts of the heart remained longer in rhythmic activity after death than others was known to Galen and to Haller, studies on the relative automaticity of different regions of the mammalian heart assumed a fresh significance after the discovery of the "nodal system", and it is on the basis of a generalisation which Demoor made regarding the relation of rhythmicity to the nodal system that his work rests.

Early studies on relative automaticity seemed to indicate that the mammalian left auricle was decidedly inferior to the right in respect of this spontaneous activity. Frédéricq (142), for example, showed that while functional separation of the right auricle from the interauricular septum abolished the effects of the right auricle on the rest of the heart, which then took up a "nodal" rhythm, separation of the left auricle from the interauricular septum usually resulted in quiescence of this chamber. Similar results were obtained by Hering (143) and by Erlanger and Blackmann (144). The latter workers in addition to investigating the automaticity of different regions of/

of the heart by producing functional separation by cutting, crushing or otherwise, employed strips of muscle from different parts suspended in a nutrient medium. They found that while automaticity was exhibited in high degree by strips from the right auricle - and the more so the nearer were the parts taken to the venae cavae - strips from the left auricle, separated from the right auricle and from the interauricular septum, rarely showed any rhythmic activity. Langendorff (145), and Mathieu (146) made similar observations with like results.*

In 1923 Demoor (148) published a paper confirming and extending these findings. He showed that while the isolated right auricle placed in oxygenated Locke solution at a suitable temperature will beat rhythmically for several hours, the left auricle under exactly similar experimental conditions behaves differently, exhibiting an irritability which differentiates it completely from the left auricle in situ and from the isolated right auricle. The left auricle under such conditions undergoes variations in tone which are followed by a period of insensibility to electrical stimuli, following which is a period when it responds to faradisation by a tetanus, or to single shocks or series of shocks by single contractions/

*See, however, Moorhouse (147).

contractions or groups of contractions which may lead to fibrillation, which latter may only disappear long after cessation of the stimulus. At the end of from half to one hour's immersion during which it remains passive, groups of irregular unequal contractions may arise spontaneously but these never become rhythmic. The left auricle as above is

insensitive to adrenaline and ^{to} changes ⁱⁿ ~~to~~ the Ca ion concentration of the irrigating fluid.

Demoor (149) as a result of his experiments on the left auricle concluded that the irregular aperiodic contractions arising in this tissue when it is isolated from the heart are the expression of the inherent automaticity of cardiac muscle, and that rhythmicity was probably to be regarded as superimposed on this inherent activity by some influence exerted by the nodal system.

This view received confirmation from later work. Porter (150), as far back as 1897, had shown that ventricular strips would beat rhythmically if suitably irrigated. Tausig and Meserve (151), and Demoor and Rylant (152) showed that this was only so if the ventricular strips contained subendocardial tissue. If this latter was removed the strips behaved after the manner of the left auricle. A similar observation had already been made by Ishara and Nomura (153), who showed that ventricular cells beat rhythmically as/

as long as they were in connection with Purkinje cells but ceased so to behave when separated from these. Finally Deloyers (154) demonstrated that if strips of the right auricle were deprived of nodal tissue they behaved in every way like the isolated left auricle.

This work, then, demonstrating as it did the fact that in nocase where any cardiac tissue was devoid of, or had been experimentally deprived of nodal tissue or of its connection with some part of the nodal system, did the spontaneous contractions which ultimately arose in such tissues become rhythmic, gave support to Demoor's contention that the irregular aperiodic activity of such preparations was in fact the expression of the inherent automaticity of cardiac muscle.

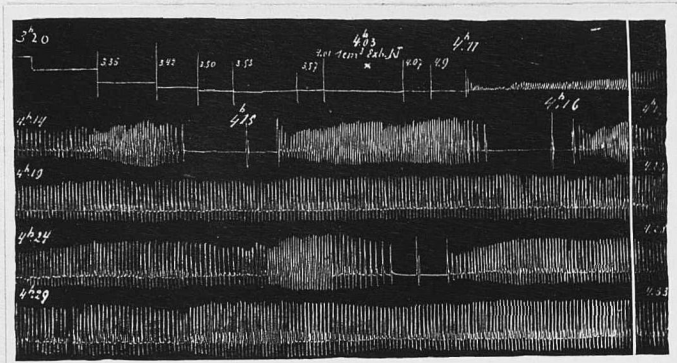
Further presumptive evidence in favour of this view was afforded by its capability of providing an explanation of Munk's classical experiment. Munk (155) showed that mechanical stimulation of the internal face of the frog ventricle produced a simple response when the stimulus was applied at the apex and a rhythmic response when applied at the base. At the apex of the ventricle is pure myocardial tissue while at the base is part of the "special" system; thus Demoor (156) interpreted Munk's findings/

findings as bearing out his view that only where myocardial tissue is found in conjunction with part of the nodal system is the production of rhythmical activity normally possible.

Discovery of the "active substances".

In 1921 Demoor (157) found that alcoholic and aqueous extracts of the ventricle, left auricle and inferior part of the right auricle of the dog's heart either did not influence or diminished the rhythm of the isolated perfused rabbit heart, whereas a similar extract of the nodal region of the right auricle intensified the beat. Experiments (158) followed on these extracts where instead of the isolated perfused heart the irrigated right auricle of the rabbit was used, and it was but a natural step to determine the action of this active substance on the left auricle the behaviour of which he was at this time studying.

Thus it was found (159), that if to a bath of Locke solution, oxygenated and at body temperature, containing the isolated left auricle of a rabbit exhibiting its typical arrhythmic activity, is added an extract of the sinus region of the heart the auricle after a variable latent period commences to beat rhythmically and behave in every way like the isolated right auricle, the rhythm being maintained with or without short intermissions until such time as

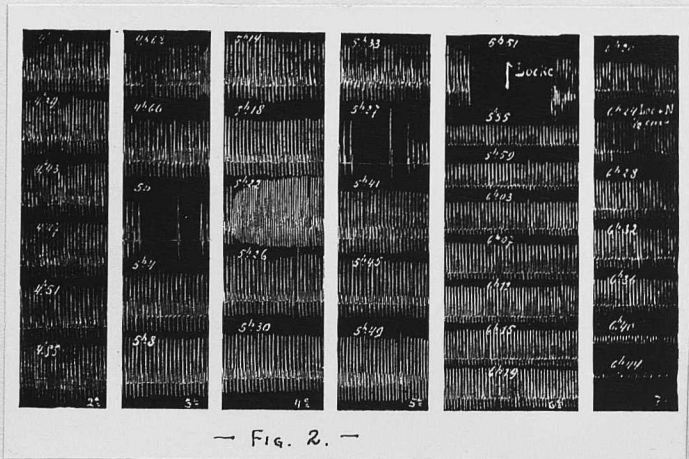


— Fig. 1. —

Fig. 1.

LEFT AURICLE OF RABBIT.

Auricle placed in bath at 3 pm: FALL in tone pronounced by 3:35. NOTE CHARACTERISTIC ARHYTHMIC BEHAVIOUR: AT 4:3 SINUS NODE EXTRACT (RABBIT) ADDED TO BATH. RHYTHMIC ACTIVITY COMMENCES AT 4:14 and continues with intermissions and with variations in chronotropism.



— Fig. 2. —

Fig. 2.

SAME EXPERIMENT AS ABOVE.

TRACING READS IN BANDS, COMMENCING AT TOP IN EACH CASE. NOTE INTERMISSIONS IN RHYTHM AND IN CHRONOTROPISM. AT 5:51 FRESH LOCKE SOLUTION ADDED TO BATH, WITH CONSEQUENT DIMINUTION IN INOTROPISM. AT 6:25 FURTHER SINUS-EXTRACT ADDED, WITH RESULTANT AUGMENTATION OF INOTROPISM. AT 6:40 OXYGEN SUPPLY STOPPED AND THE BEAT SOON VANISHES.

as the substance or substances responsible for it disappear or are destroyed, when the auricle immediately reverts to its previous behaviour. This is the fundamental experiment of Demoor.

Further work showed that the "active substances" existed elsewhere than in the region of the Keith Flack node. Thus Demoor and Rylant (160) showed that the isolated left auricle or strips of ventricular muscle devoid of subendocardium could be made to beat rhythmically by means of an extract of the ventricular subendocardium, the inference being that the active substances responsible for this result were resident in the terminal ramifications of the bundle of His - in the Purkinje fibres. Mlle. Hoebaers (161) obtained active extracts from the node of Tawara and from the upper part of the auriculo-ventricular bundle. It was found by Laubry, Walser and de Glaude (162) that extracts of the Keith Flack and of the Tawara nodes produced similar effects on the isolated perfused heart of the rabbit, and this was interpreted as confirming the identity of the substances extracted from these different sites. Demoor (164) quotes Kemal, Djenab and Mouchet (163) as having found the "active substances" in the general bundle of His, but this would appear to be an error. These authors simply showed that an extract of the bundle of His produced a fall in arterial pressure on intravenous injection to mammals.

FURTHER PROPERTIES OF THE "SUBSTANCES ACTIVES"

While the main property of the active substances derived from the sinoauricular and auriculoventricular nodes and from the Purkinje tissue of the subendocardium, is to confer rhythmicity on otherwise arrhythmic portions of myocardium, the extracts have other properties.

It was observed early in this work that the extracts were only active in initiating rhythmic activity in the left auricle preparation if they were very fresh. The active substance seemed to be very rapidly destroyed in dilute solution - within several hours - but while such extracts failed to produce rhythmic beating they permitted adrenaline to initiate such activity whereas normally the isolated left auricle is insensitive to the action of this autacoid. The response to adrenaline after "sensitisation" of the auricle by means of an otherwise inactive extract was usually very similar in nature to the normal response to an active extract, but the rhythm was generally not maintained for so long as with the nodal extract. The cessation of the rhythmic activity induced by adrenaline, was as in the case of the activity induced by active extracts, usually sudden, and a renewed activity could often be elicited by a further addition of adrenaline.

It/

It was further shown by Demoor and Rylant (165), (166), that if fresh extracts were heated to 60-63° Centigrade for some minutes the power to initiate rhythmic contractions was lost, but the ability of such extracts to sensitise the left auricle to adrenaline was only destroyed on further heating to from 70 to 73° Centigrade. From this and from the preceding observations it was concluded that in the extracts there were probably two substances of importance from the point of view of rhythmicity - hence the change in nomenclature from "substance active" to "substances actives". The adrenaline sensitising part of the extract, it should be noted, permits changes in the Ca concentration of the irrigating fluid to exert their normal effect on the left auricle whereas otherwise the preparation is insensitive to such changes.

The effects described above were obtained with alcoholic as well as aqueous extracts of nodal tissue though the latter always proved the more satisfactory. Also, while heating to 60° destroyed the property of the extract to produce rhythmic activity in the isolated left auricle, heating to 50°C. was found by Demoor to improve the normal action of the extract. This was thought to be the result of the destruction of some inhibitor or toxic substance.

We/

We may summarise the results obtained by Demoor and by Demoor and Rylant in their studies on the active substances extractable from the nodal tissue of the heart by stating that these substances have the following properties:- They have no zoological specificity; are soluble in alcohol and water but not in ether; are rapidly destroyed in dilute aqueous solution; are incompletely dialysable; they lose the property of conferring rhythmicity on the isolated left auricle when heated to 60°C ., and their property of sensitising the left auricle to adrenaline and to changes in the Ca concentration when heated to 70°C .

We must now consider the interpretations which Demoor places on his experiments, and to do so we cannot do better or be fairer than by quoting Demoor's own words from one of his latest publications (167).

Demoor's theory of the heart beat.

From the early experiments it was deduced that "le myocarde ne bat rythmiquement que lorsqu'il est morphologiquement associé au noeud de Keith, au noeud de Tawara, au faisceau de His ou aux éléments de Purkinje du sous-endocarde, soit donc à l'une ou l'autre partie du système nodal", and that if cardiac tissue is deprived of or is devoid of such connection "une capacité réactionnelle fondamentale y éclôt, donnant lieu/

lieu à des secousses apériodiques qui ne se transforment jamais spontanément en travail rythmé".

The left auricle was shown to be a tissue poor in or devoid of nodal tissue and further experiments showed that an extract of nodal tissue had the property "de rythmer, par mécanisme humoral, le travail désordonné et apériodique du myocarde de l'oreillette gauche".

Finally Demoor comes to the conclusion that:-
 "de l'ensemble de ces expériences il résulte que le sinus entraîne la modification du travail initial du myocarde* par l'intermédiaire d'un agent actif, issu de son intimité, transmis à l'ensemble des fibres contractiles, et agissant humoralement sur ces dernières. Ces substances nodales sont nommées "substances actives"..... dont la permanente intervention et la régulière distribution sont assurées par le système nodal".

The essential point in Demoor's theses and one which he reiterates time and again in his publications, is the essentially "humoral" action of the active substances. The high automaticity of the nodes and the automaticity of the Purkinje fibres is due presumably to their high content of active substances in/

* That is to say the fundamental arhythmic activity of pure myocardial tissue.

in view of their function as producers or distributors of these substances, and all other parts of the heart are only rhythmic so long as and in so far as a supply of active substances from the nodal tissues is acting on these other parts, the substances exerting their action through humoral channels. The isolated left auricle beats rhythmically when bathed with an extract of the nodal tissue because then it is under the influence of active substances as it is in the heart in situ.

This then, in outline, is Demoor's theory of the heart beat. Further discussion of this most interesting theme, and a consideration of further experiments which have been interpreted as supporting it, will be postponed until we have briefly reviewed the work of Haberlandt and Zwaardemaker and stated the results of our own few experiments.

THE SCHOOL OF HABERLANDT

THE "HERZHORMON" or "HORMON DER HERZBEWEGUNG".

Experiments with "sinus Ringer"!

Haberlandt's demonstration (168) that Ringer's solution in which the sinus venosus of a frog heart had been allowed to beat would produce a positive chronotropic effect on a frog ventricle to which it was applied, formed the starting point of his work on the heart hormone.

In the performance of this experiment the ventricle was separated from the rest of the frog's heart and suspended on a Straub cannula. The sinus venosus was freed from blood and placed in about one cubic centimetre of ringer fluid in the which it was permitted to beat. It was then found, that if to the slowly beating ventricle is added part of the "sinus-ringer" the rate of beat of the ventricle is increased. ^(Fig. 3.) While the rate of heart usually reverts to its normal value on replacement of the "sinus ringer" by the normal fluid, Haberlandt found that in some cases the chronotropic effect persists long after the sinus ringer has passed from the heart. Further, in many cases the sinus ringer produces no effect on the/

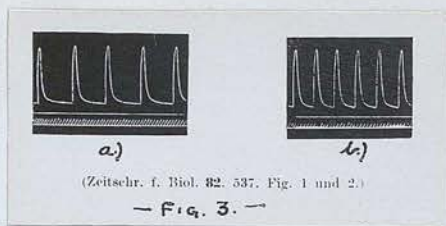


FIG. 3.
EFFECT OF "SINUS-RINGER" ON CHRONOTROPISM.
a) NORMAL. b) AFTER ADDITION OF SINUS-RINGER.

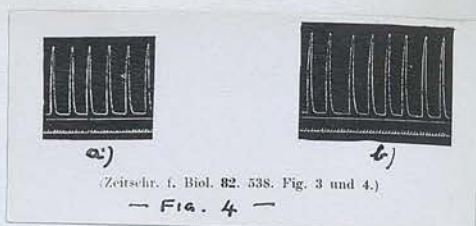


FIG. 4.
EFFECT OF SINUS-RINGER ON INOTROPISM.
a) NORMAL. b) AFTER ADDITION OF SINUS-RINGER.

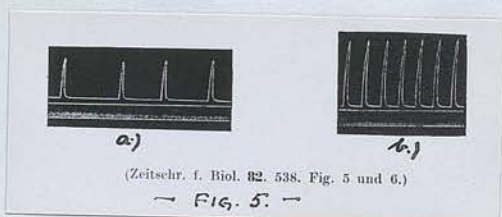


FIG. 5.
EFFECT OF SINUS-RINGER ON CHRONOTROPISM AND INOTROPISM.
a) NORMAL. b) AFTER ADDITION OF SINUS-RINGER.

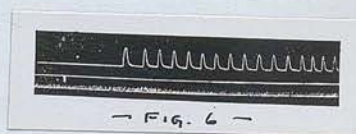


FIG. 6.
EFFECT OF SINUS-RINGER ON QUIESCENT VENTRICLE.
SINUS-RINGER ADDED AT SIGNAL.

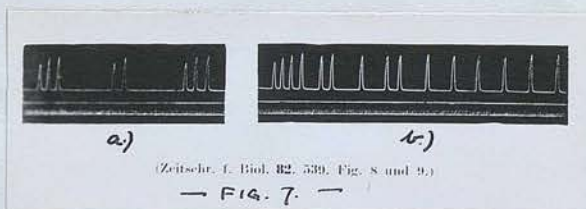


FIG. 7.
EFFECT OF SINUS-RINGER ON GROUPED BEATS.
a) NORMAL. b) AFTER ADDITION OF SINUS-RINGER.

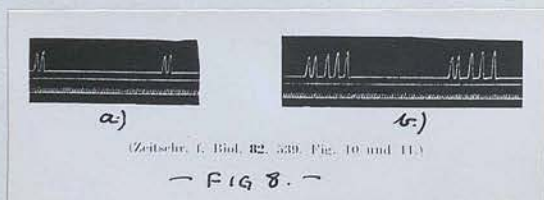


FIG. 8.
EFFECT OF SINUS-RINGER ON GROUPED BEATS.
a) NORMAL. b) AFTER ADDITION OF SINUS-RINGER.

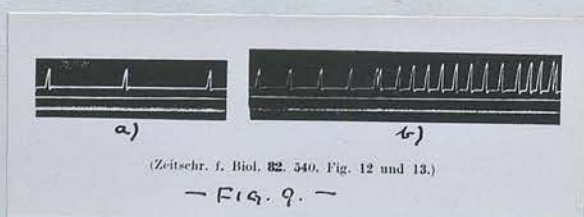


FIG. 9.
EXTRASYSTOLES INDUCED BY SINUS-RINGER.
a) BEFORE ADDITION AND b) AFTER ADDITION OF SINUS-RINGER.

the chronotropism but a pronounced increase in the force of the ventricular contractions (fig. 4), and finally, the positively inotropic and chronotropic effects are sometimes exhibited together (fig. 5).

Not only does "sinus ringer" affect the beating ventricle but it also affects the long perfused and quiescent ventricle, causing it to recommence beating. On changing to normal perfusion fluid the rhythmic activity usually ceases, but in some cases the effect initiated by sinus ringer persists. In figure 6 is shown a case where rhythmic beating was reinstated in a quiescent ventricle on addition of sinus ringer. In this case the effect had already been obtained three times.

The effect of sinus ringer on the irregularly beating ventricle - for example, on the ventricle which is giving rise to groups of beats - may be to reduce the interval between the groups, to increase the number and force of the beats in a group, or, by carrying the process still further, cause the irregularities to disappear altogether. These effects, like those previously described, may persist after replacement of sinus ringer with the normal fluid. Effects of sinus ringer on the irregularly beating ventricle are shown in figs. 7 to 9. In the last it will be noted that two extrasystoles are shown. Haberlandt found that spontaneous extrasystoles often occurred/

occurred as a result of the action of sinus ringer.

It is stated that all the experiments were controlled (168)(177). In these just described the small changes in intracardiac pressure incidental to the changing of the experimental solutions often gave rise to changes in the beat, but these were of short duration and could be easily distinguished from the specific effects of sinus ringer. The sinus ringer effects had always a longer latent period and lasted longer than did the effects due to such mechanical causes. On some occasions the pressure effects were completely lacking, while in others again the effects were in the opposite sense to those produced by the sinus-ringer. In all the experiments sinus ringer and normal ringer were used alternately and while the normal ringer might, for example, give rise to isolated beats in the quiescent ventricle the "pulse-initiating" influence of sinus ringer was expressed in the production of a still greater number of contractions.

In view of the possibility that the effects obtained with sinus ringer were due to the non-specific products of the sinus muscle metabolism such as carbonic or lactic acid, changes in hydrogen-ion concentration or of ion ratios, Haberlandt investigated the effects of such substances and changes on the non-rhythmic ventricular apex and found that although the apex was capable of entering into rhythmic activity/

activity in response to rhythmic electrical stimulation none of the substances or changes investigated would in fact initiate such activity. In view then of the "pulsauslösenden", "pulsbeschleunigenden" and "pulsverstärkenden" properties conferred on ringer solution by the presence therein of a beating sinus, and in view of the fact that the effects were presumably specific and not due to the non-specific metabolites produced by the activity of the muscle, Haberlandt came to the conclusion that in the sinus of the frog heart was produced a specific regulating substance which might be considered as a hormone, in the narrower sense of that word: "dass der im Sinus des Froschherzens entstehende Erregungsstoff von spezifischer Art und demnach als Hormon im engeren Sinne des Wortes zu betrachten ist....."

To this substance he gave the name "heart-hormone" (Herzhormon) or "hormone of the heart movement" (Hormon der Herzbewegung).

Haberlandt stresses the fact that he has never suggested that normally the "Sinushormon" acts on the ventricle in any direct manner. The ventricle is chosen as a test object simply because in the ventricle the conditions are present which permit the demonstration of the effects of the sinus hormone: in the whole heart beating at the rhythm imposed on it by the sinus it would be difficult to superimpose the/

the hormone in sufficient quantity to affect the beat.

In addition to using the ventricle and ventricular apex as a test object, Haberlandt in later experiments employed strips from the sinus venosus itself, subjecting such strips, two or three days after their isolation, to the action of sinus ringer. Twenty-two out of the thirty-five experiments gave positive results. The whole heart isolated on a Straub cannula two or three days before the experiment was also employed in some cases, and could be made to beat rhythmically after subjection to the action of sinus ringer.

Experiments with "basis-ringer".

Haberlandt's (169)(170) next advance was to show that properties similar but less in degree to those conferred on ringer by the beating sinus could be conferred by the beating base of the frog ventricle. The properties of the "basis-ringer" being practically identical with those of sinus-ringer need not further be described.

Experiments with extracts.

Extracts of the sinus venosus and of the base of the ventricle were now investigated (171). The tissue was finely minced and extracted with absolute alcohol for twenty-four hours or longer, similar extracts/

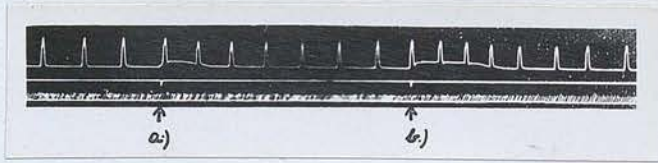


FIG. 10.
ADDITION OF UNSPECIFIC SUBSTANCES TO HEART
 a) HYDROSTATIC EFFECT OF ADDITION OF RINGER SOLUTION.
 b) HYDROSTATIC EFFECT OF ADDITION OF MUSCLE EXTRACT.

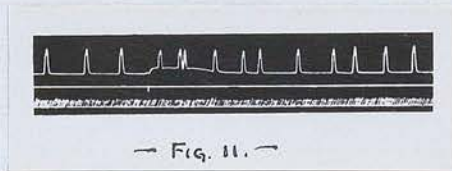


FIG. 11.
EFFECT OF EXTRACT OF VENTRICULAR APÊX.

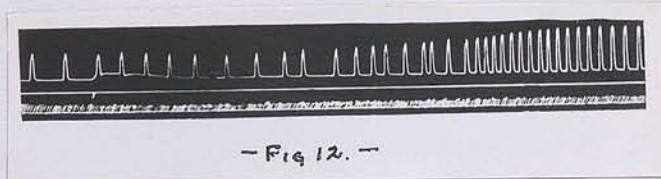


FIG. 12.
 SHOWING HYDROSTATIC EFFECT OF ADDITION OF SINUS-
 RINGER AND, LATER, SPECIFIC EFFECT OF SAME.



extracts of ventricular apices and of the gastrocnemii muscles being prepared as controls. The liquid was evaporated on a water bath and taken up in ergotamine-ringer solution. Ergotamine-ringer was used in these experiments in view of Witanowski's demonstration that the Loewi accelerating substance is present in, or at least is extractable from, the non-nerve-stimulated heart. The experiments with the alcoholic extracts of the sinus and the base of the ventricle of the frog heart showed that in some cases such extracts had the "pulse-quicken-
-strengthening and -liberating" actions previously described for sinus ringer, while the control extracts of the ventricular apex and of the gastrocnemius muscle had either no effect or had slight negative inotropic and negative chronotropic effects, due presumably to choline. As noted in the previous cases slight effects were often obtained due to pressure differences incidental to the addition of the extracts, but, as before, such effects were easily distinguishable from the specific effects of the "Herzhormon". Such pressure effects are shown in figs. 10, 11 and 12. In fig. 10 the first change is due to addition of normal ringer, the second to addition of gastrocnemius extract. The next figure shows the effect of an extract of the ventricular apex, while the last shows the effect of a sinus extract.

Finally, it should be noted, alcoholic sinus extracts/

extracts produce automatic activity in the quiescent ventricle.

The extracts, however, are not so satisfactory in producing the specific effects of the Herzhormon as is "sinus" or "ventricle-base" ringer - an observation which Demoor also made in connection with the active substances - and while with sinus-ringer positive specific effects were observed in two thirds of the experiments, good effects were obtained with extracts in less than 50% of the cases. Haberlandt considered that this result might be due to a slight solubility of the hormone in absolute alcohol or to its rapid destruction. In view of Demoor's demonstration that the active substances were rapidly destroyed in solution, Haberlandt (172) investigated this point further and showed that extraction with absolute alcohol for as long as 25 days did not give extracts which differed materially in their activity from those prepared within 24 hours.

Other properties of the Herzhormon.

In further experiments he showed that the Herzhormon is not destroyed by heating to 100°C., that it is insoluble in ether and only slightly soluble in chloroform and is thus not lipoidal in nature (172). It is dialysable. While it is adrenaline-like in some respects it is neither adrenaline nor the accelerator/

accelerator substance of Loewi, since ergotamine does not prevent its action. It differs from adrenaline in being vasodilator (172), histamine-free extracts still having this property (e.g.180). It is not histamine. It is not destroyed by irradiation with fluorescent or ultraviolet light but is inactivated to some degree by X-rays (176). It is present in small quantities in skeletal muscle (mammals)(184).

It is unfortunate that in his later experiments (178-183) Haberlandt should have made use of a commercial preparation of his heart hormone. Not only is this unsatisfactory in that its method of preparation is nowhere described but it has the additional disadvantage of being made not from the frog but from the mammalian heart!

The heart hormone in therapeutics.

Finally, in a long series of papers published in various medical journals Haberlandt (185-193)(227,228) has stressed the clinical significance of his work, and the heart hormone preparation as prepared for him is being used extensively on the continent as a therapeutic agent in various disorders of the heart. ✓

While it is not the purpose of this essay to deal with such aspects of Haberlandt's work it is difficult to refrain from making comment thereon. It will be sufficient, however, to point out that the introduction/

introduction of such products into therapeutics is not only premature but is utterly unjustifiable from every point of view, and the time spent on investigating the clinical applications of the heart hormone preparation would be much more profitably spent in elucidating the numerous physiological problems presented by this work on the chemical basis of cardiac automaticity. Once these problems have been solved it will be time enough to consider the application of the new knowledge to the problems of cardiac pathology: but, most assuredly, not before. It is indeed difficult to see how the cause of scientific medicine is to be advanced if physiologists themselves fail to be scientific, and the unjustifiable commercialisation of the heart hormone is thus the more deplorable as having been instigated by Haberlandt himself.

Summary of Haberlandt's views.

As we have already seen, Haberlandt considered as a result of his experiments with "sinus-ringer" that a specific substance was elaborated by the sinus venosus of the frog's heart which had the property of increasing the frequency and the force of contraction of the long perfused and slowly beating ventricle, or of initiating rhythmic activity in the quiescent ventricle. This property was shown to exist/

exist also in Ringer's solution in which the base of the frog ventricle had been allowed to beat for some time. It was concluded from these experiments that the sinus venosus, and to a lesser extent, the tissues of the ventricular base elaborated a specific regulating substance, which in virtue of its properties on the isolated and particularly the quiescent ventricle, must be considered as a hormone in the narrow sense of that word. To this substance the name Herzhormon was given.

Experiments with extracts of the sinus venosus and the base of the ventricle were found to have properties similar but less in degree to those of "simus" and "basis"-ringer.

The heart hormone was shown to be neither adrenaline nor histamine.

Further experiments were performed with a commercial preparation of the "heart hormone" made from the mammalian heart. This preparation was employed clinically.

Haberlandt sums up his views on the mode of action of the Herzhormon and on its physiological significance by saying, in effect, that the sinus venosus is the pacemaker of the heart in virtue of its high content of this substance - "dass jenes Herzzentrum die Oberhand hat, in dem das Herzhormon in überwiegender Menge bzw. grösserer Wirksamkeit gebildet wird".

The/

The substance itself he considers to be in fact that "inner stimulus" the chemical nature of which was postulated long ago by Engelmann and the expression of which is the automatic and rhythmic beating of the heart.

Haberlandt's early work is fully described in a monograph published by him in 1927 (177). A fuller account of his views (with special reference to the clinical applications of the "Herzhormon") is to be found in a further monograph just published (229). A short review will also be found in a recent paper in "Endokrinologie" (228).

THE SCHOOL OF ZWAARDEMAKER.Experiments on the substitution of potassium by
radioactive elements.

The observation which gave birth to the large volume of work of the Dutch school was the demonstration of Zwaardemaker (194), in 1916, that the potassium of Ringer's fluid could be replaced by an amount of uranium or some other radioactive substance the radioactivity of which was exactly equivalent to that of the potassium for which it was substituted. While non-radioactive elements would thus not serve to re-establish the beat of an isolated heart brought to a standstill by perfusion with potassium-free ringer, such elements as rubidium, thorium, uranium or ionium would so serve. Experiments on lamprey, eel, frog, toad, tortoise and rabbit hearts all gave these results (197,198).

It was shown further that not only were salts of radioactive substances capable of replacing the potassium of Ringer's fluid but that the same effect could be obtained by irradiation of the potassium-free perfused heart by means of radium, mesothorium, or the cathode rays from a Lenard tube (195,196). Figure 13, which is from a paper by Zwaardemaker, shows this effect of radium irradiation on a heart perfused/

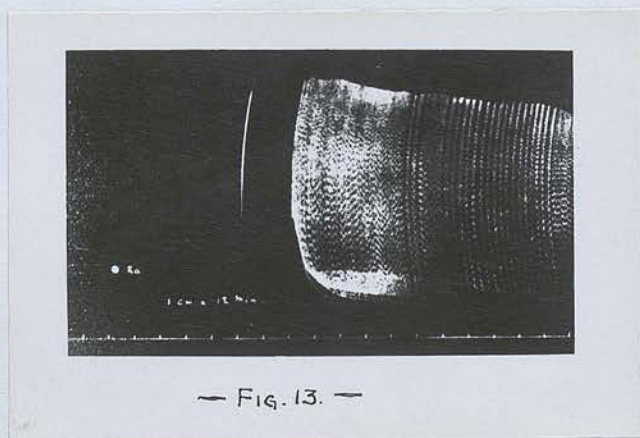


FIG. 13.

REVIVAL OF BEAT IN K-FREE PERFUSED HEART
BY EXTERNAL IRRADIATION WITH RADIUM.

Irradiation commenced at white dot. Time in 12 minute intervals. A few beats appeared one hour after commencement of irradiation. Permanent rhythmic activity commenced half an hour later still.

perfused with potassium-free ringer. It will be seen that an hour after commencement of the irradiation a few rhythmic beats appeared, permanent rhythmic activity being established half an hour later still.

The long latent period between the commencement of irradiation and the commencement of rhythmic beating is very striking and is characteristic of such experiments. Zwaardemaker found for instance that when a small glass ampoule containing 3.3 mgms. of radium was placed practically against a potassium-free perfused heart the latent period was never less than seven minutes, while at a distance of half a centimetre it varied from half to one hour.

"AUTOMATINES".

This long latency suggested to Zwaardemaker that the effect of the radiation on the heart was not, so to speak, mechanical, but was produced through the intermediary of some chemical mechanism - a supposition made the more likely by reason of the fact that once a heart had been activated by means of radium it would maintain some sort of activity for from half to one day after cessation of the "stimulus".

Following the suggestion of Benjamins, Zwaardemaker (199) adopted for this hypothetical substance produced in the heart by means of corpuscular radiations the name "automatine", and concluded from the experimental findings that the main function of K in the animal body is the production of this substance.
In/

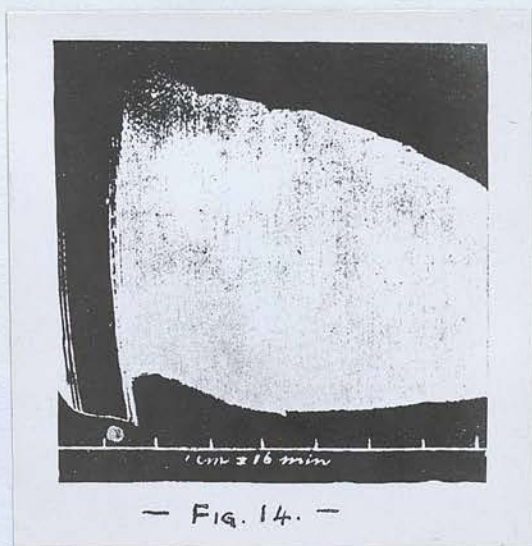


FIG. 14.
SHOWS REACTIVATION OF EEL HEART BY
MEANS OF AUTOMATINE LIQUID OBTAINED
BY IRRADIATION OF ANOTHER HEART BY
POLONIUM.

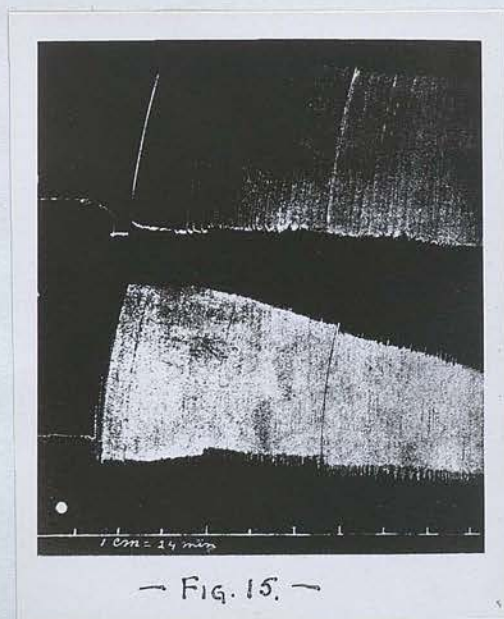


FIG. 15.
REACTIVATION BY AUTOMATINE.
TWO EEL HEARTS IN SAME PERFUSION CYCLE.
LOWER HEART IRRADIATED BY RADIUM.
UPPER HEART NOT IRRADIATED BUT RECEIVES
AUTOMATINE LIQUID FROM LOWER HEART.

In view of the fact that either α or β radiations were effective in re-establishing the beat of a K-free perfused heart (200), but that α and β irradiations when applied simultaneously in equal amount were ineffective (201), he further supposed that there were two kinds of automatines, α -automatine and β -automatine (199), and that these were antagonistic (202). Since, however, the radioactivity of K is expressed as β radiation it was supposed that the automatine normally present in the animal body is the β variety (199).

Conclusive evidence in favour of the view that chemical substance was formed as a result of irradiation was furnished by further experiments of Zwaardemaker and of Aarons (203). Two frog or eel hearts were fitted up in exactly the same manner and perfused with potassium-free ringer solution. One of the hearts was set into activity by means of external radiation by radium. It was found that if the fluid which had traversed this heart during its irradiation and for some time after the establishment of rhythmic activity, was transferred to the second heart that it too, after a short latent period of the order of half an hour, would commence to beat rhythmically like the irradiated heart, the which would not otherwise happen. Fig. 14 shows the record of such an experiment on the eel's heart, which after having been quiescent for sixteen minutes as a result of perfusion with a K-free fluid resumes its rhythmic behaviour a few/

few minutes after the addition of "automatin fluid" from a K-free perfused but radium-irradiated heart. This type of experiment we may consider the equivalent in some ways of Loewi's original experiment on humoral transmissibility of nervous effects, and the next figure shows the counterpart of Ten Cate's modification of Loewi's experiment! In this two eel hearts are perfused in series. The first heart (lower record) is irradiated and commences to beat in about half an hour. The second is not irradiated but receives automatin which has presumably diffused from the first heart into the perfusing fluid. This automatin, accumulating in the nodal tissue of the second heart ultimately reaches such a concentration that it sets this heart too into rhythmic activity. In all these experiments a small quantity - 30 to 40 cubic centimetres - of fluid is used and this is perfused and re-perfused through the heart or hearts for the whole course of the experiment.

Zwaardemaker's view, then, is that in the heart, as a result of the normal radioactive properties of the potassium in the blood, a substance, automatine, is formed to which the normal automaticity of the heart is due. Abstraction of potassium leads to stoppage of the heart because now no radiations can act on the mother substance present in the heart tissues and transform this to automatin. Addition of a radioactive/
radioactive/

radioactive salt in the perfusing fluid in place of potassium brings about revival of the beat because now once more automatine is formed in virtue of this radioactivity. External radiation by radium brings about a similar result because this too assures the formation of automatine. Further, the enormous quantity and intensity of the radiations from radium as compared with those from potassium lead to the formation of much greater quantities of automatine in the heart than obtain normally, so that on cessation of irradiation the heart may continue to beat for a day whereas on simple transference from a normal perfusing medium to a potassium free medium the heart stops almost at once. In other words, whereas under physiological conditions only sufficient automatine is present in the heart to serve its immediate needs, under experimental conditions sufficient can be manufactured to last for a day or more.

AUTOMATINOGEN.

The mother substance on which the β rays of potassium normally act to produce automatine Zwaardemaker called "automatinogen" (203,204).

While heart tissue contains large quantities both of automatin and automatinogen, it was found that these substances were present also in other parts of the body. They were found, for example, in skeletal muscle and/

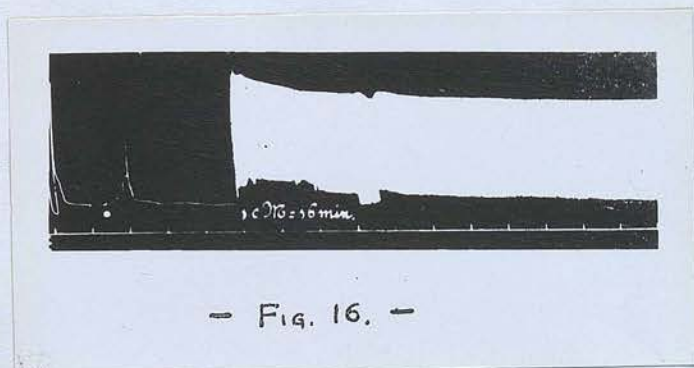


FIG. 16.

REACTIVATION OF EEL HEART BY IRRADIATED
SKELETAL MUSCLE EXTRACT.

EXTRACT ADDED AT DOT. TIME IN 16 MIN. INTERVALS.

HEART CONTINUED TO BEAT FOR 20 HRS., WHEREAS CONTROL
SUBJECTED TO NON-IRRADIATED PORTION OF EXTRACT CEASED TO
BEAT AT END OF 8 HRS.

and in blood (205,206). It stands to reason, of course, that wherever automatinogen exists there also will be automatine. But it seems that the radioactivity of potassium is so feeble that only a small proportion of available automatinogen is at a given time in process of transformation to automatin.

In order to investigate the relative effects of different automatin and automatinogen extracts Zwaardemaker and Zeehuisen (207) conducted experiments of the following type: they made up in K-free ringer solution an extract of automatinogen from the skeletal muscles, say, of the frog. A portion of this solution was irradiated. Three-eel hearts were set up and perfused, one with normal ringer, another with potassium-free ringer to which had been added the solution of automatinogen, and the last with potassium-free ringer to which had been added the irradiated automatinogen or automatin. The rate of beat and the duration in hours of the rhythm were taken to indicate the relative amounts of automatin intervening in each case. A record from an experiment somewhat similar to those noted above is shown on the opposite page. Irradiated skeletal muscle extract was added to the potassium-free ringer perfusing through an eel's heart. This particular heart beat for twenty hours, whereas another and similar heart subjected to the action of the non-irradiated extract in which automatinogen preponderated/

preponderated, ceased to beat after eight hours. Similar experiments on the guinea-pig heart have been performed by van Eldick (208).

Other properties of automatin and automatinogen.

Westenbrink and Aarons have recently made valuable contributions to this subject. Not only have they investigated the chemical and physical properties of automatinogen and automatin, but they have developed a method of extraction of automatinogen which appears to give a fairly pure product and have, finally, developed a biological method for the assay of automatine.

The automatinogen is extracted from muscle by cold alcohol and dissolved in water. It is then adsorbed on kaolin from which it is eluted by 96% alcohol. White crystals of a phosphatide are deposited, and the automatine which appears to be adsorbed on the phosphatide is released by means of radium irradiation. The quantitative test is conducted in the following manner. An eel's heart is perfused for one hour with K-free ringer plus automatin. This is followed by perfusion with K-free ringer without automatin, bringing the heart to an immediate standstill. The unit of automatin is the smallest quantity which, dissolved in 50 ccs. of K-free ringer solution causes the heart to beat again with/

with a frequency of about twenty per minute. The error in this test is of the order of 25 per cent. Expressing the results in units per kilogram of tissue used, Westenbrink and Aarons find that the sinoauricular node contains 3000 units of automatin whereas the general myocardium contains only 250 such units.

The chemical and physical properties of automatinogen and automatin may be briefly summarised.

Automatinogen, according to Westenbrink and Aarons (209), is soluble in water and in alcohol but almost insoluble in ether. It is adsorbed by kaolin. It is not dialysable. It is a phosphatide, or is automatin combined with or adsorbed on a phosphatide.

Automatin itself is soluble in water and in alcohol but practically insoluble in ether. It is not adsorbed by kaolin. It is dialysable. It is not destroyed by boiling with hydrochloric or nitrous acid, but it is destroyed by hot sodium hydroxide. It is precipitated from alcoholic solution by HgCl_2 , and from aqueous solution by phosphotungstic acid but not by silver nitrate. Zwaardemaker (210) found that automatin was produced by irradiation of vitamin B, but Westenbrink and Aarons show that it is not vitamin B itself. It is not histamine or adrenaline. While automatinogen disappears from aqueous solution in/

in about a day, automatin under similar conditions remains active for from two to three days (211).

CONCLUSION.

Such then is a brief review of the main experimental facts put forward by the Dutch school. The interpretation put on these facts by Zwaardemaker is simple. According to his view automatinogen and automatin formed in different parts of the body are carried in the blood to the nodal tissue of the heart which has a special affinity for these substances. Through the intermediation of the nodal tissue the automatin, released by the radioactivity of the potassium in the blood, exerts its effect in maintaining the automaticity of the heart. Without automatin there is no automaticity.

Fully aware of the complexity of the subject of which he treats Zwaardemaker recognises automatin to be of equal significance and importance with the ions and with the "sensibilisateurs" of the internal environment. He recognises that automaticity is maintained in virtue of a balance existing between these different sets of factors and that disturbance of this balance by alteration in the relative proportions of the constituents of one of the sets may affect automaticity in as great a degree as will abstraction of a whole set. The difficulty and indeed/

indeed the undesirability of considering any one of the sets of factors or its constituents to be of prime importance is therefore stressed and while it is recognised that "sans automatines pas d'automatisme" the view that automatin is of itself the "inner stimulus" of the classical physiologists is abhorred (212). ✓

Other papers on this subject by Zwaardemaker are listed in the bibliography (242-249). See also the monograph of van Eldick (250).

PERSONAL OBSERVATIONS.Introduction:-

The observations here recorded are concerned entirely with the "active substances" of Demoor. It was thought when contemplating research on the "heart hormones" that Demoor's experiments were much more satisfactory than those of Haberlandt, and though at the outset it did not appear that the conclusions arrived at by Demoor were necessary corollaries of his experimental findings, the phenomena described by the Brussels workers seemed of sufficient interest to warrant re-examination and, if possible, extension.

A visit was accordingly paid to the Institut Solvay with the object of becoming acquainted with the technique employed in this work on the active substances, and the experiments commenced in Brussels were continued in the Physiological Laboratory of the University of Edinburgh.

Unfortunately, the mere repetition of Demoor's fundamental experiment has itself proved a time-consuming piece of work, and very little else has been accomplished. The experiments reported here thus represent, as it were, but the beginnings of a research which it is hoped will be carried to a successful issue in the course of a year or two.

Such/

SKETCH OF APPARATUS USED IN INVESTIGATING
THE "ACTIVE SUBSTANCES" OF THE HEART.

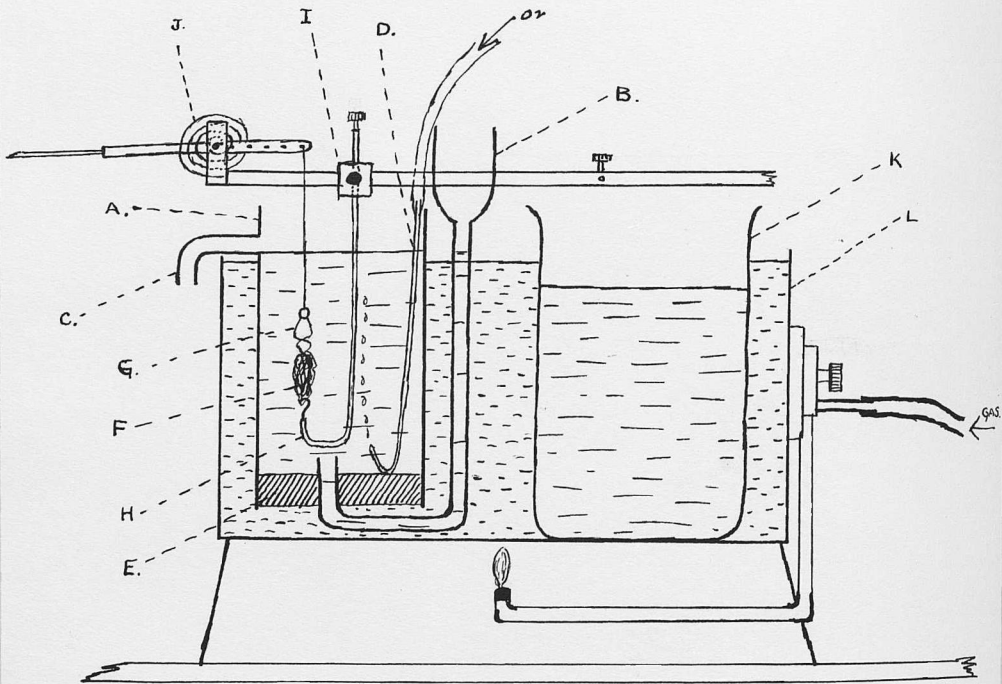


FIGURE 17.

- | | | | |
|----|----------------------------------|----|---|
| A. | - AURICLE BATH. | G. | - SILVER HOOK. |
| B. | - INPUT TUBE TO AURICLE BATH. | H. | - J piece of LEVER. |
| C. | - OVERFLOW TUBE TO AURICLE BATH. | I. | - ADJUSTABLE BOSS CARRYING J. piece. |
| D. | - OXYGEN SUPPLY TUBE. | J. | - SPRING TO INCREASE NAT. FREQUENCY OF LEVER. |
| E. | - RUBBER BUNG. | K. | - SPARE BEAKER OF LOGIC SOLN. |
| F. | - ISOLATED AURICLE. | L. | - THERMOSTAT BATH MAINTAINED AT 38°C. |

such experiments as have been performed, however, are not devoid of suggestiveness and I have presumed to permit some of these suggestions to lead to conclusions which may seem unjustifiable under the circumstances. I quite realise, however, that any conclusions which I have arrived at are little better than provisional hypotheses which must stand or fall on the results of experiments directed specifically towards that end.

METHODS.

The apparatus.

The apparatus employed was until recently similar to that used by the Brussels workers. A glass beaker of 250 cubic centimetres capacity filled with Locke solution and maintained at constant temperature by immersion in a thermostat bath forms the receptacle for the isolated auricle.

A fairly violent stream of oxygen must be passed through the solution.

The auricle is attached to a special type of lever, the design of which is apparent from the diagram opposite. The J piece as used by Demoor is nickel-plated with a silver hook soldered to its short end; but I have adopted one made from thick silver wire the terminal hook being formed by turning off one end of the wire in a lathe before forming the J. This obviates the use of solder altogether. One part of the auricle is attached to the J piece hook, the other to/

to a silver clip attached by thread or wire to the lever. The J piece is insulated from the rest of the lever, and if wire instead of thread is used for attaching the auricle clip to the lever, the J piece hook and this clip may be made to function as electrodes. A terminal is provided on the J piece and on some other part of the lever for use in such experiments.

The main disadvantage of the beaker arrangement as used by Demoor is that it is necessary to remove the auricle from the beaker when it is desired to change the fluid. A vessel has accordingly been introduced which obviates this difficulty and permits of a complete change of the fluid in which the auricle is immersed without interfering in any way with the record. The apparatus, as can be seen from the diagram, is exactly similar in principle to the plain-muscle bath of Sharpey-Schafer, differing from this only in its size and relative proportions. The capacity of the vessel is 250 ccs.

Isolated auricle preparation:-

As a rule four isolated auricle preparations were set up, either two left and two right, or three lefts and one right. In any case it is always advisable to have at least one right auricle as a control. The auricles of young rabbits were generally used, and such for the obvious reason that in old and heavy animals/

animals the thickness of the auricles is so great that simple immersion in locke fluid does not serve to maintain them for long under ideal conditions, and perfusion of the auricular vessels which would thus be necessary would involve a needless complication in technique.

The animal is killed by a blow on the neck and the heart quickly dissected out and transferred to warm locke solution. At first, and before one has become experienced in this type of work, it is an advantage to ligature the superior and inferior venae cavae before cutting out the heart, these vessels being cut through on that side of the ligatures distal to the heart. These ligatures serve the triple purpose of preventing damage to the right auricle when the heart is being cut from the animal, the which is liable to occur at first, and of giving a convenient means of holding the auricles while these are being separated from the ventricles, and, finally, of permitting easy identification of the right and left auricle after such separation has been effected and after they have been separated from one another.

The heart having been placed in a dish of warm locke, it is supported in the horizontal position, dorsal aspect upperwards, preferably under the surface of the liquid, by an assistant who holds the ventricle with the thumb and finger of one hand and the ligatures aforesaid/

aforesaid with the other, exerting a slight tension the while. A snip is made in the heart at the junction of the auricles with the interauricular septum and the ventricles, and, the heart being slowly revolved by the assistant, the scissors are run round the auriculo-ventricular junction and along the base of the interauricular septum, thus separating the auricles completely from the rest of the heart. The auricles are now separated from one another and preferably from the septum. That the irrigating fluid may more completely reach all parts of the surface of the auricle it is advisable to make a couple of radial cuts in the auricle as in diagram. Each is now transferred to one of the levers, one part of the cut edge of the auricle being attached to the J piece hook, the opposite edge to the silver clip and the whole is lowered into the beaker until the preparation is submerged.

The recording of the experiments:-

Since a continuous record of the behaviour of the left auricle is usually desired it is advisable to make use of a kymograph with a variable speed. Most of my experiments were recorded on Blix kymographs which give speeds varying from .01 mm. per second to 1000 mm. per second. The record before addition of an active substance extract can be made on a very slow rate of drum, say one revolution per two hours the/

the speed being increased to say one revolution per five to ten minutes, about the time when it is desired to make the addition of the extract. The ease with which an experiment can be recorded for long periods and yet come within the compass of a few inches square make up for the inconvenience of having to raise the cylinder at the end of each revolution.

The preparation of the extracts:-

Demoor used both aqueous and alcoholic extracts in his experiments and while for reasons which will appear later aqueous extracts were used in most of my experiments alcoholic extracts were also tried.

The aqueous extract is prepared as follows:

Sheep or other hearts are obtained from the abattoir in as fresh a condition as possible, preferably within an hour or so of the death of the animal. It may be noted here that most butchers in cutting the heart out of an animal sever the greater part of the right auricle, so that if it is desired to obtain an extract of the sinoauricular node it is advisable to inform the "killer" that the hearts must be severed as high up as possible, preferably including part of the lungs. The Keith Flack node is visible as a light band three or four millimetres broad and 2 to 3 cms. in length running in the sulcus terminalis between the inferior and superior venae cavae. It is easily dissected out/

out by means of a pair of scissors. The often large amount of fat attached to the part thus dissected is removed as completely as possible, the tissue cut into small pieces and placed in a mortar. If it is desired to obtain also the Tawara node this is visible at the junction between auricles and ventricles just above the middle cusp of the tricuspid valve. It is recognisable by its paleness and toughness as compared with the surrounding tissue, but is not as easy to identify as is the sinoauricular node nor can it be easily differentiated from the upper part of the bundle of His. I have not used extracts of Tawara node alone, but on occasion added the node to the sinoauricular extract. The tissue which, however, is most difficult to obtain satisfactorily but which when obtained, gives probably the best results is that of the ventricular subendocardium.

The tissue is obtained by a process of "peeling". The difficulty here is to obtain the proper thickness of tissue. Taking the thinnest possible layer gives merely the endocardial lining, while taking the tissue too thickly gives such an amount of myocardial tissue that the toxic effects of this latter may mask the effects of the Purkinje material.

Practice alone seems of any avail here, though as a general rule it may be said that a thickness of about one millimetre will be correct. The ventricle being/

being laid open the connections of the valves are severed and an incision about one millimetre in depth run round the base of the ventricle about $\frac{1}{2}$ to 1 cm. below its junction with the auricle. The lower border of this incision is grasped piece by piece in a pair of fine forceps and stripped down towards the apex of the ventricle until the whole ventricular surface has been bereft of a sheet of tissue approximately one millimetre thick. Both ventricles are treated similarly, the tissue is cut up into very small pieces and transferred to a mortar. The subsequent technique is of course the same for both the Keith Flack nodal extract and the subendocardial extract.

While in the earlier experiments the tissues as they were dissected were transferred to a small quantity of Locke in a mortar, it was later found that better results were obtained if they were placed in a hypotonic fluid - distilled water being used. Further, the "pure" sand used at first in the pounding of the tissue was replaced by hard glass with a striking improvement in the results. The tissues then, cut into small pieces and moistened with say one or two ccs. of distilled water are thoroughly ground with broken hard glass. For any further additions of liquid Locke is employed, but since it seems that the more dilute the extract the more rapidly/



rapidly is it destroyed, it is desirable to have as small a volume of liquid as possible. The pounding having been continued for, say, twenty minutes to half an hour, the material is transferred to a wide-mouthed stoppered bottle, the mortar washed out into the bottle with a small quantity of Locke, and the whole placed in a mechanical shaker for two hours. The fluid is now centrifuged and the clear supernatant liquid is the desired extract.

Extracts of tissues other than the heart which were employed in some of my experiments were prepared in exactly the same way as and usually at the same time as the heart extracts.

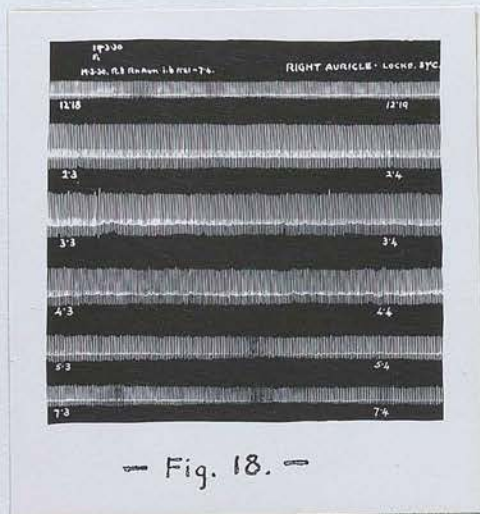
A few experiments only were done with alcoholic extracts.

Demoor's original method for the preparation of such extracts is as follows: The tissue is cut into small pieces, moistened with acetone and well pounded with pure infusorian earth. It is then shaken for 24 hours with acetone, three successive acetone extractions being made. The pulp is dried and extracted thrice with absolute alcohol. The alcoholic extract is evaporated and the residue remaining after such evaporation is dissolved in a small quantity of distilled water at the time of experiment. The pulp remaining after decantation of the alcoholic extract may/

may be dried and an aqueous extract of this made to compare with the alcoholic extract.

In the later experiments Demoor, while following out the same general plan of extraction, reduced the times occupied by the various processes and obtained more satisfactory results. Thus the shaking with acetone was reduced to three hours, the pulp remaining after centrifugation and decantation of the acetone being dried and subjected to two extractions with absolute alcohol.

In my experiments this first method was employed on one occasion and the later and shorter method on others. Extracts were also made using 90% alcohol and rectified spirits instead of the absolute alcohol. Extraction of the pulp with ether after the acetone extraction and before the alcohol extraction was tried, as was ether extraction of the dessicated alcoholic extract, this latter with a view to further purifying the final product. As has been indicated, however, little was actually done with alcoholic extracts due largely to the time which had perforce to be spent in getting the aqueous extracts to work, and it is entirely within the bounds of probability that could the same time and trouble have been taken with the alcoholic as was taken with the aqueous extracts, the results obtained with the former would have been other than reported below. Further work will decide this point.



— Fig. 18. —

FIG 18.

TYPICAL BEHAVIOUR OF ISOLATED RIGHT AURICLE.

RIGHT AURICLE OF RABBIT PLACED IN BATH OF OXYGENATED LOCKE SOLN. AT 11.41 A.M. RECORDS FROM ABOVE DOWN SHOW RECORDS OF BEAT BETWEEN 12.18 and 12.19; 2.3 and 2.4; 3.3 and 3.4; 4.3 and 4.4; 5.3 and 5.4; and 7.3 and 7.4 respectively. The preparation was immersed in 200 ccs. of Locke which was not changed during the course of the experiment. Compare this record with that of Fig 19, which shows the behaviour of the LEFT AURICLE of the same animal.

RESULTS.The behaviour of the isolated right auricle:

That the isolated right auricle suspended in oxygenated locke solution at a temperature of from 35 to 38° Centigrade will beat regularly for many hours, whereas the left auricle under exactly similar conditions will not so behave, is confirmed. I have observed in my experiments with the right auricle that there is usually a pronounced improvement in the amplitude of the beats during the first thirty or sixty minutes of immersion. In some cases the maximum beat may be recorded as soon as 15 minutes after immersion or it may only be attained at the end of 2 hours. Thereafter the amplitude may be maintained for two or three hours or it may commence to diminish slowly almost as soon as it has attained its maximum. In any case there is usually a diminution in the extent of contraction towards the end of five hours (though the frequency of the rhythm may be maintained) and though the beat may be considerably revived by the addition of fresh locke to the bath diminution quickly sets in once more until the beat becomes very feeble. I have observed such feeble beats continuing rhythmically as long as thirty hours after the preparation was set up.

Fig. 18 shows hourly records of a right auricle preparation/

preparation which may be taken as an average case though here the attaining of the maximum beat took just less than two hours. It must not be supposed that all preparations will behave with absolute regularity - various types of disturbed rhythm are met with, one of the most interesting being where regular cyclic increase and diminution of amplitude - and sometimes of rhythm - are met with, but it is not the purpose of this paper to deal with these.

Addition of adrenaline to the bath containing the right auricle gives immediately a pronounced increase in both frequency and amplitude of the contractions*, histamine having a somewhat similar effect but in much larger doses.

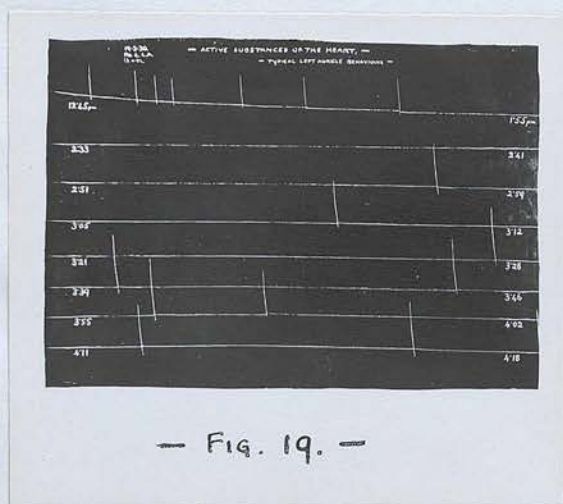
The behaviour of the isolated left auricle.

The left auricle behaves in a manner entirely different ^{from} ~~to~~ that of the right. As we have seen, Demoor describes it as undergoing changes of tonus which are followed by a period of 30, 50 or 70 minutes during which it remains entirely quiescent, this period being followed by one of many hours during which the auricle gives rise at irregular intervals to isolated ~~one~~ contractions which never become rhythmic. That this is so in many cases is indubitable, but it is not the invariable behaviour of such preparations.

In the first place the only "tonus change" which

I/

* See figure 26, facing p. 65.



— Fig. 19. —

FIG. 19.

SHOWING TYPICAL BEHAVIOUR OF ISOLATED LEFT AURICLE.

LEFT AURICLE OF SAME ANIMAL AS FIG. 18. AURICLE PLACED IN BATH AT 11:52. RECORD SHOWS BEHAVIOUR OF AURICLE BETWEEN THE TIMES MARKED ON EACH LINE. NOTE THE ENTIRE ABSENCE OF RHYTHMIC ACTIVITY, AND THE UNEQUAL CONTRACTIONS WHICH ARISE AT IRREGULAR INTERVALS. IN MANY CASES EVEN THESE ARE ABSENT AND THE PREPARATION REMAINS ENTIRELY QUIESCENT FOR MANY HOURS.

I have noted is a fall in tone which follows the initial immersion of the preparation. This is often followed by a period of quiescence but as often the irregular arhythmic contractions appear at once without such a quiescent interval intervening. Further, the frequency of these "secousses" is very variable - from one or two per hour to as many or more per minute and, finally, such secousses may never appear at all. Demoor does not mention such behaviour as this last but I have found it frequently in the course of some ninety experiments, and, what is of some importance, such auricles which have remained entirely quiescent for as long as four hours are just as readily activated by the active substance as are the others. Fig. 19 shows the record of a left auricle which gave rise to contractions at irregular intervals as occurred in approximately half of my experiments.

While I have confirmed Demoor's observation that adrenaline by itself, while it has a pronounced action on the right auricle, will not rhythmicise the left auricle, I have in several cases found that it increased the rate of appearance of the aperiodic contractions when these are present. Further in view of the identification of the heart hormones with histamine the action of this substance on the left auricle was tried and it was found to be entirely without effect. ✓

Unsuccessful experiments with extracts:

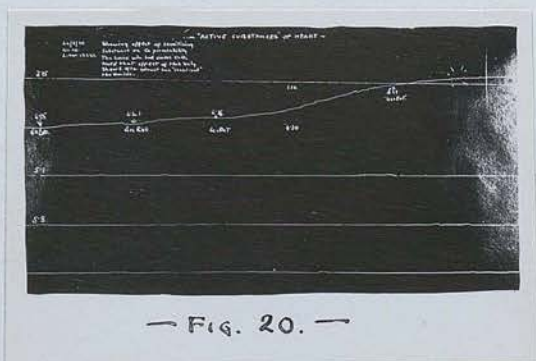
Attempts to obtain extracts which would rhythmicise the left auricle, were at first, despite the help of Professor Demoor and Dr. Rylant, entirely unsuccessful. Unfortunate as this appeared at the time it was nevertheless a happy circumstance since the consequent elimination of disturbing factors which culminated in the success of the experiments gave an insight into the necessary conditions for the preparation of active substances which could not have been obtained otherwise. On returning to Edinburgh difficulties again cropped up which took some time to overcome. While these difficulties will be referred to later it seems proper perhaps to refer to the significance of them here.

While no one outside of Demoor's own laboratory has, so far as I know, repeated his experiments, except myself, many have sought to criticise them. Leaving such critics aside it may be expected that sooner or later publications will appear giving results of experiments which fail to confirm Demoor's and it is thus perhaps unfortunate that Demoor's publications should give the impression that his experiments are easy to perform. My experience is that they are not - unless certain very definite conditions are observed - and as a result of this experience I am bound to say that negative results in such/

such experiments can be of little significance. I hope, however, to lay down conditions, the observation of which should enable any experimentalist to repeat with success the fundamental experiment of Demoor. This, however, is a digression.

The early failures to obtain rhythmic beating in the left auricle by the addition of an aqueous extract of Keith Flack node or of the Purkinje tissue of the subendocardium permitted frequent demonstrations of the adrenaline sensitising properties of such extracts. Thus whereas addition of adrenaline to the bath previous to the addition of the extract produced no effect or a slight increase in the frequency of appearance of the characteristic isolated contractions, addition subsequent to the addition of the extract determined the onset of rhythmic contractions which continued for some time in a regular manner, or suffered periodic intermissions. After complete oxidation of the adrenaline the rhythmic contractions ceased abruptly, the auricle thereafter resuming its typical behaviour. The action of adrenaline is much more prolonged when added to the left auricle sensitised by extract than when a similar amount is added to the right auricle.

This has been noted continually and is of interest in view of Wiltshire's (236,266) recent observations/



— FIG. 20. —

FIG. 20.

EFFECT OF EXCESS CALCIUM UPON THE ISOLATED LEFT AURICLE.

The auricle was set up in the bath at 2:42. The Locke solution had an excess of Calcium. The auricle showed no unusual tension change on immersion in this solution. At 4:15 (beginning of second line) 4ccs of an extract of KEITH-FLACK node (sheep) are added, further portions being added at 4:21, 4:26, & 4:36. After the first addition of extract the tone of the heart muscle increased, and continued to do so until about 4:40. In other words the auricle only "reacted" to the excess Calcium after the addition of the extracts, and therefore presumably because of their action.

observations. It should also be noted that if too large an amount of extract is added the auricle does not respond at all to adrenaline, this presumably being due to a toxic action exerted by some factor in the extract such as, for example, potassium.

Not only does this "sensitising" substance permit the action of adrenaline but it also permits alterations in the calcium content of the fluid to affect the auricle, whereas otherwise this ion has no effect. This is shown in a striking manner in fig.20. (By an error on the part of the "preparateur" the Locke solution used had twice the normal content of calcium). It will be noted that the auricle on being placed in this solution suffered no untoward change in tone, but as soon as the extract had been added the typical effect of excess calcium was exhibited. The fact that the auricle under normal conditions when set up suffers a slight fall in tone might be interpreted as due to an excretion, as it were, of calcium, and since the initiation of rhythmic beating by the agency of the active substances is almost always immediately preceded by a slight but abrupt and definite rise in tone, the initiation of this beating might at first be thought due to the re-establishment of normal permeability to calcium which is apparently seriously impaired in the isolated left auricle. The fact however that this resumption of normal permeability to/

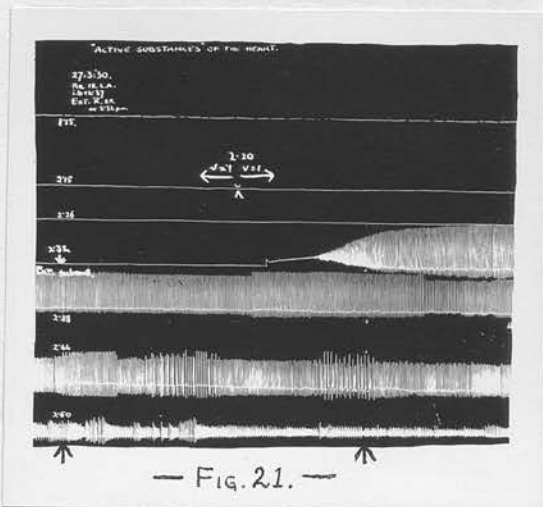
to calcium is due to the adrenaline sensitising part of the extract and not to the automaticity initiating fraction shows that the rhythmic beating is not due to this permeability change alone though the change is necessary for rhythmic beating to be established.

SUCCESSFUL EXPERIMENTS WITH EXTRACTS.CONFIRMATION OF DEMOCR'S FUNDAMENTAL EXPERIMENT.

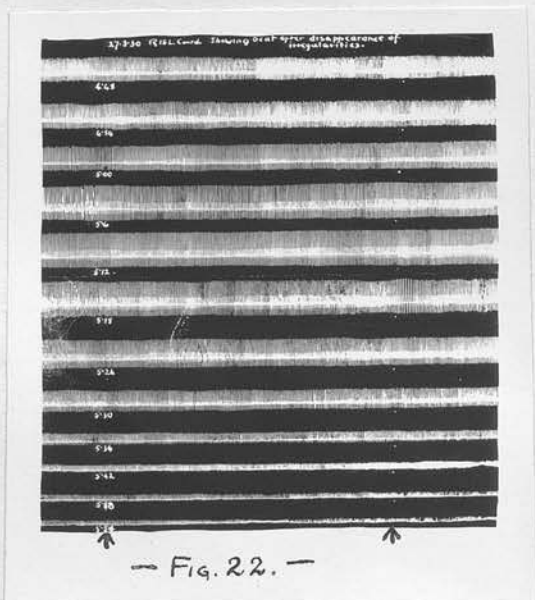
The addition to the bath containing the left auricle of an active aqueous extract of the sino-auricular node, or of the ventricular subendocardium, determines the onset, after a variable latent period, of rhythmic activity which may continue for some minutes though more usually for some hours. The period intervening between the addition of the extract and the onset of activity varies from about half a minute to a quarter of an hour, seven or eight minutes being about the average time. The onset of the effect, like its cessation, is usually abrupt and is nearly always preceded by a sudden though small rise in tone. In some cases a few arrhythmic and unequal contractions may immediately precede the rhythmic activity. The first few contractions may be remarkably strong, the amplitude gradually becoming constant, or, on the other hand, the first contractions may be almost invisible, gradually increasing to a constant value, but in most cases the initial amplitude is not excessive and is maintained at a practically constant level for the duration of the experiment. Finally it must be observed that irregularities in the rhythm often develop at the outset or after some time, these being presumably due to toxic or depressant substances/

substances in the extract. The appearance of irregularities can usually be correlated with the amount of extract added, and it may be that potassium is one at least of the substances which might be responsible. However, it seems that if the toxic substances are not present to too great an extent the heart establishes an equilibrium, as it were, with the disturbing factors in its environment, because it often after a period of irregular behaviour once more comports itself perfectly rhythmically and suffers no further alteration in its mode of action. On the other hand the toxic action of an extract may lead to a stoppage after a short period of, in such a case, usually irregular activity. A distinction can be made methinks between extracts which are excessively toxic and those which are merely slightly active by the fact that after the cessation of the activity induced by the former rhythmic activity can not be re-established by means of adrenaline*, whereas in the latter case such activity can be re-established by this means. In view of this toxicity exhibited by the extracts as they are at present prepared, in some experiments the addition of the extract to the bath containing the left auricle was made in stages. Thus if a first small dose did not produce an effect within say five minutes, a further small quantity was added, and so on until rhythmic activity was obtained. The results obtained by such a procedure were much more/

* See figure 25, facing p. 65.



LEFT AURICLE OF RABBIT. — ACTION OF "ACTIVE SUBSTANCES."
 AURICLE PLACED IN BATH AT 12:37. NO ACTIVITY WHATSOEVER EXHIBITED BY PREPARATION UNTIL 1:31 $\frac{1}{2}$, AN EXTRACT OF THE VENTRICULAR SUBENDOCARDIUM OF THE SHEEP HEART HAVING BEEN ADDED $\frac{7}{16}$ MIN. PREVIOUSLY. THE LATENT PERIOD BETWEEN ADDITION OF EXTRACT AND COMMENCEMENT OF RHYTHMIC ACTIVITY IS HERE VERY SHORT. THE ARROWS AT THE LOWER EDGE OF THE PHOTOGRAPH INDICATE AN INTERVAL OF ONE MINUTE. (They do not apply to the top 2 lines)



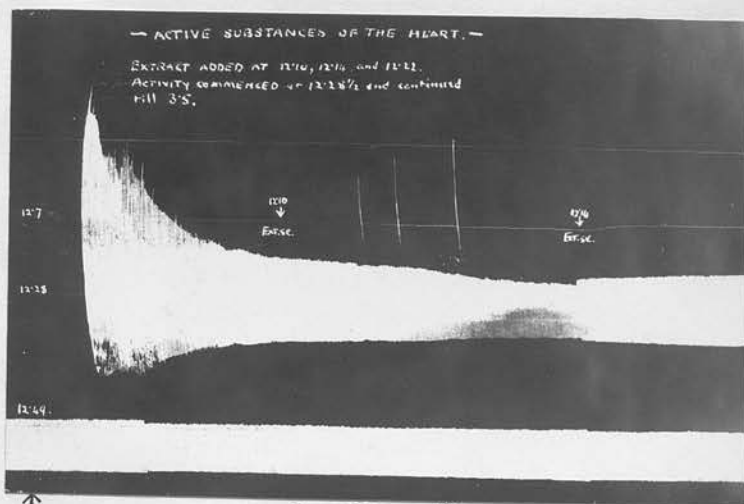
LEFT AURICLE OF RABBIT: SAME EXPT. AS ABOVE (Fig. 21).
 THIS FIGURE SHOWS THE BEAT AFTER DISAPPEARANCE OF THE IRREGULARITIES EVIDENT IN THE UPPER FIGURE. AN INTERVAL OF ONE AND THREE QUARTER HOURS ELAPSES BETWEEN THE END OF THE RECORD IN FIG. 21. AND THE COMMENCEMENT OF THIS RECORD. TIME INTERVAL INDICATED BY ARROWS — ONE MINUTE.

FROM AN EXPERIMENT BY THE AUTHOR.

more satisfactory than in those cases where a large dose of extract was added at one time, the auricle presumably being enabled to come more gradually into equilibrium with its surroundings, and as often happened, having a much smaller total amount of the extract administered than by the other method.

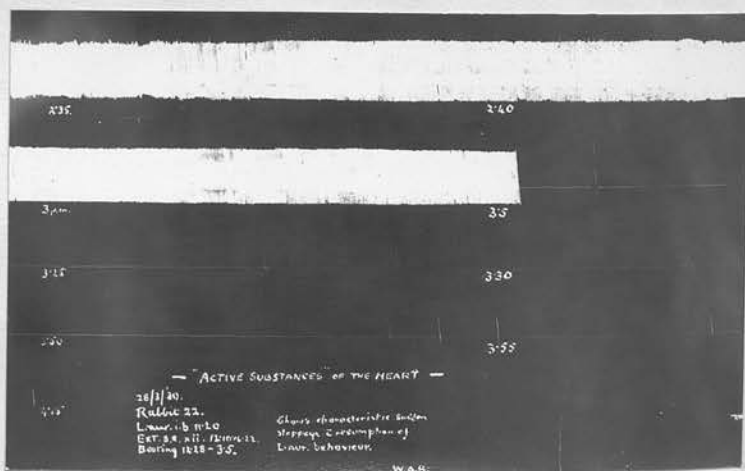
We must now consider some actual experiments chosen particularly because of the manner in which they illustrate some of the points which we have mentioned. Fig. 21. shows part of the record of a successful experiment. The left auricle was placed in the bath at 12.37. The kymograph speed was changed from .1 mm. per second to 1 mm. per second at 2.20. At 2.32 - the auricle having been in the bath for one hour fifty-five minutes, and having given not so much as a single contraction during that period - part of an aqueous extract of the sub-endocardium of four sheep hearts was added to the bath. After about three quarters of a minute there is a small but sharp increase in tone, and from what has the appearance of an incomplete tetanus a regular rhythmic beat gradually arises attaining its maximum amplitude in about a minute. The beat is perfectly regular from about 2.36 until about 2.39 when there is a slight increase in amplitude. This disappears, but by 2.44 the beat has become quite irregular.

This/



— FIG. 23. —

LEFT AURICLE OF RABBIT. Action of Active Substance Extract. NOTE MODE OF ONSET OF RHYTHMIC ACTIVITY IN THIS CASE. Compare with Fig 21. NOTE ALSO THE LONGER LATENT PERIOD HERE. ARROWS INDICATE INTERVAL OF 5 MINUTES.



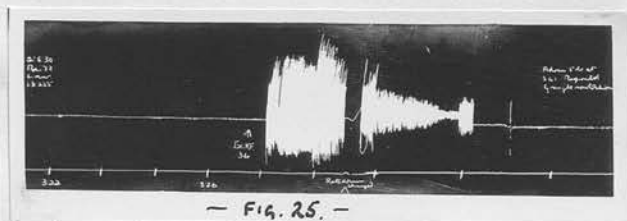
— FIG. 24. —

SAME AS ABOVE — CONCLUSION OF EXPERIMENT.

NOTE SUDDEN CESSATION OF RHYTHMIC ACTIVITY ABOUT 3:54. THE PREPARATION THEN RESUMES THE BEHAVIOUR CHARACTERISTIC OF THE ISOLATED LEFT AURICLE. — NOTE THE ISOLATED CONTRACTIONS WHICH ARISE AT IRREGULAR INTERVALS.

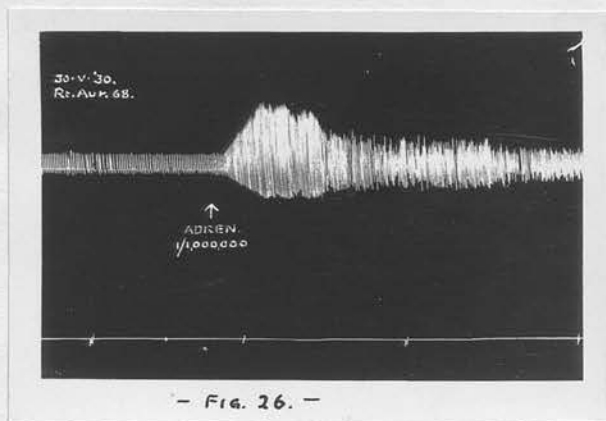
This behaviour is continued, with a decrease in amplitude, the condition shown in the last line of the tracing actually continuing till 3.50 after which a gradual improvement sets in. The second part of the figure ^(Fig. 22.) which gives the record from 4.48 till 5.55 shows the later phases of the recovery of amplitude and the subsequent regular decrease towards the end of the experiment. The experiment was actually concluded at 6.18, the auricle having been in the bath for five hours forty-one minutes and having exhibited more or less rhythmic activity for three and three quarter hours as the result of the addition of the single dose of extract.

The next figures illustrate the commencement and termination of another similar experiment (figs. 23, 24). The auricle was set up at eleven thirty. At 12.10 a small quantity of extract was added. Within three minutes of this addition three contractions of the auricle occurred, another three having been recorded previously. At 12.14 further extract was added without effect and at 12.22 a final addition of extract was made. About 12.29 rhythmic activity was initiated by violent contractions of the auricle - a striking contrast to the mode of commencement illustrated in the previous figure. The latent period between the last addition of extract and the commencement of the beat is, it should be noted, considerably longer/



- Fig. 25. -

LEFT AURICLE OF RABBIT. "TOXIC" ACTION OF NODAL EXTRACT.
 AN EXTRACT OF KEITH-FLACK NODE ADDED TO THE BATH ABOUT 317.
 AFTER A VERY SHORT LATENT PERIOD THE AURICLE COMMENCED TO BEAT.
 THE BEAT WAS IRREGULAR AND LASTED FOR ONLY THREE MINUTES.
 THEREAFTER ADRENALINE WAS ADDED TO THE BATH, BUT WITHOUT EFFECT.

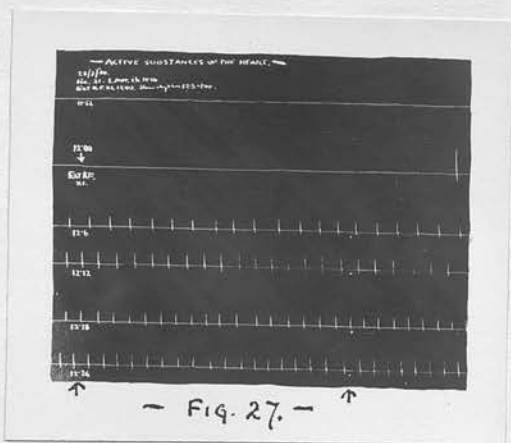


- Fig. 26. -

RIGHT AURICLE OF RABBIT. ACTION OF ADRENALINE.
 ADRENALINE, 1 PART IN 1,000,000, WAS SUBSTITUTED FOR
 THE ORDINARY LOCKE SOLUTION AT THE POINT INDICATED.
 TIME IN ONE MINUTE INTERVALS.

longer than in the previous case - seven minutes in this case as against seventy-five seconds in the other. The activity introduced in this violent manner was continued in the manner shown in the second line until 3.6, when as shown in the second part of the photograph the beats ceased abruptly. It will be seen that the auricle then resumes its typical behaviour, giving rise at irregular intervals to unequal contractions which do not become rhythmic. This activity is continued until the end of the experiment at 4.15.

2.64



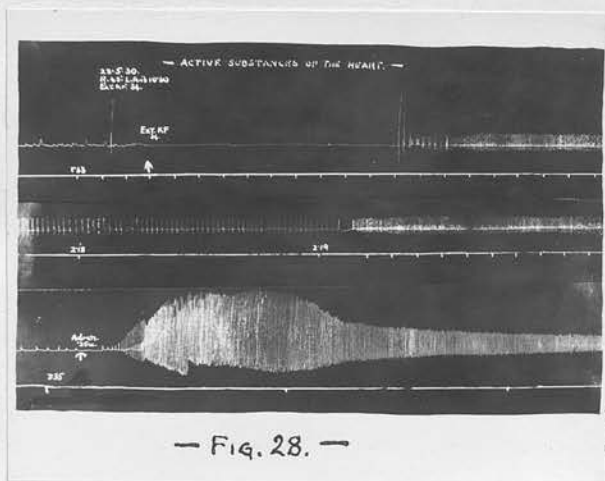
LEFT AURICLE OF RABBIT.

RHYTHMIC ACTIVITY INDUCED BY AQUEOUS EXTRACT OF KEITH-FLACK NODES ADDED AT 12 MORN. NOTE THE SLOW RHYTHM. ARROWS INDICATE INTERVAL OF ONE MINUTE.

FURTHER SUCCESSFUL EXPERIMENTS.

Another type of record which has not been reported by Demoor, but which must be considered of some importance, is reproduced in figure 27 and in figure 28. Each of these shows the effect of adding an extract of Keith-Flack node to the left auricle of a rabbit. In the first case the auricle was set up in the bath at 11.16. At 12 noon an aqueous extract of the Keith-Flack nodes of three sheep hearts was added. A minute later the auricle gave an isolated contraction.- the first it had given during the period of its immersion - and at 12.3 it commenced to beat rhythmically. The most striking characteristic of the beat, however, is its frequency which is but nineteen per minute at first, increasing gradually to twenty-five. This automatic activity ceased at 1 p.m. and was not resumed.

The next figure shows the record of a similar experiment. In this case the auricle was set up at 10.30 a.m. and gave several "secousses" before 1.36, at which time an aqueous extract of six Keith-Flack nodes was added. Ten minutes later a few aperiodic contractions heralded the appearance of rhythmic beating which was maintained until 3.30. In the second line of the tracing the speed of the kymograph is increased ten times and it is seen that the frequency of the beat is forty-six per minute. The bottom/



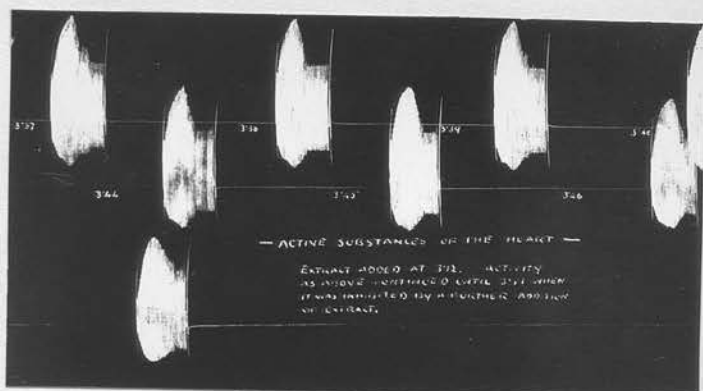
LEFT AURICLE OF RABBIT.

EXTRACT OF KEITH-FLACK NODE ADDED AT 1:36. ACTIVITY COMMENCES 1:46. (TOP LINE). NOTE SLOWNESS OF RHYTHM — SHOWN IN FIRST PART OF SECOND LINE, WHERE SPEED OF DRUM IS INCREASED 10 TIMES. BOTTOM LINE SHOWS BEAT AT 3:35 (DRUM SAME SPEED AS IN FIRST PART OF SECOND LINE) WHEN IT IS WEAK AND SOMEWHAT IRREGULAR. ADRENALINE IS NOW ADDED (CONC. 1 IN 1,000,000). NOTE THE ENORMOUS INCREASE IN AMPLITUDE AND FREQUENCY OF THE BEAT.

bottom section of the tracing taken on the same rate of drum as the first part of the second section shows the effect of adding adrenaline to the auricle bath. The concentration of adrenaline in the Locke solution was 1/1,000,000 and the enormous increase in amplitude and frequency of the beats as compared with the previous line is very obvious.

Such results as the two foregoing make it very unlikely that the active substance is adrenaline. I have never been able to obtain any such slow rhythms in a sensitised left auricle with adrenaline. Either the adrenaline added in such experiments is insufficient to give any effect at all, or when it does give an effect it is always to produce a very rapid rhythm the duration of which varies in a rough way with the amount added. Even allowing for the considerable slowing up of the oxidation of adrenaline which appears to obtain when that substance acts in presence of tissue extracts it is difficult to see - assuming that the slow rhythm is due to an exceedingly small concentration of adrenaline or, indeed, of the accelerator substance of Loewi - that its effects could be exerted for as long as an hour or an hour and a half. Even fairly large doses of adrenaline rarely act on the left auricle for such a time.

Abnormal/



- Fig. 29. -

LEFT AURICLE OF RABBIT.

For description see text - p 68.

Abnormal reaction of the left auricle.

It has already been noted that a left auricle does not respond to an extract either because the extract is inactive or because it has been added in such amount as to be toxic to the tissue. On three occasions, however, a peculiar result has been obtained for which at present there is no explanation so far as I know. Part of one of these records is reproduced (fig. 29)

The auricle was set up at 12.33 and remained quiescent from that time until a few minutes after the addition of a dose of extract at 3.12. From 3.20 until 3.51 groups of contractions arose. Each group consists of about 70 beats and each group is separated from the preceding by an almost equal interval of time. The amplitude of the corresponding beats of each group is practically identical as can be seen, and the whole forms a beautiful if not very instructive picture. This particular activity was inhibited by a further addition of extract at 3.51, a single group following about a minute and a half after this addition. It was found after stopping the record at 4.3, that though all spontaneous activity of the auricle had ceased a group could be very easily elicited by the slight mechanical stimulus afforded by tapping the recording lever. Once the group of contractions ceased, however, the auricle was refractory to further stimulation and

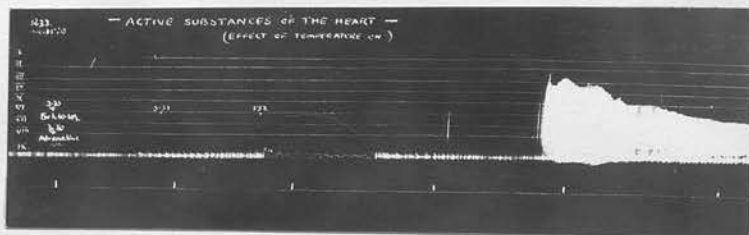
as/

as soon as this refractory period disappeared a further group of contractions could be produced as before. The groups obtained in this subsidiary experiment varied in their duration, and the post-group refractory period was found to be roughly proportional to the duration of the group.

Effect of temperature on the active substances.

Demoor showed that the active substance responsible for the initiation of automatic activity in the isolated left auricle was destroyed or lost its activity when heated to 60 or 63° Centigrade, while the substance responsible for the sensitisation of the left auricle to adrenaline was only destroyed on further heating to 70 or 73° Centigrade. As both Zwaardemaker and Haberlandt had shown that the substances described by them resisted heating to 100° Centigrade, Demoor reinvestigated this question in respect of the active substances and confirmed his previous findings. ✓

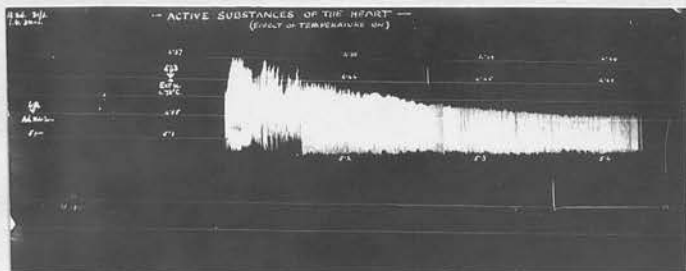
On the next page will be found the records of one of my experiments designed to test this point. An aqueous extract of the ventricular subendocardial tissue of six sheep hearts was prepared and divided into four lots. Two of these were maintained at room temperature; another was heated to between 60 and 63° and/



- FIG. 30. -

LEFT AURICLE. EFFECT OF PORTION OF EXTRACT HEATED TO 63°C.

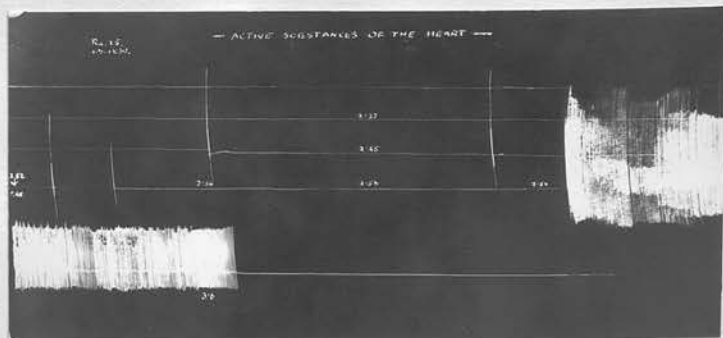
EXTRACT ADDED AT 3:30 - line VI. NO EFFECT BY 3:52. AT 3:52 ADRENALINE ADDED. ABOUT 3:57 RHYTHMIC ACTIVITY COMMENCED. THE AMPLITUDE OF THE BEATS DIMINISHES RAPIDLY. ACTIVITY CEASED AT 4:13, THE BEATS HAVING BEEN VERY SLIGHT AND IRREGULAR FROM 4:3 UNTIL THAT TIME.



- FIG. 31. -

LEFT AURICLE. EFFECT OF PORTION OF EXTRACT HEATED TO 73°C.

EXTRACT ADDED AT 4:43. (2nd line, 1/3 along). NO EFFECT BY 4:54, AT WHICH TIME ADRENALINE WAS ADDED. (Beginning of 4th line). THIS TOO WAS WITHOUT EFFECT. FINALLY, ABOUT 5 P.M. AN UNHEATED PORTION OF THE EXTRACT WAS ADDED. THIS PRODUCED RHYTHMIC ACTIVITY WHICH LASTED ABOUT 3 MINUTES.



- FIG. 32. -

LEFT AURICLE. EFFECT OF UNHEATED PORTION OF EXTRACT USED IN ABOVE EXPTS.

EXTRACT ADDED AT 2:52. SHORTLY AFTER 2:58 RHYTHMIC ACTIVITY COMMENCED, AND CEASES IN A CHARACTERISTIC MANNER AT 3:02, HAVING LASTED 3 MINUTES.

and maintained at this temperature for twenty minutes, while the fourth lot was heated to between 73 and 75° for the same time.

The first record shows (line VI) that the extract heated to 63° was added to the auricle bath at 3.30. No effect having appeared by 3.52 adrenaline was added. About 3.57 rhythmic beating commenced, the amplitude of the beats diminishing rapidly. From 4.3 to 4.13 when activity ceased the beats were very slight and exceedingly irregular - a type of behaviour noticed only in the activity induced by adrenaline.

The next figure shows the effect of that part of the extract heated to 73°. The extract was added at 4.43. At 4.54, no effect having become apparent, adrenaline was added. This too was without effect. Finally, about 5 p.m. one of the normal unheated portions of extract was added, this producing rhythmic activity which lasted for three minutes.

The bottom tracing shows the effect of adding an unheated portion of the extract to a left auricle. This gave rise to rhythmic activity which lasted for 8 ~~minutes~~ minutes. This last record was actually made first in order to determine whether or not the extract to be used in the other experiments was active. The reaction obtained with the normal extract in the middle tracing is comparable with that obtained in the last figure. The shortness of the response is probably/

probably due to the age of the extract (the animals were killed at eight in the morning) and to the small amount added.

It is thus seen that there is some justification for the view that the "automaticity initiating" property of active substance extracts is lost when the extract is heated to 63° , and that the adrenaline sensitising property is only destroyed on further heating to 73° .

The question of specificity.

In view of the fact that Demoor had not performed experiments with tissues other than from the heart, it was thought desirable to investigate extracts of other organs.

These observations may be summarised by saying that while none of the tissues investigated (with one exception) would initiate rhythmic activity in the isolated left auricle, extracts of skeletal muscle, both red and white, would sensitise the left auricle to the action of adrenaline. With brain and liver extracts the results were negative. With blood serum and with defibrinated blood the adrenaline sensitising reaction was obtained. A similar result was obtained with serum albumen and with cholesterol.

These findings made it appear that the adrenaline sensitising properties of nodal tissue extracts were not/

not necessarily due to a substance specific to the nodal tissues. Further, the fact that such a sensitising action could be produced by lipoid substances made it likely that initiation of rhythmic activity in the isolated left auricle could be brought about by an extract of at least one tissue of the body outside the heart, viz. the suprarenal, because though adrenaline by itself would not produce such a result the lipoids of the suprarenal cortex would serve to sensitise the auricle to the adrenaline of the medulla. It was found that such indeed was the case, and that rhythmic activity of the left auricle could be readily induced by means of an aqueous extract of the whole suprarenal.

The fact, noted earlier in this work, that the action of adrenaline on a left auricle subjected to the sensitising action of an otherwise inactive extract was more prolonged than the action of a similar dose of adrenaline on a right auricle, or as was shown later, that a dose applied to a right auricle subjected to the action of an animal extract was more prolonged than a similar dose applied to an auricle not so treated, taken together with the demonstration that this prolongation in activity is due actually to the substance or substances responsible for the sensitisation of the isolated auricle and that substances having this action exist in the suprarenal/

suprarenal cortex, deserves further study, for it indicates the possibility of a hitherto unrecognised significance of the suprarenal cortex in relation to the physiological actions of adrenaline.

The question of the nature of the active substances.

That the active substances of the nodal tissue was in fact adrenaline combined in some way with a substance which prevented its rapid oxidation, was early considered. Presumptive evidence against this view is afforded by those experiments which I have described where the rhythm obtaining after addition of an active extract was exceedingly slow. It was thought that further evidence bearing on this point would be obtained by ascertaining whether or not ergotamine would affect the action of the active substances. Experiments were accordingly instituted with a view to determining the dose of ergotamine which would abolish the response of the right auricle to adrenaline. The ergotamine used was that put out by Sandoz Co. under the trade name of "Femergin". Ergotamine phosphate (B.D.H.) was also tried. To my surprise neither of these substances in the doses used (up to 2 mg. ergotamine tartrate per 250 cc. solution) abolished, or indeed had any appreciable effect on the response of the auricle to adrenaline. Though I (234) had already obtained this same effect on/

on the heart of the various decapod crustacea, where it was found that the only effect of ergotamine was a general toxic one on the myocardium, it was hardly expected in the mammalian heart. Professor Gunn of Oxford, however, informed me that he had obtained, in the isolated perfused mammalian heart, results exactly similar to my own on the isolated auricle, and the proposed experiments on the effect of ergotamine on the action of the active substances were accordingly abandoned. Evidence, however, against the view that the active substance is adrenaline is furnished by experiments of Demoor and Rylant (235). They show that the chronaxie of the left auricle, driven electrically at a constant rate, is the same before and after the addition of active substances. In other words, the active substances have no effect on the chronaxie of the heart, whereas adrenaline increases the chronaxie as does the accelerator substance of Loewi.

If it can be shown of course that adrenaline can exist bound in some way with, say, lipoid substances, and that adrenaline so bound does not affect chronaxie as does the pure substance, the argument from these experiments of Demoor and Rylant falls to the ground.

A criticism might also be levelled at those experiments of my own which are interpreted as supporting the view that the rhythmicising factor is neither adrenaline nor the accelerator substance.

There is a possibility that the slow rhythm which obtained/

obtained in these was due to the presence of acetylcholine ("vagus-substance") in the extracts. That this is unlikely, however, seems indicated by the very duration of the rhythm as well as by the age of the extracts. It is difficult to see that acetylcholine could remain active for so long under the conditions of the experiment, although the fact that in one of the cases described the chronotropism actually increased slightly during the course of the experiment might be taken as presumptive evidence that acetylcholine was indeed present and was moreover being gradually destroyed, as one would expect, this circumstance accounting for the progressive increase in frequency of the auricular beats.*

* Whether or not the substances extracted from the mammalian heart by Drury and Szent-Györgyi (238) have any relation to this work on the active substances remains to be determined. See, however, p 97.

DISCUSSION.

There can be no doubt but that Demoor has written of his experiments and built up his theory of the humoral action of the active substances in a manner which makes convincing reading. Nevertheless it does not appear to me that - granting the truth of the experiments and the absolute specificity of the "substances actives" - the conclusions arrived at by Demoor are the necessary or even the most likely ones to be drawn from the experimental evidence which he puts forward in support of them. Such experiments as I myself have performed serve only to confirm this view.

I agree that the isolated left auricle does not exhibit rhythmic activity when isolated, whereas the right does: I agree that the arrhythmic behaviour of the right may indeed be the expression of the fundamental irritability of mammalian myocardial tissue - in fact I might even go further and suggest that since the degree of spontaneous activity exhibited by any isolated cardiac tissue is probably a function of the amount of nodal tissue present, pure mammalian myocardial tissue may not exhibit any spontaneous activity at all. I agree further that such arrhythmic or entirely quiescent myocardial tissue may be made to beat/

beat rhythmically by irrigating it with an aqueous concoction of the nodal tissue of some other heart, and that this action is humoral. But here Demoor and I part company, for while he holds that the beat of the heart in situ depends on the continuous action of the active substances on all parts of the myocardium - an action exerted through humoral channels - I do not see that this is at all necessary; that, indeed, it needlessly complicates the issue; that, finally, it is arrived at by a fault of reasoning and must be discarded until better proofs can be adduced in its favour.

I believe that the heart beat depends on the primary motor activity of the nodes, that this primary motor activity is exhibited in virtue of a chemical substance or substances, loosely bound in some way with these special tissues, and capable of being transferred under the special conditions of experiment to an otherwise non-rhythmic portion of myocardium causing this to assume rhythmic activity. While the exact mode of action of the active substances in such an experiment is not of course known, it is probable that they act on some minimal quantity of nodal tissue present in the preparation and by increasing the concentration of active substances in that nodal tissue cause it to assume the physiological properties/

properties of a node. In any case it appears that a higher automaticity than exists normally in a given fragment of myocardial tissue can be conferred on it by increasing the concentration of active substances in contact with it. But in applying to the heart in situ such considerations as arise from a study of isolated fragments methinks that the considerations must be altered to suit the altered conditions. And this is what Demoor has failed to do.

Apart from his own experiments with the isolated left auricle subjected to the action of the active substances, Demoor adduces two other sets of experiments in support of his view that the heart rhythm is maintained in virtue of the constant action on all the heart fibres of these active substances.

The first is a series of experiments by Deloyers (227). Deloyers showed that if a left auricle were immersed in a bath of Locke in such a way that one part could be subjected to the action of an experimental solution and the other maintained in the original environment, then that part subjected to the action of the active substances behaved in every way like a right auricle whereas the other, though driven by the first part which now functioned as an "artificial node", maintained in respect of its reactions to Ca and to adrenaline etc., the behaviour typical of the left auricle. Hence, according to Demoor, it was proved/

proved that the actual presence of active substances in contact with all parts of the myocardium was essential for the normal regular activity of the heart. Failure of active substances to come into contact with any given part of the myocardium would cause that part to exhibit the abnormal reactivity to Ca and to adrenaline characteristic of the left auricle, and would thus seriously embarrass cardiac activity.

Since it has been shown, however, by myself that the sensitising factor in active substance extracts is probably quite distinct from the rhythmicising factor and in any case exists in skeletal muscle and in blood itself, the significance of Deloyer's experiment for Demoor's theory falls to the ground, because the necessity for considering the normal reactivity of the myocardium as being due to active substances poured from the nodal tissues and transmitted to the myocardial fibres disappears when it is shown that the lipoids of the blood can perform this function. And there is no evidence to show that any of the lipid constituents of the blood are formed in the nodal tissues.

While Deloyer's experiment thus offers no support to Demoor's contention, it may be interpreted as giving some support to my own view that the active substances act, under the experimental conditions employed by Demoor and myself, by causing some part of/

of the auricle to function as a "node".

Other experiments which Demoor considers as giving support to his view are the grafting experiments by Rylant. Rylant (225) showed that if the Keith-Flack node were excised from its normal site and grafted on some atypical auricular site, that it would still act as pacemaker though no anatomical connection had been established between the graft and the tissue to which it was applied. In further experiments (226) he destroyed the Keith Flack node of one animal, and grafted the node from another animal in this site. The grafted node acted as pacemaker and conferred on the heart which it now regulated a rhythm almost identical to that which obtained in the heart which furnished the graft. These conditions were demonstrable electrocardiographically 2-6 hours after the operation, and persisted until the degeneration of the graft, the coronary node then acting as pacemaker.

While these grafting experiments are taken as giving presumptive evidence in support of the humoral theory of the heart beat, once more the evidence can be interpreted otherwise. It is quite likely, for example, that the graft acts in a purely physical manner in driving the heart to which it is applied. Even, however, if it did act by pouring out active substances on to the other heart this does not mean that/

that such must be considered a physiological mechanism. A graft being a damaged tissue it is indeed possible that active substances would be transferred from it to the heart, but even so the action of these substances would presumably be a local one: in any case such an action would not serve to prove that normally the action of the active substances is humoral. The fact, moreover, that the electrocardiogram of the experimental animal was similar to that of the donor probably favours the view that the action of such grafts is purely physical. Experiments which I have in view will probably settle this question.

Having abandoned that part of Demoor's thesis which views the active substances as exerting their effects by humoral channels, and having thus taken the main prop as it were from his theory, it is necessary to offer an alternative interpretation of the experimental findings.

My own view, in brief, is: that automaticity is a function of nodal primitive tissue; that nodal tissue is automatic in virtue of the presence therein of some chemical substance or substances; that this substance is probably but not necessarily formed by such tissue; that this substance is probably not an actual component of the tissue but something acting on it - as witness the fact that it can be extracted/

extracted from one tissue and utilised by a similar tissue deficient in respect of it -; that the higher the concentration of this substance in a given mass of nodal tissue the higher the automaticity of that mass; that in the heart in situ the part of highest automaticity acts as pacemaker, the impulse initiated in this part being conducted to other parts of the heart by the ordinary conducting channels. In other words, the heart beats because of the high automatic activity of the nodes the which depends on the high local concentration of an "active substance". I do not believe that the heart beats in virtue of all its parts being bathed by substances poured out from the nodal system.

One of the most striking experiments in favour of the view that the active substances exert their effects locally and that the action of the nodes is none other than a "driving" action on the rest of the heart, is performed every time one separated the right from the left auricle. If Demoor's view that the active substances act humorally on all parts of the heart were true, surely sufficient of these substances would be present in the left auricle to maintain its rhythm for a short time after its isolation a few seconds after the death of the animal. But such is not so. In separating the left auricle from the right it is found that the two beat synchronously/

synchronously until the last bridge of tissue between them is severed. Immediately the severance is complete the left auricle ceases to beat.

It might be thought that the results of tissue-culture experiments deal a serious blow to the view that automaticity exists only in the tissues of the nodal system and that the heart is thus simply "driven" by that part of the nodal system exhibiting automaticity in highest degree. It must be remembered, however, that the tissues of the nodal system are fairly widely distributed in the heart and that it is most likely that in excising some part of the heart for such an experiment ~~the~~ nodal as well as myocardial tissue is present. This being so the nodal tissue having been removed from the influence of more highly automatic centres will itself function as a node and thus cause the myocardial fragment to beat rhythmically as if it possessed this inherent power. Such considerations are the more likely when one considers how often fragments of tissue from the inner surface of the ventricles are used in these experiments.

When, on the other hand, the tissues are obtained from the embryonic heart, while similar considerations might apply it would probably be more correct to admit that here indeed the rhythmic activity was inherent in the myocardial tissue itself. But this would not militate against the view that automaticity is/
is/

is subserved almost entirely by the nodal tissues in the adult heart to which alone the theory is meant to apply.

It can hardly be doubted that in the elementary heart all the fundamental attributes of cardiac tissue are subserved in more or less equal degree by each individual cell. Thus the physiological differentiation of labour seen in the vertebrate heart, where the different functions are subserved mainly by distinct tissues, is a product of evolution. This differentiation is expressed in greatest degree in the mammalian heart, in which as has been shown, pure myocardial tissue is practically devoid of automaticity: its main - or perhaps its only - function is contractility.

Ontogeny, however, is but a shortened recapitulation of phylogeny, and this must be true of the physiological as well as of the morphological. This being so it is obvious that the embryonic heart cells in their earliest stages will subserve all those fundamental attributes of cardiac tissue in some if not in equal degree. Only as ontogeny proceeds will the gradual separation, as it were, of these functions be sufficiently far advanced to permit of one obtaining cardiac cells which do not exhibit some degree of spontaneous rhythmicity.

GENERAL DISCUSSION.Criticisms of work on the heart hormones.

Though there are no good a priori grounds for setting aside the possibility that the automaticity of the heart is due to the presence of some chemical substance in the primitive or nodal tissues, there is a good deal of justification for some of the criticisms which have been levelled against the work of those who have sought to show that this is so.

Criticisms of Haberlandt's experiments.

By far the most of this criticism has been directed against the work of Haberlandt. It is needless to go into these criticisms in detail. Suffice it to say that numerous workers have shown that various tissue extracts will give effects exactly similar to those described for the "Herzhormon", and that therefore the substance described by Haberlandt is not specific to the nodal tissues of the heart and has thus no claim to be considered a hormone*. Among those who have come to this conclusion are Rigler(217) Rigler and Singer (213,214), Katz and Liebenson (215) Pawlenko (218), Weichart (216), Winternitz (219) and Oppenheimer (220). Despite Haberlandt's contention that/

* See also Zuelzer (239) on the "heart hormone" of the liver, and Frey et al. (237) on a "heart hormone" extracted from urine.

that his heart hormone is not histamine, Oppenheimer, among some of the others named above, points out anew the striking similarity between the action of histamine and of the so-called heart-hormone and other tissue extracts.

The main criticism of Haberlandt's experiments is to be found, however, in the fundamental paper of Clark (222) published in 1913. Had Haberlandt at the outset realised the significance of this work of Clark he would never, methinks, have permitted himself to be so uncritical of his own experiments and to have thus produced so much that is valueless. On his own showing he has worked largely with ^{the} hypodynamic* frog heart and yet has not even troubled to make himself acquainted with ordinary reactions of this preparation.

At the same time it must not be supposed that because Haberlandt's experiments are unsatisfactory the theory which he bases on them is wrong. It may be quite otherwise. If the work of Demoor is of any significance - and Demoor's experiments are infinitely more satisfactory than Haberlandt's - it is indeed likely that the basis of cardiac automaticity is chemical and that therefore "active substances" are to be found in the frog as well as in the mammalian heart/

*The similarity which exists, in certain respects, between the isolated left auricle of the mammal and the hypodynamic frog heart deserves further study and may prove of great interest and value. This should have been noted in a previous section.

heart; but - and this is the main point in this argument - all the evidence is against Haberlandt having obtained these substances. So many different factors affect the hypodynamic heart - and indeed the degree of differentiation of function in the frog heart is so slight - that to demonstrate a substance on which the automaticity of cardiac tissue depends is almost impossible using the frog heart itself as a test object. Further, while we may consider the extract experiments as quite unconvincing it might be thought that the sinus-ringer and basis-ringer experiments were more satisfactory. But here again the possibility of unspecific factors diffusing from these tissues and affecting the heart to which they are applied is a factor which must be eliminated before the experiments can be considered convincing.

It must not be imagined, of course, that such destructive criticism is necessarily justified. It can only be justified by experiment. The crucial experiments would be to determine whether or not the heart hormone preparation as made for Haberlandt would rhythmicise the isolated mammalian left auricle. If it did not then the criticisms regarding the extract experiments would be justified. Experiments on the effect of sinus-ringer on the mammalian left auricle preparation would be rather difficult, but supposing/

supposing that aqueous concoctions of the sinus regions of a number of frog hearts would rhythmicise the isolated left auricle then it might be considered that, indeed, the Herzormon of Haberlandt did exist. Experiments such as these indicated will be performed in the near future by myself.

Criticisms of Demoor's work.

Demoor's work has come in for but little criticism - little, at least, that is based on experiment.

Haberlandt, peculiarly enough, was so rash as to state that Demoor's experiments were opposed by the work of Abderhalden and Gellhorn. These workers showed (224) that numerous substances and tissue extracts would affect the beat of the isolated frog and mammalian heart. It is obvious, of course, that the experiments of Abderhalden and Gellhorn have infinitely more significance for Haberlandt's own work than for Demoor's.

Mrs. Oppenheimer (220) has offered experimental evidence to support the view that the action of the active substances is not specific and is due to histamine. There are two fallacies in her work. In the first place the method of extraction employed is such that no active substances could be present in the extracts/

extracts. In the second place she investigates the action of these extracts on the isolated perfused mammalian heart! And somewhat similar experiments, which led to similar conclusions, have been performed by Rigler and Tiemann (221). It is obvious, however, that such experiments have no bearing whatever on the phenomena described by Demoor and myself and are thus valueless as evidence against us.

Criticisms of Zwaardemaker's work.

The work of Zwaardemaker has come in for the most damaging criticism. Clark (251), for example, showed that uranium, while it will excite a heart arrested from potassium lack, cannot be said to replace potassium in the manner in which rubidium will do so. It thus appeared that if K acts in virtue of its radioactivity it must have some other effects also apart from this. Similar results were obtained by Peters (252) in experiments on the substitution of uranium for potassium in growth media. A further paper of Clark's (253) dealt a still more serious blow to Zwaardemaker's contentions. He shows that the contractions initiated by radioactive substances applied to the K-free perfused heart are always abnormal in character; that the typical effects of excess K on the frog's heart and other organs are not produced by radioactive/

✓ a. J. 2

radioactive elements; and that the effects of K lack upon the isolated rabbit auricle and upon the isolated gut and uterus of the rabbit, effects which are typical and immediately remedied by addition of K, have neither their onset prevented nor their progress inhibited by the addition of radioactive elements.

Libbrecht, in a short series of papers (255-257), shows that Zwaardemaker's views are untenable, as do Zondek (258) and Niederhoff (259) who repeated Zwaardemaker's experiments and obtained completely negative results. Among others who failed to confirm Zwaardemaker are Verzar (260), Hamburger (261), and Kawashima (262), while he has been criticised on other grounds by Fröhlich (263), Viale (264), and Arboretus and Zotterman (265).

How far the varied criticisms of these different workers are justified remains to be seen. Purely negative results in attempts to repeat Zwaardemaker's fundamental experiment probably mean as little as do negative results in attempting to repeat Loewi's experiment or Demoor's experiment. Be this as it may and granting the accuracy of Zwaardemaker's experimental results, there are still in my view grave difficulties in the way of accepting his theory. Two only need be mentioned.

The/

The first of these is, peculiarly enough, of Zwaardemaker's own making. Having shown that known radioactive substances would replace potassium, he showed that caesium acted similarly (249). He interpreted this result as a proof of the radioactivity of caesium, and in subsequent publications lists this element under other β -radioactive elements. The most careful physical research has, however, failed to show any trace of radioactivity in caesium, and so the main point in Zwaardemaker's whole thesis is put in serious jeopardy.

A second difficulty is the fact that after stoppage of the heart by perfusion with a potassium-free solution the heart still contains large amounts of potassium which can only be very insignificantly reduced by even prolonged perfusion. (253). This being so, it is difficult to see why the radiations from the potassium still present in the heart should not suffice to maintain a beat if Zwaardemaker's view is correct. In other words, it seems certain that the function of potassium is exerted by the potassium ion as such, and not in virtue of the radioactive principle of the element. It is more than conceivable - and the suggestion is made for what it is worth - that the results obtained by Zwaardemaker may be partly accounted for by the liberation, from the potassium reserves/

reserves of the heart, of diffusible (ionic) potassium as a result of more or less destructive changes in the heart cells brought about by the applied radiations.

The question of the identity of the substances of Demoor, Haberlandt, and Zwaardemaker.

While each of the three leaders in this work on the heart hormones has in turn identified the substance described by the other two with that described by himself, this is more the expression of a pious opinion than of an experimental fact. It is apparent that this aspect of the problem would repay experimental investigation; for if it could be shown that the substances of Demoor, Haberlandt and Zwaardemaker were indeed identical then the value and significance of this work would be immeasurably increased.

The type of experiment which would establish the identity of the "Herzhormon" and the "substances actives" has already been indicated, and I would suggest that the "automatine" of Zwaardemaker be investigated in the same way - that is to say on the isolated left auricle. There can be little doubt, methinks, that the isolated left auricle should be used in such experiments rather than the whole heart preparations/

preparations as used by Zwaardemaker and Haberlandt - Indeed, it is difficult to see how any definite conclusions could be drawn from experiments carried out on any other lines.

Assuming that Haberlandt's "Herzhormon" did in fact rhythmicise the left auricle the interpretation of the phenomena observed in the tissue before and after its subjection to the extract, and the application of these considerations to the entire heart, would be simple and straightforward. On the other hand, if Zwaardemaker's "automatine" acted similarly an interpretation of the behaviour of the left auricle in terms of Zwaardemaker's theory would require to be possible if the theory itself were to remain tenable. This is, however, possible.

Zwaardemaker considers the stoppage of the heart when perfused with K-free ringer to be due to the absence of the β rays of potassium necessary to the formation of automatine on which rhythmicity depends. In the fluid bathing the isolated mammalian left auricle irrigated after the manner employed by Demoor and myself, potassium is, however, present. Why then does not the left auricle beat? The reason is that automatinogen and automatin act in virtue of their concentration in the nodal tissue, and there being but little nodal tissue in the isolated left auricle the/

the concentration at any point is insufficient for the establishment of rhythmic activity. If addition of automatin or of automatinogen were to determine the onset of rhythmic activity the interpretation of the result is then simple:- the concentration of automatin has now been increased in some part of the nodal tissue - thanks to the special affinity of such tissue for automatine - which thus assumes the functions of a node.

It would be of interest in passing from a consideration of this question to speculate as to how far the results which I obtained with skeletal muscle extracts were due to automatin produced from the automatinogen of the muscle. This is of especial interest in view of the demonstration of Westenbrink and Arons that automatinogen is automatin adsorbed on or combined with a phosphatide. The fact, however, that similar results were obtained by me with such substances as saponin and lecithin complicates the question which must thus await further experimental treatment.

On heart-hormone research.

In concluding this brief review of certain aspects of the experimental work on the heart hormones it is well to point out just to what stage this work has/

has actually got. And this aspect can be dispensed with in a word.

Leaving aside for the present the experiments of Haberlandt - these have been treated of sufficiently in the foregoing pages - and leaving aside also the work of Zwaardemaker - for of this I have no personal acquaintance - a sufficiently clear idea of how things stand is afforded by a consideration of the work of Demoor and of myself.

Demoor has never pretended that his experiments are ideal. He upholds - and upholds rightly, methinks, his method of investigating the basis of automaticity by means of the isolated left auricle preparation, and maintains that it is superior in every way to the method employed by Haberlandt and Zwaardemaker - and that it is, in fact, the only method by which the basis of rhythmicity can be investigated -; but he does not hold that the extracts as employed by him represent anything like pure extracts of the active substances. With these views I am in entire agreement. The very fact, however, that the extracts as at present used contain such a heterogeneous collection of substances is a very unsatisfactory state of affairs, and it certainly appears that any conclusions drawn from the results of experiments with such must be at least guarded. Now, while I have confirmed Demoor's fundamental experiment, and have moreover been unable to/

to show that the rhythmicising factor is other than specific to the nodal tissues, I cannot but feel that before any of the conclusions based on this work can be justifiably accepted the work must be put on a sounder bases. And this can only be done when the active substance or substances of Demoor has been obtained in a much purer state than hitherto and its physiological properties determined by what will then be scientific experiments. The significance of this work seems fundamental and an attempt to put it on a sound basis should certainly be made.

ON THE BASIS OF CARDIAC AUTOMATICITY.

Appendix: On the relationship of adenosine to the heart hormone of Haberlandt.

As has already been noted Haberlandt put a preparation of his heart hormone on the market for therapeutic use. This preparation which was called "Hormocardiol" was an extract prepared from beef heart. Despite the fact that Haberlandt confined his experimental work entirely to the frog heart it appeared that he had in this extract an active principle similar to that which he had found in the frog, since the commercial extract gave results identical with those obtained from frog heart extracts. (This, in point of fact, is not surprising in view of what has already been pointed out regarding the reactions of the hypodynamic heart.) Hormocardiol, however, appears to have had but a short existence, and was soon replaced by an extract of skeletal muscle called "Carnigen", which again had all the properties of the heart hormone — "Es wirkt pulsauslösend, pulsbeschleunigend und pulsverstärkend". The name of this preparation was later changed to "Lacarnol" and as such is on the market today.*

That/

*On this subject see Fahrenkamp and Schneider (267).

That "Lacarnol" will indeed revive a hypodynamic heart is shown in fig. 33 which is from one of my experiments. A heart was rendered completely hypodynamic by prolonged perfusion. In this particular case perfusion was continued from 4.20 p.m. until the commencement of the experiment at 2.30 on the following day. By this time the ventricle had ceased to beat entirely and the auricles were beating irregularly at the rate of about once per minute. A drop of "lacarnol" (1/100) was added to the perfusion cannula at the point indicated. Almost immediately the auricular contractions increased to 15 per minute. The next part of the figure shows the beat at the end of 10 minutes; the rate is halved, having attained this value 3 minutes after the application of lacarnol. Adrenaline, 1/100,000, is now added and abolishes the beat. When the heart recommences the rate is the same as before and is maintained for a further seven minutes, when histamine, 1/100,000, is added. Once more the contractions cease, to be resumed a short time before washing out the experimental solution.

The lacarnol, then, served to re-establish the activity of this heart, which had been more or less quiescent for some hours. The main interest of the record, however, lies in the fact that neither adrenaline or histamine served to improve the beat. These substances/

substances either have no effect on the long perfused heart or have a depressant action. This is a point in favour of Haberlandt's view that his hormone is not in fact adrenaline or histamine.

The question as to which of the presumably numerous components of lacarnol its action in re-establishing activity in a hypodynamic heart might be due, seemed one which might be difficult of solution. A possible clue, however, was afforded by work along entirely different lines, and it is hoped that as a result the whole problem of the Haberlandt hormone may soon be solved.

In a paper published in 1929 Drury and Szent-Györgyi (238) reported the isolation of a substance from extracts of heart muscle which had a marked effect upon mammalian heart, producing a transient high grade heart block and pronounced coronary dilatation. The substance was identified as adenylic acid, and while it was obtained from sources other than the heart the yield from this organ was the highest. They showed further that adenosine, prepared from yeast nucleic acid, had properties identical with those of adenylic acid.

Despite the marked effect of this substance in producing heart block in the mammalian heart it seemed/



FIG. 33.
ACTION OF "LACARNOL", ADRENALINE AND HISTAMINE ON HYPODYNAMIC HEART. (*Rana esculenta*)

Heart perfused 27 hours before observations. I) ADDITION OF LACARNOL, 1 drop $\times 100$ with resumption of beat. II) 7 mins. later - ADDITION OF ADRENALINE $1/10000$ - depression of beat. III) 6 mins later than II. - ADDITION OF HISTAMINE $1/10000$ - INHIBITION AND, LATER, RECOVERY.

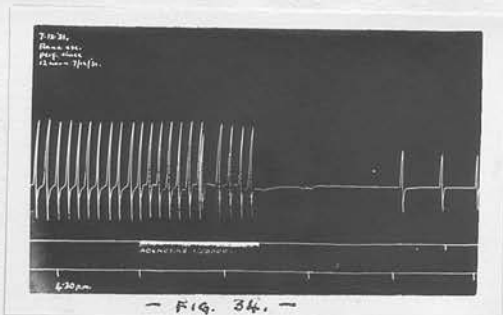
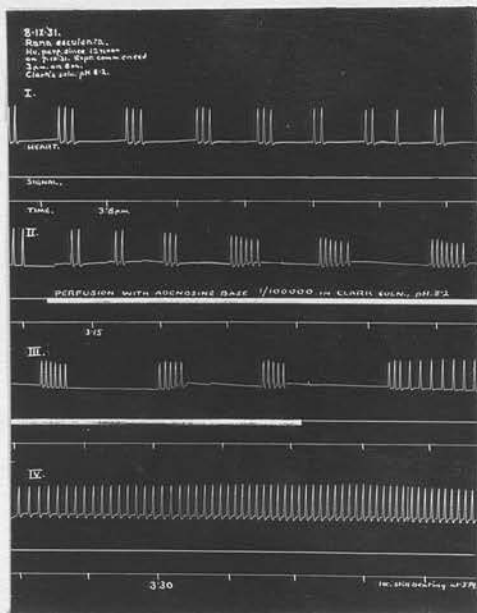


FIG. 34. ACTION OF ADENOSINE ON FROG-HEART.

ADENOSINE, $1/50000$, PERFUSED FOR ABOUT ONE AND HALF MINUTES. NOTE INITIAL TRANSITORY SLIGHT INCREASE IN FREQUENCY AND "TONE", AND THE SINGLE EXTRASYSTOLE. ON RETURN TO NORMAL PERFUSION LIQUID HEART IS COMPLETELY INHIBITED FOR OVER $1\frac{1}{2}$ MINS. THEREAFTER BEAT GRADUALLY RESUMED.

seemed desirable to investigate its relationship to the effects described by the different workers on the heart hormone, especially as adenylic acid must be a constituent of practically every extract as used by these workers. Up to the present some experiments have been made in connection with Haberlandt's work and one of these will be described.

In the first place it must be noted that the action of adenosine on the normal frog heart in no way resembles that of a cardiac stimulant. In a concentration of $1/250,000$ it may (1) have no visible effect; (2) cause a slight increase in the rate and/or amplitude; or (3) - and this is the most usual action - have a depressant effect. In higher concentrations it is decidedly depressant, but the initial action may be slightly accelerator and the depressant effect appear after the perfusion fluid has been changed once more to normal. The figure (34) on the opposite page shows such a result. In this case the heart had been perfusing for four and a half hours previous to the observation. Adenosine base, $1/50,000$, was perfused for about one and a half minutes. During the perfusion with this solution the only change noticeable is a very slight initial quickening of the beat and the occurrence of an extra systole/



— Fig. 35. —

ACTION OF ADENOSINE IN REVIVING BEAT OF HYPODYNAMIC HEART.

HEART OF *R. ESCULENTA* PERFUSED FOR 17 HRS. BEFORE COMMENCEMENT OF OBSERVATIONS.
 LINE I. "NORMAL" BEAT. LINE II. COMMENCEMENT OF PERFUSION WITH ADENOSINE BASE 1/100,000. — NOTE EFFECT ON GROUPS OF BEATS (CF. FIG.)
 LINE III. CESSATION OF PERFUSION WITH ADENOSINE. SEVENTY SECONDS LATER NOTE RESUMPTION OF REGULAR BEATS (IDIOVENTRICULAR).
 LINE IV. AT SECOND BEAT AURICULAR CONTRACTIONS COMMENCE, AND AT 3:30 $\frac{1}{4}$ SINUS ALSO COMMENCES (SINUS CONTRACTIONS DO NOT SHOW ON RECORD— NOTE, HOWEVER, INCREASED CHROMATROISM.) THE VENTRICLE ONLY WAS STILL BEATING WHEN THE EXPT. WAS DISCONTINUED AT 3:59.

systole. Almost immediately the normal perfusion fluid is substituted for the experimental one the heart stops completely, the beat being resumed gradually.

The effect of adenosine on the long perfused heart is shown in fig. 35. This heart had been perfused for 27 hours before the commencement of the experiment, and though still beating the beats were idioventricular and appearing in groups (line 1). In other words the heart was in the same condition as were most of those on which Haberlandt worked. The second and third lines show the commencement and finish respectively of perfusion with adenosine base, 1/100,000. This was perfused for slightly over ten minutes. After two minutes the number of beats in each group was increased and this behaviour continued until perfusion with the normal fluid was resumed. About a minute and a quarter thereafter the ventricle commenced to beat regularly (line 3), and about two minutes later the auricles also recommenced (beginning of line 4), to be followed about three minutes later still by the sinus (line 4 towards middle). The different parts of the heart ceased to function in the reverse order, the sinus stopping about 3.37 and the auricles about 3.40. The ventricle, however, was still/

still beating slowly when the preparation was left at 3.59.

While more experiments require to be made before the time arrives for an interpretation of these results to be attempted, the fact that adenosine will revive the beat of a quiescent or almost quiescent and long perfused heart is probably significant, especially when it is remembered that this substance is a known constituent of cardiac tissue. That adenosine, or a compound formed from it, is the substance to which Haberlandt's results are due, is made the more likely by the fact that adenosine is known (268,269) to be a constituent of those commercial preparations which Haberlandt himself assures us have all the properties of his heart hormone.*

* It is probably to the dilator action of adenosine on the coronary arteries that any therapeutic effect which lacarnol may possess is due.

ON THE MODE OF ACTION OF THE
EXTRINSIC CARDIAC NERVES.

Part I. Critical Review.

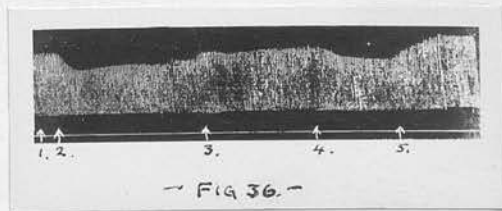
Introductory:

While the presence of nerves leading to the heart seems to have been known from early times - Galen (2) was certainly aware of their existence, and satisfied himself by experiment that they were not essential to the regular beating of the heart - and although the functions of these nerves had by the later part of the nineteenth century been fairly well worked out, thanks to the pioneer observations of Thomas Willis and Richard Lower which paved the way for the work of the Webers and the Cyons, and although the work on the extrinsic cardiac nerves gave rise to a general theory of excitation and inhibition, which saw the former as an increase in dissimilative metabolism or katabolism, and the latter as an increase in assimilative metabolism or anabolism, any view backed by experimental evidence as to the actual mechanism by which such phenomena were brought about, was non-existent.

In/

In 1921, however, it was announced by Loewi, Professor of Physiology in the University of Graz, that the effects obtained on the frog heart by stimulation of the cardiac nerves were due to the production of specific chemical substances in the heart by such stimulation and the subsequent action of these substances on the cardiac tissues. It is with this fundamental observation and the others which followed that the first part of the present paper will deal.

Discovery of the Humoral Transmissibility of the Effects of Cardiac Nerve Stimulation. Loewi's fundamental discovery was made in the following manner:- A small quantity of Ringer's fluid was permitted to irrigate the heart of a frog or toad suspended on a Straub cannula, the heart having its vago-sympathetic trunks attached. A sample of such liquid taken after faradisation of the vagus and transferred to a second heart fitted up in the same manner as the first (or replaced in contact with the first heart after a preliminary wash with fresh Ringer) caused this second heart to behave as though its vagus was being stimulated. A sample taken from the first heart during inactivity of its nerves had on the other hand no effect on the/



— FIG. 36. —

FIG. 36.

R. ESCULENTA.

- 1) NORMAL. 2) RINGER FROM 15' VAGUS-STIMULATION PERIOD. 3) RINGER FROM 15' NORMAL PERIOD. 4) SAME AS 2) ABOVE. 5) 0.1 MG. ATROPINE ADDED.

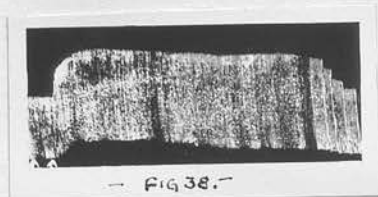


— FIG. 37. —

FIG. 37.

R. TEMPORARIA.

- 1) RINGER FROM 15' VAGUS STIMULATION PERIOD. 2) NORMAL. 3) SAME AS 1). 4) NORMAL.

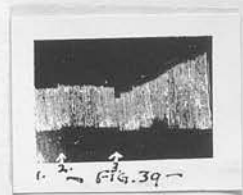


— FIG. 38. —

FIG. 38.

TOAD NO. 1.

EFFECT OF VAGUS STIMULATION IN TOAD.



— FIG. 39. —

FIG. 39.

TOAD NO. 1.

- 1) NORMAL. 2) 25' NORMAL PERIOD. 3) 25' VAGUS STIMULATION PERIOD.



— FIG. 40. —

FIG. 40.

TOAD NO. 2.

- 1) RINGER FROM 25' NORMAL PERIOD OF TOAD NO. 1.
2) RINGER FROM 25' VAGUS STIMULATION PERIOD OF TOAD I.

the rhythm of another heart to which it was applied.

In view of the fact that the sympathetic fibres to the heart are in close conjunction with the vagus during most of its course Loewi obtained on some occasions a sympathetic effect on stimulation of the nerve trunks of the first heart, and when such was the case transference of the fluid in contact with the heart to a second heart determined the appearance in the second heart of the effects characteristic of sympathetic stimulation. The figures from Loewi's original paper with their legends are reproduced on the opposite page.

These results immediately suggested to Loewi that the effects obtained by stimulation of the cardiac nerves were due to the production of chemical substances by such stimulation and their subsequent action on the tissues, and not as had been previously supposed to a direct action of the nerves (38)(42). As Loewi himself admits, the experiments are difficult to perform; the percentage of positive results in a given run of experiments is often low, and while positive results are definite they are not as a rule strikingly so.

One of the main difficulties of the experiment arises from the fact that it is not easy to dissociate completely the two effects, but by using weak/

weak stimuli Loewi was able to obtain a predominant vagal effect, and by using very strong stimuli a predominant sympathetic effect (43). While a slowing was sometimes obtained in the second heart as a result of vagal stimulation in the first - Loewi shows such a result in his first paper - more usually only a negative inotropic effect was observed. This has been noted also by nearly all subsequent workers. Better results were obtained by Loewi in those cases where he "paralysed the sympathetic fibres" by means of ergotamine, thus allowing free play of the vagi (44).

The announcement of these facts and the interesting interpretation put on them led many investigators to attempt by like and by different techniques to obtain similar results. As far back as 1913 Hemmeter (45), working on dogfishes with crossed circulation had obtained negative results in respect of heart rate and force in one animal on stimulating the vagi of the other, and many who attempted to repeat Loewi's experiment obtained similar negative results. Others, again, though obtaining a percentage of positive results were unable for various reasons to accept Loewi's thesis, and while we can disregard entirely the purely negative findings of such workers as Bohnenkamp (46), Asher (47), Nakayama (48), Patrizi (49), Heymans and Heymans (50), Hermann and Malmejae (51), and Tournade/

Tournade, Chabrol and Malmejac (52), we must examine later the evidence against Loewi's view afforded by the work of those of his critics who were fortunate enough to perform more or less positive experiments.

Most of those who attempted to confirm Loewi's findings departed from his technique, for the most part with a view to obtaining a self-recording experiment. Brinkman and van Dam, for instance, connected the hearts in situ of two frogs A and B in such a manner that the liquid perfusing through heart A passed immediately to heart B. They found that effects on heart A produced by stimulation of the nerves leading to that heart were reproduced after a short latent period in heart B. There is, however, an obvious criticism to the technique employed here. Since the connection between the two hearts is continuous a change in the rate and force of A might determine a corresponding change not only in amount of liquid passing to the second heart but what is perhaps of greater importance might by changing the rate of incidence of the purely hydraulic stimuli afforded by this connecting column of liquid produce a change in the rate and force of heart B. That the results obtained by Brinkman and van Dam were not necessarily due to such effects, however, was shown by Ten Cate (54), who obtained similar results with a modified technique. He interposed between the two hearts a small reservoir which served the double/

double purpose of breaking the continuity of the connection between the two hearts, thus eliminating the purely mechanical effect exerted by one heart on the other and at the same time permitting of the maintenance of a constant head of pressure for the second heart.

Nemoto (55), while his experiments are not of the "self recording" variety, has recently obtained good "vagus substance" effects in experiments on toads. He cut the sympathetic two to three weeks previous to the experiment. Vagus stimulation which produced complete stoppage of the heart was maintained intermittently from ten to twenty minutes, a circulation of Ringer being maintained by joining the right aorta with the posterior vena cava. The fluid collected after such stimulation always produced a negative inotropic and a slight but definite negative chronotropic effect when reintroduced to the heart after this had been irrigated with fresh Ringer. Oliveira Frias (56) obtained chronotropic effects in ten out of thirty-six experiments on the turtle (*Clemmys* sp.) heart, this being the highest average for such an effect that seems to have been recorded. Riccitelli and Vita (57) obtained vagal substances from the vagus stimulated hearts of tortoises and though they obtained chronotropic effects in some cases on transference of the fluid to a toad heart they showed that with tortoises as with frogs and toads the inotropic effect is more easily/

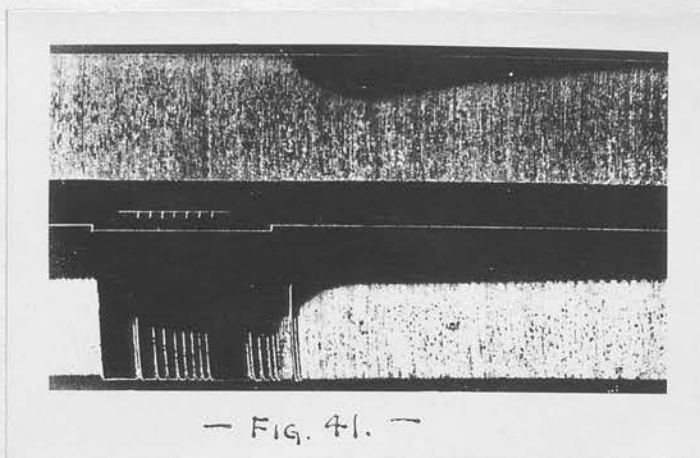


FIG. 41.

Rana esculenta.

Lower record is of vagus-stimulated heart. Note decreased inotropism of second heart as result of vagus stimulation in the first.

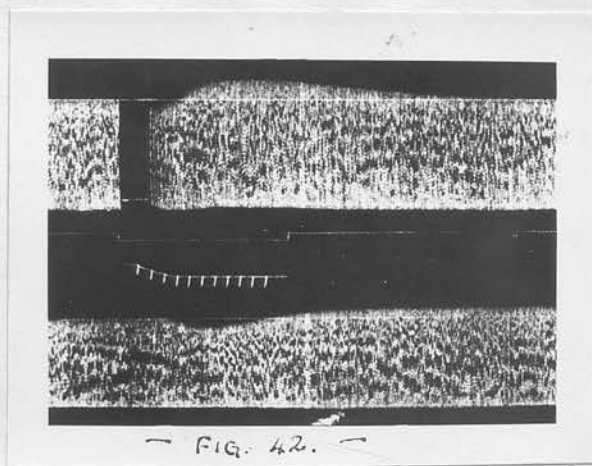


FIG. 42.

R. esculenta.

Upper record is vagus-stimulated heart. Note that characteristic sympathetic effect which follows vagus-stimulation shows also in the second (non-nerve-stimulated) heart.

easily elicited.

Perhaps the most definite results of all, however, have been obtained by Kahn (58). Kahn used a double-nosed cannula carrying two frog hearts each with its double vagus supply intact. By stimulating the nerve of one, the vagus or sympathetic effect, whichever predominated, showed in the second heart after a brief latent period. The two hearts could be used alternately to produce the vagomimetic or sympathomimetic substances, and the effects obtained were, of course, reversible. Two of Kahn's records are reproduced on the opposite page and show the striking type of result obtained by his technique. Lambert, Hennequin and Merklen (59), and Asher and Scheinfinkel (60), have repeated Kahn's experiment, and while they have obtained some positive results they are unable, for reasons which will be stated later, to accept the views of Loewi and Kahn. Samojloff (61), on the other hand, using the same technique, made the interesting observation that the heart supplied by the vagus substance showed the electrocardiographic characteristics of a vagus-stimulated heart.

The mode of action of atropine and
ergotamine.

In 1924 Loewi and Navratil made a fundamental contribution to the subject. They showed that if
the/

the heart whose vagus was to be stimulated was atropinised, though no vagal effect could now be produced in this heart by stimulation of the vagus nerves, nevertheless, if the fluid bathing it during such stimulation was transferred to a second heart, it would produce typical vagomimetic effects in this. Similarly, it was shown by Navratil (63) that if the first heart were ergotaminised, while sympathetic stimulation would produce little or no sympathetic effect on this heart, transference of the fluid obtained during such stimulation would produce sympathomimetic effects in a second heart. Loewi and Navratil concluded from these results that atropine did not "block" the parasympathetic nerve endings, or ergotamine the sympathetic endings; that, in other words, atropine and ergotamine did not prevent the production of the vagomimetic or sympathomimetic substances of Loewi, but that they exerted their effects by preventing the substances produced by stimulation of the nerves from exerting their effects on the myocardium.

Extension of Loewi's findings to
the mammalian heart.

As was but natural, experiments were soon attempted with a view to ascertaining if what held for the frog and toad held also for the mammal.

The/

The earliest of such experiments are those of Duschl (64) and Duschl and Windholtz (65). Using rats and sometimes cats and dogs, with crossed circulation, they showed that excitation of the cardiac vagus in one animal led to a slowing of the heart in the second. It is manifest, however, that with such a technique the results obtained are open to numerous interpretations, though the later demonstration of Duschl (66) that blood withdrawn from the heart of one animal during vagus stimulation and injected into a second animal produced in such vagomimetic effects, might be considered more satisfactory. This last observation, made on cats and rabbits, was confirmed later by Brinkman (67) and by Brinkman and van de Velde (68), who obtained vagal blood from the carotid artery instead of from the heart. Zunz and La Barre (69) obtained vagal fluid from the isolated perfused heart of one animal and on injecting this into the ear vein of another animal with reduced circulation obtained a slowing of the heart. These workers made the further observation that the vagal fluid when mixed with blood rapidly loses its inhibitory qualities - an observation also made by Plattner (70) in his transfusion experiments with dogs. Somewhat similar experiments, using isolated perfused heart as a source of the vagus substance, were performed by/

by Foa (71) and Mochizuki (72). The latter, in addition to determining the effect of vagal fluid on other intact animals, used a second isolated perfused heart to which he applied the fluid obtained from the first, thus eliminating most of the fallacies of the previous experiments. Riccitelli and Vita (57), using a similar modified Loewi technique, demonstrated the production of the vagal substance in dogs, rabbits and cats, and, like Zunz and La Barre and Plattner, found that "vagal Locke" was much more effective in producing the result than was "vagal blood".

The most striking results obtained with the mammalian heart, however, appear to be those of Rylant (73) (109). Not only did he show humoral transmission of the vagal and sympathetic effects in the isolated perfused hearts of cats and rabbits, but he also obtained with these animals similar effects to those described by Navratil for the frog in respect of the action of atropine and ergotamine. Thus while an atropinised rabbit heart would show none of the effects characteristic of vagal stimulation when its vagi were in fact being stimulated, the fluid passing from this to a second heart would nevertheless produce typical vagomimetic effects in this. Similarly with ergotamine and sympathetic stimulation. Finally Rylant showed that if defibrinated blood be used for the perfusion instead of Locke, while the same effects may be obtained they are not exhibited to/

to such a degree, indicating that the substances responsible for the effects are destroyed more or less rapidly in contact with blood - a result which has, as we have seen, been obtained by most other workers.

Rylant's technique is of interest. The heart of a cat or rabbit - preferably a rabbit - is isolated with its vagus and sympathetic supply intact and is attached to a glass cannula about one metre in length. A second heart is fitted up in exactly the same way but without its nerve supply. The two cannulae are placed in the same vertical line so that the fluid issuing from the heart whose nerves are to be stimulated falls into a thistle funnel at the open end of the cannula carrying the other heart, so supplying this latter with fluid. It is advisable to have as large a heart as possible to act as donor, otherwise it may be necessary to permit extra fluid to flow down the outside of the upper cannula in order to keep the lower cannula full. The great simplicity of this whole arrangement is made possible by the fact that the heating devices usually employed in heart perfusion work are dispensed with, the whole laboratory in which the experiment is carried out being heated electrically to about 40 degrees Centigrade and maintained at/

at this temperature by an electric thermostat, the atmosphere being kept saturated with water vapour and duly agitated by means of fans.

Other properties of the neuromimetic substances of Loewi.

The effect of the neuromimetic substances on chronaxie.

Observations of great interest concerning the action of the Loewi substances on the chronaxie of the heart were made by Henri Frédéricq. Having previously shown (74) in the dog, frog and tortoise, that stimulation of the vagus produced a decrease in the chronaxie of the heart while stimulation of the sympathetic produced an increase in the chronaxie - an observation confirmed by Mme. Lapique and Mme. Veil (75) among others - Frédéricq demonstrated (76, 77, 78) that such effects on chronaxie were humorally transmissible to a second heart, and that such effects were moreover obtained in this second heart when the concentration of mimetic substances arriving there was such that they produced no effect which was visible. Two different techniques gave comparable results. In the first, two frogs were arranged in series, the perfusate from the heart of one passing directly to the second. Faradisation of the vagosympathetic trunk of the first heart produced/

produced a change in the chronaxie of the second in a direction depending on whether the parasympathetic or sympathetic effect predominated in the first heart. In the second method the perfusate from a frog or tortoise heart in situ was collected during rest and during stimulation of the nerve. Another heart was immersed successively in such liquids and its chronaxie with respect to an extrasystole determined. It is found to be modified by the mimetic substances as before. In these experiments, as in Loewi's, the vagal effect was the one usually obtained, the sympathetic appearing but seldom. It is of interest to note that the chronaxie of the heart is diminished not only by vagal stimulation but also by parasympathomimetic substances such as choline, acetylcholine, arecoline, pilocarpine and eserine, while as with sympathetic stimulation it is increased by sympathomimetic substances such as adrenaline and strophanthin (79). That the vagus substance shortens the chronaxie of the frog heart has also been shown by Lambrechts (111).

Similar experiments of great beauty have lately been performed on mammals by Rylant (235). Using whole isolated perfused hearts as in his original Loewi experiments, or an irrigated auricle receiving mimetic substances from an isolated perfused heart, he showed that the vagomimetic substance reduced the chronaxie/

chronaxie while the sympathomimetic substance increased it, similar results being obtained with acetylcholine and adrenaline. By using the irrigated right or left auricle and driving it electrically at a constant rate he showed that the changes in chronaxie on addition of the mimetic substances still obtained though the substances were now unable to affect the rate of beat of the preparation. According to Rylant, then, with the normal chronaxie of the mammalian auricle at about 2 sigma the substance resulting from sympathetic stimulation will raise this figure to 2.5 or more, adrenaline producing the same effect; while the vagus substance will bring the chronaxie down to slightly over 1 sigma, acetylcholine acting similarly.

Effects of the cardioneuromimetic substances on the stomach and intestine.

Using a technique of similar principle to that employed in their original experiments, Brinkman and van Dam (53) made the interesting observation that the fluid passing from the heart during vagal stimulation in the first frog produced motor effects on the stomach of the second. If a sympathetic effect were obtained in the heart an inhibitor effect was observed in the stomach. Brinkman and Van de Velde (81) made similar observations on the stomach of the rabbit/

rabbit, Ten Cate (82) on the intestine of the frog, Jendrassik (83) on the intestine of the rabbit, and Viale (92) on the intestine of the guinea-pig. Finally Granit and von Bonsdorff (84), using curarised rabbits under ether anaesthesia, showed that stimulation of the cardiac vagus gave rise to motor effects in the denervated stomach of the same animal - an effect which from the conditions of the experiment must have been due to a humoral transference of the vagus substance from the heart.

The question of the specificity of the
Loewi substances.

That the vagus substance is not species specific has been shown by several workers. Popper and Russo (85) showed that the vagus substance obtained from the rabbit's heart would, when made up in a suitable medium, affect the frog heart in a similar manner. This was confirmed by Granit and von Bonsdorff (86) who showed further that the effect on the second heart was prevented by atropine, and somewhat similar experiments were performed by Plattner (87). Oliviera Frias (88) showed that serum of dog's blood obtained during vagal stimulation produced a negative inotropism and chronotropism in the turtle heart.

Chemical/

Chemical and physical properties of
the Loewi substance.

Other properties of the vagus substance may be briefly described. It was found by Loewi (89) to be destroyed by heat and by alkali and to be dialysable. Witanowski (90) and also Gondin (91) found that it was soluble in alcohol but precipitated by ether; Atzler and Muller (93) that it was not absorbed by colloids. Brinkman and van Dam (94) found that it lowered the surface tension of blood serum but Plattner and Galehr (95) and Lambrechts (111) deny that this is so. As we have already seen it lowers the chronaxie of the heart (Fredericq, Demoor and Rylant), and is rapidly destroyed in contact with blood (Plattner, Riccitelli and Vita, Zunz and La Barre, Rylant, Viale).

As regards the sympathetic substance less is known. According to Gondin (91) it is soluble in ether and alcohol (both substances are of course water soluble), raises the surface tension of blood serum (Brinkman and van Dam (94)) and raises the chronaxie of the heart (Fredericq, Rylant). According to Lanz (96) it has all the properties of adrenaline.

The/

The nature of the cardioneuromimetic
substances.

We must now examine the views which have been put forward as to the nature of these substances. As long ago as 1905 Howell (97) and Duke (98) showed that stimulation of the vagus in the perfused heart of the frog led to an increase in the potassium content of the perfusate, and since the action of potassium when present in excess in a perfusing fluid is in fact vagomimetic (Botazzi, 99) it was suggested that the liberation of K in the heart might be the mode of action of the vagus (Henrijean)(100)*. A few years later it was shown by Zondek (101) that the faradisation of the cardiac sympathetic caused an increase in the calcium content of the perfusate. Howell's results were confirmed by Bouckaert (102), Yasatake (103), and by Scheinfinkel (104), and Zondek's by Buskuet (105).

Among/

* It may be observed in passing that Dixon (106) in 1906, arguing along lines suggested by the then recent work on secretin suggested that the heart contained a substance "pro-inhibitin", which on stimulation of the vagus was transformed into "inhibitin", which on combining in some way with the cardiac muscle brought about slowing or stoppage of the heart. This hypothesis does not appear to have been made the subject of experimental inquiry by Dixon, though Loewi's work might be considered as upholding in some degree his view.

Among the principal grounds for setting aside the possibility that the vagus substance of Loewi is potassium is the fact that atropine does not abolish the effects of potassium, whereas it does prevent the action of the "Vagusstoff". The powerful action of the vagus substance on the stomach and intestine offers further presumptive evidence against the potassium hypothesis. In point of fact nearly all the properties of the substance which have been described point definitely to its organic nature.

The similarity between the effects of the Vagusstoff and of choline were early recognised and Loewi was able to show that the choline content of the heart was indeed increased as a result of vagal stimulation (107). This increase, however, was not sufficient to account for the effects obtained and the very much greater vagomimetic effect of acetyl - choline immediately suggested that this derivative of choline might be the vagus substance. The fact that acetylation of the vagus substance did not increase its activity (Plattner) was presumptive evidence in favour of this view.

Loewi soon demonstrated that the behaviour of acetyl-choline and of the vagussubstance was practically identical, one of the most striking similarities being the ease with which each of these substances/

substances was destroyed or its activity diminished, in contact with the heart or with blood. The cause of this rapid destruction of acetylcholine when in contact with the heart - a phenomenon noted by several workers including Clark (110) - Loewi showed to be due to the presence in the heart of an extractable esterase (107). This esterase is destroyed by heat, fluorescent and ultraviolet rays. Physostigmin, according to Loewi (112) inhibits the activity of the esterase and so a greater vagal effect can be obtained in a heart which has been treated with physostigmine than in one which has not. That the "sensitisation" of the vagus in the frog heart by physostigmine is due to a failure of the heart to destroy the vagus substance may, however, be only part of the truth, for Granberg (113) has shown that physostigmine has an ergotamine-like action in respect of sympathetic effects and thus it may be that physostigmine affects the vagal response in two ways - firstly, by preventing dilution of the vagal effect by sympathetic substance and secondly, by preventing the destruction of the vagus substance.

However, be this as it may, it is now generally accepted that the effects of vagal stimulation on the heart are due to the peripheral release of acetylcholine by such stimulation, and we will see in/

in a later section how work along other lines tends to show that this same mode of action is true also for other parasympathetic nerves.

As regards the nature of the sympathetic substance less is definitely known. While the great similarity between the action of the sympathetic substance and the action of adrenaline has been apparent to all who have obtained this effect, only Lanz (96) has gone so far as to assert definitely that it is in fact adrenaline. Much further work is needed before one would be justified, however, in accepting Lanz's conclusion, attractive as it is, and at present the safest course is to say merely that the sympathetic substance has several properties in common with adrenaline: it is an adrenaline-like substance.

Criticisms of Loewi's views.

We have already noted that many workers on attempting to repeat Loewi's experiments obtained entirely negative results and thus came to doubt Loewi's views. Others again, who repeated his experiments, obtained some positive results, but for various reasons were unable to accept the conclusions of the pharmacologist of Graz.

Among these latter are Lambert, Hennequin and Merklen/

Merklen (114). They first of all repeated Loewi's original experiment with moderate success. Later they repeated Kahn's experiment and were successful in demonstrating the humoral transmission of both "vagal" and "sympathetic" effects, i.e., of both negative and positive inotropic effects (59). In the vagus experiments they never obtained an effect on the chronotropism, and their last paper (115) records a final attempt to obtain such. The attempt failed, and they oppose Loewi mainly on account of their consistent failure to obtain this effect which, they hold, is the indication par excellence of vagal activity and hence of the presence of the proposed vagus substance. They hold that the different (opposite) effects on inotropism often obtained by them - and which were sometimes in an opposite sense to that expected - are due to the different pharmacodynamic effects of different concentrations of a non-specific substance such as acetylcholine (sic) liberated by the stimulation.

Antoine (116) mainly on the basis of this work of Lambert and his pupils has come to similar conclusions. He considers that the variable results obtained by many workers who have repeated Loewi's original experiment are not due, as Chiba (117) supposed/

supposed, to differing amounts of the neuromimetic substances in the perfusing fluid but are due to modifications in excitability and conductivity of the heart tissue under the conditions of the experiment, and he concludes that any generalisation regarding the humoral transmission of nervous effects is premature.

Atzler and Müller (93) have put forward the view that the humoral effects are brought about by changes in the hydrogen ion concentration of the perfusate as a result of the nerve stimulation, increase of pH giving a sympathomimetic effect, decrease a vagomimetic effect. This view, however, lacks experimental support.

Adachi (118) thinks that the results obtained in such experiments are due to the accumulation of lactic and phosphoric acids and not to a specific substance set free by stimulation of nerves. He finds that the perfusate from a quiescent heart or from skeletal muscles will give effects similar to those described by Loewi.

Finally, Asher and Scheinfinkel (119), while confirming Kahn's results by Kahn's original method, found on "improving" the method that they obtained negative results. They suggest that the apparently positive results obtained by Kahn's original method are due to one or more of a number of factors such as mechanical/

mechanical influences, the formation of non-specific substances due to disturbed metabolism of the heart, variations in sensitivity of the test heart, or to spread of the stimulating current.

Conclusion.

While purely negative experiments in such work are of little value, it might appear at first sight that criticisms of Loewi's viewpoint arising from experiments that were not entirely negative might merit serious consideration. As things stand at present, however, this is hardly so. Not only will these workers require to produce much more conclusive data to support their negative attitude than they have hitherto succeeded in doing, but they will have to disprove a large mass of the evidence accumulated on the other side. While, for example, it may be granted that the experiments on the frog heart are open to some fallacies due mainly to the close anatomical relations of the vagus and sympathetic nerves, how are the clear cut results obtained with the mammalian heart to be explained away? How also are the effects on the chronaxie of the heart as a result of the action of the "vagus" or "sympathetic" fluids to be correlated with the production of non-specific substances as a result of the nerve stimulation, and how, finally, are the effects/

effects of atropine and ergotamine on the production and action of the proposed non-specific substances to be explained? At present the "anti-Loewians" cannot answer such questions and it is difficult to see that they will ever be able to do so.

We are thus justified in considering that the thesis of Loewi and his school is proved: that the cardiac nerves produce their effects not directly as had been previously supposed, but by the liberation of chemical substances and the subsequent action of these on the heart; that the substance produced on vagal stimulation is almost certainly acetylcholine while that liberated in response to activity of the sympathetic has many properties in common with adrenaline: that, further, atropine and ergotamine do not produce their effects by preventing the production of these substances but by preventing their subsequent action on the cardiac tissue.*

* Viale has recently (231) contested the view that the substance produced by vagal stimulation is acetylcholine. See, however, the work of Lanczos (232) and of Engelhart (233) which appears to offer further evidence that the substance in question is indeed acetylcholine.

ON THE MODE OF ACTION OF THE
EXTRINSIC CARDIAC NERVES.

Part II. Personal experiments.

Introductory:

A glance at the records reproduced on the previous pages - records which are the most striking that have been published in support of this work on the humoral transmissibility of the effects of nervous stimulation - shows that though the effects are definite enough they are almost all of an inotropic nature: all workers are indeed agreed that chronotropic effects are but rarely seen. Further all workers on this subject agree that it is difficult to obtain positive results at all, and that long runs of negative experiments often crop up without apparent cause. The numerous opponents of Loewi's thesis point triumphantly to these facts as supporting their contentions, and it seemed desirable to develop a technique by means of which the humoral transmissibility of the effects of nervous stimulation would be more easily and more definitely demonstrated than hitherto, and, in particular, a technique which would permit of chronotropic effects manifesting themselves.

An investigation, the results of which are here reported/

reported, had this as its main aim. A subsidiary object was the determinations of the conditions which had to be satisfied to procure positive results in the largest possible number of cases.

The argument for the method.

Loewi considered that the first essential in experiments designed to demonstrate the humoral transmission of nervous effects was the employment of the smallest possible quantity of fluid in the nerve-stimulated heart, his idea being that the smaller the quantity of fluid in contact with the heart the more concentrated would be the resulting neuromimetic fluid, and thus the more definite the effects on the heart to which this was applied. The experiments of some other workers, however, notably Rylant, appeared to show that a concentration of vagus substance sufficient to affect a second heart is more easily obtained than Loewi supposed.

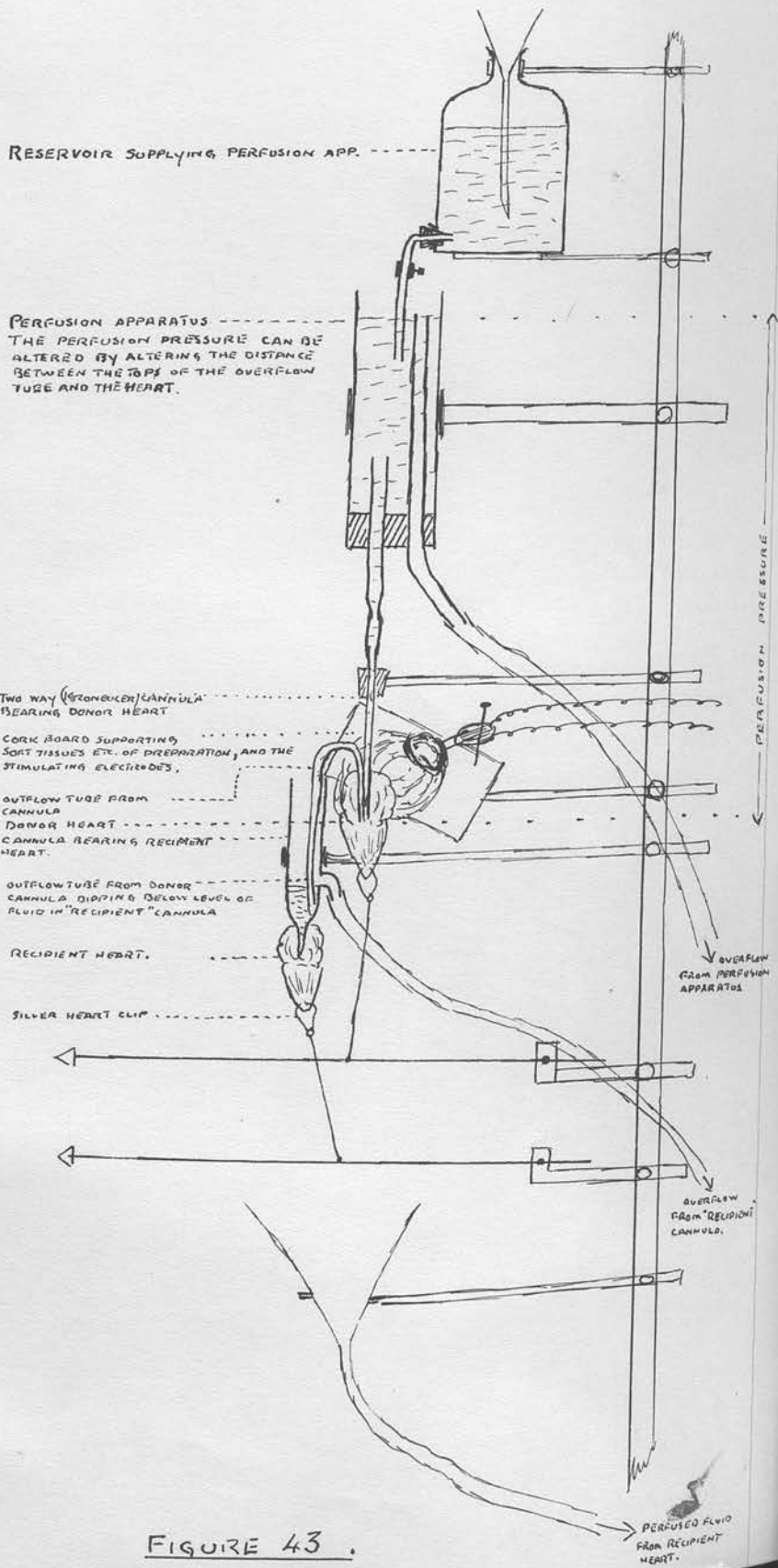
In any case, it appeared to the writer that in the method adopted by Loewi a considerable amount of the vagus substance formed during stimulation must be destroyed by the mechanism known to exist in the heart for, as it were, that purpose. From these considerations it appeared possible that good results/

results might be obtained by a method in which the irrigating fluid is passed more or less rapidly through the heart in the hope that the vagus substance, almost as soon as it is formed, would pass into the irrigating fluid and thus away from the major factor operating for its destruction. As will be seen, this hope was realised.

It was desired at the outset that the method should be a self-recording one. In no technique which has previously been described has it been possible to obtain complete stoppage of the donor heart without interference with the supply of ringer to the recipient heart. Loewi, of course, in his experiments, collected fluid from a quiescent vagus-stimulated heart and applied this to another heart, but his method did not permit of ringer flowing continuously from a quiescent donor directly to the recipient heart. In Kahn's experiment too the vagus-stimulated heart might stop entirely, but in such circumstances the circulating action of the heart having entirely stopped a transference of vagus substance to the recipient heart has to rely largely, if not entirely, on simple diffusion. In Ten Cate's experiment stoppage of the donor heart involves/

THE HUMORAL TRANSMISSION OF THE EFFECTS OF VAGUS STIMULATION.

DIAGRAM OF APPARATUS USED.



involves a complete cessation of the supply of fluid to the recipient heart. In other words, vagal stoppage of the donor heart has had to be avoided by previous workers who have used self-recording experiments, and this has probably been a factor militating against their obtaining dramatic results.

The method.

The method consists in washing Clark's solution through a donor heart by means of a double cannula such that the fluid enters by one limb, irrigates part of the inner surface of the heart - usually the sinus venosus and auricles - passes out of the heart by means of the second limb of the cannula and is then immediately applied to a recipient heart. The cannula is depicted on the opposite page in a diagram which shows the general arrangement of the experiment.

At first a simple preparation consisting of a frog heart with its vagosympathetic trunks intact was used. This is in some respects unsatisfactory in view of the relations of the vagus and sympathetic in the frog, and in later experiments a more complicated preparation was employed. This consisted of the heart, the medulla oblongata with its bony covering, and the tissues carrying the nerves between the/

the medulla and the heart. The nerves were not cleared in this preparation as they were more easily kept moist and less liable to damage by being left in situ. The vagus was stimulated by shielded electrodes placed in the medulla. ✓

The main necessity in obtaining and fitting up the preparation is speed, as the nervous components fairly soon lose their irritability. In my experience it is unusual to get a good response from stimulation either of the medulla or of the vago-sympathetic trunks after thirty minutes have elapsed from the commencement of the dissection. Whether this is due to an effect on the nerve trunks themselves, the terminations of the nerves, or something peripheral to these, has not been determined. ✓

The venae cavae having been tied off, a metal cannula, such as is depicted in the diagram, is inserted into the sinus venosus and, if possible, secured there. Otherwise it is carried into the left auricle. Alternatively the cannula may be placed in the left auricle through an incision in the wall of this latter, the interauricular septum having been severed, the sino-auricular valves obliterated, and the venae cavae tied off as before.

The input tube of the cannula is attached to a perfusion/

perfusion apparatus as in diagram, such that the hydrostatic pressure applied to the donor heart can be adjusted from about 5-30 cms. and maintained constant at any desired level between these limits.

The perfusion apparatus is supplied in a large reservoir. Clark's solution is supplied to the donor heart and the perfusion pressure adjusted to such an extent that fluid falls at a regular rate from the output limb of the cannula, and, even when the heart is completely stopped, continues to fall in amount not appreciably different from before.

A second heart - the recipient heart - is attached to a small nosed cannula inserted in the sinus venosus. The fluid issuing from the donor heart is led to the recipient heart by means of fine drainage tubing, the end of which dips below the surface of the fluid in the glass cannula.

In order to make certain that hydrostatic effects do not contribute towards the effects obtained, this second cannula is provided with an overflow tube such that the capacity of the cannula is maintained constant at about cm.

Each of the hearts is attached to a lever by means of a thread, connection between thread and heart being effected by means of a small silver clip.

When/

number?

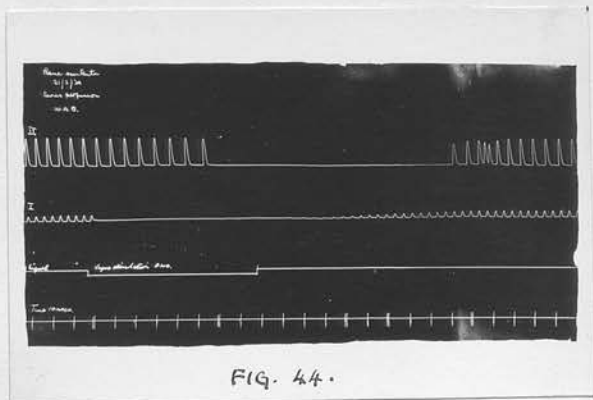


FIG. 44.

RANA ESCULENTA.

Upper line (marked II) RECIPIENT HEART. Second line (marked I) VAGUS STIMULATED (DONOR) HEART, Third line SIGNAL. Fourth line time marking in 10second intervals. VAGUS STIMULATION IS CARRIED OUT FOR 80seconds. THE DONOR HEART IS IMMEDIATELY BROUGHT TO A STANDSTILL. THE RECIPIENT HEART SLOWS SLIGHTLY AND THEN STOPS COMPLETELY AFTER STIMULATION OF THE DONOR'S NERVES HAS BEEN CONTINUED FOR 50seconds. THE DONOR RECOVERS COMPLETELY FROM THE EFFECTS OF THE STIMULATION ABOUT 90 seconds after this has been discontinued, the recipient recovering more suddenly about the same time. The recipient gives two EXTRASYSTOLES JUST AFTER THE FIRST THREE BEATS FOLLOWING THE INHIBITION.

When both hearts are functioning regularly the vagi of the first may be stimulated. If a good vagus effect is obtained in the donor an effect usually shows in the other after a short latent period. On the opposite page is a record of the type of results obtained by this method. The top line shows the contractions of the recipient heart, the next those of the donor; the third line is the signal, and below that is the time marking in 10 second intervals.

It will be seen that the effects of vagus stimulation of the donor heart are humorally transmitted to the recipient, complete stoppage of the recipient heart being obtained. The effect is much more striking than that illustrated in any of the previous records, thus justifying the views which led to the elaboration of the technique.

In carrying out an experiment the main precaution which must be taken is to ascertain that the donor heart supplies sufficient fluid to cause a steady drip from the overflow of the recipient-heart cannula, even when the donor heart is completely quiescent as the result of vagal stimulation.

The same method of perfusing the donor heart may be used to obtain a transmission of the effects of vagus stimulation to tissues other than the heart, suitable/

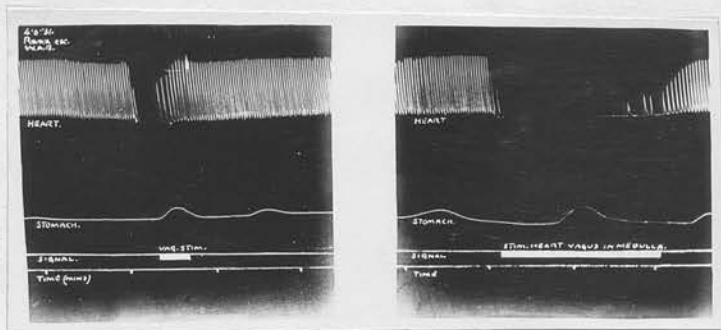


FIG. 45.

EFFECT OF VAGUS SUBSTANCE ON FROG'S STOMACH.

Top line — contractions of donor heart. Second line — contractions of isolated frog stomach. Third line — signal. Bottom line — TIME IN ONE MINUTE INTERVALS. NOTE THE AMPLITUDE AND RATE OF CONTRACTION OF THE MOVEMENTS DURING VAGUS STIMULATION OF THE HEART AS COMPARED WITH THOSE ARISING OTHERWISE. NOTE ALSO THE TENDENCY FOR THE TONE OF THE STOMACH MUSCLE TO BE INCREASED IMMEDIATELY AFTER A "VAGUS" CONTRACTION.

suitable precautions being taken to ensure that the test tissue receives a constant supply of fluid during the experiment.

In fig. 45 is reproduced the record of such an experiment where the frog stomach was used as a test tissue. The top line shows the heart contractions, the next the movements of the stomach, and the third and fourth lines the signal and time marking respectively. The stomach had given a contraction just at the beginning of the record. The contraction was slight, and typical of the previous behaviour of the tissue. The heart vagus is now stimulated and the stomach almost immediately gives a contraction which is of large amplitude and which attains its maximum rapidly. The vagus stimulation is discontinued during the relaxation of the stomach. About a minute after the relaxation is complete - and it will be noted the tone of the stomach is now maintained at a slightly higher level than before - another spontaneous contraction arises. This has an amplitude slightly greater than that shown in the first contraction, but less than half that of the contraction which took place when the vagus substance was in contact with the stomach. The rate of contraction and relaxation is also very much less than in the "vagus substance contraction". In the second part of the tracing is a repetition of the observation about six minutes later than the first. Here again, both the rate and amplitude of the stomach contraction initiated during vagus stimulation are much greater than in the normal. The vagus stimulation was in this case continued after the contraction had passed off, and during this time the tone of the muscle tended to remain at a higher level than before.

The method having been shown to be satisfactory experiments embodying the same principle were designed with a view to extending the observations to other nerves. Before considering these experiments, however, certain factors, which are of importance if the maximum number of positive results are desired, may be briefly summarised/

summarised.

In the first place the stimulated heart may not exhibit a good vagus response. This happens most often in male frogs, and so female frogs should always be used as donors. If female frogs fail to give the desired result the simplest way to overcome the difficulty is to raise the hydrogen ion concentration of the perfusing fluid with hydrochloric acid to a pH value of about 6.5. This not only improves the vagus response considerably but increases also the sensitivity of the recipient heart to the vagus substance. Finally, to increase the sensitivity of the recipient heart to the vagus substance the heart may be perfused continually for some hours before the experiment proper commences. It will then be in a more or less hypodynamic state and will respond well to even slight concentrations of the substance passing over from the donor heart.

A positive result, with complete stoppage of the recipient heart, can almost be guaranteed with the technique described above if (1) the heart of a female frog is used as donor; (2) the pH of the Clark solution used for perfusion is slightly on the acid side of neutrality and (3) the recipient heart has been rendered hypodynamic before it is subjected to the action of the vagus substance.

ON THE EXTENSION OF THE PRINCIPLES
ENUNCIATED BY LOEWI.

Introductory:

The demonstration of the production or liberation by the action of the cardiac nerves of chemical substances, which apparently brought about the actual effects previously attributed to a direct action of the nerves, immediately led to investigations the aim of which was to determine whether or not this mode of action of the cardiac nerves could be demonstrated for other nerves and thus be considered general.

It would appear that such studies would have been advanced far beyond their present state had a publication of Demoor (120) in 1913 received the attention which it merited. Demoor showed that if a quantity of the saliva resulting from stimulation of the chorda tympani in the dog were injected intravenously to the same animal it determined the onset of a pronounced ^{increase} in the activity of the gland. If injected into another animal it caused a copious flow of saliva. This was the first demonstration of the humoral transmissibility of the effect of nervous activity and the significance of the observation was recognised.

"Nous reconnaissons à de véritables hormones les activités/

activités attribuées jusqu'à présent au système nerveux lui-même" said Demoor. But the subject was not further pursued.

Following on Loewi's experiments various investigators attempted to show the humoral transmissibility of nervous effects other than those produced by stimulation of cardiac nerves.

Using two dogs and an ingenious crossed circulation technique, such that the output and input of each animal was maintained constant, Zunz and Govaerts (121) were able to induce a fall in the blood pressure of one animal as the result of vagal stimulation of the other., this fall being independent of the heart rate of the animal in which it was induced. Gautrelet (122) had previously obtained somewhat similar results, but with a technique much less refined than that of Zunz and Govaerts. These latter workers had already in earlier experiments (123) obtained a rise in blood pressure in the second animal as a result of stimulation of the splanchnic nerves in the first, but the result in this case is complicated by the possibility of an increased adrenaline secretion in the first animal consequent upon such stimulation.

Hess and von Neergard (124) and Brinkman and Ruiter (125, 126) claim that faradisation of the motor nerve of (the) frog muscle irrigated with ringer solution confers/

confers on the ringer excito-motor properties for the stomach of the frog. Somewhat similar experiments have been performed on mammals by Cannon (127) and by Houssay (128). They have shown that excitation of the mammalian sciatic nerve liberates chemical substances which pass into the blood and cause a rise in blood pressure by stimulating the vasomotor centre and the suprarenals. The interpretation of such experiments is, however, difficult, in view of the large number of factors which may be involved in bringing about the observed results.

Inhibition in smooth muscle:

Finkleman (129) has recently demonstrated the humoral transmissibility of sympathetic inhibition in the intestine. Two pieces of gut are suspended in a moist chamber and ringer-locke fluid is permitted to flow over the surface of the first piece of gut, which has its nerves intact, on to the second piece. Stimulation of the nerves supplying the upper portion of the intestine causes immediate inhibition of the movements, similar effects but slightly less in degree being produced in the lower portion of gut after a short latent period.

Finally, Cannon and his co-workers (240,241) have shown that in the cat, with heart, suprarenals and/

and liver denervated, stimulation of the sympathetic supply to the smooth muscle of the tail hairs causes the passage into the blood of a substance which causes a slight but definite acceleration of the heart, and sometimes a slight increase in the blood pressure. They consider the substance a specific hormone, and, since it is derived from structures under sympathetic control, under conditions of sympathetic activity, they suggest that it be called "sympathin". This work, like that of Finkleman, awaits confirmation.

Mode of action of parasympathetic vasodilator nerves.

Other work of an entirely different nature to the foregoing has recently appeared, which not only lends valuable support to the theory of nervous action enunciated by Loewi but which goes far to prove - in an indirect manner though it be - that parasympathetic nerves in general exert their effects by liberating acetylcholine at their terminations. This latest work has developed in the following manner.

In 1863 Vulpian^{and} Philippeaux (130) showed that after section and degeneration of the hypoglossal nerve the voluntary muscles of the tongue respond to stimulation in a manner different to the normal, a slow contraction of tonus wave following peripheral stimulation of the chorda lingual nerve, or of the separated/

separated chorda tympani. Heidenhain, (131) who investigated this phenomenon, considered it to be due in some way to the vasodilator fibres and showed that nicotine produced a similar effect.

An observation somewhat similar to Vulpian's was made in 1894 by Sherrington (132). He showed that when the muscles of the hind limb had been deprived of their motor nerves by degeneration following spinal root section, stimulation of the sciatic nerve caused an abnormal slow and weak contraction of the leg muscles. It was presumed that this effect was due to antidromic stimulation of sensory fibres.

The nerves responsible for the Sherrington reaction were definitely traced to the blood vessels of the limbs and not to the muscle fibres (Hinsey, 133) and were later shown to be in fact the fibres responsible for vasodilatation (Hinsey and Casser, 134). As regards the Vulpian reaction itself it is reasonable to suppose that here also the contracture response is brought about by vasodilator fibres as, indeed, Heidenhain concluded.

It was shown by Frank, Nothmann and Hirschkauffman (135) that the Vulpian reaction in the denervated tongue could be brought about by the intravascular/

intravascular injection of acetylcholine. Dale (136) had already shown that acetylcholine had both a "muscarine" and a "nicotine" action, and Dale and Gasser (137) showed that it was the nicotine action which was responsible for the effects produced by acetylcholine on ~~an~~ denervated muscle.

That the contracture reactions of denervated muscle were due to the liberation of some substance having both vasodilator and contracture-producing properties was first suggested by Bremer and Rylant (138) and later by Hinsey and Gasser (139). But none of these workers produced any evidence to indicate the nature of the hypothetical substance.

The demonstration by Dale and Dudley (140) that acetylcholine exists as a normal constituent of the body and is thus probably of physiological significance, led these workers to consider afresh the reaction of denervated muscle with a view to ascertaining whether or not the reactions when they occurred as a result of nerve stimulation were not, in fact, due to the liberation of acetylcholine, which was now known to satisfy the requirements of the hypothetical substance of previous workers, being vasodilator under normal conditions and contracture-producing in the denervated muscle.

As/

As a result of exhaustive researches comparing the normal reactions of denervated voluntary muscle with those induced by acetylcholine Dale and Gaddum (141) finally came to the conclusion that the vasodilator effects of parasympathetic nerves and of sensory nerves stimulated antidromically, and the contractures of denervated muscles accompanying these actions, are due to the peripheral liberation of acetylcholine. ✓

PERSONAL EXPERIMENTS.

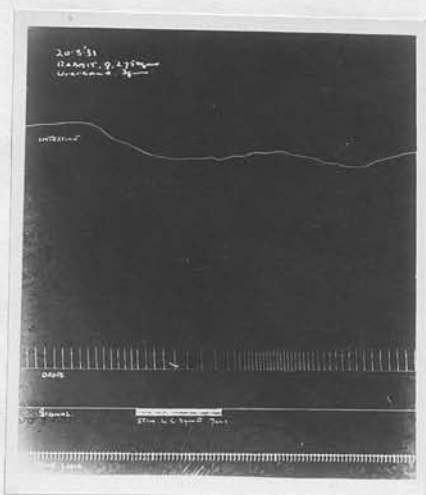
Introductory:

The last work mentioned is of particular interest, and, while it may be accepted as proving that vasodilator nerves do indeed produce their effects in the manner suggested, the proof is an indirect one. Experiments were therefore instituted with the object of determining whether or not it was possible to demonstrate the production of specific substances on stimulation of vasomotor nerves - both sympathetic and parasympathetic - the mode of demonstrating the existence of the substances to be their humoral transmission from the site of production to some test tissue.

Experiments on the rabbit's ear.

Method and results:

The first experiments were made in an attempt to demonstrate/



— FIG. 46. —

PERFUSION OF RABBIT'S EAR.

TOP LINE—RECORD OF MOVEMENTS OF ISOLATED INTESTINAL STRIP. SECOND LINE—RECORD OF DROPS FROM EAR. 3rd. line—SIGNAL. BOTTOM LINE—TIME IN 2sec. intervals.

THE PERIPHERAL END OF THE CERVICAL SYMPATHETIC WAS STIMULATED FOR THE PERIOD INDICATED BY THE SIGNAL. NOTE THE DECREASE IN THE NO. OF DROPS DUE TO THE VASO-CONSTRICTION; ON CESSATION OF THE STIMULATION, NOTE THE GREATLY INCREASED FLOW. FROM THE BEHAVIOUR OF THE INTESTINE NO CONCLUSIONS CAN BE DRAWN, SINCE THE ACTIVITY OF THIS TISSUE WAS SO DEPENDANT UPON THE AMOUNT OF FLUID REACHING IT. IN LATER EXPTS. ON THE DOG'S TONGUE THIS DIFFICULTY WAS OVERCOME, THE FLOW THROUGH THE TONGUE VESSELS BEING SUFFICIENTLY GREAT TO PERMIT OF THE TEST TISSUE BEING PLACED IN A BATH AND YET RECEIVE FLUID IN SUFFICIENT AMOUNT TO PERMIT OF THE CONCENTRATION OF NEUROMIMETIC SUBSTANCES IN EFFECTIVE AMOUNT.

demonstrate the humoral transmission of the effects of sympathetic stimulation of the blood vessels of the rabbit's ear, a piece of the same animal's intestine being used as a test tissue.

A large rabbit was anaesthetised with urethane 1.4 gms. per kilo. and a cannula inserted into the great auricular artery, another cannula being placed in the great vein. The ear was perfused from a large reservoir of Dale's solution and the fluid issuing from the venous cannula was permitted to run down the outside of a specially designed glass tube on to an electric drop recorder and thence to a piece of intestine suspended in a moist warm atmosphere. The cervical sympathetic of the same side had been isolated previously. When all was in order this nerve was stimulated. An immediate constriction of the blood vessels of the ear ensued. As a consequence of this the flow of fluid through the ear was immediately greatly reduced and this usually to such an extent that no fluid reached the intestine. Attempts were now made to increase the perfusion pressure during stimulation to such an extent that the flow of fluid through the ear was not appreciably reduced. With the apparatus at our disposal, however, this was not found to be possible and so the experiments were discontinued/

THE HUMORAL TRANSMISSION OF THE EFFECTS OF
VASOMOTOR NERVE STIMULATION.

ROUGH SKETCH OF APPARATUS EMPLOYED.

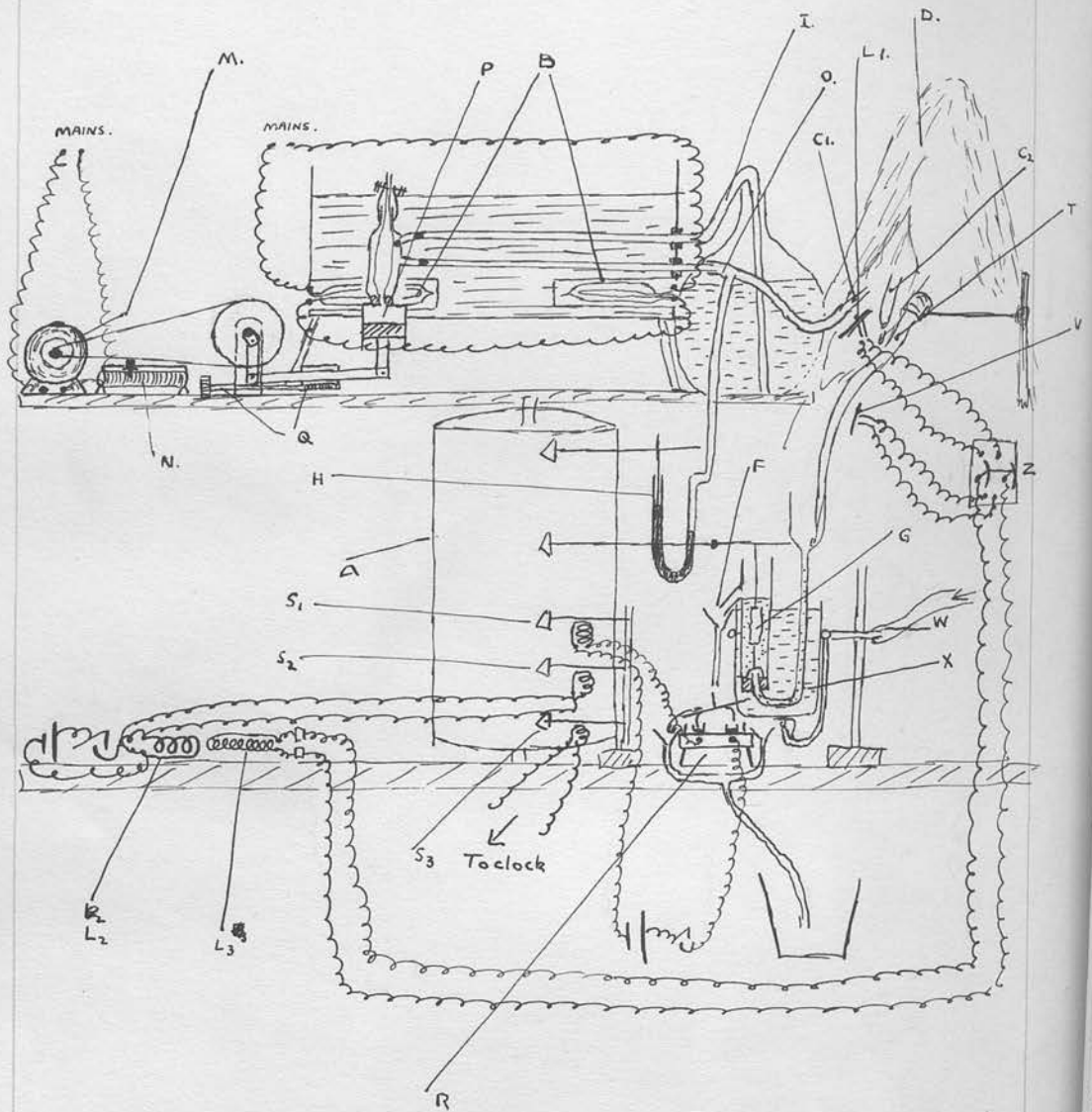


FIGURE 47.

- | | |
|---|---|
| A. KYMOGRAPH. | M. MOTOR DRIVING PUMP. |
| B. HEATING LAMPS. | N. VARIABLE RESISTANCE TO MOTOR. |
| C1. CANNULA IN LINGUAL ARTERY | O. OUTPUT TUBE FROM PUMP. |
| C2. CANNULA IN LINGUAL VEIN. | P. PUMP CYLINDER. |
| D. DOG. | Q. STROKE ADJUSTMENT TO PUMP. |
| F. OUTFLOW FROM INTESTINE BATH. | R. SCHAFFER FLOW RECORDER. |
| G. INTESTINE IN SCHAFFER BATH. | S1. SIGNAL OPERATED BY FLOW RECORDER. |
| H. MANOMETER RECORDING PERF. PRESSURE. | S2. SIGNAL OPERATED BY PRIMARY COIL. |
| I. INPUT TUBE TO DALE-SCHUSTER PUMP. | S3. SIGNAL OPERATED BY CLOCK. |
| L1. LINGUAL N. WITH ELECTRODES IN PLACE | T. TUBE, DOWN OUTSIDE OF WHICH FLUID |
| L2. PRIMARY COIL, WITH S2 IN IT. | FROM TONGUE FLOWS TO INTESTINE BATH. |
| L3. SECONDARY COIL LEADING TO STIMULATING | V. ELECTRODES ON VAGO-SYMP. TRUNK. |
| ELECTRODES ATTACHED TO ZWAY SWITCH, Z. | W. GAS SUPPLY TO HEATER X, FOR INTESTINE BATH |
| | X. HEATER FOR INTESTINE BATH. |
| | Z. POHL COMMUTATOR USED AS TWO-WAY SWITCH. |

discontinued.

One result only seems worthy of mention before passing to the later experiments. In many cases though not in all stimulation of the cervical sympathetic is followed by pronounced vasodilatation. This is illustrated in fig. 46 which also demonstrates the entirely unsatisfactory nature of the experiment for the purpose for which it was intended. A description of the figure is given in the legend.

Experiments on the dog.

Method:

For these experiments the dog's tongue was chosen. An organ of considerable size, it has a parasympathetic supply to the blood vessels in the lingual nerve and a sympathetic supply in the vagosympathetic trunk, each of which can be readily isolated for stimulation. In principle the experiments consisted in perfusing one side of the tongue through the lingual artery by means of a Dale-Schuster pump, the perfusate being led from the lingual vein to a bath in which was immersed a short length of rabbit intestine as a test tissue. The general lay-out of the experiment, though somewhat complicated, will be appreciated from the sketch on the opposite page.

A large dog is anaesthetised with chloralose, .08 gm. per kilo. A long skin incision in the mid-ventral/

ventral line, reaching from 2 or 3 cms. (~~from~~) behind the symphysis of the lower jaw to 5 or 6 cms. below the larynx, is made. The left common carotid is cleared for as great a distance as possible and strong ligatures placed in position round this. A strong ligature is also placed, ready for securing, round the left external carotid. On the right side the vagosympathetic trunk is cleared for a few centimetres. All the branches of the external carotid on the right side are now successively identified and ligatured loosely, two ligatures being placed round the lingual artery. The lingual nerve is now exposed and cleared as much as possible. Finally the right lingual vein is cleared and double ligatured, and a ligature placed round the anastomosing vein between the linguals of the two sides.

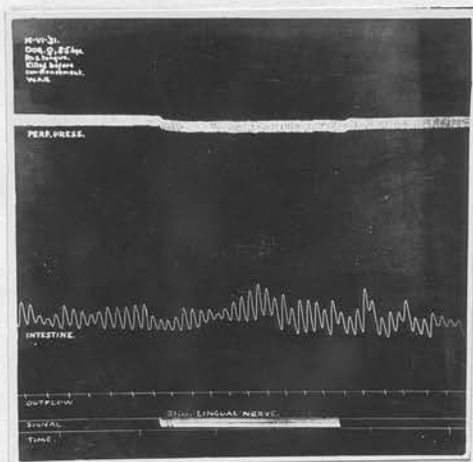
When all is ready the ligatures round the left external carotid and the branches of the right external carotid are tightly tied, the animal moved to the experimental table and placed in position there. The centremost ligature round the right lingual artery is secured, the artery clipped distal to this and a cannula attached to the output tube of the Dale-Schuster pump is inserted and secured by the second ligature. The vein is now ligatured and a short glass/

glass cannula inserted. The pump being in action the blood is soon washed from the tongue and a clear perfusate results. This drops on to the outside of a specially designed glass tube down which it rapidly passes and so, with the minimum possible loss of time, arrives at the intestine bath. When the output from the tongue is constant and the intestine is contracting regularly, the nerves may be ligatured and their peripheral ends prepared for stimulation.

It should be noted that the output tube of the pump is connected with a mercury manometer as well as with the lingual artery. By this means the pressure of fluid applied to the tongue vessels can be read directly and can be recorded. Further, the rate of the pump can be altered by means of a resistance in the driving motor circuit, while the amount of fluid delivered by the pump can be altered instantaneously by means of an adjustment on the pump itself. Thus the pump can be made to maintain an artificial circulation in the tongue which imitates, in respect of pressure, flow and frequency, the conditions which are maintained normally in the animal. Also, any change in the resistance to the flow applied through the tongue, whether brought about by spontaneous changes in the calibre of the blood vessels, by changes imposed by stimulation of nerves, or by mechanical obstruction, will immediately show in the perfusion pressure record, which thus affords a criterion of the conditions obtaining as to the state of the blood vessels at any given moment.

In order, however, to have a more exact idea of the extent of the changes brought about by nerve stimulation it was arranged that the perfusate, as it overflowed from the intestine bath, should operate a flow recorder, so that the absolute changes in the rate of flow through the tongue could be measured. This arrangement is shown in the diagram.

One of the main difficulties of the experiment -
apart/

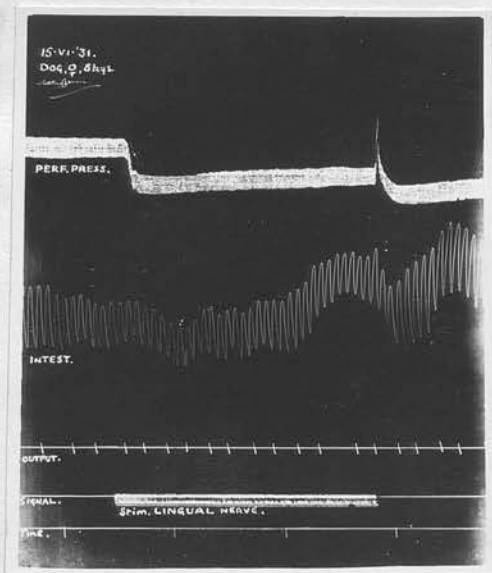


— FIG. 48. —

PERFUSION OF DOG'S TONGUE.

Top line — perfusion pressure. 2nd line — record of CONTRACTIONS OF ISOLATED PIECE OF INTESTINE. 3rd. line — RECORD OF OUTFLOW FROM TONGUE. 4th & 5th lines — SIGNAL, & TIME MARKINGS (1 MIN.) RESPECTIVELY.

NOTE INCREASED TONE ETC. OF INTESTINE ENSUING AFTER A SHORT LATENT PERIOD, FROM STIMULATION OF PERIPHERAL PART OF LINGUAL N.



— FIG. 49. —

PERFUSION OF DOG'S TONGUE.

NOTE FALL IN PERFUSION PRESSURE RESULTING FROM STIM. OF PERIPHERAL PART LINGUAL NERVE, AFTER INITIAL FALL IN TONE, INTESTINAL TONE RISES. THE VASODILATION PERSISTS AFTER CESSATION OF STIMULATION. The interruption in the perfusion pressure record is due to a CONVULSIVE MOVEMENT OF THE ANIMAL.

apart from the difficulty of ensuring that all the mechanical arrangements will function smoothly - is associated with the dog, and consists in obtaining a bloodless perfusate.

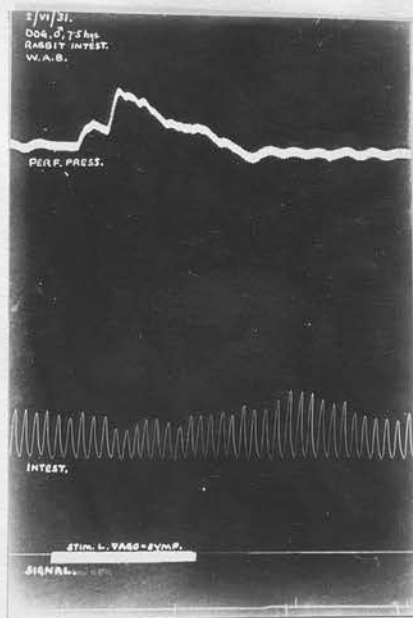
From what has been said it is evident that the animal is intended to be alive during the course of the experiment, and this was aimed at in order to determine whether or not spontaneous changes in the calibre of the blood vessels, brought about by changes in the vasomotor centre itself, would produce effects upon the test tissue. When a bloodless perfusate could be obtained after carrying out the procedures detailed above, usually no spontaneous changes in vasomotor tone exhibited themselves. In one case, however, which will be described, such did appear.

If ligaturing the whole common carotid on the left side still fails to prevent blood appearing in the perfusate, the right external carotid branches should be religatured. If this fails the only cure is to kill the animal by bleeding. This we effect by clipping the common carotid, severing high up and allowing the blood to spurt into a large basin. Approximately half our experiments were performed on animals which had just been killed in this manner.

Results:

Stimulation of lingual nerve.

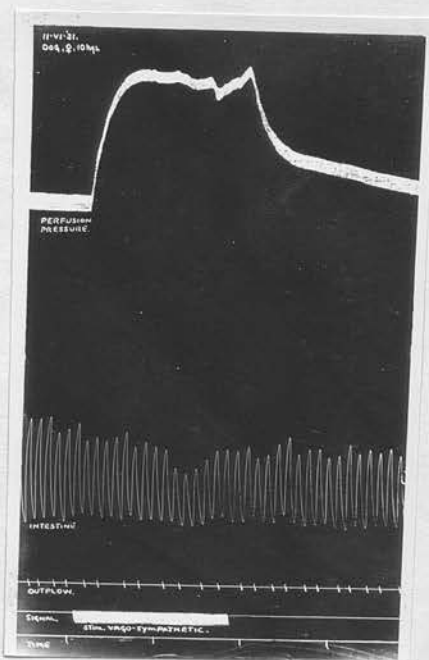
Fig. 48 is the record of an experiment in which the lingual nerve was stimulated. It can be seen that about 45 seconds after stimulation commenced the tone of the intestine rose, and though this fell again slightly/



— Fig. 50. —

PERFUSION OF DOG'S TONGUE.

STIMULATION OF VAGO-SYMPATHETIC TRUNK. NOTE DIMINUTION IN AMPLITUDE OF INTESTINAL MOVEMENTS WHICH SETS IN AFTER A SHORT LATENT PERIOD. NOTE "REBOUND" AFTER CESSATION OF STIMULATION. IS THIS DUE TO AN INCREASED PARASYMPATHETIC ACTIVITY IN THE TONGUE, OR TO AN INHERENT PROPERTY OF THE TEST TISSUE? Cf. the record of sympathetic stim. of rabbit's ear vessels, Fig. 46.



— Fig. 51. —

PERFUSION OF DOG'S TONGUE.

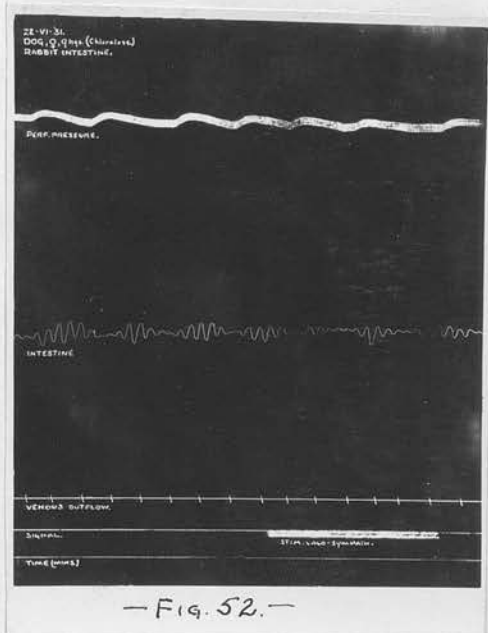
HUMORAL TRANSMISSION OF EFFECT OF SYMPATHETIC STIMULATION. NOTE CHANGES IN AMPLITUDE AND TONE OF INTESTINE CONSEQUENT UPON STIMULATION OF PERIPHERAL PART OF VAGO-SYMP. TRUNK. NOTE ABSENCE OF "REBOUND" AND CONTINUED STATE OF VASOCONSTRICTION (WHICH GRADUALLY PASSES OFF) AFTER CESSATION OF STIMULATION.

slightly the amplitude of the contractions was greater than before. In this case the animal was killed just before the commencement of the experiment.

The next figure ^(Fig. 49) shows the record of a similar experiment on the living animal, where the effect was more delayed and was preceded by a fall in tone of the intestine. Towards the end of the period of stimulation the animal gave a convulsive movement. The perfusion pressure rose momentarily, the intestinal tone fell, rose again, then rapidly returned to normal. The fall in perfusion pressure due to the vasodilatation brought about by stimulation is well marked here, and amounts to 16 mm. Hg.

Stimulation of vagosympathetic.

In the experiment of which fig. 50 is a record, stimulation of the peripheral end of the vagosympathetic trunk caused an irregular rise of perfusion pressure the maximum extent of which was 15 mm. Hg. By 30 seconds after the commencement of stimulation the amplitude of the intestinal contractions was much reduced and the tone fell slightly. On cessation of stimulation the movements increased in amplitude to greater than normal and then reverted once more to their original height. Though the perfusion pressure record gives no clear indication of the state of affairs/



PERFUSION OF THE TONGUE — SPONTANEOUS CHANGES IN VASOMOTOR TONE WITH CHANGES IN TONE, OF SIMILAR PERIOD, IN TEST PIECE.

The tracing explains itself. (THE VAGO-SYPATHETIC TRUNK WAS STIMULATED AT SIGNAL WITHOUT APPARENT EFFECT — STIMULUS PROBABLY TOO WEAK.)

affairs in the blood vessels, and though unfortunately the flow recorder was out of order on this occasion, it is possible that here we have a case of "rebound" similar to that observed in the rabbit's ear after sympathetic stimulation, the effects of this having been transmitted humorally from the tongue to the intestine.

The next figure (fig. 51) is a record of a similar experiment where, however, there is a complete absence of "rebound" and where vasoconstriction is maintained after cessation of the stimulation and this continuance of sympathetic activity is shown in the behaviour of the intestine. The amplitude of the intestinal contractions at the beginning of the record averages 35 mm. This is reduced by sympathetic stimulation of the tongue vessels to a minimum of 19 mm., the tone of the intestine falling by 3 mm. at the same time. After cessation of stimulation the amplitude averages 27 mm. The rise of perfusion pressure in this case was 45 mm.

Finally, in fig. 52, is shown a record of spontaneous changes in vasomotor tone which are accompanied by rhythmic changes in the behaviour of the intestine. The animal was alive with left common carotid tied off, but right internal carotid patent as usual. Allowing for the latent period due to transmission of fluid from the tongue and its attaining an effective concentration/

concentration in the intestine bath, the record shows an almost perfect agreement between calibre of vessels, i.e., between the activity of the vasomotor nerves, and the behaviour of the intestine.

Conclusions:

While experiments along the line of those described above are being continued, the results so far have afforded some direct evidence in favour of the view that vasomotor nerves produce their effects, like the cardiac nerves, by the production at their endings of specific chemical substances. Further, the fact that in no case where blood has entered the perfusate in appreciable amount have definitely positive results been obtained, tends to support the view that the substances produced by nerve stimulation are rapidly destroyed by the blood.

And indeed this is what one would expect. It may be that the substances are not even passed out of the tissues into the blood at all under normal circumstances.

Finally, it seems to me unfortunate that the humoral transmissibility of these substances has ever been stressed. This is unfortunate because it has led a large number of workers to attach great physiological significance to this humoral transmissibility for/

✓

for the which under physiological conditions there is no good evidence whatever. It is true that the effects of nervous stimulation are transmissible humorally under the conditions of experiments expressly designed with that end in view, but it is extremely doubtful if any effects transmitted humorally under physiological conditions are, or could be, of much significance. The main point about the work of Loewi is not that the effects of nervous excitation are transmitted humorally but that the effects are due to production of chemical substances at the nerve endings. That the effects are humorally transmissible is the manner - indeed the only manner - of demonstrating that the nerves do act thus, and the very difficulty of such experiments is surely one of the main arguments against such transmissibility being of much, if any, physiological significance when compared with the effects produced by the substances at their site of production. Moreover a mechanism exists which expressly tends to prevent such transmission - the rapid destruction of the neuromimetic substances by the blood. As far as I can see, then, the substances produced by the activity of nerves exert their effects locally at the site of production and/

and any humoral transmission of these substances is probably incidental and unimportant.

SUMMARY.

1. A historical résumé of work and views on the automaticity of the heart is given.
2. Demoor's work on the active substances of the heart is reviewed.
3. Haberlandt's work on the heart hormone is reviewed.
4. Zwaardemaker's work on automatine is summarised.
5. Personal experiments on the active substances of the heart are described.
6. It is shown that the isolated right and left auricles behave in the manner described by Demoor.
7. Demoor's fundamental experiment is confirmed.
8. Evidence is offered in support of the view that the rhythmicising factor in active substance extracts is not adrenaline or the accelerator substance of Loewi.
9. The effects of active substance extracts are not due to histamine.
10. Demoor's views as to the effects of temperature on the active substances are confirmed.
11. It is shown that extracts of skeletal muscle have an action similar to the "sensitising" action of active substance extracts.
12. Blood serum and defibrinated blood have an action similar to skeletal muscle extracts.
13. Lecithin, saponin, and serum albumen sensitise the left auricle to calcium and to adrenaline.
14. The similarity of the left auricle to the hypodynamic frog heart is pointed out.
15. Aqueous extracts of whole suprarenal will rhythmise the left auricle.
16. The theory of the humoral action of the active substances is criticised and an alternative interpretation of the experimental results is offered.
- 17./

17. Work on the heart hormones is discussed generally and lines for future investigation indicated.
 18. In an appendix to the foregoing sections some evidence is brought forward which shows that adenosine, or a related compound, may be the substance responsible for the effects described by Haberlandt.
 19. The work of Loewi and his school is reviewed.
 20. A technique by means of which the humoral transmission of the vagus substance may be demonstrated in a much more dramatic way than hitherto is described.
 21. Further experiments are described which demonstrate directly, for the first time, that vasomotor nerves produce their effects by the peripheral liberation of specific chemical substances.
 22. It is shown that stimulation of the vasodilator nerves of the perfused tongue in the dog leads to the liberation of a substance which can be transmitted humorally to an isolated piece of intestine and produce augmentation of the tone and/or contractions of this last.
 23. It is shown similarly that stimulation of the sympathetic supply to the blood vessels of the tongue liberates a substance which diminishes the tone and/or contractions of an isolated piece of intestine.
 24. It is suggested that, under normal circumstances, these substances exert their effects locally, and that any humoral transmission to other sites is probably of little significance.
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THE ACTION OF ADRENALINE AND OF CERTAIN DRUGS
UPON THE ISOLATED CRUSTACEAN HEART.* By W. A.
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the Department of Physiology, University of Edinburgh. (With
eleven figures in the text.)

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INTRODUCTION.

THE following experiments, which include a study of the action of certain drugs and autacoids upon the hearts of crabs, were undertaken as a preliminary to a reinvestigation of the nervous supply to the crustacean heart and its relationship to the heart-beat. The results obtained are of interest in view of the paucity of our knowledge regarding the effects of such substances on the heart of invertebrates.

Since the investigation of the action of adrenaline and ergotoxine formed the starting-point of the present study, the literature relating to this subject will first be dealt with, other work being noted and references given as the occasion arises.

It would seem that CARLSON (1) was the first to record a positive action of adrenaline on the heart of an invertebrate, observing an excitatory effect on both the cardiac ganglion and myocardium of *Limulus*, but giving no graphic record of his experiments. ELLIOT (2) obtained negative results in a single experiment on the crayfish (? *Astacus*), in which he dropped adrenaline on to the surface of the exposed heart without effect. He does, however, state that "movements of the animal followed as the drug was carried round in the circulation and irritated the nervous ganglia," this being observed also by BRÜCKE and SATAKE (3) in an experiment on *Homarus*, a rise in blood-pressure and increased heart-rate (following an initial fall in blood-pressure after an injection of adrenaline) coinciding with the commencement of these movements. More recently HOGBEN and HOBSON (4) have studied the action of adrenaline on a wide selection of invertebrate preparations, the Crustacea being represented by the decapod *Maia squinado*. In the isolated heart of this animal, perfused with artificial sea-water of suitable reaction, these authors recorded an increase in tone of the heart-muscle accompanied by acceleration of the beat in response to adrenaline in a concentration of 1 in 40,000 parts of the perfusing fluid. They state that the latent period between the

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addition of the autacoid and the onset of the characteristic response was usually somewhat protracted, a minute at least intervening in most experiments, and they consider this evidence that the heart-muscle absorbs the autacoid slowly. Epinine was found by these workers to produce an effect similar to that obtained with adrenaline, but was effective in much greater dilution.

The effect of ergotoxine on the response to adrenaline does not seem to have been investigated hitherto in an invertebrate.

MATERIAL AND METHODS.

The heart has been studied in three genera of the decapod Crustacea—*Maia squinado*, *Cancer pagurus*, and *Carcinus mœnas*. The technique of perfusion was the same in all cases. With *Carcinus* considerably greater care has to be taken in obtaining the preparation than is necessary in the larger genera, but the additional trouble is worth taking, the *Carcinus* heart being the most satisfactory of the preparations investigated.

The method of dissection ultimately adopted was as follows:—¹ The limbs having been removed, an opening is made on the dorsal aspect of the cephalo-thorax and a large circular portion above the cardiac region excised with the aid of bone forceps, care being taken to separate the chitinous covering from the subjacent pigmented dermis. The latter is then dissected off, the roof of the pericardium removed, and the pericardial cavity flushed out with the perfusing fluid. A glass cannula is inserted in the *bulbus arteriæ dorsalis* through an incision in the mid-line at a level between the median posterior and the posterior alæ of the heart, and is tied in position by a ligature round the extreme posterior part of the heart. This ligature should include the posterior heart-tendons. The heart is immediately flushed out with perfusing fluid and a second ligature, for attachment of the heart to the recording lever, is applied. This includes the dorsal antero-lateral alæ, but passes under the cephalic artery, which is cut close to the heart before the ligature is secured. It will be seen that with this technique the cephalic and hepatic arteries, and often the sternal and lateral arteries as well, are left patent, and thus efficient perfusion is assured. The remaining attachments of the heart are now severed and the preparation connected with the perfusion apparatus. This is on the same principle as that adopted by HOGBEN (6) in his later work, but was modified to permit the perfusion pressure being adjusted to any desired level—a useful modification where preparations of such differing sizes are employed. Rapid changes could be made from one solution to another with a minimum of mechanical disturbance.

¹ The description applies particularly to *Cancer*. An account of the anatomy of the heart in this genus is available in the monograph of PEARSON (5).

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Various artificial media suitable for the perfusion of the isolated heart in different species of crustacea have been proposed, but after submitting several of these to trial it was found that the most satisfactory medium was sea-water reduced to a suitable pH, and that the hearts of all three genera would beat for many hours in it without either the rhythm or the amplitude undergoing any marked change. Sea-water, the pH of which had been reduced to about 7.5 by the addition of hydrochloric acid, was accordingly used in all the experiments. Several litres of sea-water were prepared for each experiment, the experimental solutions being made up in the same medium in every case and the pH of these further adjusted if necessary. The pH values were determined colorimetrically.

The adrenaline employed was that of Parke, Davis & Co., and was similar in its effect to the synthetic adrenaline chloride of British Drug Houses, which was also tried. The other drugs—ephedrine hydrochloride, pilocarpine hydrochloride, atropine sulphate, and ergotoxine phosphate—were supplied by the latter firm. A 1 per cent. solution of ergotoxine phosphate in distilled water was made up every few days and measured quantities of this solution added to a known volume of the perfusing fluid to give the desired concentration.

EXPERIMENTAL DATA.

The Action of Adrenaline.—The response of the *Maia* heart to adrenaline is shown in fig. 1, which is from a typical experiment. Perfused with sea-water at pH 7.5 and temperature 21° C., this particular heart beat in a regular manner with a frequency of seventeen per minute. The fall of the signal indicates the commencement of the change over from sea-water alone to sea-water plus adrenaline at a similar temperature and pH, the concentration of the autacoid being 1 to 40,000 of the perfusing fluid. The rise of the signal indicates the return to pure sea-water, the adrenaline solution having been perfused in this case for about twenty seconds. It will be seen that four or five seconds after the admission of adrenaline there is a marked acceleration of the heart-rate, the frequency of the rhythm increasing from seventeen to seventy-four per minute, this being accompanied by a progressive increase in the tone of the muscle.

The increase in tone is greatly exaggerated when the autacoid is in higher concentration, the heart generally going into a state of strong tonic contraction (fig. 2), from which it gradually recovers as the excess of the drug is washed out, the beat then becoming regular but showing a pronounced increase in frequency and tone over the normal.

The heart of *Carcinus* responds to adrenaline in a manner similar to that just described for *Maia*, although the increase in tone is usually less well marked. Fig. 3 is the record of a typical experiment.

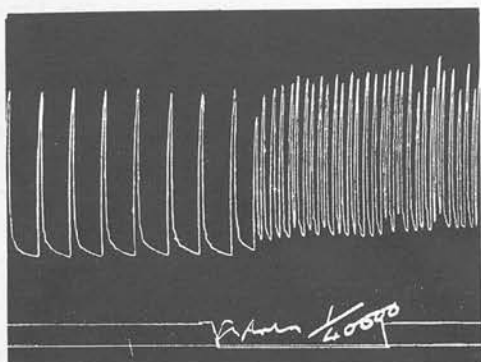


FIG. 1.—Heart of Maia: action of adrenaline. Adrenaline (1/40,000) at signal. Effect appears in 4 seconds. Normal rate 17 per minute. After adrenaline, 74 per minute. pH 7.5. Temp. 21° C. Time in minutes (the same in all except where otherwise stated).

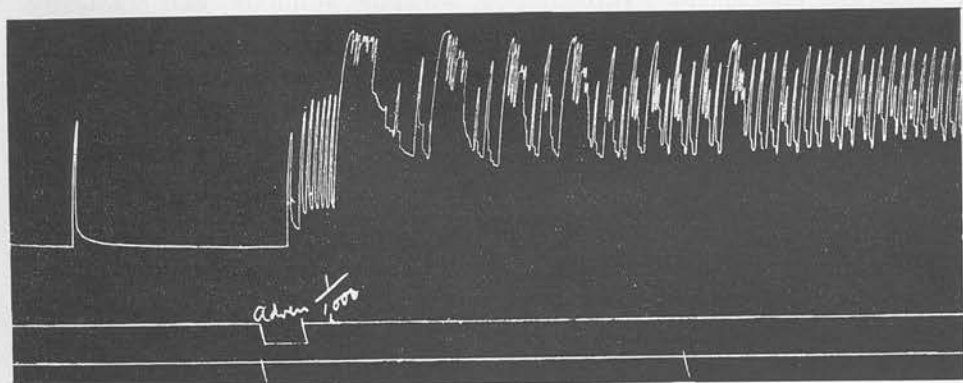


FIG. 2.—Heart of Maia: action of adrenaline. Adrenaline (1 c.c. of 1/1000) added to perfusion apparatus at signal. pH 7.6. Temp. 18° C.

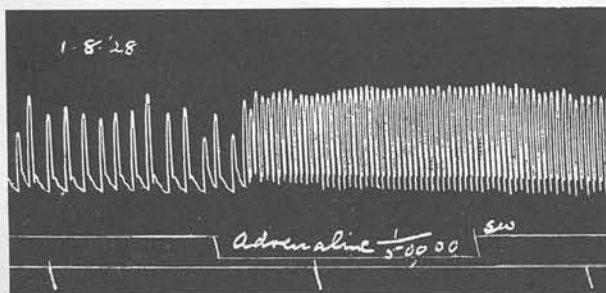


FIG. 3.—Heart of Carcinus: action of adrenaline. Adrenaline (1/50,000) added at signal and perfused for about half a minute. Normal rate 32 per minute. After adrenaline, 100 per minute. pH 7.6. Temp. 19° C. Time in half minutes.

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Adrenaline (1 in 50,000) was perfused for thirty seconds. In less than four seconds the characteristic acceleration commenced, the rate per minute increasing from thirty-two to over a hundred.

An attempt was made in this genus to determine whether or not adrenaline has a chalonic action when perfused in great dilution. It was found that when it gives any effect at all, that effect is always excitatory. In a dilution of 1 in 100 millions this excitation was just perceptible, while with 1 in 10 millions the effect was sufficiently obvious. This is shown in fig. 4, where the heart-rate was increased from thirty-six to forty-eight per minute, and the average amplitude of the beats increased.

In *Cancer* the effect of adrenaline on the frequency of the beat is similar to that described for *Maia* and *Carcinus*. A characteristic difference, however, is the effect on the amplitude of the beat. In neither *Maia* nor *Carcinus* is much increase often observed, but in *Cancer* a marked improvement in the extent of the contractions is always present (fig. 5). Another difference worth noting is, that while adrenaline usually abolishes any irregularities in the heart rhythm of *Maia* and *Carcinus*, this is not often seen in *Cancer*.

Ephedrine hydrochloride, perfused in a concentration of 1 in 50,000, has no effect whatever on the heart-beat in any of the three genera examined.

Effect of Ergotoxine on the Response to Adrenaline.—Ergotoxine phosphate (1 in 10,000) causes diastolic stoppage in all three genera. Considerable variation exists, however, among individual hearts in their response to similar concentrations of the drug. While in some the stoppage occurred without a preliminary slowing, in others it was preceded by progressive decrease in frequency. In most cases both the rhythm and amplitude of the slowed beat were irregular, and often two or more successive beats fused into a short "tetanus." With ergotoxine in smaller concentration (1 in 20,000 to 1 in 40,000) the decrease in frequency of the beat is usually the most marked effect, although the irregularities noted above are seldom entirely absent.

An attempt has been made to determine whether ergotoxine, when perfused in such a dilution that none of the above effects is visible on the record, would nevertheless influence the action of adrenaline. The record of such an experiment is given in fig. 6. In this heart perfusion

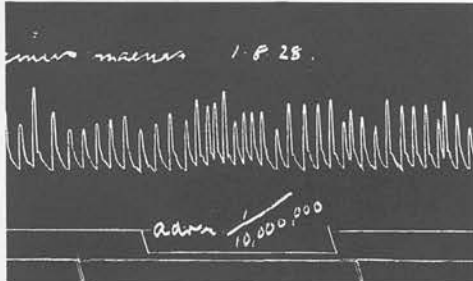


FIG. 4.—Heart of *Carcinus*: action of adrenaline.

Adrenaline (1/10,000,000) at signal.
Frequency increased from 36 to 48
per minute. pH 7.6. Temp. 19° C.
Time in half minutes.

with ergotoxine, 1 in 40,000, produced marked slowing. The concentration of the drug was accordingly reduced to 1 in 80,000, and this was perfused for two minutes. At the end of this period

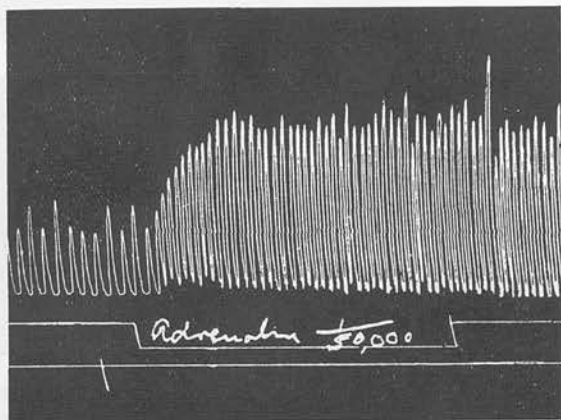


FIG. 5.—Heart of Cancer: action of adrenaline. Adrenaline (1/50,000) at signal. Marked increase in amplitude and frequency. pH 7.5. Temp. 18.5° C.

(towards which the record commences) the fluid was changed to one containing, in addition to ergotoxine (1 in 80,000), adrenaline (1 in 50,000); this was perfused for one minute. Thereafter a return

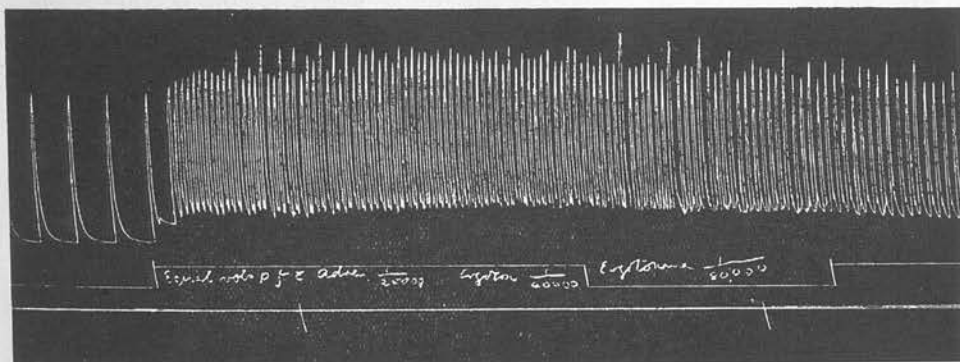


FIG. 6.—Heart of Maia: effect of ergotoxine on action of adrenaline.

Ergotoxine phosphate (1/80,000) perfused for two minutes. Rise of signal—commencement of perfusion with equal volumes of adrenaline (1/25,000) and ergotoxine (1/40,000). Fall of signal—return to ergotoxine without adrenaline. Rate before adrenaline, 13 per minute. Rate after adrenaline, 74 per minute. *I.e.* ergotoxine has no effect on adrenaline response. pH 7.5. Temp. 21° C.

was made to the ergotoxine for half a minute before the original sea-water was finally substituted. It will be seen that neither the previous perfusion with ergotoxine nor the presence of ergotoxine during

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the adrenaline perfusion prevents the appearance of the characteristic adrenaline effect. A similar result was obtained when ergotoxine was used in concentrations which produced great slowing of the heart. Even when complete stoppage had been brought about by perfusion of the drug in still higher concentration, the beat was immediately revived by adrenaline. Fig. 7 is the record of a case in which the heart had been quiescent for four minutes after two minutes' perfusion with ergotoxine (1 in 10,000). No sign of recovery was visible after four minutes' perfusion with sea-water. Adrenaline (1 in 40,000) was now perfused, and the beat

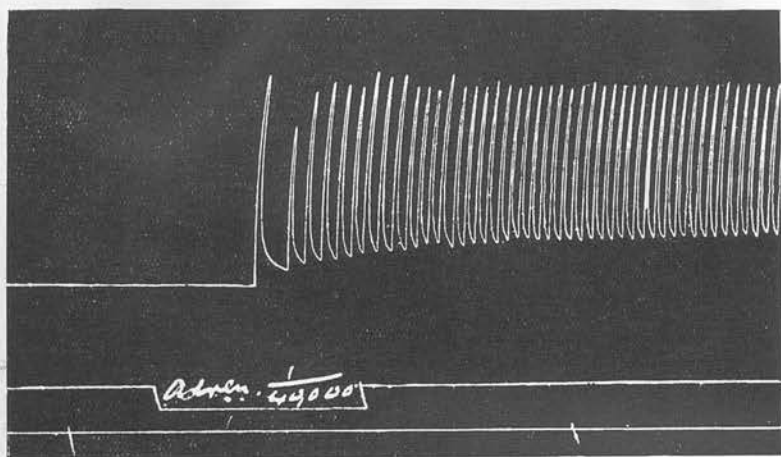


FIG. 7.—Heart of Maia: effect of ergotoxine on action of adrenaline. Heart brought to complete standstill for four minutes after two minutes' perfusion with ergotoxine (1/10,000). Adrenaline (1/40,000) added at signal. Recovery of beat. pH 7.5. Temp. 21° C.

recommenced at once. A similar result is shown in figs. 8 and 9 from an experiment on Maia. The heart had been brought to a standstill by ergotoxine (1 in 50,000). After three and a half minutes adrenaline was perfused for sixty seconds, followed by ergotoxine in concentration one and a half times greater than that which had previously produced complete stoppage. This solution was perfused for ninety seconds. It produced a slight slowing, which was immediately counteracted by further perfusion of adrenaline. These experiments show that the action of ergotoxine upon the crab heart does not furnish a true physiological antagonism to adrenaline, the action of which is neither abolished nor reversed by ergotoxine.

The Action of Pilocarpine.—While there is nothing in Crustacea which corresponds morphologically with the sympathetic nervous system of Vertebrata, accelerator and inhibitor nerves are known to supply the heart. JOLYET and VIALLANES (7) first described augmentor and inhibitor centres for the heart of *Carcinus*. They state that these

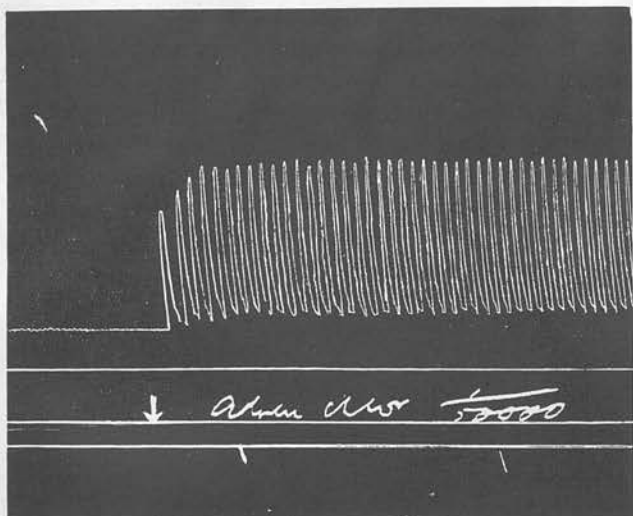


FIG. 8.—Heart of Maia: effect of ergotoxine on action of adrenaline.

Heart brought to standstill by two and a half minutes' perfusion with ergotoxine (1/50,000). After three and a half minutes' stoppage adrenaline (1/50,000) added (arrow). Recovery of beat. After one minute perfusion with adrenaline, ergotoxine (1/20,000) added and perfused for one and a half minutes.

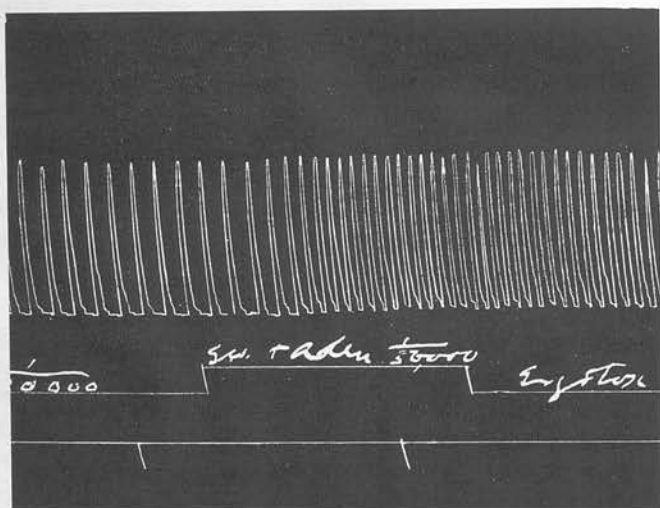


FIG. 9.—Same heart three minutes later.

Shows slowing produced by the ergotoxine. At rise of signal adrenaline added. This immediately counteracts the effect of the ergotoxine. pH 7.7. Temp. 19° C. Time in half minutes in both tracings.

lie in the anterior portion of the thoracic ganglion. Shortly afterwards CONANT and CLARKE (8) succeeded, in *Calinectes*, in tracing the nerves from these centres to the heart itself. The results of these workers were confirmed by BOTTAZZI (9) (*Maia squinado*), and by CARLSON (10) (*Cancer*, *Palinurus*).

In view of the above facts and of the definite results obtained with such a typical sympatho-mimetic substance as adrenaline, it was thought of interest to investigate the effects of a typical para-sympatho-mimetic drug; for this purpose pilocarpine was chosen.

Several observers, using methods other than that of perfusion, have recorded a depressant action of muscarin on the hearts of certain invertebrates; this seems to have been the only para-sympatho-mimetic drug investigated in invertebrate material. Such experiments on Crustacea seem, however, to be scarce. PICKERING (11), who immersed whole *Daphniæ* in a saturated solution of muscarin, found it to have no action on the heart of this genus; but NUKADA (12) obtained inhibition of the *Limulus* heart with muscarin, the action being antagonised by atropine. With atropine sulphate (.03 per cent.) PICKERING obtained an increase in the frequency of the beats in *Daphnia*, while PLATEAU (13) got pronounced slowing of the *Homarus* heart in response to strong doses of atropine.

Pilocarpine hydrochloride, in a concentration of 1 in 10,000, produces an effect which is very similar, both qualitatively and quantitatively, to that obtained with adrenaline in concentration 1 in 50,000. As with adrenaline, a second dose of the pilocarpine, given after the effect of the first has passed off, reproduces the original effect; similarly with subsequent doses. In *Cancer* the marked increase in amplitude of the beat which is observed to occur with adrenaline is present also during perfusion with pilocarpine. In fact, the action of the two substances is so alike in every respect that further description is unnecessary.

The Effect of Atropine on the Response to Pilocarpine.—Perfusion with atropine sulphate (1 in 250,000) has no visible effect on the heart-beat, but almost immediately abolishes the pilocarpine effect when this is present, and prevents its reappearance in response to a second dose for some minutes after the change back to sea-water. This is shown in fig. 10, which is from an experiment on *Cancer*. Pilocarpine (1 in 10,000) was perfused for slightly over a minute, followed immediately by atropine (1 in 250,000) for slightly less than that time. The pilocarpine effect was abolished in twenty seconds. A return to sea-water was made for one and a quarter minutes, after which pilocarpine was again perfused—this time without effect. It was noted that although atropine when perfused in such low concentration does not produce a slowing of the heart, the heart-rate after the abolition of the pilocarpine effect by atropine is often slower than normal. This is illustrated in fig. 11. The heart-rate in this case was twenty-nine per minute at the

beginning of the experiment. This was increased to over a hundred by 1 in 10,000 pilocarpine, the effect coming on some four seconds after the first addition of the drug. Atropine (1 in 250,000) reduced the rate to twenty-six in less than half a minute. Perfusion with pilocarpine for twice as long as before brought the rate to thirty-three, and five and a half minutes after the return to sea-water the rate was still thirty-three.

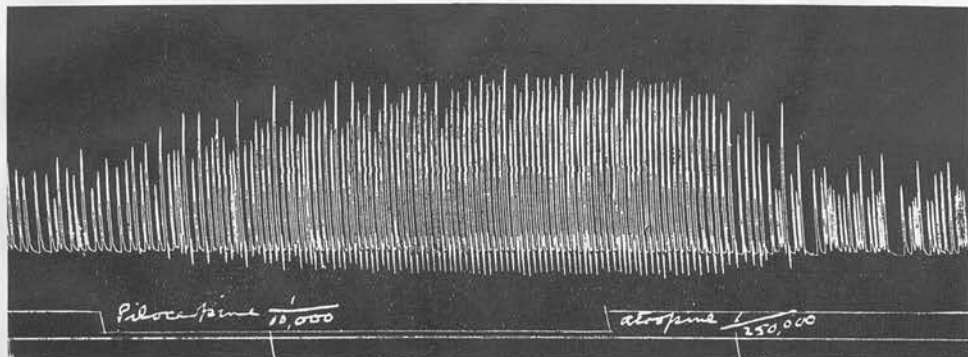


FIG. 10.—Heart of *Cancer*: action of pilocarpine and of atropine.

Pilocarpine (1/10,000) added at first signal and perfused for one minute. Atropine (1/250,000) abolishes effect in less than half a minute. One minute and a half after cessation of atropine-perfusion addition of pilocarpine had no effect on the heart. pH 7.4. Temp. 20° C.

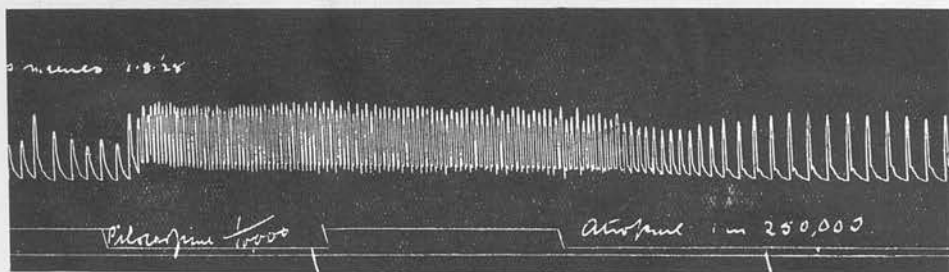


FIG. 11.—Heart of *Carcinus*: action of pilocarpine and of atropine.

Pilocarpine (1/10,000) added at first signal. At second signal atropine (1/250,000) added. The atropine abolishes the pilocarpine effect. pH 7.5. Temp. 20° C.

It is of interest to note, in view of the hormonal action of pilocarpine in these cases (and the belief that such drugs and their antagonists act directly on the cell-substance and not on any hypothetical "receptive substance" produced by the agency of nerves), that MATHEWS (14), and later SOLLMANN (15), obtained an increased rate of development of the fertilised eggs of the echinoderms *Asterias* and *Arbacia* with this drug, and showed that the action was antagonised by atropine.

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Effect of Atropine on the Response to Adrenaline.—The close similarity between the adrenaline and pilocarpine effects and the ease with which the latter is antagonised by atropine, suggested that the former too might be abolished by the same means. But experiments made to test this have yielded negative results. Even with the concentration of atropine as high as 1 in 40,000, no effect on the response to adrenaline could be obtained. When adrenaline and pilocarpine were given simultaneously, a greater effect was obtained than when each was perfused separately at the same concentration as in the mixture. On addition of atropine only part of the effect was abolished, the heart continuing to beat at a greater rate than normal and in an increased condition of tone, but less than that obtaining before the addition of atropine.

The very close similarity of response obtained with adrenaline and pilocarpine respectively immediately suggests a similar mechanism responsible for each. The failure of atropine, however, to antagonise the effect of adrenaline, shows that this can hardly be so; further, the similar failure of ergotoxine demonstrates that the conditions are not strictly comparable with those obtaining in Vertebrata.

SUMMARY.

1. The action of adrenaline, ephedrine, ergotoxine, pilocarpine, and atropine has been studied on the heart of the crabs *Maia squinado*, *Cancer pagurus*, and *Carcinus maenas*.

2. A method for the perfusion of the isolated heart in these genera is described.

3. Adrenaline produces a marked acceleration of the rhythm and an increase in tone of the heart-muscle in all cases.

4. In *Cancer*, in addition to the above effects, there is a pronounced increase in the amplitude of the beats.

5. The action of adrenaline is not reversible. In the lowest dilution in which it gives any effect, that effect is excitatory.

6. Ephedrine has no action on the crab heart.

7. Ergotoxine has a depressant action, but does not antagonise or reverse the action of adrenaline.

8. Pilocarpine produces an effect similar to that obtained with adrenaline.

9. Atropine has by itself no action on the heart in the dilutions employed, but antagonises the effect of pilocarpine.

10. Atropine has no effect on the response to adrenaline.

The author's acknowledgments are due to Dr E. J. ALLEN and Mr C. F. A. PANTIN for placing the facilities of the Marine Biological Laboratory at his disposal, and to Miss B. B. SMITH for assistance in conducting the experiments.

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II.—The Employment of Intracardiac Injection of Adrenaline in Asphyxia. By Sir E. Sharpey-Schafer, F.R.S., President, R.S.E., and William A. Bain, B.Sc. (From the Department of Physiology, University of Edinburgh.) (With Six Plates.)

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ASPHYXIA FROM OCCLUSION OF THE TRACHEA.

THE efficacy of intracardiac injection of adrenaline to assist recovery from asphyxia having been recently disputed,* we have instituted a number of experiments with the object of determining what effect, if any, is produced by such injection in asphyxiated animals in which the respirations and/or the heart had entirely stopped, and the blood-pressure had become reduced almost or quite to zero.

Since the differences in the mode of causation of asphyxia might influence the result, it seemed desirable to investigate the effect of intracardiac injection in asphyxia produced under different conditions. In the present paper we deal with its effect in asphyxia produced by occlusion of the trachea; in future communications we shall deal with its effect in asphyxia produced by drowning, by electrocution, and by inhalation of various gases and vapours. Lastly, we propose to consider its application to asphyxia neonatorum, which is not amenable to ordinary methods of promoting artificial respiration.

Methods.—In the present experiments we have used cats exclusively. The animals have been anæsthetised with urethane, administered hypodermically in a dose of 1·5 gm. per kilogram. When completely anæsthetised the animal is placed on a warm table, a Y-shaped trachea tube inserted, and the blood-pressure and respirations recorded on a smoked surface driven at a moderate rate. The blood-pressure is recorded by a mercury manometer connected with the femoral artery; the respirations by a double tambour applied to the lower part of the chest and connected with a recording tambour. Asphyxia has been produced by closing the trachea tube, which was so arranged that at any given moment artificial respiration by intermittent admission of compressed air could be commenced. Or, alternatively, artificial respiration by

* *Journ. Amer. Med. Assoc.*, Jan. 11, 1930. See also Yandell Henderson, *Brit. Med. Journ.*, Oct. 17, 1931.

intermittent manual compression of the thorax could be substituted. In the cat such manual compression also involves intermittent compression of the heart, and produces a pumping action which mechanically promotes an artificial circulation and even, if the condition of asphyxia is not too advanced, provokes cardiac contractions which may be efficient enough to cause the blood to pass from the venous to the arterial system.

RESULTS.

The result of causing complete asphyxia by tracheal occlusion without any attempt at resuscitation by artificial respiration and without administration of adrenaline may first be described (see fig. 1).

The immediate effect upon the respirations of closing the trachea (in this instance it was closed shortly after the beginning of an expiration) is to slow them. The extent of each respiration is at first diminished, but as asphyxia becomes more pronounced the movements of respiration become gradually more extensive and ultimately exhibit well-marked inspiratory spasms. After a little more than two minutes the respirations suddenly and completely stop; the arrest occurring in the expiratory phase. Although the respiratory movements cease, the tracing does not show a horizontal line, for after about a quarter of a minute an inspiratory tone slowly develops, persists for a few seconds, and then gradually subsides. About a minute after the cessation of respiration the so-called "agony respirations" begin. They are from three to ten in number, and succeed one another at intervals of 15 to 30 seconds. If the heart has stopped before they begin they are usually ineffective in restoring the circulation and in reviving the animal. But if the heart is still beating and the trachea is no longer occluded, they may admit enough air for spontaneous recovery to occur (see p. 144 and fig. 6). Sometimes the respirations become much deeper as asphyxia proceeds, and before it is complete assume a convulsive character; but in the tracing shown they are at no time excessive.

The immediate effect on the arterial pressure of closing the trachea is to cause a slight fall. The respiratory waves are accentuated upon the tracing and are of course at first slower than before. After a short time the pressure begins to rise; the rise is gradual, and in the case illustrated, not marked. The heart-beats are slowed, and therefore the pulse-waves are accentuated. Shortly before the respirations cease, the heart-beats again become fast; the blood-pressure becomes more rapidly depressed on stoppage of the respiratory movements and gradually descends to zero, a position attained about two minutes after the

cessation of the respiratory movements, by which time the heart-beats are no longer visible on the tracing.

If the occlusion of the trachea is now removed, it is still possible to revive the animal by intermittent manual pressure on the thorax, for, as already explained, this action not only renews air in the lungs, but also pumps blood through the heart and into the arteries, and starts an artificial circulation. The blood driven through the heart by this pumping action is oxygenated in the lungs, the air of which is being renewed by the artificial respiration, and passing to the respiratory centre, which had become paralysed from want of oxygen, restores its activity: always provided the cessation of respiration and circulation has not been too prolonged.

It is not easy to estimate the time which may elapse if such recovery is to be possible, for it appears to vary in different individuals and under different circumstances. Instances from the human subject have been cited in which recovery has been obtained by artificial respiration even half an hour after natural respiration has ceased; but this is exceptional. Ten minutes is probably the limit under ordinary circumstances.

The combination of artificial respiration with heart massage may succeed when the former alone is inadequate. In the human subject heart massage is difficult; it involves the insertion of the fingers through an incision in the epigastrium and compression of the heart through the diaphragm. In performing such massage the heart is liable to be bruised by the pressure of the fingers. In the smaller animals, such as the cat and rabbit, and in young dogs, the thorax is sufficiently yielding for it to be possible to compress the heart through the chest wall. Mere renewal of the air in the lungs is not effectual by itself. This is illustrated in fig. 2, which shows the result of an experiment on resuscitation of a cat from asphyxia, in which respiration is practised not by manual pressure on the thorax but by driving air intermittently into the lungs through the trachea tube. The procedure was started four minutes after natural respirations had ceased, and after the blood-pressure had descended to zero, with disappearance of any sign of heart-beats, the animal being to all intents and purposes dead.

The respiration curve shows nine "agony respirations," which began one minute after cessation of the ordinary respirations, and occurred during the next two minutes, but were ineffectual for recovery. One minute later, *i.e.* four minutes after cessation of the ordinary respirations, the trachea was connected with the artificial respiration apparatus usually employed in laboratories, and for rather more than one minute air was

pumped intermittently into the lungs. This produced no sign of recovery either of natural respiration or of the heart or of blood-pressure. A dose of adrenaline (0.5 c.c. of a 1/10,000 solution of adrenaline chloride) was then injected into the myocardium. The heart immediately resumed its activity. The blood-pressure rapidly rose from zero to 180 mm. Hg, and continued at a level not much below this; and then gradually fell to the level it occupied before asphyxia commenced. Following the rise of blood-pressure, natural respirations were resumed. They were at first slow and shallow, but gradually became faster and deeper, so that in two or three minutes they had acquired their normal character. There can be no doubt that the recovery was due to the action of adrenaline upon the arrested heart, which was thereby caused to resume its activity, so that blood was pumped through the now aerated lungs, and the asphyxiated centres in the bulb were restored to their normal condition.

Although in such a case as this the commencement of recovery is actually due to the action of adrenaline in stimulating the heart to increased contraction, there would be no permanent recovery unless the pressure in the arteries were not only raised, but maintained at a sufficient level. This could only be brought about by contraction of the arterioles, owing to the action of the adrenaline upon them. It is their contraction that has caused the great rise in blood-pressure observed in the tracing, and which is maintaining the arterial pressure at a high level. In this way a steady flow of blood is kept up in the bulb; its centres (vasomotor, respiratory, etc.) resume their lost activities, and the carotid sinus reflexes, which had disappeared, reappear.

If the heart is still beating, although the respirations may have completely ceased and the blood-pressure may have fallen almost to zero, the administration of adrenaline is obviously more likely to be effective than if there is no circulation at all. In the experiment illustrated by fig. 3, 0.5 c.c. of 1/5000 adrenaline chloride was injected into the heart. The effect on the heart itself was not immediate: from this we infer that the injection was not into the muscular substance—in which case it would not fail to produce an immediate effect—but into one of the heart cavities, probably the left ventricle. About a quarter of a minute elapsed before the rise in blood-pressure began. This rise is caused both by increased force and frequency of the heart and by contraction of the systemic arterioles. The adrenaline-containing blood has passed out of the ventricle, and is now reaching the capillaries of the coronary and aortic systems. Simultaneously with the rise of blood-pressure and resumption of the circulation through the bulb, natural respirations are resumed. They at

first show apnoeic intervals; but after certain fluctuations of blood-pressure and heart-rate, due probably to carotid sinus reflexes, both circulation and respirations settle down into normal conditions.

Fig. 4 illustrates the advantage of artificial respiration together with heart massage (by chest compression) in facilitating the passage into the blood-vessels of adrenaline which has been injected into one of the cavities of the heart. In spite of the injection of 0.55 c.c. of 1/1000 adrenaline chloride into the beating heart, the latter showed no increase in rate or force; indeed its beats were diminished. Presumably the injection was into one of the cavities, probably into the right ventricle. Half a minute after the injection, the chest was compressed intermittently so as to permit artificial respiration, and at the same time to massage the heart. This had no immediate effect on the blood-pressure. The tracing of the compression is interspersed with a few deep respirations at intervals of about fifteen seconds: they are probably "agony respirations." Half a minute later the blood-pressure suddenly rose, the heart-beats becoming stronger, and the arterioles contracting. The massage was then stopped, natural respiratory movements being resumed. The rise in blood-pressure was very sharp, and rapidly attained the very considerable height of 184 mm. Hg. The natural respiratory movements, at first very deep and marked by apnoeic intervals, became in a minute or two regular and of normal character. The blood-pressure continued very high for some minutes, and showed strongly marked irregularities. These were probably the effect of the large dose of adrenaline, the solution employed being ten times as strong as those used in most of our experiments.

An adrenaline effect may be obtained in cases of recovery without the artificial administration of adrenaline. Such a case is illustrated in fig. 5. Asphyxia was, as before, induced by occlusion of the trachea tube. Two minutes after closure the natural respirations, which were at first slow but later more rapid and convulsive, stopped. One minute later the "agony respirations," five in number, began, and succeeded one another at intervals of about twenty seconds, showing, as is usual, a well-marked "staircase." Two minutes after stoppage of the natural respirations the trachea tube was reopened, and half a minute later the chest was intermittently compressed at a rapid rate in order to promote artificial circulation by heart massage. No adrenaline was administered. The blood-pressure had fallen to zero, and showed no heart-beats: it seemed as if there were no chance of effecting recovery. But three minutes after the respirations had ceased and after the heart massage had been practised for half a minute, the heart-beats were again visible. The blood-pressure began to rise, at

first gradually and then very rapidly, and soon attained a height of 160 mm. Hg. The effect was exactly like that seen when a dose of adrenaline is administered by intravascular injection (*cf.* figs. 2, 3, 4). The pressure soon fell somewhat, and gradually settled down to its normal level. Natural respirations were resumed, being at first slow and deep, then showing marked alternations in depth and extent, but gradually becoming more regular. Four and a half minutes after cessation of the massage and commencement of recovery, a dose of 0.5 c.c. of 1/10,000 adrenaline chloride was injected into the heart. The effect of this was gradually to produce a steady and maintained rise of blood-pressure, accompanied by a resumption of regularity of respirations, followed by complete recovery.

The explanation we offer of the result of this experiment is as follows: During asphyxia the centre for secretion of adrenaline—along with the other centres in the bulb—is stimulated, and an unusual amount of adrenaline passes into the suprarenal vein. With the failure of the circulation which ultimately results from the asphyxia, the blood of the suprarenal vein does not pass into the inferior vena cava and through the heart; the secreted adrenaline accumulates in the vein. But the intermittent pressure on chest and abdomen has in this case caused it to pass into the inferior vena cava and right auricle, and through the pulmonary circulation into the left side of the heart and aorta, producing as it reached the coronary and systemic arterioles the rapid and great rise of pressure characteristic of intravascular administration of adrenaline. In this instance the respirations were slow in attaining regularity, although there was no question that the animal had recovered from asphyxia. It was to ascertain whether the recovery would be accelerated by intracardiac administration of adrenaline that we subsequently injected the 0.5 c.c. of a 1/10,000 solution into the myocardium. This had the effect of steadying both respiration and blood-pressure, and evidently proved helpful towards complete recovery.

In fig. 6 we give an illustration of spontaneous recovery without the assistance of massage of the heart, or artificial respiration, or adrenaline. Natural respirations ceased about two and a half minutes after occlusion of the trachea. The cessation was followed by the usual gradual fall of blood-pressure almost to zero, the heart-beats being, however, just visible on the tracing. The trachea tube was opened about half a minute after respirations had stopped. Less than a minute later the "agony respirations" began. In this case they were effective not only in drawing air into the lungs, but apparently also in causing the adrenaline-containing blood of the suprarenal vein to pass towards the heart, thus promoting

its passage through the pulmonary circulation into the coronary and systemic vessels. In consequence the blood-pressure rose significantly (from zero to 50 mm. Hg), although not in so striking a manner as in the last experiment. Simultaneously natural respirations were resumed. At first slow and deep, and somewhat irregular, they soon became regular and normal in extent. Although an adrenaline effect is less obvious in this case than in the experiment illustrated in fig. 5, the difference is probably due to the fact that the passage of the adrenaline-containing blood through the heart must have been less rapid than when the circulation was promoted by heart massage. Unfortunately the arterial cannula was partly obstructed by a clot, which prevented the heart-beats from showing themselves on the rising blood-pressure curve.

LITERATURE.

The literature of intracardiac administration of adrenaline has of late years become extensive. It relates mainly to the recovery of a heart which has been arrested, whether as the result of shock, or from the administration of anæsthetics, or from indeterminate causes. The effect in simple asphyxia does not seem to have been previously investigated, although it has been employed as an auxiliary method in cases of failure of heart and respiration such as occurs in electrocution and drowning. Other drugs (camphor, ether, strophanthine, strychnine) have also been recommended for intracardiac injection, but none approach adrenaline in effectiveness and rapidity of action. It is, moreover, *the* physiological stimulant of the heart, as well as of the whole vascular system.

Most of the published papers on the subject merely relate one or two cases; but some authors have taken the opportunity to collect all the known clinical evidence up to the date of their article, and thus furnish a useful bibliography. The latest writer, Hyman (3), collected 250 cases of intracardiac injection, with 25 per cent. successes. This proportion of successes is the more remarkable when we consider that an intracardiac injection is usually administered when the patient is moribund; sometimes when he is to all intents and purposes dead, and could not otherwise recover. Hyman rightly recommends that the injections should be made into the substance of the myocardium* and not into the cavities, nor into the pericardium. The last is no more effective than a hypodermic injection. Even intravenous injection is not to be recommended, being both difficult and uncertain. Hyman's own cases are nine in number. In one—a man

* By injecting into the substance of the heart, the drug at once gets to every part through the extensive lymphatic system which pervades the myocardium.

of thirty-six—there was complete cardiac arrest on the operating table, and all the usual remedies had been ineffectually essayed. Adrenaline was injected intracardially eleven minutes after the heart had stopped; it produced complete recovery. Another case of a child injected fourteen minutes after heart stoppage gave a similar result. He cites another case in which the heart had completely stopped for thirty minutes; another of twenty minutes' arrest; and others of twelve and ten minutes.

Hyman comes to the curious conclusion that it is the mechanical prick of the hypodermic needle which is effective, and recommends a succession of pricks of the right auricle (the *primum movens*). But in view of the unquestionable benefit of adrenaline both on cardiac activity and in promoting contraction of the systemic arterioles, as well as its beneficial effect on the respiratory system—effects which are clearly demonstrated in the tracings given in this paper—Hyman's conclusion is unacceptable.

Frenzel (2) mentions a case of heart arrest under anæsthesia, treated successfully by intracardiac injection of 1 c.c. of 1/1000 adrenaline solution. He also cites a number of other cases in which heart massage had been tried unsuccessfully, but in which adrenaline produced rapid recovery. He expressly states that in the event of recovery there are no after-consequences, and recommends that adrenaline should always be kept ready for injection at an operation.

Vogt (4) also strongly recommends intracardiac administration in cases of heart failure. He describes it as the simplest and best method, far more effective than heart massage; which is, moreover, liable to bruise the heart.

In an interesting paper in the *Lancet* of 1923, Bodon (1) describes the case of a man, aged fifty-six, with syphilitic disease of pulmonary arteries, who became moribund and completely unconscious. The respirations and heart stopped, the corneal reflexes disappeared, the eyes became glazed, the pupils widely dilated, the face cyanosed. Urine and fæces were passed involuntarily. The patient was to all intents and purposes dead. A few seconds after 1 c.c. of 1/1000 adrenaline solution had been injected into the heart, its action recommenced, the radial pulse could be felt, and respirations were resumed. About two hours later the man recovered consciousness; he remembered nothing about his collapse. Half an hour later he could walk across the room with assistance; he eventually recovered completely. Bodon gives results in 90 cases which he had collected. Of these, 24 were permanently successful. He recommends the employment of the method in all cases of heart failure, whether from surgical shock, anæsthesia, electrocution, obstetrical hæmorrhage and

shock, acute diseases of the circulatory system, effect of drugs; in anaphylaxis, and in asphyxia neonatorum. He is insistent that the injection should be made with as little delay as possible. All authorities are agreed that no deleterious after-effects are to be anticipated.

The method recommended by Vogt and Bodon for man is to make the puncture with a fine hypodermic needle, 8 cm. (about 3 inches) long, in the fourth left intercostal space, just above the fifth rib and about a finger-breadth from the edge of the sternum. This avoids the internal mammary artery, and pierces the heart where it is close to the chest wall. The needle can be felt to penetrate the myocardium, and if the heart is still acting, it will be moved to and fro.

SUMMARY AND CONCLUSIONS.

1. The effect of intracardiac injection of adrenaline in assisting recovery from asphyxia produced by occlusion of the trachea has been investigated in cats anaesthetised with urethane.

2. It is shown that such injection has a striking effect in promoting recovery of the circulation. Even if the heart has ceased to beat, it may start contracting rapidly and strongly as the result of such injection, the blood-pressure being rapidly raised. The rise is due not only to the action of adrenaline on the heart, but also to its causing contraction of the systemic arteries.

3. The effect in promoting restoration of respiration is no less striking. Breathing recommences at the same time that the circulation is restored, and although at first slow and deep, the respiratory movements gradually resume their normal character and rate.

4. If the injection is into one of the heart-cavities instead of into the myocardium the effect is slower in manifesting itself, because the adrenaline has in the case of injection into the left ventricle to pass into the capillaries of the aortic and coronary systems, and in the case of injection into the right ventricle, also to traverse the capillaries of the pulmonary system. The passage is assisted by heart massage and artificial respiration such as can be produced in animals by intermittent pressure on the abdomen and lower part of the thorax.

5. Rarely an adrenaline effect is obtained with heart massage and artificial respiration without an actual injection of adrenaline. This appears to be due to the passage towards the heart of adrenaline-containing blood which has stagnated in the suprarenal veins, in consequence of the failure of the circulation, and, as a result of the intermittent pressure

applied to the thorax and abdomen, becomes driven towards and through the heart and pulmonary circulation, and thus eventually into the aortic and coronary capillaries.

6. Still more rarely spontaneous recovery may occur, without either heart-massage or artificial respiration or adrenaline administration. In such a case the passage of the adrenaline-containing blood of the suprarenal veins towards the heart seems to be facilitated by the "agony respirations," which begin, in all cases of asphyxia, about a minute after the natural respirations have ceased. If the occlusion of the trachea has been released, these respirations introduce air into the lungs, and at the same time draw and press the adrenaline-containing blood of the suprarenal veins towards the heart, and thus facilitate its passage into the circulation.

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DESCRIPTION OF FIGURES.

Fig. 1. Effect of occluding the trachea without any attempt at resuscitation.
Cat, ♂. 3200 grms.*

The trachea was occluded at the beginning of expiration: the occlusion lasts for rather more than three minutes. Respirations cease in a little more than two minutes. The "agony respirations" begin about one and a half minutes after cessation of the ordinary respirations, but are ineffectual in promoting recovery. After cessation of respirations the blood-pressure gradually falls to zero, and the heart-beats, which are at first increased as the asphyxial condition develops, become gradually less obvious, until they are no longer visible on the tracing.

* In this and every other case the anaesthetic employed was 1.5 grms. of urethane per kg, given subcutaneously.

In all the tracings the uppermost line shows the respirations as recorded by a tambour connected with a pneumograph attached to the thorax-abdomen, the up-stroke being inspiratory; the second line shows in millimetres of Hg the blood-pressure in the femoral artery; the third line, the period of occlusion of the trachea and any other recorded events; and the lowest line the time in minutes.

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Fig. 2. Effect of intracardiac administration of adrenaline in promoting the recovery of heart, blood-pressure, and respirations after complete cessation of heart and respirations had occurred several minutes previously; artificial respiration by intermittent inflation having failed to effect any recovery.

Cat, ♂. 2650 grms.

The trachea is occluded at the end of an expiration: the occlusion lasts for five minutes. The respirations stop after two minutes after having been much increased in extent, although slower in rate. Blood-pressure, which had slowly risen, falls, at first more abruptly, afterwards gradually, and is at zero four minutes after respirations had ceased. (This record is interrupted by the formation of a clot in the cannula, which was removed.) The heart-beats become gradually weaker, and at last can no longer be detected. The "agony respirations" (nine in number) begin one minute after the natural respirations have ceased and continue for two minutes. They exhibit a well-marked "staircase," but have no influence in promoting recovery; nor could this be expected, since the trachea is still occluded. The animal is to all intents and purposes dead. The trachea having been released, an injection of 0.5 c.c. of 1/10,000 adrenaline chloride was then given, but produced no effect; probably it did not enter the heart. Artificial respiration was now started by a pump, the strokes of which are recorded on the respiration curve. This had no result. Five minutes after the natural respirations had ceased, 0.5 c.c. of 1/10,000 adrenaline chloride solution was injected into the heart, and artificial respiration was stopped. Recovery of the heart and blood-pressure rapidly followed; the effect was magical. The blood-pressure rose to 180 mm. Hg, was maintained for a short time at a high level, and gradually fell to normal. In less than a minute natural respirations were resumed. At first slow and shallow, they gradually increased in rate and regularity. Complete recovery of respirations and blood-pressure was soon obtained, but is not shown in the part of the tracing reproduced.

Fig. 3. Rapid recovery of respiration and blood-pressure as the result of an early intracardiac injection of adrenaline, without the assistance of artificial respiration or heart massage.

Cat, ♂. 3200 grms.

In this animal the trachea was occluded for rather more than three minutes. Cessation of respiration is seen to occur in about two minutes. The blood-pressure, after rising somewhat towards the end of asphyxia, gradually falls almost to zero, but heart-beats are continued strongly. One and a half minutes after cessation of respiration 0.5 c.c. of 1/5000 adrenaline chloride solution is injected into the heart. No effect is observed for about a quarter of a minute; there is then seen a rapid rise of blood-pressure and increase in force of the heart's action, with the vagal effects characteristic of adrenaline. The increase in the heart's action combined with arterial constriction has produced a supernormal blood-pressure; but, after some fluctuations, this gradually subsides to the normal pre-asphyxial level. Accompanying the rise in blood-pressure, the respirations recommence: they are at first deep and slow and irregular, with apnoeic intervals, but soon become regular and of normal rate and depth.

Fig. 4. This is another illustration from the same animal of the effect of injecting adrenaline intracardially in promoting recovery from asphyxia.

One and a half minutes after occlusion of the trachea the respirations, after becoming greatly exaggerated, stop abruptly. The injection of adrenaline, which in this case was 0.5 c.c. of 1/1000, was administered about three-quarters of a minute after the respirations had stopped. Blood-pressure had fallen considerably, but the heart was still beating strongly, although slowly. The adrenaline at first produced no effect, having probably not been injected into the substance of the myocardium, but into the right ventricle. Since after half a minute more no recovery was apparent, massage of the heart by compression of the thorax was practised. At the end of another half minute or so an adrenaline effect on the heart and blood-vessels shows itself by the production of an enormous rise of blood-pressure accompanied by resumption of natural respirations; these are at first slow, deep, and irregular, but soon become regular. The dose of adrenaline was considerably larger—ten times as large—as in most of the other experiments. Apparently in consequence of this there is produced not only a very great excitatory effect on the heart and arteries, but considerable irregularities both of respiration and blood-pressure. These are no doubt due to the intense action of the large dose of adrenaline upon the bulbar centres, which would be affected both directly and reflexly (through the carotid sinus).

Fig. 5. Recovery from asphyxia produced by manual massage of the thorax, showing an adrenaline effect without artificial administration of the autacoid.

Cat, ♂. 3350 grms.

In this experiment the trachea was occluded for four minutes. The respirations, at first slow, gradually increase in amplitude and in rate and cease abruptly in two minutes. One minute afterwards five "agony respirations" begin, and succeed one another at intervals of about twenty seconds. Although the last two occur after the trachea is opened, they fail to effect recovery. Blood-pressure is at zero, and no heart-beats are visible (but there is some evidence of the presence of a clot in the arterial cannula). Manual massage of the thorax, and through this of the heart, is then practised. This soon has the effect of causing the heart to resume beating, and in about three-quarters of a minute the blood-pressure rapidly rises to 150 mm. Hg, exactly as if adrenaline had been injected into the vessels, although none has been administered. After falling again somewhat, an average pressure of about 130 mm. Hg is assumed. With the rise of blood-pressure the respirations recommence: at first they are infrequent and deep, soon to be alternated by shallower movements; but eventually they become more regular, although for a time irregular and exaggerated. A dose of 0.5 c.c. of adrenaline chloride 1/10,000 is now injected into the heart: this rapidly steadies both the blood-pressure and the respirations, and rapidly leads to complete recovery.

Fig. 6. Asphyxia from closure of trachea. Spontaneous recovery without either artificial respiration or adrenaline injection.

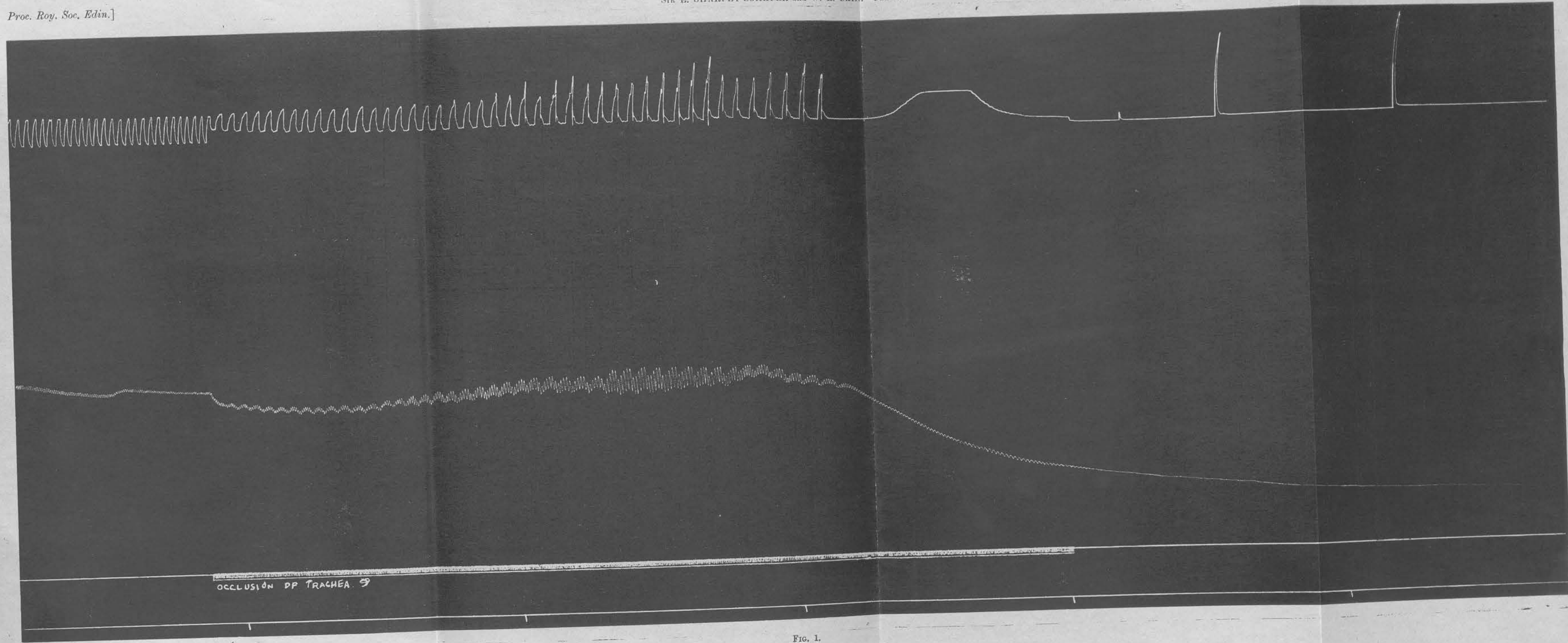
Cat, ♂. 3200 grms.

In this case respiration stops two and a half minutes after occlusion. The trachea is then released, and the animal left undisturbed. The blood-pressure

falls gradually almost to zero; perhaps owing to a partial clot in the arterial cannula, the heart-beats are only shown indistinctly upon the tracing. The "agony respirations" begin about a minute after the natural respirations have ceased: the trachea being now open, they prove effective in causing recovery; ordinary respirations are quickly resumed, at first deep and somewhat irregular, but soon becoming normal. Resumption of respiration is accompanied by recovery of blood-pressure; but the recovery is less abrupt and the rise less marked than in the experiment illustrated in fig. 5, where the circulation was assisted by manual massage of the thorax.

The spontaneous recovery in this case was probably brought about by the mechanical effect of the "agony respirations" in forwarding towards the heart adrenaline which had stagnated in the suprarenal veins. Further, since the trachea tube had been opened, the "agony respirations" would introduce air into the lungs and re-oxygenate the blood in the pulmonary capillaries: this, being forwarded to the systemic circulation, would revive the heart and blood-vessels as well as the reflex centres of the bulb.

(Issued separately February , 1932.)



OCCLUSION OF TRACHEA. 9

FIG. 1.

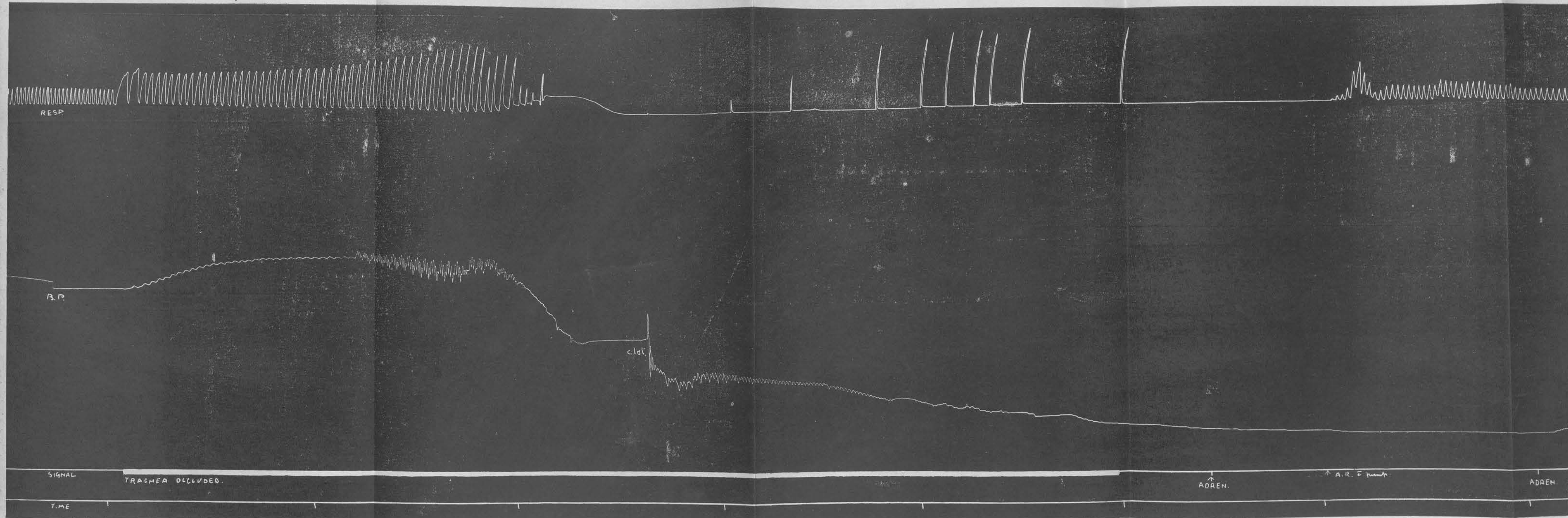


FIG. 2.

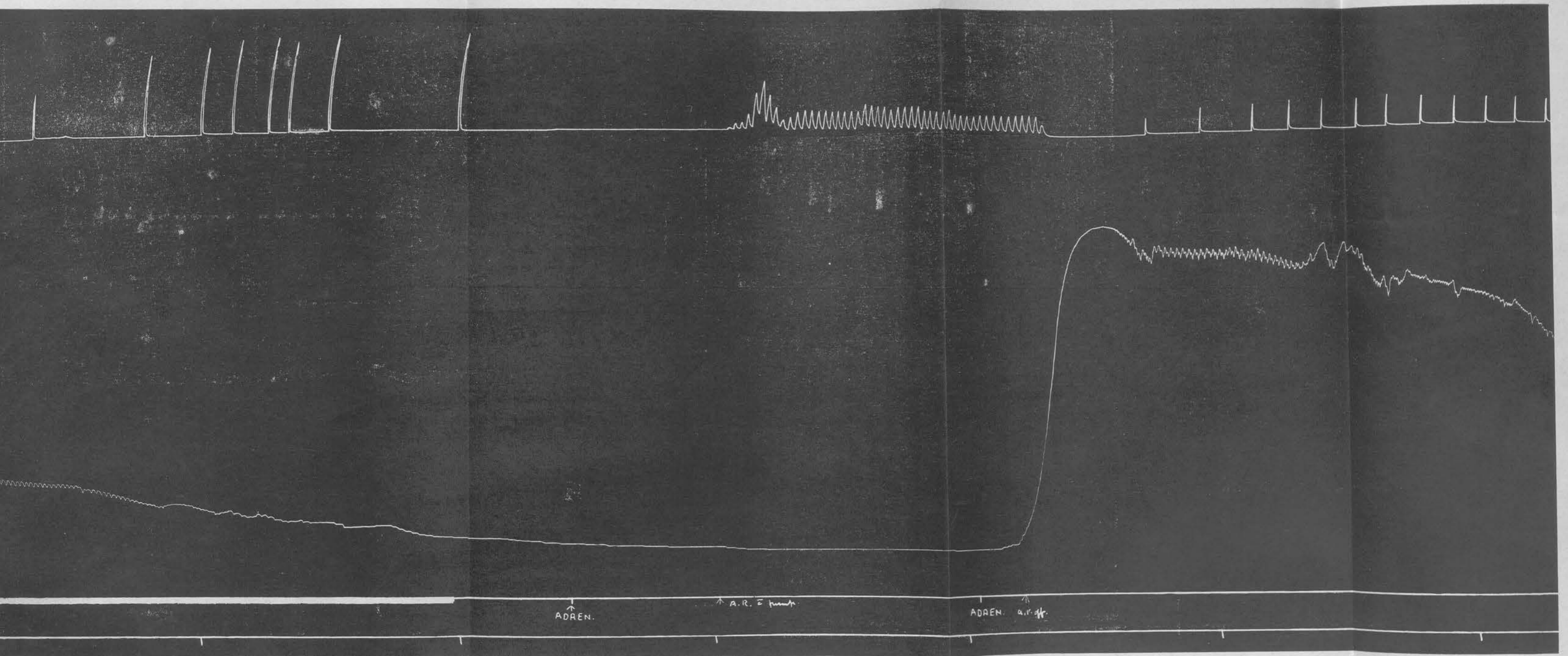


FIG. 2.

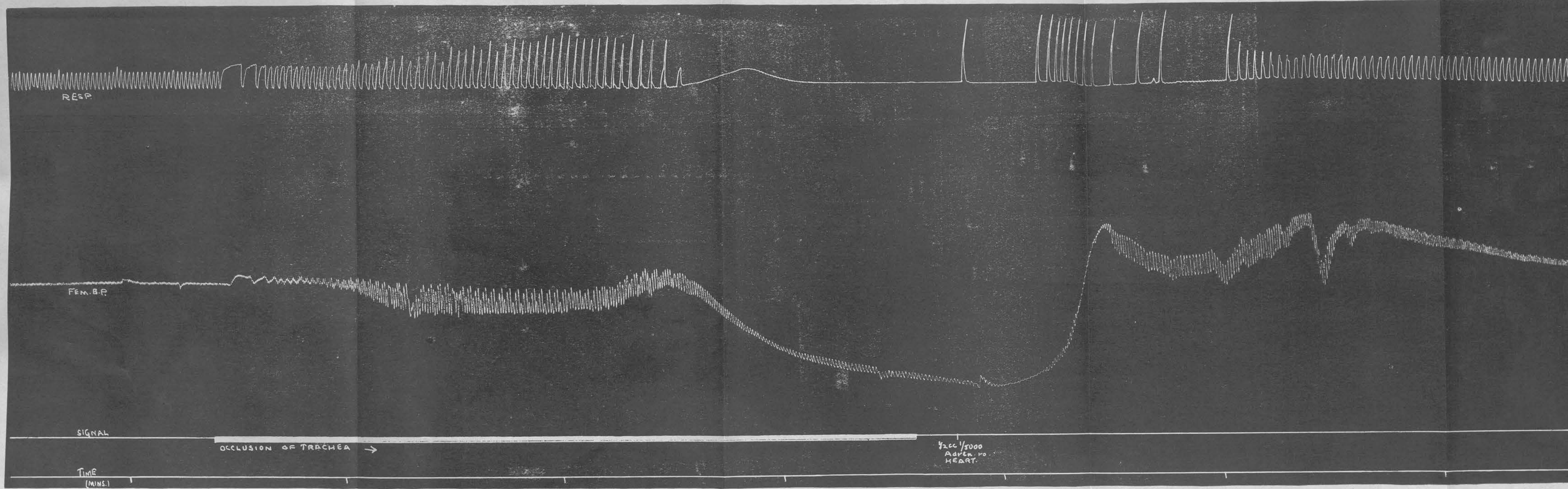


FIG. 3.

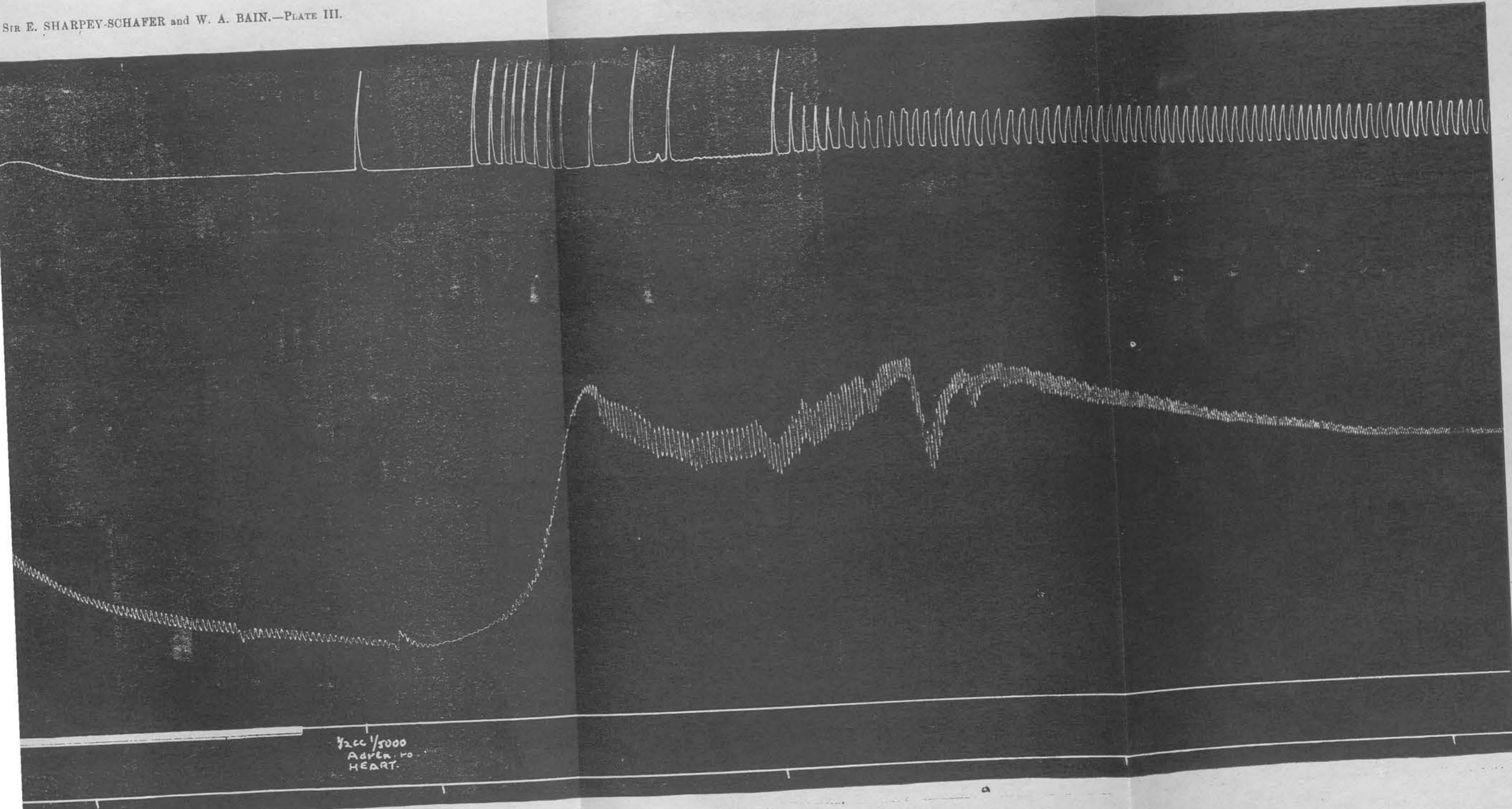


FIG. 3.

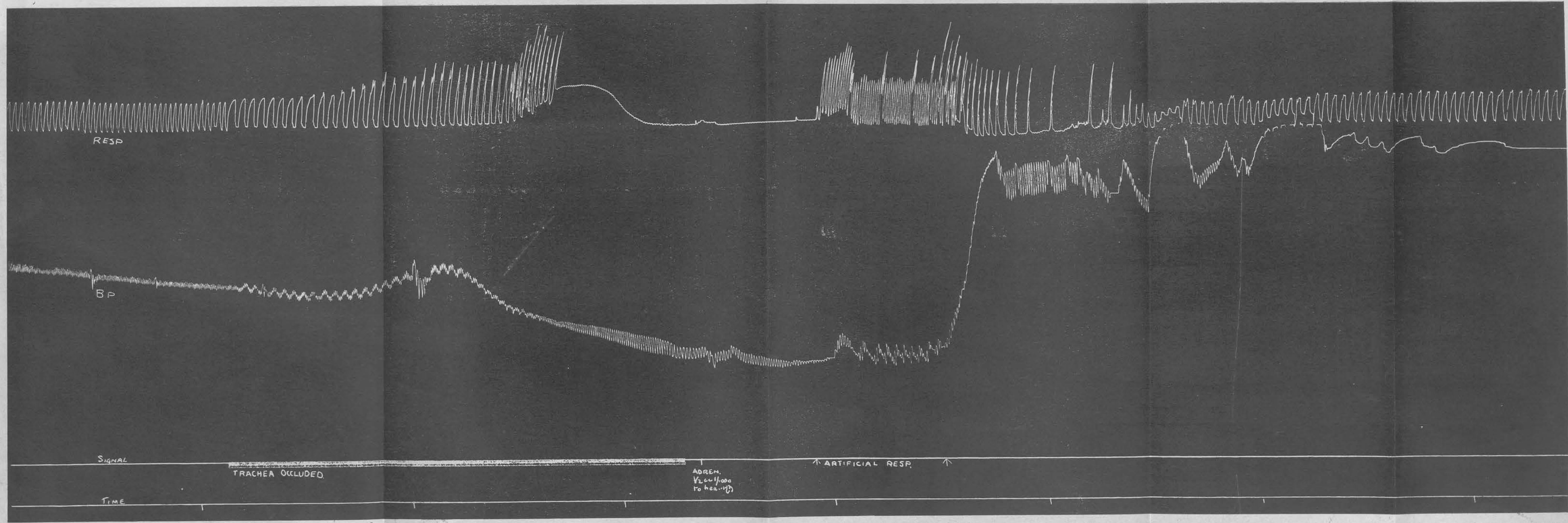


FIG. 4.

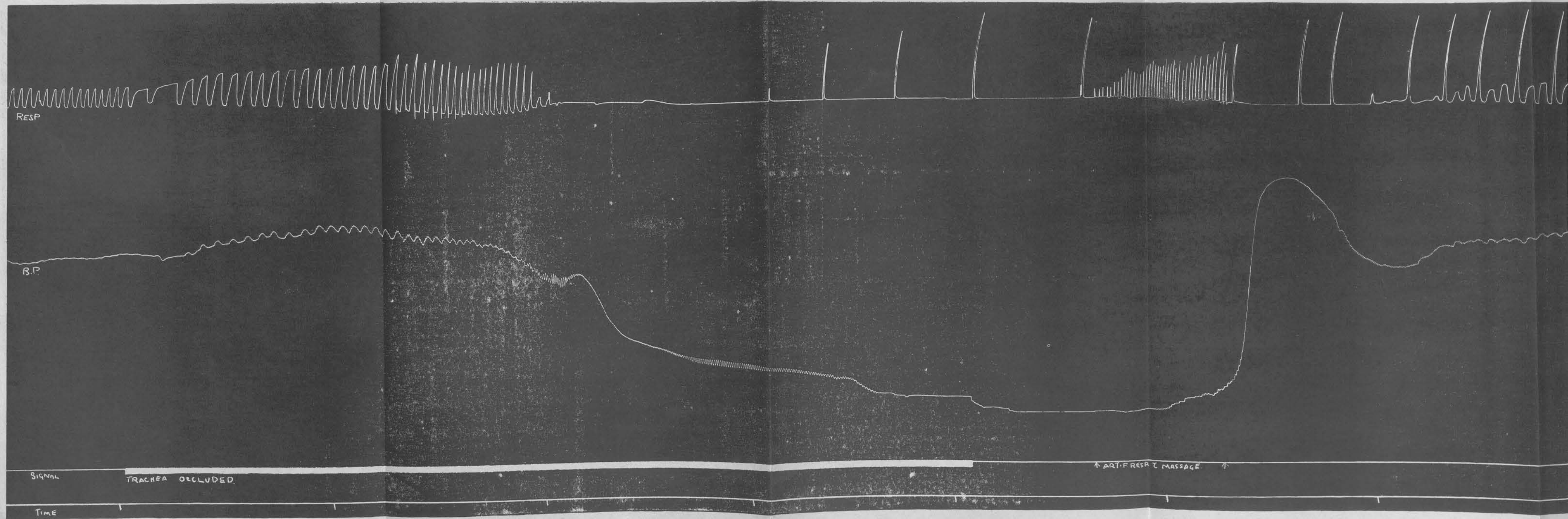


FIG. 5.

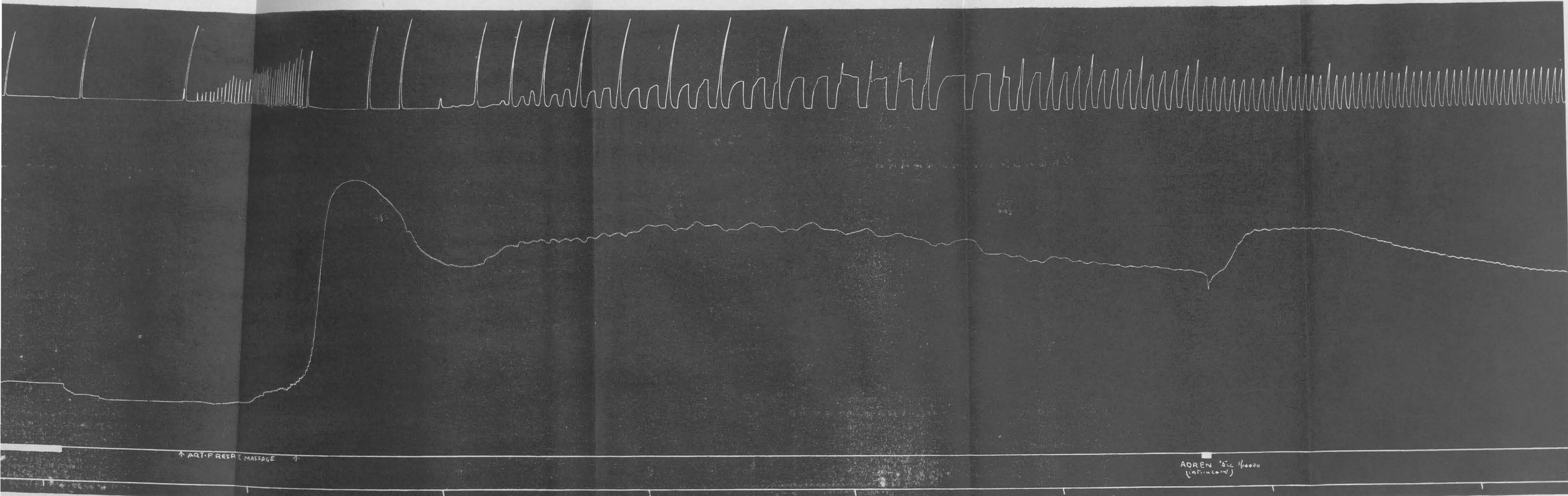


FIG. 5.

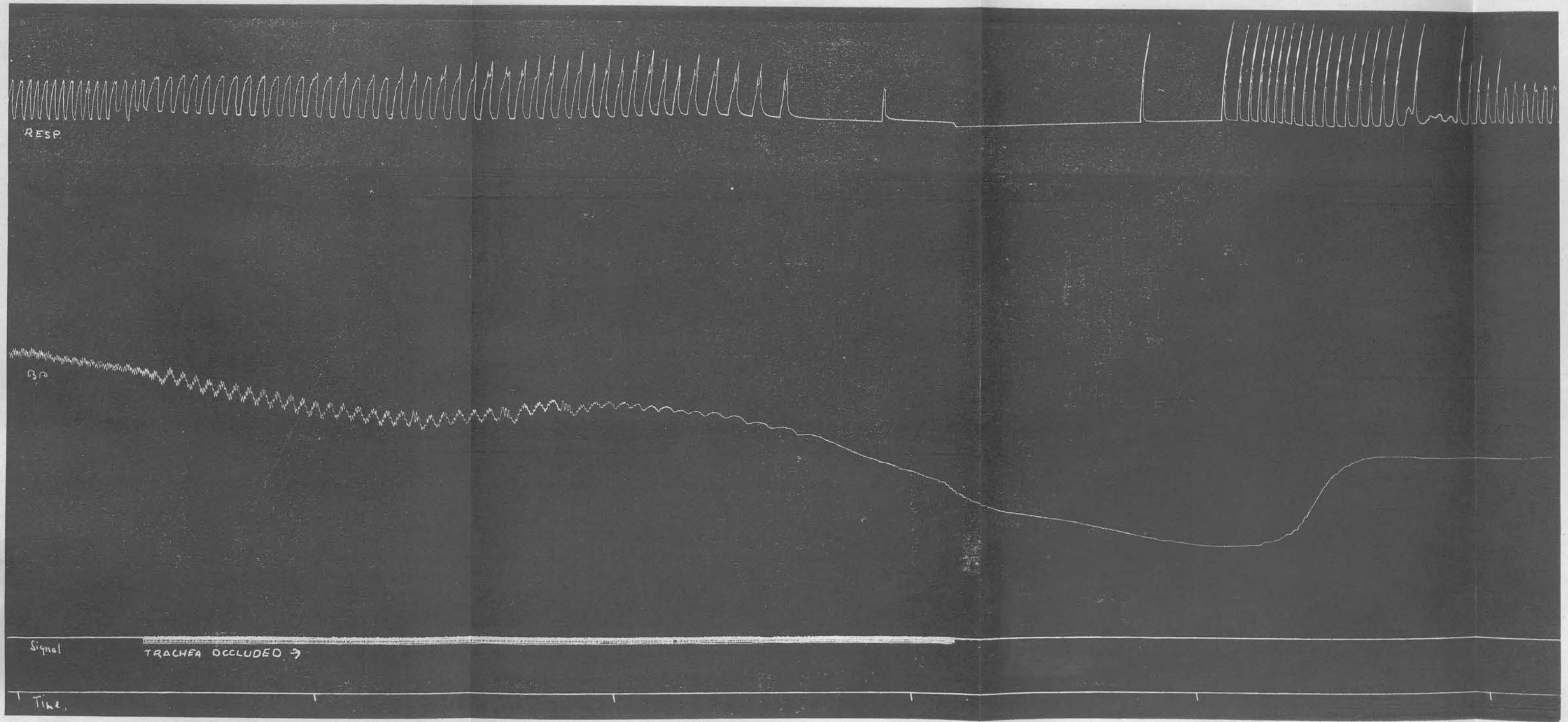


FIG. 6.