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THE AUTACOID SUBSTANCES

in relation to

THE AUTONOMIC NERVES.

THE THYROID GLAND.

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by

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C O N T E N T S.

THE AUTACOID SUBSTANCES IN RELATION TO THE AUTONOMIC NERVES.

THE THYROID GLAND.

INTRODUCTION - CHIEFLY HISTORICAL.

The investigation of the action of the thyroid gland has occupied the attention of physiologists for many years. Its function has been studied by noting the effects resulting from congenital deficiency, or post-natal atrophy or removal; and these observations have been supplemented by the investigation of the results following "substitution therapy", or those

which occur in consequence of the administration of the gland or its preparations to intact animals.

Some observers have used either the entire gland or extracts prepared from it, whilst others, in an effort to discover the active principle, have concentrated their attention more particularly upon the physiological effects of the administration of specific bodies prepared from the gland.

CHEMISTRY OF THE THYROID.

The earliest attempts to investigate the chemistry of the thyroid gland were made by Bubnow (1884)(1) who found that aqueous or saline extracts of this organ yielded a precipitate on the addition of acid. He was able to show that this substance was a protein, and in view of its origin he named it thyreoprotein.

Gourlay (1894)(2) in an attempt to recognise the chemical nature of this protein, concluded that it was a nucleo-albumin, because of the presence of phosphorus in its molecule. His conclusion however was scarcely justified, owing to his omission to test for the presence of purin bodies, and by his failure to observe if it gave a reducing substance after acid hydrolysis.

Baumann(1895)(3) obtained evidence to show

that the gland contained iodine, and to the compound in which this was present he gave the name thyroiodin. He obtained this by hydrolysing the whole gland with 10% sulphuric acid, and dissolving the alcoholic extract of the precipitate so produced, in 1% caustic soda solution. This was made faintly acid, and the resulting precipitate was the thyroiodine. It contained 0.5% of phosphorus and 10% of iodine.

We are indebted however to Hutchison (1896)(4), working in the Department of Physiology, of Edinburgh University for the first systematic examination of the chemical nature of the thyroid. He was able to classify the ingredients of the thyroid as follows:-

1. Nucleo-albumins, from cells lining the acini,

2. Colloid material, containing a large amount of iodine, and a small amount of phosphorus. This gave no reducing substance on boiling with mineral acids, and yielded no purin bodies,

3. Extractives, Xanthin, hypoxanthin, lactic acid, creatin.

On subjecting the colloid material to peptic digestion he was able to divide it into two components:-

a. Non-proteid, containing much iodine

and a small amount of phosphorus,

b. Proteid, containing some iodine and no phosphorus.

The colloid material was the only one effectual in combating the effects of thyroidectomy, and he found this property depended almost entirely upon its non-proteid component, which he considered to be identical with Baumann's thyroiodin.

In further investigations (1898)(5) Hutchison found that the activity of the colloid was proportional to the amount of the iodine present. The artificial addition to this of iodine as such, produced no more effect. In control experiments using thymus extracts with iodine added, no "thyroid" effects were obtained. The conception of iodine as an important ingredient of the thyroid is strengthened by the presence of that element in this gland in Elasmobranchii, Amphibia, Reptilia, Aves and Mammalia.

Many other workers have investigated these substances, and it was shown by Oswald (1899)(6) that the iodine present is contained in a globulin, to which he gave the name iodothyroglobulin. Digestion with trypsin completely dissolved the compound, and the resulting solution contained no iodine, but much tyrosin. From this he concluded that the iodine was not present originally in the tyrosin group.

In later investigations (1909)(7) he

formed the opinion that the iodine is contained in the indole radicle of the protein.

Finally, Kendall (1919)(8) as a result of eight years research on the chemical nature of the gland, was able to isolate a crystalline compound of iodine of definite composition, which he named thyroxin. This substance was obtained by alkaline hydrolysis of the fresh glands, filtering the solution and then acidifying. The resulting precipitate is purified by re-precipitation with acid. It is then air-dried, dissolved in alcohol, purified with baryta, and the sodium salt repeatedly re-precipitated to purify it. Finally, the addition of weak acid throws down the thyroxin in a pure condition.

Thyroxin contains 65% of iodine, and this latter represents 25% of the total iodine contained in the gland. This ratio between thyroxin-iodine and the remaining iodine has been found to be constant. Chemically it is a derivative of tryptophane, and is supposed to be trihydro-triiodo-oxybeta-indole propionic acid.

It may exist in three forms with either a keto-, enol, or open-ring formation. The last is the condition in which it is supposed by Kendall to be present in the body.

Kendall considers that the iodine of the foodstuffs is gradually built up into iodothyroglobulin, which product forms the non-toxic method of storage. As the iodine is required it is liberated

into the bloodstream in the form of thyroxin.

Experimental evidence (9) supports the conclusion that thyroxin can replace the whole thyroid in respect of its power to relieve all the symptoms of cretinism and myxoedema. Kendal is of opinion that it acts as a catalyst.

Whether it reproduces all the functions of thyroid secretion still remains to be solved, and no finality will be possible until the actual functions of the thyroid gland have been more fully delineated.

Sammartino (1922)(10) claims to have isolated another constituent of the thyroid, obtained by fractional precipitation by picric acid, of a protein free acetic acid extract of the gland. This differs from other thyroid components by having calcium in its molecule.

THE ACTION OF THYROID UPON NERVE.

Evidence is not wanting to show that the thyroid gland has a controlling influence on the metabolism of the body; but whether it has any specific action upon the activities of nerves, is more difficult of demonstration.

To investigate the latter point, it has been customary to administer either the whole gland, or one of the preparations just enumerated. Alternatively, some workers have attempted to increase the amount of thyroid secretion in the circulating blood by stimulating the afferent nerves of the gland.

Subsequent to such preparatory treatment, the tonicity of certain nerves were investigated, either by noting the alterations if any, in their normal functioning; or by observing any alteration in their degree of response to artificial stimuli.

Amongst the earliest workers in this field was Heinatz (1894)(25) who consistently found in dogs,

as the result of thyroid injections, increased pulse rate and increased bloodpressure. Section of the vagus nerves had no effect upon these results. His findings however have not been confirmed.

Cyon's Work.

Cyon (1898)(11) investigated the action of the thyroid secretion upon the depressor nerve, and on the cardiac fibres of the vagus. His method consisted in removing the thyroid glands from animals and observing what effect this had upon the cardio-vascular system. This was followed by injections of thyroid substance. Frequently he administered the injections to intact animals.

The preparation which he used was iodothyrin freshly prepared and dissolved in normal saline.

He found that in rabbits thyroidectomy gave rise to high bloodpressure, tachycardia and slowed respiratory movements, with a great diminution in the tone of the vagus centre. The evidence which he produces in support of the latter conclusion is however not convincing. After the injection of iodothyrin, the sensitivity of the vagus and depressor were greatly increased. In the normal rabbit injection of iodothyrin increased the sensitiveness of the depressor and vagus fibres to stimulation.

Dogs treated similarly exhibited the same phenomena.

In all the animals the injection of iodo-

thyrin was accompanied by a fall in the bloodpressure, frequently associated with a slowing of the heart. Cyon was able to demonstrate this slowing of the heart in dogs whose vagi had been cut previous to the injection. He suggested from this observation that the drug acted either on the nerve trunk or on the endings of the nerve in the heart. Whilst the former possibility could not be excluded, it was unlikely, and he concluded that the latter suggestion was the correct explanation.

Action of alkaline iodides.

As these effects bore a proportionate relation to the amount of iodine present in the iodothyrin, Cyon suggested to Barbera that he might investigate the responses obtained by the injestion of alkaline iodides under like conditions.

The results obtained were the direct antithesis of those obtained by Cyon with iodothyrin. Barbera (12) found that the injection of 2cc. of 2% sodium iodide into a rabbit gave a large rise in bloodpressure, and acceleration of the pulse, due to stimulation of the sympathetic nerves. On further increasing the dose, the depressor nerves and the cardio-inhibitory fibres in the vagus were rendered ineffectual, and the animal developed "Krampfe". Injections of sodium phosphate neutralised these effects.

Cyon's conclusions.

Cyon concluded that the thyroid had a protective influence in safeguarding the cardiac and vasomotor nerves from the poisonous effects of inorganic iodides.

Observations by other workers.

Boruttau (1899)(13) was able to confirm the sensitising effect of thyroid injections upon the vagi, but Asher's pupil (14) was unable to confirm Cyon's statement regarding the lessened tone of the vagi by sodium iodide injections.

Fürth and Schwartz (1908)(14a) could not agree with Cyon's findings regarding the action of iodothyrin in the vagus endings. In cats during injection they obtained a fall in bloodpressure, and retarded pulse rate, but they concluded that this was due to an action on the vagus centre as it ceased on section of the vagus nerves. Such a deduction is however scarcely warranted on the basis of this experiment. It is possible that the injection acted on the vagus centres and increased their tone, or that it acted upon the nerve endings and made them more responsive to the normal impulses reaching them. Section of the nerves would prevent any impulses from reaching the endings and hence any increased sensitiveness there would be rendered ineffectual because of the inability to utilise it in the conditions of this experiment.

They confirmed Cyon's observations that in

the rabbit, thyroidectomy caused a loss of tone in the depressor nerves, partially recovered from after iodothyrin injections. In dogs and rabbits they failed to find any unmistakable effect upon the circulatory apparatus, as a result of injection of iodothyrin.

Asher (1911)(15) could not get this paresis of the depressor, neither could Gerhardt (1911)(16), and they concluded that it was not a regular phenomenon.

Kocher (1906)(17) states that neither complete nor incomplete functioning of the thyroid gives rise to variations in the rhythm or type of the heart's activity. Asher however states (loc.cit.) that thyroid secretion partially restores the excitability of the vagus, which has previously been depressed on adrenalin injection. He could not accept Cyon's statement regarding increased sensitivity of the depressor after iodothyrin, but found that he got this result after injection of thyroid extract.

Kraus (1906)(18), Friedenthal (19) and Oswald (1915)(20) could not observe any effects such as Cyon obtained during the injection of iodothyrin. The latter however agreed that it increased the sensitivity of the depressor, cardiac vagus and sympathetic nerves to stimulation.

The apparently divergent views of other workers could be partly explained by his conclusion that the thyroid secretion stimulates the ehtire

autonomic nerves. Contrary to the observations of previous workers he found the sensitivity of the vagus increased to atropine. Investigating the extent of these responses in relation to the iodine content of the iodothyroglobulin he found that they ran pari passu. Iodothyroglobulin without iodine was not quite without action, but the addition of iodine to this artificially, did not increase the response. Ionised iodine or iodo- casein did not have the above properties.

Recent observations.

More recent workers are equally divergent in their views. Albertoni (1916)(21) fed his animals on fresh thyroid glands and found that small doses increased, and large doses decreased the sensitiveness of the vagus. The latter effect was likewise obtained on prolonged administration of the drug.

Levy (1916)(22) using thyroxin found that there was no appreciable effect upon the cardio-vascular system as a result of its injection. He found, however that after a latent period of 5 to 20 minutes that it increased the response to adrenalin. Stimulation of the cervical sympathetic nerve, which he assumed contained secretory fibres to the thyroid gland, gave increased response to adrenalin after a latent period of about an hour.

Marine and Lenhart (1920)(23) using the variations in the respiratory exchanges after adrenalin injections as an indirect method of investigation,

failed to find any effect from thyroid injections within the limits of an acute experiment.

Asher (1920)(24) in some later work states that active thyroid extracts have absolutely no direct effect upon the strength or the rate of the heart beat, or upon the bloodpressure. He still maintains however that the thyroid substance increases the excitability of all the autonomic nerves, and concludes that the ultimate effects depend upon whether the sympatetics or parasympathetics are predominant.

Backmann working at Berne (26) found that thyroid extracts had a dilating effect upon the kidney vessels. Richardson and Kakei (27) found no difference between the surviving mammalian hearts of a thyroidectomised and a normal animal; and got no influence upon the former with thyroid substance.

Eiger (28) investigating the reflex function of the isolated spinal cord, found that small doses of thyroid substance increased the responses, whilst large doses had an inhibitory effect.

THIS RESEARCH.

GENERAL.

We have seen from the literature just quoted, that there is no uniformity in the results of the various workers regarding the action of thyroid preparations upon certain of the autonomic nerves. There are many possibilities of error in such investigations, and the conditions under which the experiments are conducted lead to the introduction of many variables. The object of the present research was a re-investigation of some of the experiments to which referemce has been made, with a technique less open to criticism.

The significance of the different factors has received a varying degree of recognition, and evidence is not lacking to show that much of the work has been rendered less valuable through failure to

adopt recognised methods, by means of control experiments, for the evaluation of the effects due to certain of the variables.

Leaving out of consideration at present, the nature of the preparation administered, a large majority of the experiments have consisted in finding the amount of response obtained from the electrical stimulation of a nerve, before and after the administration of the substance. Frequently, the effect of such administration upon the response of the nerves to some other drug, has also been utilised as an indication of the action of the thyroid.

For the former method of enquiry, to be of value, a stimulus of constant strength must produce an unvarying degree of response in a nerve-effector combination when the conditions remain unaltered.

Even if this premise be assumed it will then be necessary to produce evidence to show that there has been no alteration in the conditions of the experiment before and after the administration of the drug, other than those caused directly and specifically by such drug.

The possible causes of variations affecting the condition are many, and in the course of this research, attention was directed more particularly to the following.

The degree of anaesthesia.

The depth of anaesthesia has a bearing upon

the response obtained from the stimulation of a nerve.

This has been long known, but its significance seems to have been overlooked by some of the workers whose papers I have quoted. During the course of a prolonged experiment, when a volatile anaesthetic is used, it is practically impossible to keep the animal in the same condition throughout unless special means are adopted for the administration of the anaesthetic.

The strength of stimulus applied.

In all the experiments recorded, the stimulus applied to the nerve for comparative effects was electrical. This has been obtained from an inductorium, an interrupted induced current being used. It has been assumed by all the workers, that provided mo voluntary alteration was made in the adjustment of the coils, the strength of the stimulus remained constant. That this is not so, I have been able to demonstrate. For the purpose of obtaining the interruptions in the primary circuit, it is usual to employ a flattened piece of metal firmly clamped at one end. This metal is springy in nature and when it is set in vibration the primary circuit is made and broken at the end of each period of its oscillation. In some of the coils the necessary contact is made by a piece of platinum attached to the spring, striking a platinum pin firmly fixed to a support. In others, a

platinum projection on the spring makes contact by dipping into a cup of mercury suitably connected in the circuit. This method is the one adopted generally in the Kroenecker inductorium, in which the surface of the mercury is kept clean by means of alcohol or a running stream of water.

In neither of those methods can one be assured that the same strength of stimulus is always being obtained from the secondary coil. The causes of variation are several:- the presence of subsidiary vibrations in the springs; varying degrees in the duration of "make" and of "break"; and the production of waves on the surface of the mercury (where used) causing inequality in the period and duration of contact.

These factors are known to physicists, but there does not seem to have been a due appreciation of their importance in much of the work to which reference has been made.

The manner in which this difficulty was surmounted, will be discussed later.

The method of applying the stimulus.

In all the papers which I have read, no special emphasis is laid on the method by which the exposed nerves were stimulated, nor is there any indication as to what precautions, if any, were taken to maintain the physical condition of these nerves constant. Evidence will be adduced to show that the

omission to standardise the method of application of the stimuli, may account for many of the phenomena observed.

METHODS EMPLOYED.

General.

So that due allowance might be made for the idiosyncrasies of the animals, it was decided to spread the observations over as large a number as possible. Over ninety animals have been experimented upon, the bulk of these being cats. The remainder was composed of rabbits and dogs. So that seasonal variations, if any, might be noted the experiments were conducted throughout the course of two years.

All the experiments were "acute" in nature. That is, a fresh animal was taken for each experiment, to it was administered the preparation to be examined, and the observations were completed in the course of a few hours, after which the animal was destroyed.

Anaesthetics used.

All the animals were put under general anaesthesia. In order to ascertain whether the anaesthetic had any bearing upon the response, various drugs were tried. In the course of this research there were used ether, chloroform,urethane, and

Anaesthetics used.

paraldehyde. For the dogs, ether with and without morphia was the anaesthetic of choice. It was found that when the volatile anaesthetics (ether etc.) were being used variations in the responses were obtained, corresponding to differences in the degree of anaesthesia. For this reason preference was given to the non-volatile type (e.g.urethane). It was assumed that if this anaesthetic had any effect upon the response of the nerves, its effect would be progressive and unidirectional.

Thyroid preparations used.

For these investigations I used extracts made from the dried thyroid glands of the sheep; and solutions of thyroxin. The glands were obtained fresh from the slaughter house; immediately on receipt I freed them from any adherent fat, or parathyroids, and decapsulated them. They were then cut into thin slices, and immediately placed in a hot air oven to remove rapidly the bulk of the water. The residue was then reduced to a rough powder and heated at 80°C until it ceased to lose weight. After being finely powdered it was placed in a dessicator for 24 hours, and then transferred to a closely stoppered wide-mouthed glass bottle, and collected until a sufficient bulk had been obtained. This was stored in a cool dark place and sufficient extract made up as occasion arose, to last a few days.

Preparations used.

The extracts were made either by triturating the dried powder with warm distilled water and filtering under pressure in a Buchner funnel, or by boiling such a mixture for five minutes, making up to volume, and then filtering. In each case the finished product was of such a strength that 1 cc. of the liquid represented the extractives from 1 gm. of fresh thyroid gland.

The same batch of thyroid powder was used throughout the whole series of experiments. Its iodine content was estimated by Kendall's (1914)(29) method, and the amount of this element present was found to be 0·21%.

The thyroxin used was manufactured by Squibb, New York, and was obtained from them in the crystalline condition. This was dissolved in a weak solution of sodium hydroxide (0·002N) as recommended by the makers. Sufficient of this solution was made for a large number of observations, it was then filled into ampoules, sterilised by heat, and hermetically sealed.

THE STIMULUS EMPLOYED.

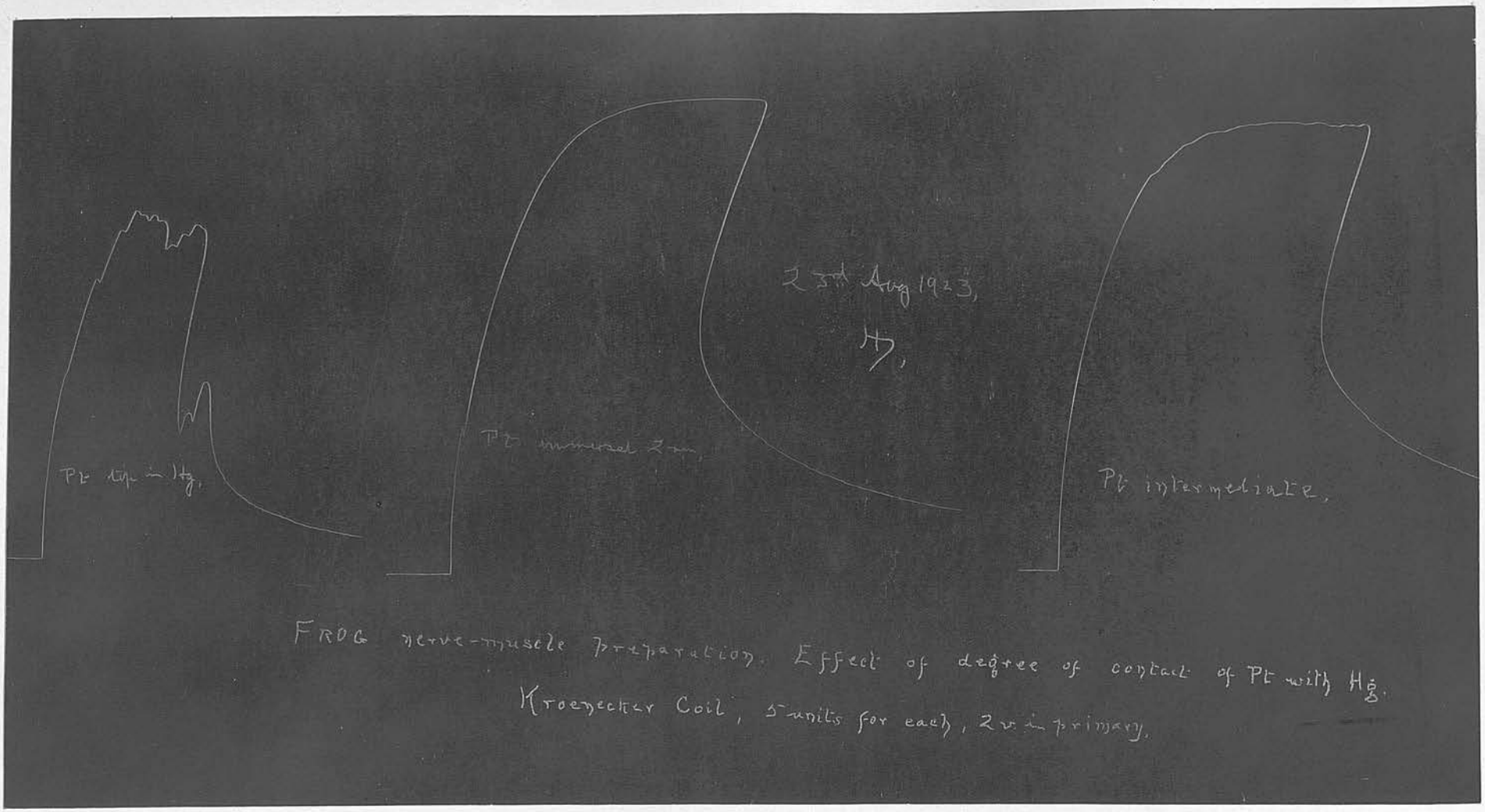
The choice of a suitable source of electrical stimulus caused considerable trouble and delay in the earlier part of this work. It was decided to utilise a Kroenecker inductorium activated by a single cell lead accumulator. The interruptions to the

primary circuit were effected by means of a vibrating steel spring carrying a platinum pin which dipped into a cup of mercury. The spring was fixed at one end to a rigid boss screwed to the base of the inductorium and the mercury cup was held in a mount, the height of which might be altered by means of a rack and pinion.

It was essential for the success of these experiments that there should be no alteration in the interval of time between successive stimuli. For this purpose it is not sufficient that the number of contacts in a given period of time should be constant. The explanation of the fact that the rate of contacts may remain constant while the rate of stimuli varies is as follows.

It will be kept in mind that a secondary current and, therefore, a stimulus occurs each time that the platinum in its downward movement makes contact with the mercury ("make"), and that a secondary current and stimulus also occur each time that the platinum on its upward journey leaves the surface of the mercury ("break").

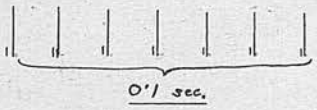
Let it be assumed that the spring is giving 60 double vibrations per second, and further that the mercury cup is placed at such a distance below the platinum point that the latter at the extreme limit of its excursion is just enabled to make contact with the mercury. Under these circumstances the interval between "make" and "break" may be less than the re-



FROG nerve-muscle preparation. Effect of degree of contact of Pt with Hg.
Kroeyecker Coil, 5 units for each, 2v in primary.

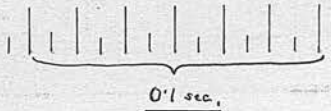
TRACING No. 1.

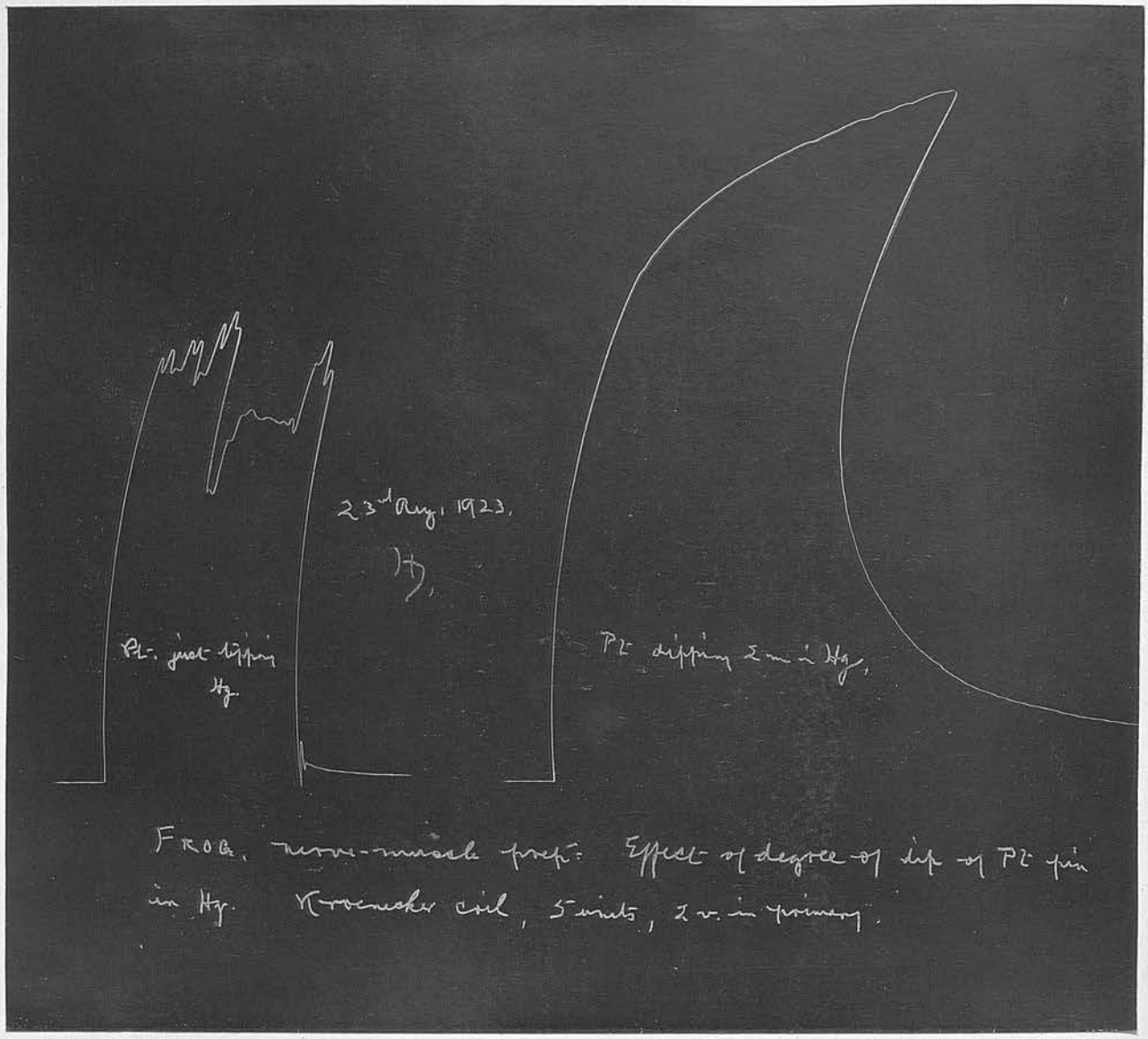
fractory period of the nerve, and so the stimulus at "break" may be rendered ineffectual if the "make" stimulus be of an effective strength. This would result in 60 effectual stimuli being given every second. Graphically these stimuli might be shown thus;-



If now the mercury cup be raised to a small extent, the interval of time between the successive instants at which the platinum on its downward journey reaches the surface of the mercury will be, as before, $\frac{1}{60}$ sec. But the time between "make" and "break" will be increased by the time spent by the platinum point in moving within the mercury from the surface to the limit of its vibration and back again; and the time between "break" and "make" will be decreased by the same amount. The interval between "make" and "break" may now exceed the refractory period of the nerve and there will be an effective stimulus at "make" and also at "break", not, as in the previous case, at "make" only. Whereas under the previous conditions only 60 effectual (and available) stimuli per second were being delivered, there will now be 120.

This might be represented thus:-





FROG, nerve-muscle prep: Effect of degree of dip of Pt pin in Hg. Krocneker coil, 5 units, 2 v. in primary.

TRACING No. 1, (additional).

TRACING No.1 shows the results obtained in a nerve-muscle preparation which was subjected to stimulation under the varying conditions just enumerated. Examination of the graphs will show that an appreciable difference is caused in the responses.

Causes of variations in the stimuli.

During the course of the earlier experiments it was discovered that there were at least two means by which such alterations in the effectiveness of the stimulating current might accidently arise. The mercury cup was held in position by rack and pinion, and it was found that during the course of an experiment, the constant vibration of the spring tended to cause an alteration in the level of the cup. The other factor which modified the time interval between the contacts was the production of waves upon the surface of the mercury.

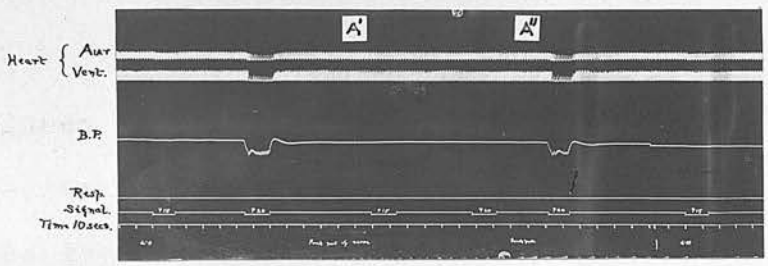
To overcome these difficulties, I replaced this means of interruption by a mercury turbine interrupter. This depended for its action upon a mass of mercury being sprayed in fine jets upon metal segments. The mercury and metal plates were immersed in alcohol to prevent undue oxidation. This proved satisfactory, and the interrupter was introduced into the primary circuit of the Kroenecker coil.

ELECTRODES.

The application of electrical stimuli to an exposed nerve in order to test its excitability, presents difficulties if the experiment is a prolonged one, and if fallacies are to be avoided. This point seems to have been ignored in the papers quoted. In my earlier experiments I used shielded platinum electrodes, composed of two platinum wires lying upon a bed of vulcanite, and having a moveable cover of like material, but it was obvious that the mechanical irritation which they produced, caused eventually a loss of function in the nerve at the site of stimulation.

Plain platinum electrodes emerging from the end of a glass holder were then tried, but difficulties were encountered with these also. Owing to the length of each experiment, the nerve if left on the electrodes did not maintain an even degree of moisture, even when precautions were taken to assure this. If it became drier than normal it was either more irritable or quite irresponsive. In the latter event a fresh portion had to be utilised, and I considered this totally unsatisfactory for comparative results.

If the nerve was moistened with warm Ringer's solution the degree of moisture affected the conductivity between the platinum points, and this was made evident by wide variations in the responses obtained to stimuli of identical strength. To overcome these difficulties I tried the method of removing the nerve



TRACING No. 2.

A' and A" electrodes moved to fresh part of nerve.

Stimuli, in order of application: -

15, 20, 15, 20, 20, 15, Kroen: units.

from the electrodes by means of its attached thread, between each set of stimuli, and laying it on the adjacent tissues which were kept moist with Ringer's solution.

This procedure did not satisfy me because there was no assurance that the nerve was of the same degree of moisture throughout an experiment, or that it was replaced in exactly the same position each time, or that the same degree of contact with the electrodes was obtained on each application.

TRACING No.2 shows the variations in results obtained by placing different portions of the cervical vagus nerve upon the electrodes. It will be noticed that previous to the first adjustment (A'), a stimulus of 20 units has an appreciable effect upon the heart, after the adjustment the same strength of stimulus has no effect. On again shifting the nerve (A''), a stimulus of 20 units gives a response comparable with the first.

Inequalities in the degree of moisture give variations in the excitability of a nerve. This very important point does not seem to have been stressed by the other workers in this field. It is conceivable that in many of the experiments quoted where progressive excitability of a nerve was found, it was due to the progressive drying of this tissue.

This problem presented difficulties in the earlier part of this research, but, I have overcome

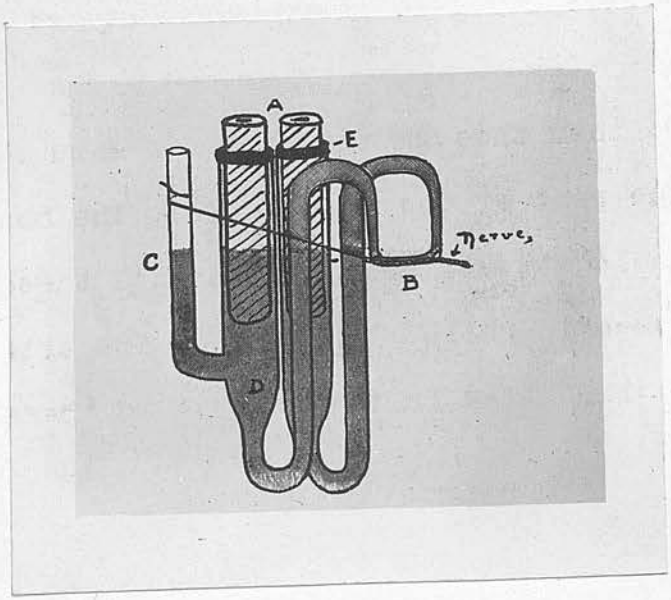


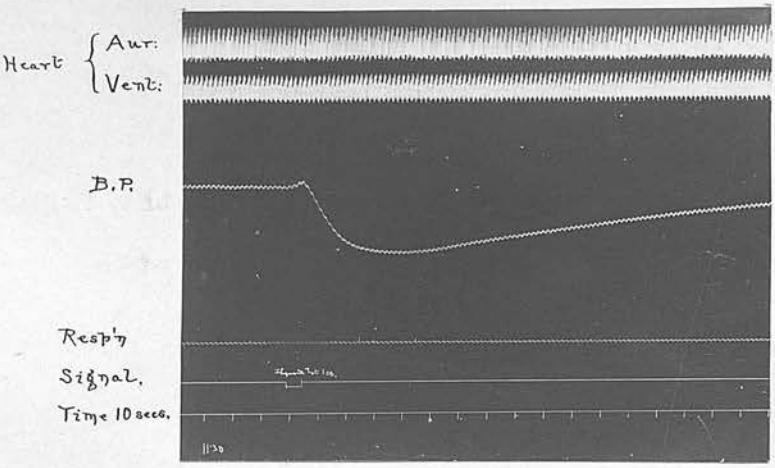
FIGURE 1.

these by designing a new type of electrode of the "fluid" variety. These electrodes are fully described elsewhere (1923)(30), but the illustration opposite shows the principle. The instrument is made of glass, with the exception of the two hollow clay plugs A. These are closed at their lower extremities, and contain a saturated solution of zinc sulphate. Into these dip amalgamated zinc terminals and the current is conducted between them through the Ringer's solution which fills the rest of the apparatus. The horizontal limb B has a hole at each end, and through these holes the divided nerve is pulled by means of its attached thread. This limb lies in the tissues in the space normally occupied by the nerve.

By means of these electrodes the same portion of the nerve is stimulated throughout the course of the experiment, the moisture is maintained constamt and sufficient, and any risk of polarisation is entirely eliminated.

THE PREPARATION OF THE ANIMALS.

In all the experiments the animal was placed under a general anaesthetic. After the insertion of a tracheal tube, the left vagus was exposed in the neck and (in the case of the cats and rabbits) dissected clear from the sympathetic nerve. The vagus was divided, and the central and peripheral ends dissected clear for about 2 cm. The animal was now put on art-



TRACING No. 3.

Showing no effect upon heart.

ificial respiration, and after ligature of the internal mammary arteries the thorax was opened by removing the sternum and the sternal portions of the ribs between the levels of the fourth and seventh ribs. The pericardium was opened and the ventral portion removed. The right auricle and the right ventricle were attached respectively to the recording levers of a Schafer's cardio-myograph. The opening in the thorax was covered lightly with a thick layer of cotton wool.

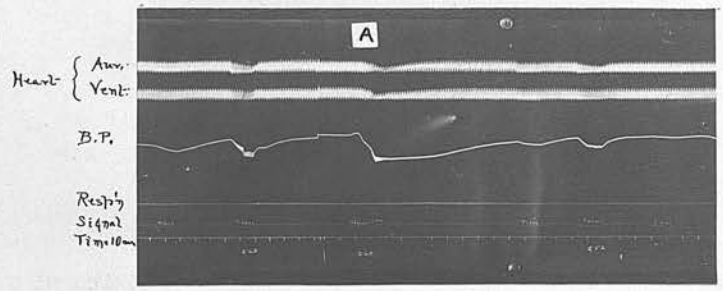
The insertion of a vein-tube into the left external jugular vein, and the linking up of the right carotid artery with a recording mercury manometer completed the preparations.

EFFECT OF INJECTION OF THYROID EXTRACT, UPON THE BLOODPRESSURE.

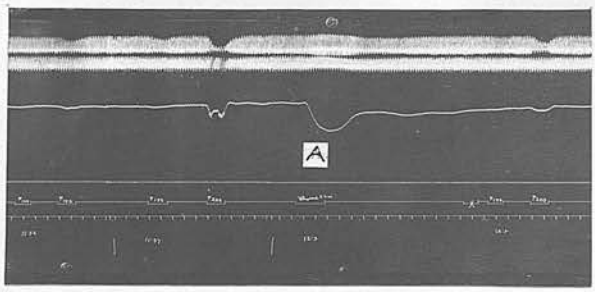
In rabbits.

The effect upon the bloodpressure during and immediately following the injection of thyroid extracts, varies with the species of animal which is being experimented upon. In rabbits there may be a slight rise; and occasionally a fall of about 3 mm. may be noted.

No evidence was obtained from the experiments with these rodents which would indicate that their bloodpressures are modified to any great extent by such injections.



As above.



TRACINGS Nos, 4 and 5.

No. 4, showing diminished rate and amplitude,

No. 5, showing increased rate and amplitude.

"A", injection of thyroid extract.

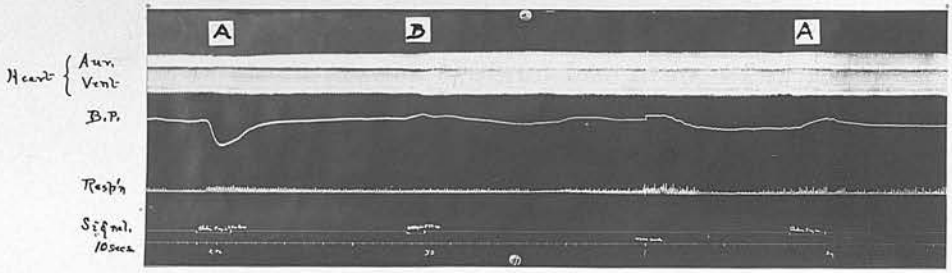
Bloodpressure - in cats.

In cats however the results were different, and, in one particular, were consistent. In every instance the injection of thyroid extract was accompanied by a fall in the bloodpressure, usually preceded by a slight rise. This fall was observed in 40 animals so treated. Although this fall in bloodpressure was always obtained, the effect upon the heart varied. Of the above cats 13 showed a slowing of the heart concurrent with the fall in bloodpressure, 26 showed no change in the rate, whilst 1 indicated an increase in the heart rate. This is dealt with more fully later on.

TRACINGS Nos. 3, 4, 5, illustrate these varying effects upon the heart. The first tracing shows no effect on the heart as a result of the injection; the second shows a decrease in rate and amplitude; whilst the third record indicates an increase in the heart rate.

These phenomena are not uncommom during the injection of tissue extracts. The fall in the bloodpressure has been attributed partly to choline. Lohmann (1907)(31), and Fürth and Schwartz (1908)(32) state that this is the responsible substance for the depressor effect.

On the other hand Popielski (1909)(33) is of opinion that choline is not responsible for the depressor action of organic extracts in general, and Modrakowski (1908)(34) states that pure choline



TRACING No. 6.

A. Injection of 5 mg. choline,

B. Injection of 0·33 mg. atropine.

causes a rise, and not a fall in the bloodpressure.

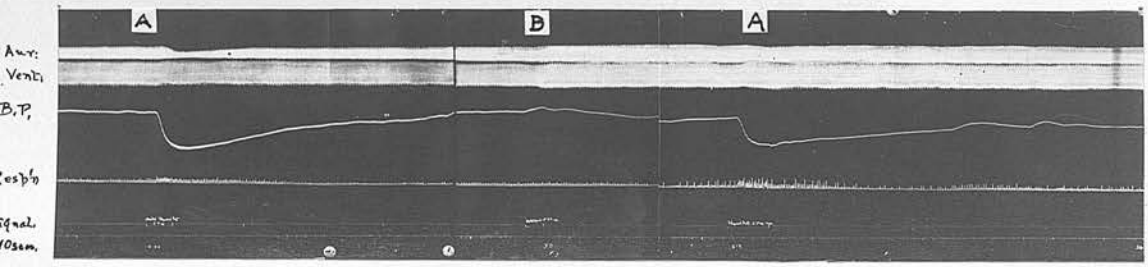
INVESTIGATION OF THE DEPRESSOR PROPERTY.

This point was considered worth further investigation, and I procured a sample of pure choline hydrochloride manufactured by the Hoffmann la Roche Chemical Company. This extremely hygroscopic preparation was accurately weighed under appropriate conditions and dissolved in normal saline solution.

Action of choline.

5 mg. of the drug was injected into the external jugular vein of a cat. This was accompanied at once by an acute and pronounced fall in the bloodpressure, the recovery to normal being almost as rapid. The experiment was repeated on several cats with the same result. That this effect is due to an action upon the vessels may be observed from an examination of the records, whereby it is seen that the rate of the heart beat remains umaltered.

It has been recorded by other workers that this depressor effect of choline is abolished by atropine. In order to confirm such observation I repeated the injection of a similar dose of choline, after having injected 0°33 mg. atropine. The result of this procedure was a rise in the bloodpressure,



TRACING No. 7.

A. Injection 2·5 cc. thyroid extract.

B. Injection 0·33 mg. atropine.

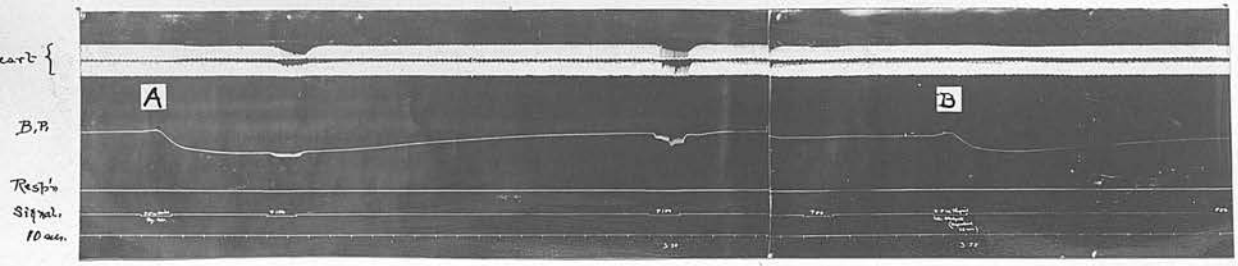
instead of a fall.

TRACING No.6, is one of such records obtained by me. It will be observed that the injection of the choline is accompanied by increased respiratory movements. That this is not due entirely to anaemia of the brain may be inferred from the fact that the same phenomenon occurs even when the choline causes a rise in the bloodpressure.

It was now decided to see if atropine had such an effect upon the depressor properties of thyroid extract. The experiment was conducted under similar conditions to those just detailed for the choline injections.

TRACING No.7, is typical of the results obtained. It will be observed that atropine lessens to a slight degree the depressor effect of the thyroid, the greater part of this property however still being retained. This experiment does not negative the suggestion that choline is one of the depressor principles present, but seems to indicate that it is not the most active of these substances in thyroid extracts.

With a view to investigating the properties of this depressor substance I submitted various batches of the thyroid extract to treatment by hydrolysis, dialysis, and incineration respectively.



TRACING No. 8.

A. Injection 2·5 cc. thyroid extract,

B. Injection 2·5 cc. acid hydrolysed thyroid.

EFFECT OF HYDROLYSIS.

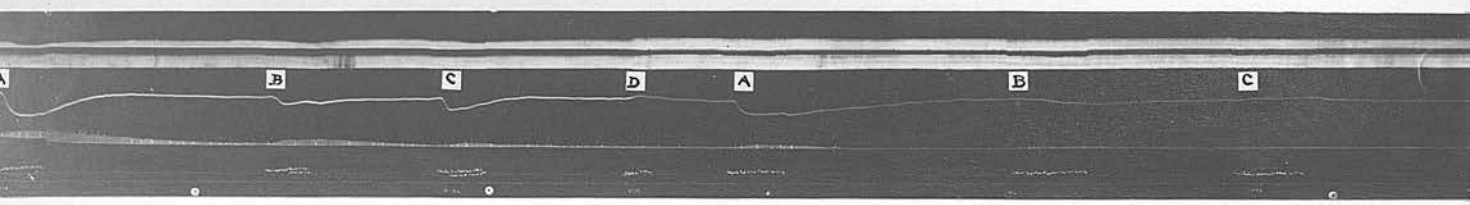
Hydrolysis was effected at 100°C by two different means - by acid, and by alkali. That was effected by boiling a measured volume of the extract with the addition of 5% hydrochloric acid for five hours under a reflux condenser. The alkaline hydrolysis was conducted similarly using 2% sodium hydroxide. These preparations were carefully neutralised by the addition respectively of sodium hydroxide and hydrochloric acid.

It was found that the acid hydrolysis had no appreciable effect upon the depressor response, which still occurred during the injection even after the administration of atropine.

TRACING No. 8, shows the effect of such an injection, compared with untreated thyroid extract.

It is stated that choline is readily destroyed by alkaline hydrolysis, being broken up into trimethylamine and glycol. It was not known what physiological effects these might have, and so, as a control to use with the alkali hydrolysed thyroid extract I submitted some choline to alkaline hydrolysis under conditions similar to those applied to the thyroid. This solution likewise was carefully neutralised with hydrochloric acid.

In these neutralised hydrolysates there was of necessity an appreciable excess of sodium chloride. The amount was calculated, and there were



TRACING No. 9.

A. Injection thyroid extract with NaCl.

B. Injection alkali hydrolysed thyroid,

C. Injection alkali hydrolysed choline,
(All equivalent doses.)

D. Injection 0·33 mg. atropine.

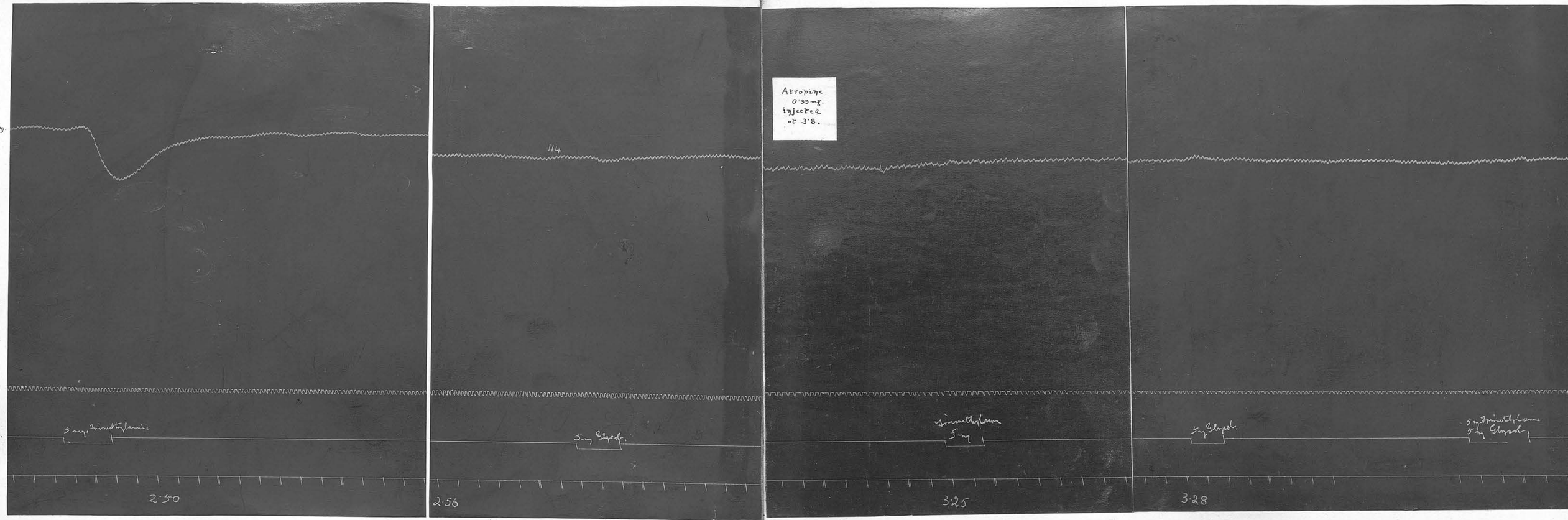
used as additional controls unhydrolysed thyroid extract with the appropriate amount of sodium chloride added, and also a solution of the latter substance in water.

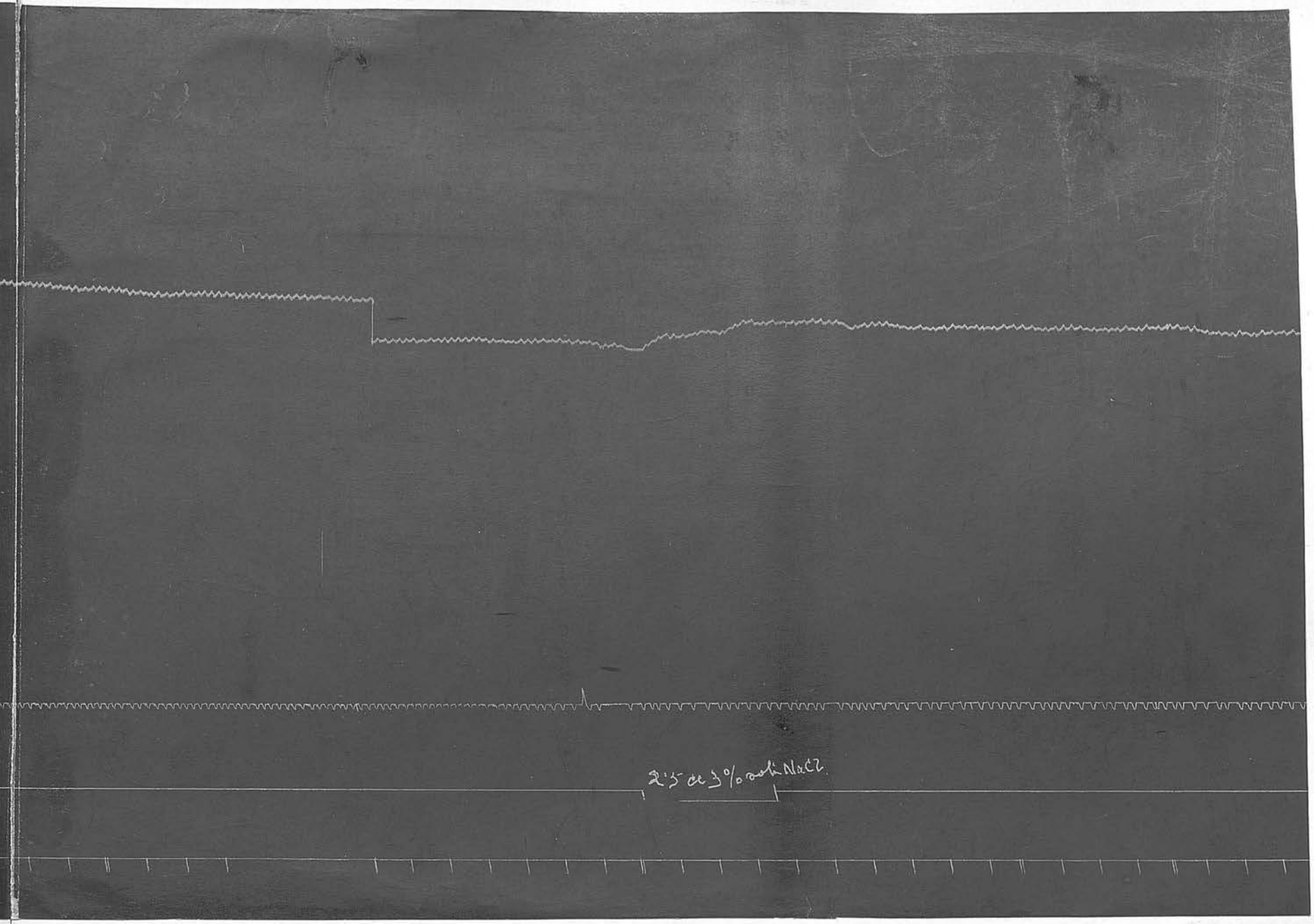
These solutions were then injected into a cat in the following order; unhydrolysed thyroid with sodium chloride, thyroid extract hydrolysed with alkali, choline similarly treated, and the solution of sodium chloride. The results from these having been noted, there was then injected 0·33 mg. of atropine, and this was followed by a series of injections similar to, and in the same order as, those given previously.

TRACING No. 9, is a record of such an experiment. It will be observed that the alkaline hydrolysis has reduced considerably the depressor effect of the thyroid extract. On the other hand, whatever changes have occurred in the choline as a result of such treatment, the solution retains pronounced depressor properties. The administration of atropine is seen to lessen to a slight degree the fall in bloodpressure resulting from the injection of the untreated thyroid extract - this result being similar to those of previous experiments. But with the other injections changes are noticed.

An examination of the tracing will show that after the atropine injection the administration of the alkali - hydrolysed thyroid and the similarly treated choline now give a slight rise followed by

TRACING No 10.




2·5 ce 3% soln NaCl

a scarcely perceptible fall in the bloodpressure.

We have seen from the records of previous experiments, and from the action of the untreated thyroid in this one before and after atropine, that the more active depressor principle present cannot be choline. On the other hand, when such extract is hydrolysed by boiling with alkali, it gives results on injection both before and after atropine, which are comparable to those obtained from choline so treated.

At least two possibilities might be offered in explanation of these phenomena; (a.) choline is not destroyed, as stated, by alkaline hydrolysis, but the other depressor substances present in thyroid are,(b.) some of the products of hydrolysis of the depressor substances present in thyroid have properties similar to those of hydrolysed choline, amd some are inert.

Products of hydrolysis.

In order to investigate this, the solution of the hydrolysed choline was examined for the presence of choline by Rosenheim's test (35), and no evidence of it could be obtained. It was thus apparent that the depressor effect of the hydrolysed choline was due to the trimethylamine or the glycol, or both.

Into a cat weighing 2770 grm. there was injected 5 mg. of trimethylamine dissolved in 2°5 cc. Ringer's solution. This was followed immediately by a fall in the bloodpressure of 34 mm., with a quick recovery. The heart rate was not affected. A

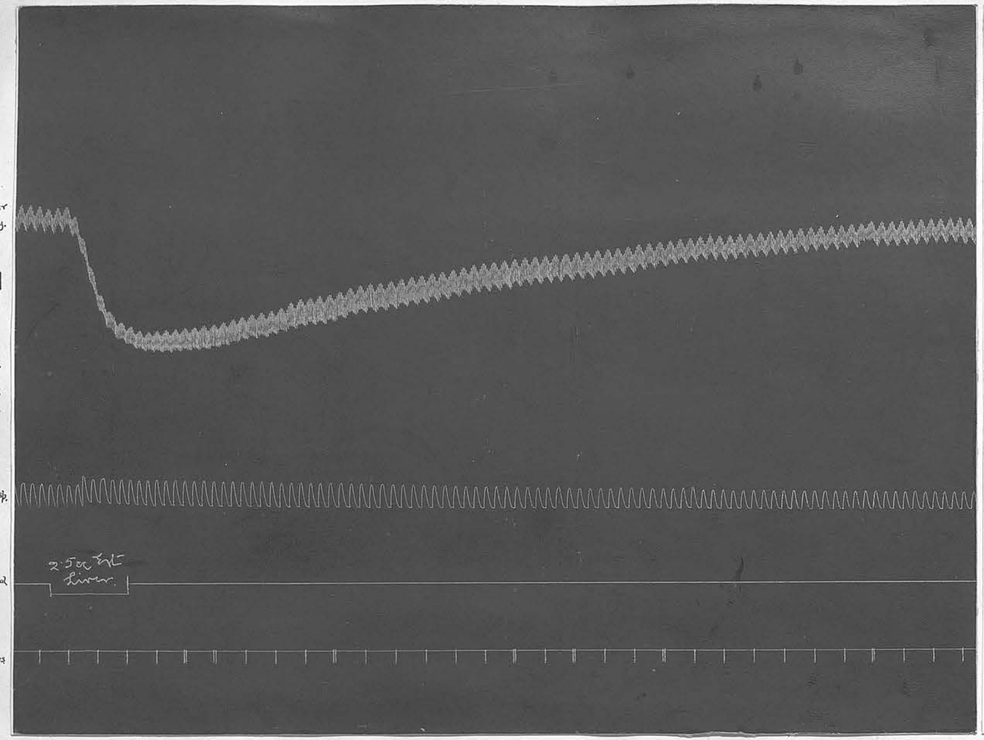
Car Ay.

TRACING No. 11.

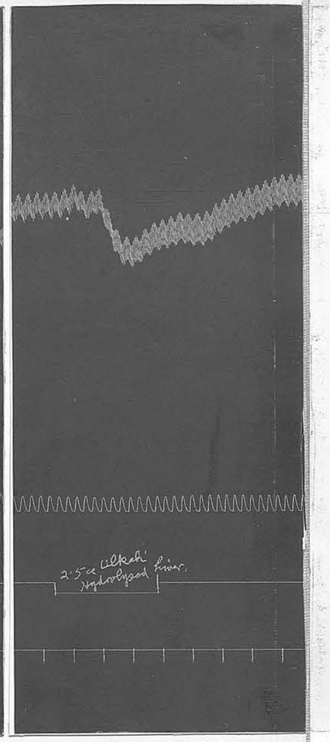
Resp.

Signal

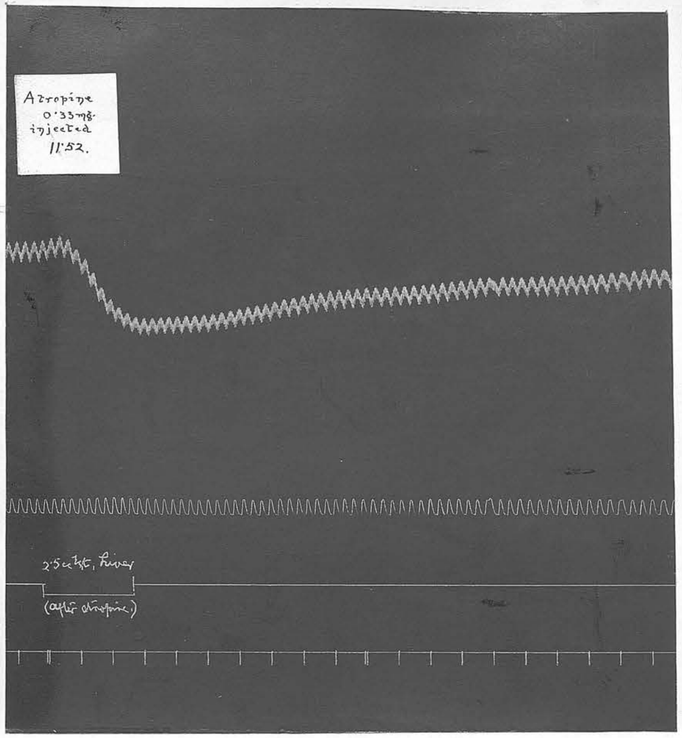
10 Secs



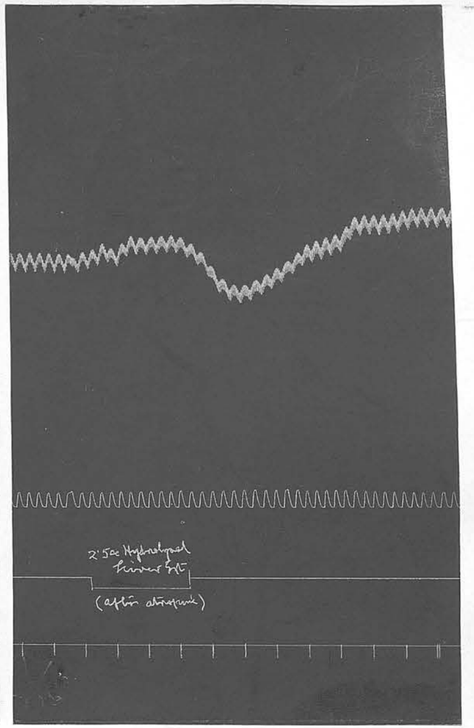
11·31



11·40



12·9



12·15

similar dose of glycol had no effect upon the blood-pressure.

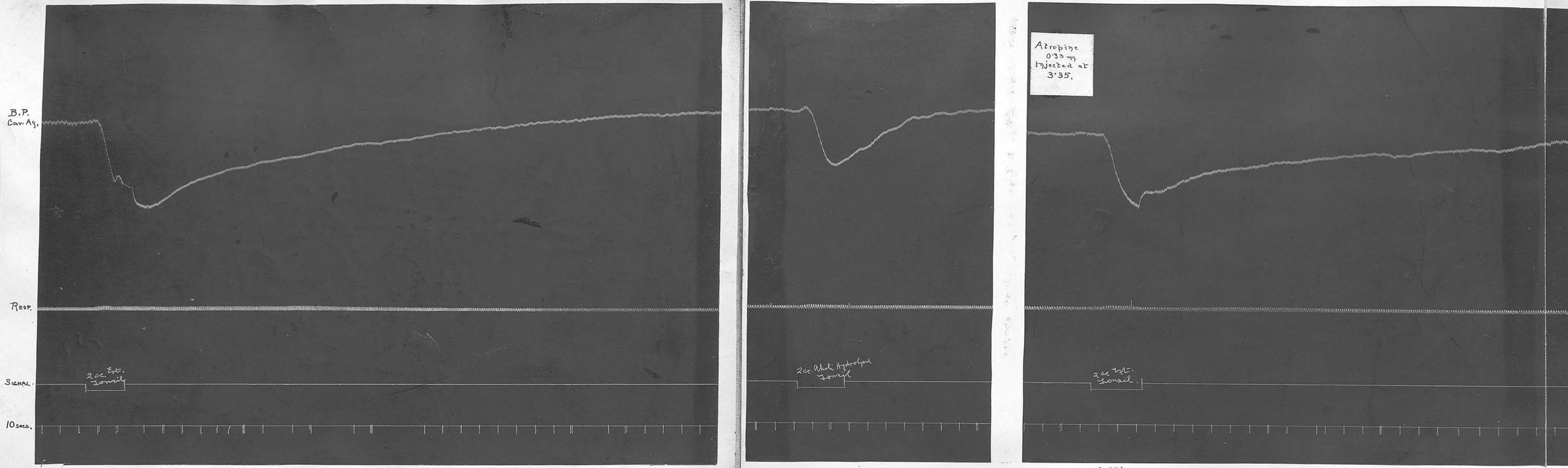
Atropine was next administered, and the previous injections repeated. They were without effect upon the bloodpressure. Both trimethylamine and glycol were then injected simultaneously with a like result. Finally, the injection of a solution of sodium chloride of a strength similar to that present in the hydrolysed thyroid (or choline) was followed by a slight rise in the bloodpressure similar to those obtained previously from the hydrolysed preparations.

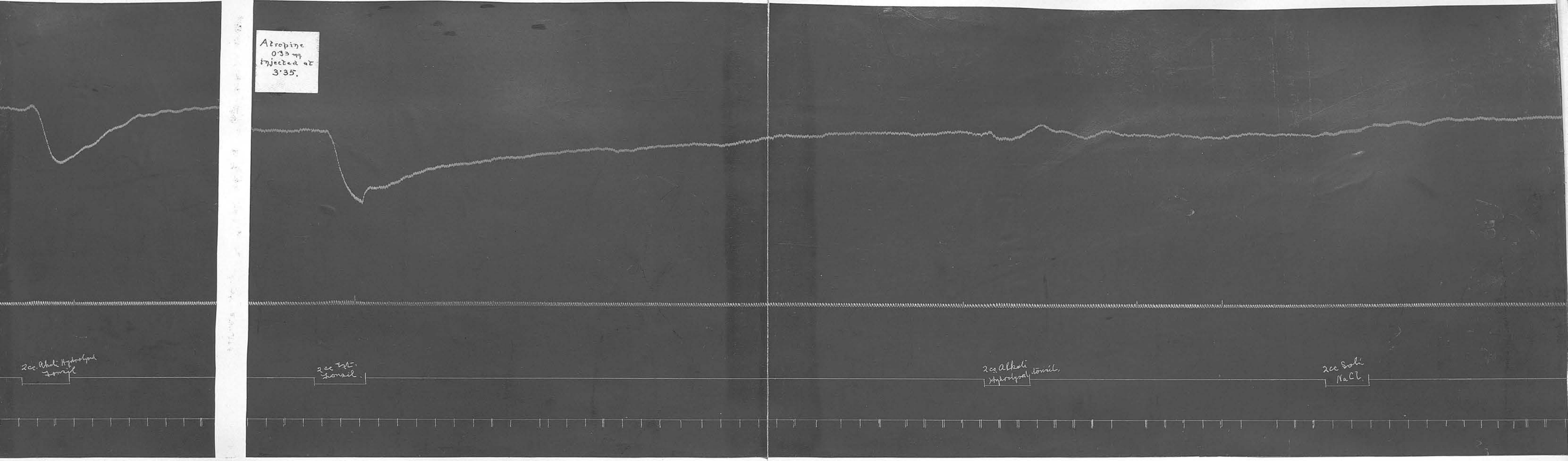
TRACING No.10, shows these results. From them it may be inferred that alkaline hydrolysis has the effect of reducing the depressor bodies in thyroid to a substance or substances having physiological properties similar to those of trimethylamine.

OTHER TISSUE EXTRACTS.

Control experiments were undertaken with extracts of liver and of tonsil, both untreated and hydrolysed. In these also, the effect of hydrolysis was to diminish considerably their depressor properties. When however atropine was administered, and the injections were then repeated, a difference was observed in the response obtained from hydrolysed liver as compared with that from hydrolysed tonsil. The latter had its depressor properties almost abolished, and in

TRACING No. 12.




Atropine
0·33 mg
injected at
3·35.
2cc. Alkali Hydrolysed Tonsil
2cc Alkali Tonsil.
2cc Alkali Hydrolysed tonsil.
2cc Soln NaCl.
3·30
3·38½
3·44

this respect resembled thyroid extract. The hydrolysed extract of liver however, gave as great a fall in the bloodpressure, when it was injected after the administration of atropine, as that which occurred before the atropine.

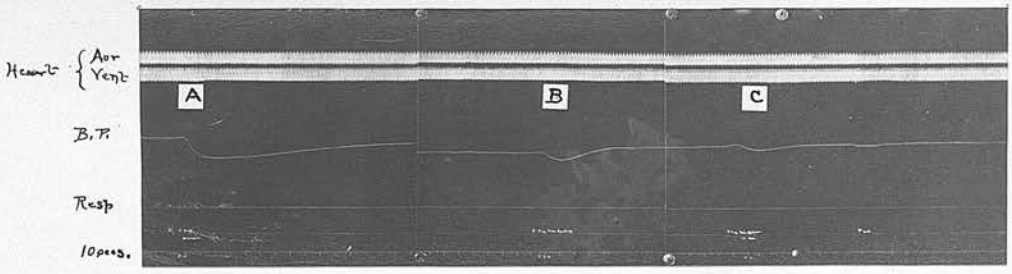
Summarised, it may be stated that atropine has the effect of abolishing the fall in bloodpressure which occurs on the injection of hydrolysed extracts of thyroid and of tonsil, but that it fails to prevent the fall in bloodpressure resulting from the injection of liver extract so treated.

TRACING No. 11, shows these results as obtained with liver extracts, and TRACING No.12, is the record of a similar experiment, using extract of tonsil.

DIALYSED EXTRACTS.

A portion of thyroid extract was dialysed through a collodion thimble, which I made specially resistant to the passage of protein, verifying this resistance by appropriate tests. The dialysis was carried out against distilled water, and it was observed that during the process a sediment appeared in the solution contained within the tube. The distilled water was changed several times until examination gave no indication of any further dialysis proceeding. These batches were mixed together and evaporated under reduced pressure until of correct volume.

Three solutions were obtained from such dialysis; the liquid surrounding the tube (the dialysate), the liquid within the tube cleared by centrifuging (the dialysed extract); and the sediment within the tube dissolved in Ringer's solution.



TRACING No. 13.

A. Injection 2·5 cc. "dialysate",

B. Do. 2·5 cc. "dialysed extract",

C. Do. 2·5 cc. solution of residue.

The strengths of these three solutions were so adjusted that equal volumes of them represented the equivalent in volume of the original extract. These solutions were examined qualitatively for the presence of iodine and this was found to be present in the dialysate and in the solution of the sediment, but it was absent from the dialysed extract.

Effects during injection.

They were injected into a cat in the following order, in each case equivalent doses being given; dialysate (A), dialysed extract (B), and solution of sediment (C). The effects upon the bloodpressure were as follows:-

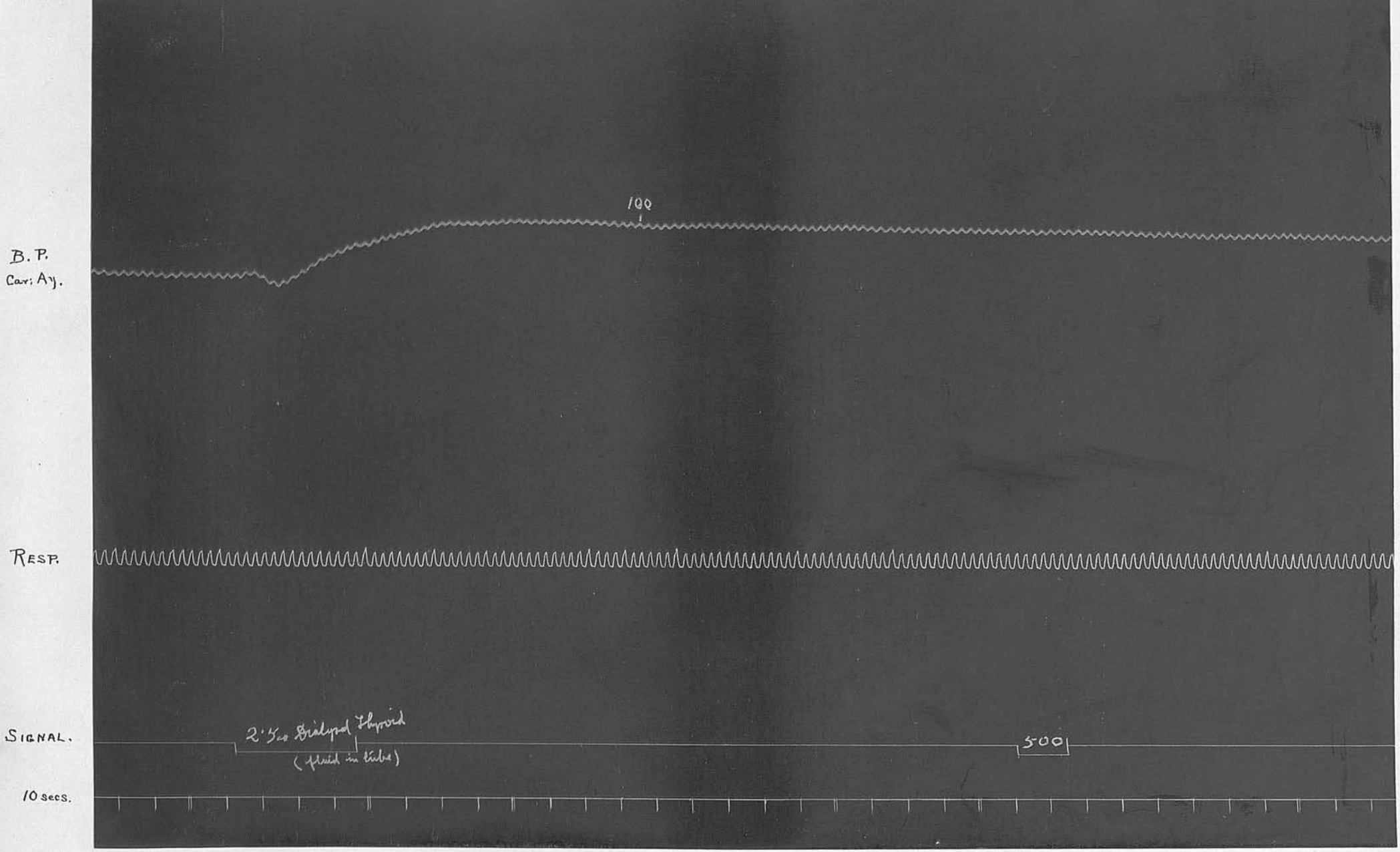
A. Fall in B.P. from 60 mm.to 25 mm. recovering to 52 mm.in 180 secs.

B. Fall in B.P. from 36 mm.to 22 mm. recovering to 44 mm.in 55 secs.

C. Rise in B.P. of 4 mm.,then a fall of 4 mm. below normal,recovering in 45 secs.

This experiment was repeated upon another cat, but with a variation in the order of the injections. In this instance the order was B - A - C. The results were qualitatively the same, and confirmed the previous experiment. From these it may be seen that the dialysate has the greatest depressor effect, the solution of the sediment the least, whilst the dialysed extract occupies an intermediate position.

TRACING No. 13, shows a record of such an experiment. It was frequently observed, and this



TRACING No. 14.

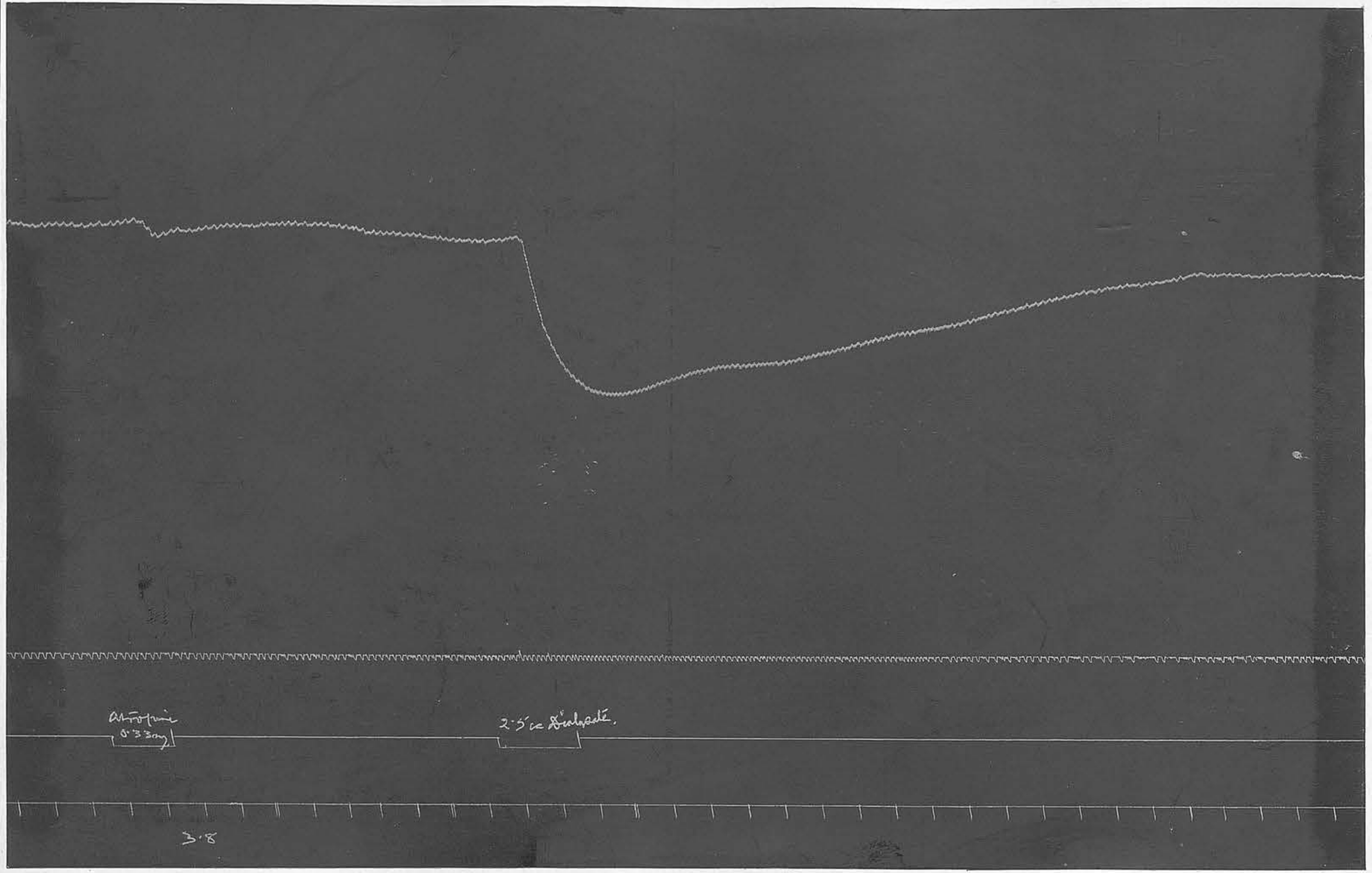
Showing slight pressor effect of "dialysed extract."

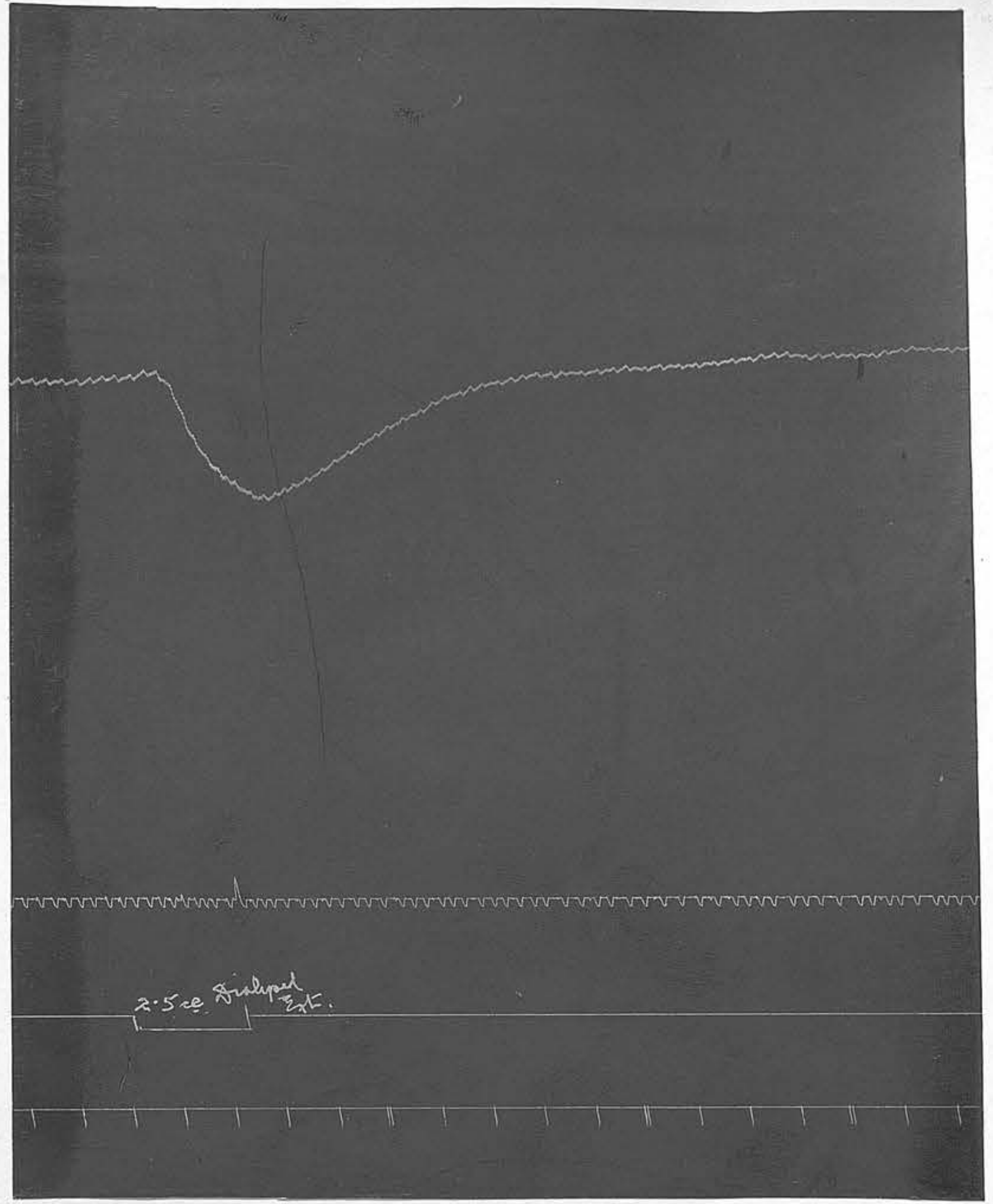
tracing shows it, that after the fall caused by the dialysed extract (B), the bloodpressure returned to a higher level than that which it had before the injection. In one experiment the dialysed thyroid extract gave a rise with scarcely any preliminary fall. TRACING No.14 is from an experiment in which such an effect was obtained.

It is possible that this pressor effect may be due to the presence of putrefactive products. The collodion tubes are somewhat difficult to render sterile, and the prolonged dialysis (in some cases two days) are favourable for bacterial growth. It was suggested to me by Professor Sir E. Sharpey Schafer, that I might try adding a little chloroform to the freshly boiled extract in order to inhibit putrefaction during the dialysis. This precaution was adopted with a fresh batch of thyroid, and the injections were repeated upon fresh animals.

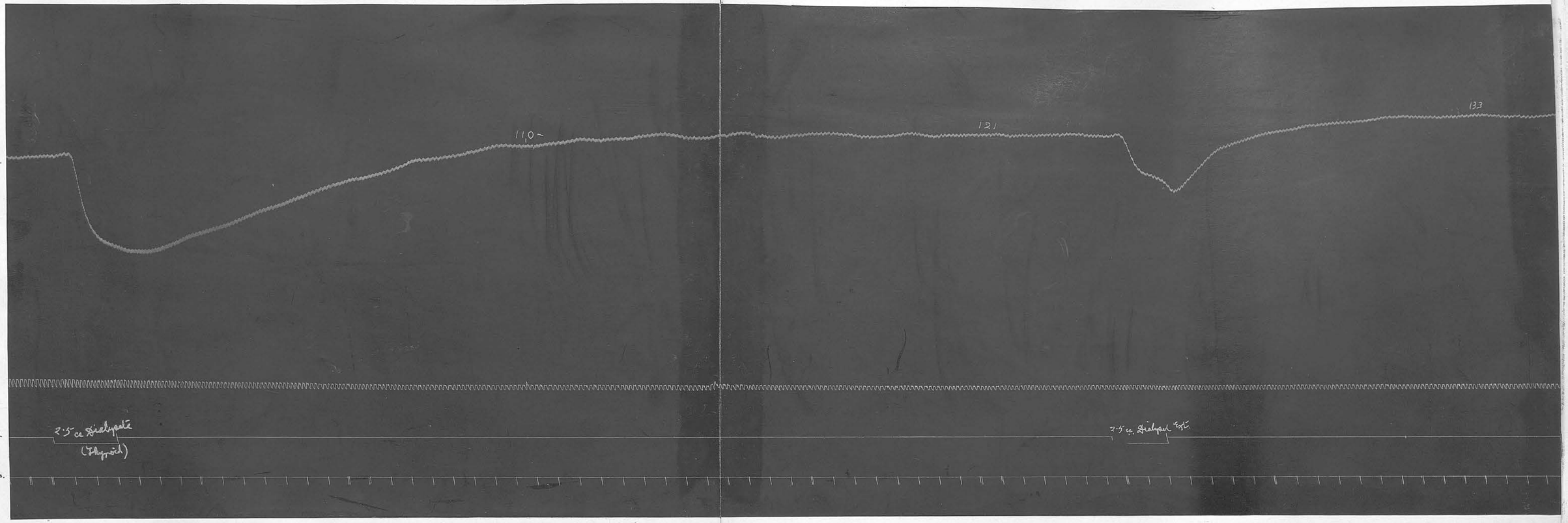
In two cats so treated, both gave a fall in the bloodpressure after the dialysed extract, in one case returning to the old level, and in the other again attaining a level above that preceding the injection. In the latter experiment the increased height was certainly not very much, reaching 133 mm. in place of 121 mm; but it is of note to observe that this gain in the level was again obtained when the injection was made after the administration of atropine.

It was inferred from these observations


Atropine
0.33 mg
2.5 cc Dialysate.
3.8


2.5 cc Dialysed Ext.

TRACING NO. 15.



that the more potent of the depressor substances are dialysable, and it remained to be seen if any of these solutions depended upon choline for its depressor properties.

Effect of atropine on dialysed solutions.

This was investigated by repeating these injections upon another animal, administering atropine, and again giving the injections in the same order.

TRACING No. 15, shows the results obtained from such an experiment. It will be observed that the atropine has little effect upon the depressor properties of the dialysate, the fall in the blood-pressure caused by this before and after the administration of the atropine being 62 mm. and 60 mm. respectively. The dialysed extract however has its potency more affected, giving a fall of 24 mm. after the atropine, in place of a previous 38 mm.

In view of these findings it became of interest to see if choline itself was dialysable. Accordingly a collodion tube was set up, and a solution of choline hydrochloride was dialysed against distilled water. Its presence was investigated by means of Rosenheim's test (u.a.) and it was found that the drug passed freely through the membrane.

EFFECT OF INCINERATION.

A portion of thyroid extract was evaporated to dryness and ignited. The residue was extracted with distilled water, and this solution after filtration was injected into a cat. A very slight rise in the bloodpressure was observable, due no doubt to the saline contents of the injection.

EFFECT OF THE INJECTION OF THYROXIN UPON THE BLOODPRESSURE.

In the course of these enquiries, 21 animals received injections of thyroxin. Of these, 18 were cats and the remainder were rabbits.

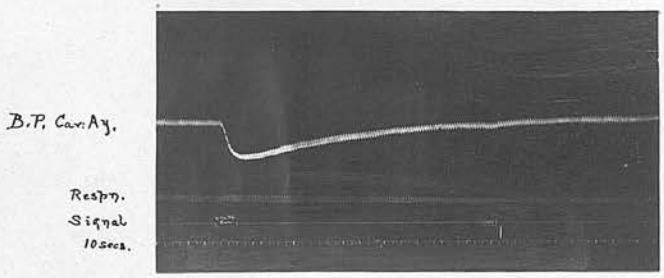
It was observed that this drug differed materially from thyroid extracts as regards its result upon the bloodpressure. In general, no effect was seen, but in several instances slight alterations in the level occurred. I submit herewith the results in tabular form, the only outstanding instance being where a rise of 20 mm. was recorded in one of the cats. This rise was maintained throughout the greater part of the experiment.

It will be observed that one of the rabbits showed a slight rise after the injection of the thy-

Effect of thyroxin upon bloodpressure.

roxin solution. When however a control injection was made with a solution of NaOH similar in strength and amount to that present in the thyroxin solution, a similar rise was obtained.

	RISE.		NO CHANGE.	FALL.	
	4 mm.	20 mm.		4 mm.	
CATS.	3	1	11	3	
RABBITS.	1		2		
Totals,	4	1	13	3	21.



TRACING No. 16.

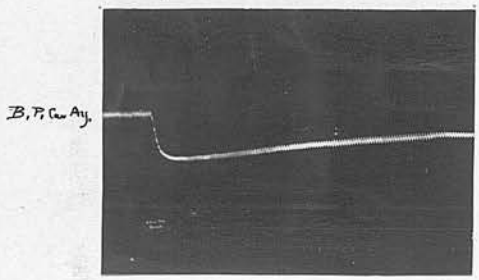
A. Injection of liver extract.

THE EFFECT OF THYROID EXTRACTS UPON THE SENSITIVENESS OF THE CARDIAC FIBRES OF THE VAGUS.

Reference has already been made to the effect upon the heart which occurs during the injection of thyroid extracts. It is intended here to discuss this more fully, and also to analyse the results obtained from stimulation of the cardiac fibres of the vagus after such injections.

DURING THE INJECTION.

An examination of the records which have been reproduced in order to show the effect which injections of thyroid extract have upon the blood-



TRACING No. 17.

A. Injection of extract of tonsil.

During injection.

pressure, shows that occasionally there was some modification in the heart's action. Sometimes there was a diminution of the amplitude only, but more often when this occurred it was accompanied by a retardation in the rate. This has been noticed by previous workers, and has been interpretated by them as indicating an increase in the sensitiveness of the cardio-inhibitory fibres of the vagus specificially caused by the thyroid substance. In my experiments this slowing of the heart beat was not a consistent feature of such injections. In 35 cats so treated, the slowing occurred in only 13. It did not occur in any of four rabbits similarly injected. These results do not support the conclusion that thyroid injections have the effect noted above, and further experiments have shown that this slowing of the heart is not a property specifically belonging to the thyroid.

EFFECT OF OTHER TISSUE EXTRACTS.

This point was demonstrated by making extracts of liver, and of tonsil, and injecting them under conditions similar to those used with the thyroid experiments.

TRACINGS Nos. 16 and 17 are typical of the results obtained. An examination of these will show that in both, the heart was slowed during the injection

of the extracts.

As already noted certain workers contend that thyroid preparations have a special affinity for the cardiac fibres of the vagus, but my experiments do not bear this out.

Oswald (1915)(36) however explains these variations in response, by the hypothesis that thyroid stimulates not the para-sympathetics alone, but the entire autonomics. The result of an injection will therefore, according to him depend upon the relative preponderance of the two main divisions of the autonomic nerves. Whilst this view is attractive, it is difficult of proof when applied to the regulating nerves of the heart. So far I have been unable to find any substance which will paralyse the sympathetic nerves to the heart, without affecting also the cardio-inhibitory fibres.

EFFECTS OF STIMULATION OF THE CARDIAC FIBRES OF THE VAGUS.

General.

In order to test the effects which the injection of thyroid preparations might have upon the subsequent response to stimulation of the cardiac fibres of the vagus, it was necessary to fix upon a standard.

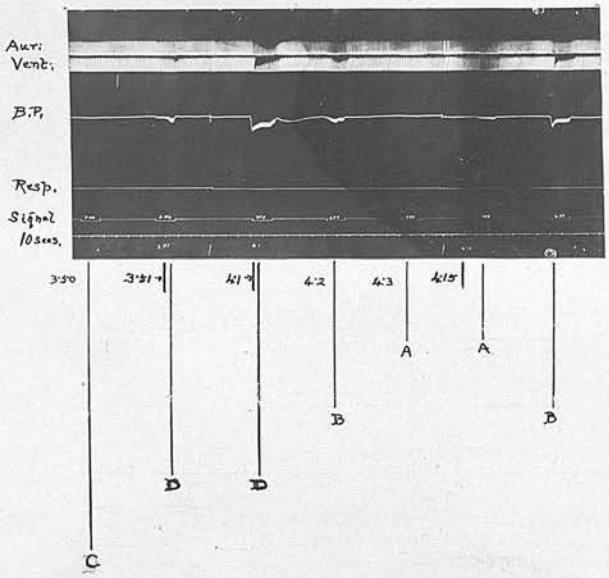
Effects of stimulation of the vagus.

As the result of trial experiments two standards were adopted. One method was to find the minimal stimulus which, when applied to the peripheral portion of the divided cervical vagus, was just sufficient to cause a slight inhibition of the heart, with a consequent fall in the bloodpressure. The injection was then administered, and a stimulus of exactly the same strength applied to the vagus and the effect upon the heart noted. The alternative method was to find the minimal stimuli necessary to give equivalent inhibitions of the heart, before and after administration of the thyroid.

Determination of a standard stimulus.

The fixing of a standard caused no little trouble, and I am convinced that many of the results of earlier workers are rendered less valuable as a result of want of precaution in this respect. The routine followed was to pass a stimulus for 15 seconds and note the result ; the strength was adjusted if necessary and after a lapse of 40 seconds another stimulus was applied. This was repeated until a suitable strength had been found.

After this had been obtained, a space of time was allowed to lapse which would be equivalent to that occupied in the administration of an injection. The stimulus was then repeated, and very frequently it was found that the response of the heart had altered



TRACING No. 18.

Showing variations in response at different periods of time.

A. 300 Kroenecker units,
B. 400 do. do.
C. 500 do. do.
D. 600 do. do.

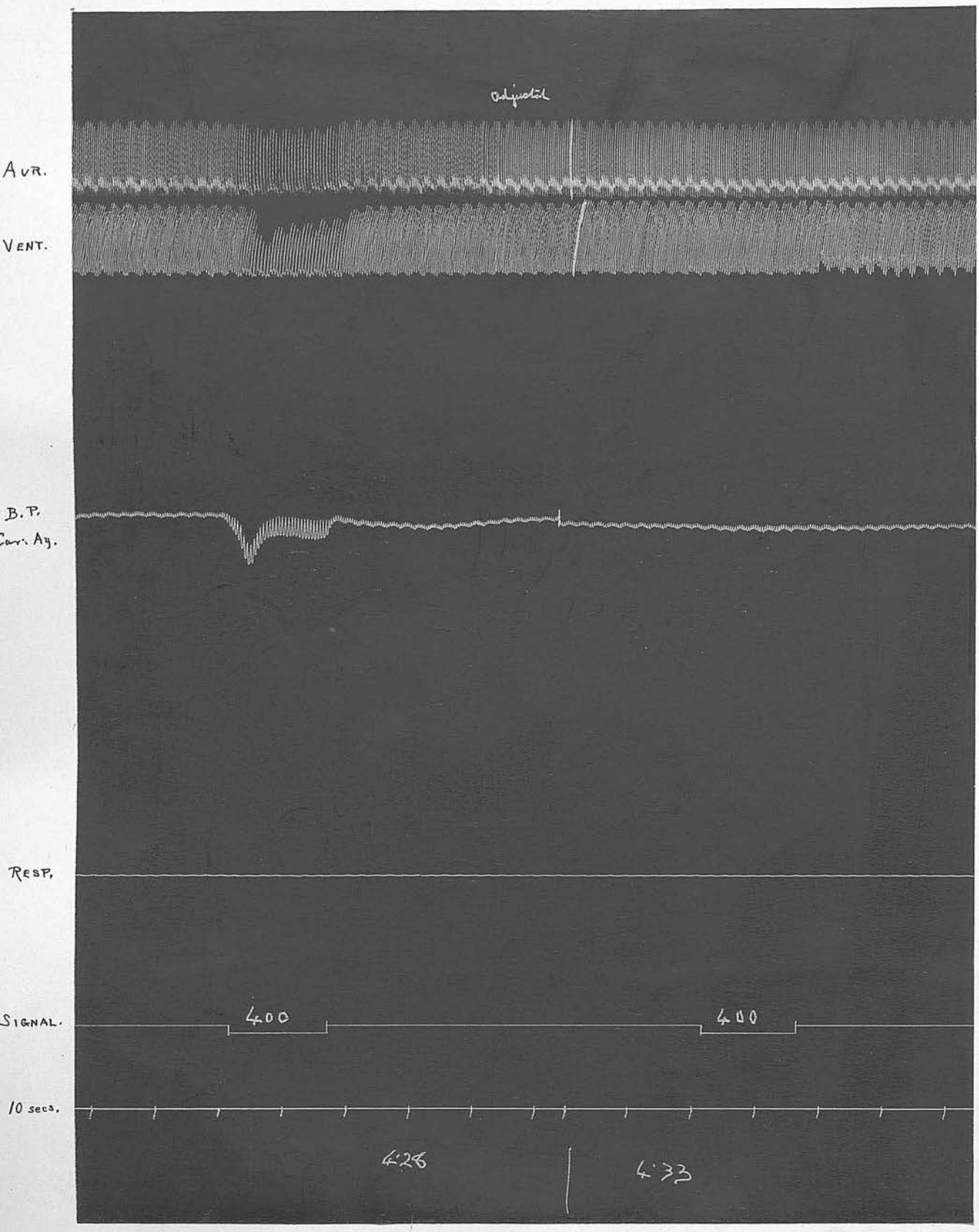
considerably. The strength was again adjusted, and the procedure repeated until there was a reasonable consistency in the results.

Variations in the response.

Examination of the records of some of the work quoted shows an entire absence of this precaution. The response of the heart to stimuli of similar strength applied to the cervical vagus in the cat is not always identical. In illustration of this point I submit herewith TRACING No. 18. Examination shows that at 3·50 p.m., the passage of 500 Kroenecker units failed entirely to affect the heart, whilst at 4·2. 400 Kroenecker units both slowed the heart and decreased its amplitude. Likewise 600 units at 3·51 had only a slight effect upon the heart and bloodpressure, whilst at 4·1 a stimulus of similar strength had a pronounced effect upon both. A similar increased response may be observed from the 300 units applied at 4·15 as compared with that at 4·3.

The effects were not always those of an increased response. TRACING No. 19, taken from the same experiment shows that at 4·28 p.m., 400 units gives an appreciable result, whilst the same strength of stimulus applied five minutes later has no effect whatever upon the heart.

All the animals did not show these phenomena - some exhibited them more so than others.



TRACING No. 19.

Showing effect of "time" upon the response.

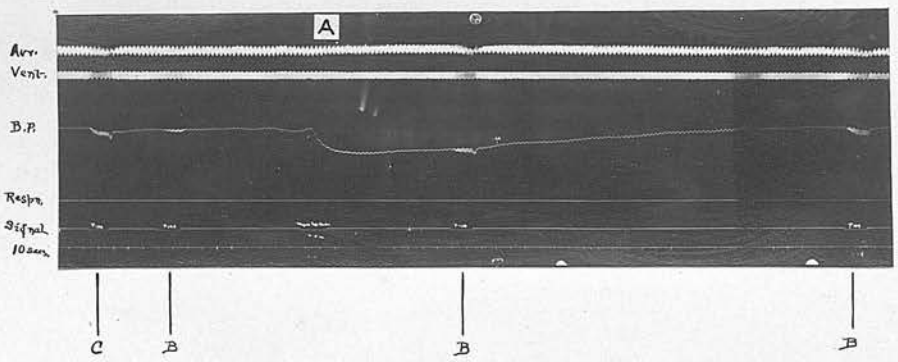
Variations in the response.

If any animal was found particularly irregular, the experiment was either not proceeded with, or if completed, the results have not been included in the summing up.

I have already indicated many of the factors which no doubt contributed to these irregularities in the earlier experiments, but although they have been individually and severally remedied this "vagus phenomenon" still appears, and requires further investigation.

Method of observation.

During the course of the experiments to be described, the procedure was as follows. After the "standard" stimulus had been found, and verified as detailed above, the drug was injected via the external jugular vein, and the stimulus was then repeated. As it was possible that the effect of the thyroid might not be manifest for some appreciable time, the stimuli were repeated at intervals of 5 - 10 minutes for the remainder of the experiment. This usually lasted for 1½ hours after the injection had been given. Occasionally a second, and sometimes a third injection was administered. Experience showed this prolonged recording to be necessary. Any transitory variations in the response, unless they were always in the same direction, were not considered of value.



TRACING No. 20.

A. Injection 2·5 cc. thyroid extract.

B. 100 Kroenecker units,

C. 150 do. do.

RESULTS OBTAINED WITH THE CARDIAC VAGUS.

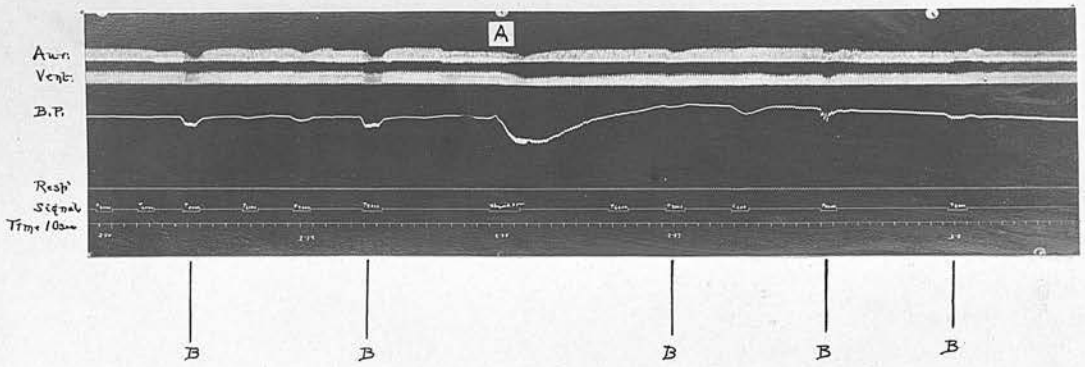
Increased response.

The results derived from the stimulation of the cervical vagus after injection of thyroid extracts were widely divergent.

Some animals showed an increased response after such treatment, indicating inferentially a greater sensitiveness of the vagus as the result of the thyroid administered. This increase in the response was often manifest immediately after the injection was completed, more frequently its onset was delayed. In the latter event it did not usually appear until about twenty minutes later. TRACING No. 20, is typical of such an effect following the administration of thyroid extract.

Decreased response.

These results however were never consistent, and the opposite effect happened just as frequently, namely an apparent decrease in the sensitiveness of the cardiac vagus. The record of such an experiment is shown in TRACING No. 21, wherein it may be seen that a stimulus of 8000 units applied to the vagus nerve after the injection gave only a slight inhibition of the heart, whereas before that was administered, an appreciable effect was noticed by a stimulus of the



TRACING No. 21.

A. Injection 3·5 cc. thyroid extract.

B. 8000 Kroenecker units.

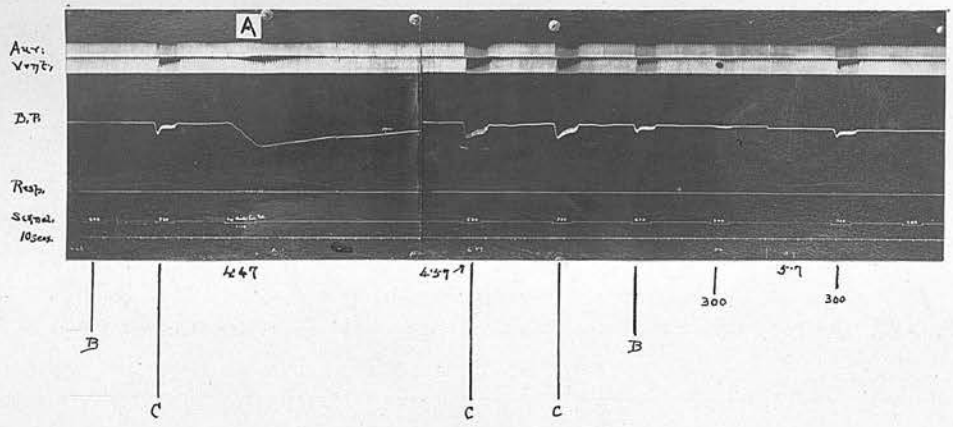
same strength.

No alteration.

In a large percentage of the animals however, no variation in the sensitiveness of the vagus could be demonstrated with certainty. There are many possible explanations which might be produced to explain these varying results. During this research, as each hypothesis was formulated, an effort was made to test its value experimentally. The results of such investigations will now be considered in detail.

CONTROL EXPERIMENTS.

The object of these investigations was principally to see if support could be given to Asher's findings. We have seen that he holds the view that thyroid extract has a specific sensitising effect upon the cardiac fibres of the vagus. So far I had been able to observe in some animals an increased response to stimulation of the vagus, following such injections. This was discounted however by finding that in a larger number of experiments no such increased response could be obtained. Further, in those instances in which greater sensitiveness was shown, there was no evidence that such variation was due specifically to the thyroid preparation.



TRACING No. 22.

A. Injection liver extract.

B. 400 Kroenecker units,

C. 500 do. do.

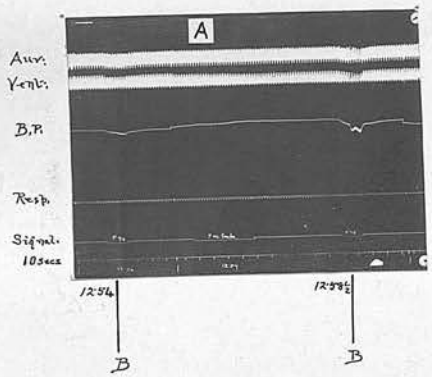
Control experiments.

A large number of control experiments was therefore undertaken in order to test this point.

Effect of injection of tissue extracts, upon the response to stimulation of the vagus.

Extracts were made by boiling minced tissues in Ringer's solution, and using the filtrate from such decoctions. The organs were obtained from freshly killed animals, usually cats. Extracts were made of liver, and of tonsil. These were administered under conditions similar to those used for the thyroid, and the stimuli applied to the vagus as in those experiments.

It was found that with these extracts effects could be obtained on the vagus similar to those which Asher states follow thyroid administration. I submit a photograph of TRACING No. 22, in which an apparent sensitising of the vagus occurs after the injection of liver extract. Here also it may be seen that the maximum effect is obtained, not immediately after the injection, but twenty minutes later, Examination shows that before the injection, 400 Kroenecker units had no effect on the heart, twelve minutes after administration 400 units had a decided effect, and nine minutes after this a response even greater, was obtained by the application of only 300 units.



TRACING No. 23.

A. Injection 5 cc. Locke's solution.

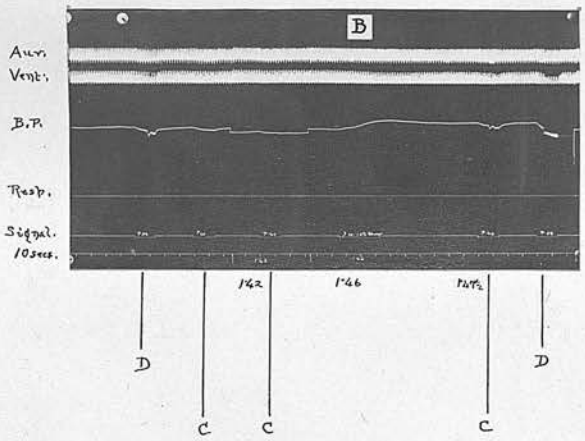
B. 70 Kroenecker units.

Effect of Locke's solution, etc.

These controls were extended to the investigation of the condition of the vagus consequent on the injection of Locke's solution, and of glucose. All the animals used were cats, four were examined for the effects of Locke's solution, and nine for the action of glucose. This was made into a 16% solution in Locke. The doses were so adjusted that each injection raised by 0·2% the glucose content of the animals' blood.

Three of the animals receiving the Locke's solution showed an increased sensitiveness of the vagus, and one a decreased. In the experiments with glucose, six indicated a greater susceptibility, one had no effect, and two were doubtful. TRACING No. 23, is illustrative of those wherein an increase in the sensitiveness of the vagus was obtained after an injection of Locke's solution. At 12·54, 70 units were just able to show an effect upon the heart and bloodpressure, whereas at 12·58½ (after injection of Locke's solution) the same strength of stimulus had a much more powerful effect upon both.

The effect of an injection of glucose increasing the response of the vagus is seen in TRACING No. 24. The method of investigation was similar to that employed for the last experiment, and the necessary data are given under the tracing.



TRACING No. 24.

B. Injection 3 cc. 16% glucose solution,

C. 40 Kroenecker units,

D. 50 do. do.

Effect of Locke's solution.

These experiments show that the injection of a relatively simple substance may be followed by alterations in the sensitiveness of the vagus. Results such as these, are sufficient to cast doubt on the conclusions formed by Asher and his co-workers. It was decided however to investigate the action of thyroid extracts still further, by subjecting them to treatment before injection.

EFFECT OF DIALYSED THYROID EXTRACTS.

On the assumption that these variations in the sensitiveness of the vagus were due to, and not merely coincident with, the administration of the thyroid extracts, those might be caused by the presence in these of different active substances having antagonistic physiological effects. For example one ingredient might be a stimulant of the cardio-inhibitory fibres, another of the cardio accelerators. Or again, there might be only one active ingredient which stimulated all the autonomics - as suggested by Oswald, and the varying responses might be due to the relative effectiveness of the sympathetic and parasympathetic nerves in the various animals.

As a substance could not be found which would paralyse the accelerator nerves to the heart, and at the same time leave the inhibitory fibres intact, it

was decided to see if it were possible by dialysing the thyroid extract, to separate the active principles, and thus obtain preparations which would have consistent effects upon the sensitiveness of the cardiac fibres of the vagus.

The dialysis was carried out as already detailed, and the three solutions so obtained were injected in the usual manner.

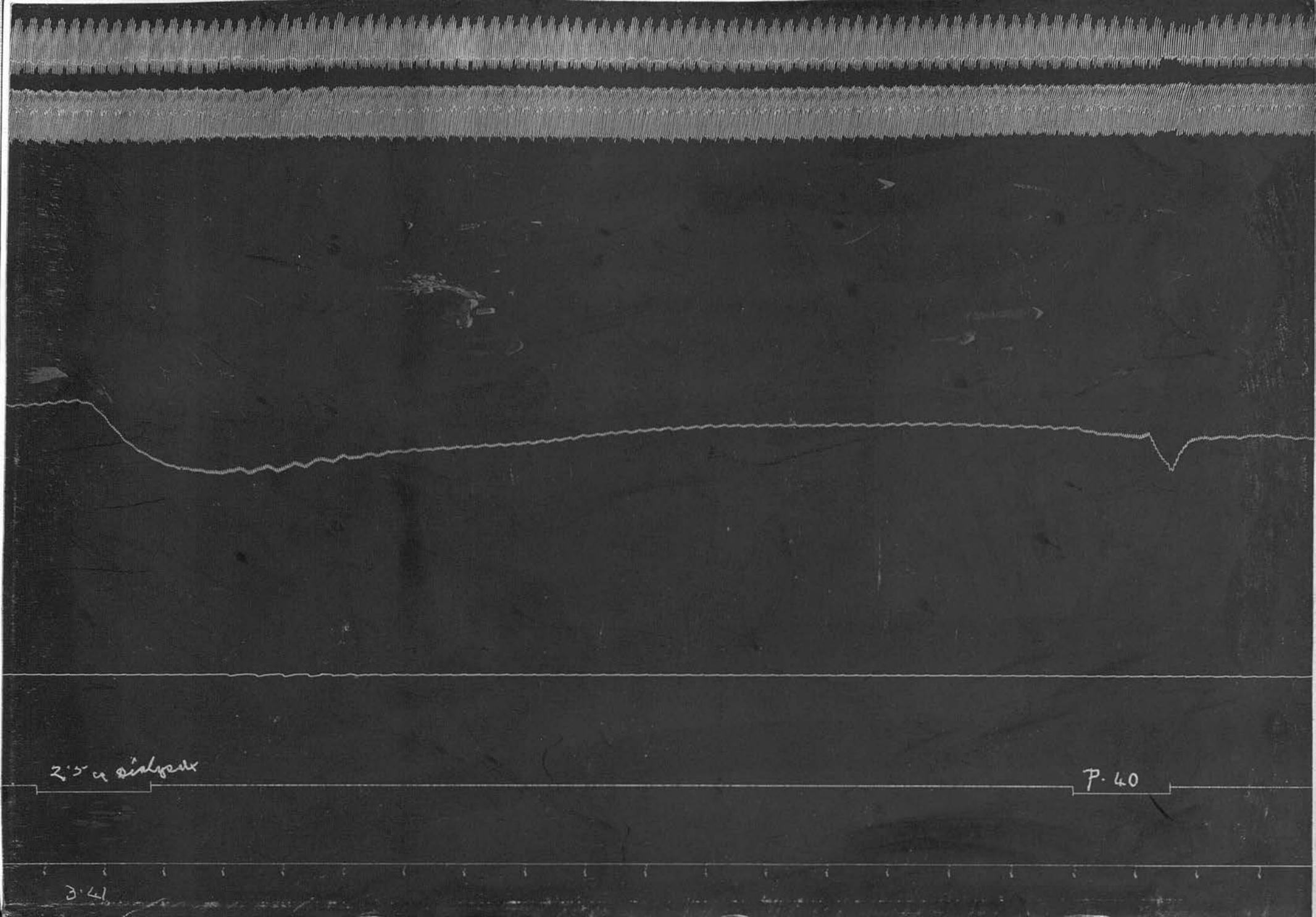
It was conceivable that the results following the injection of any of these solutions might be due to the cumulative effects of the substances administered, and not to the immediately preceding injection. In order to avoid erroneous deductions from this cause, the order in which the injections were given, were varied in the different animals.

Effect of the dialysate.

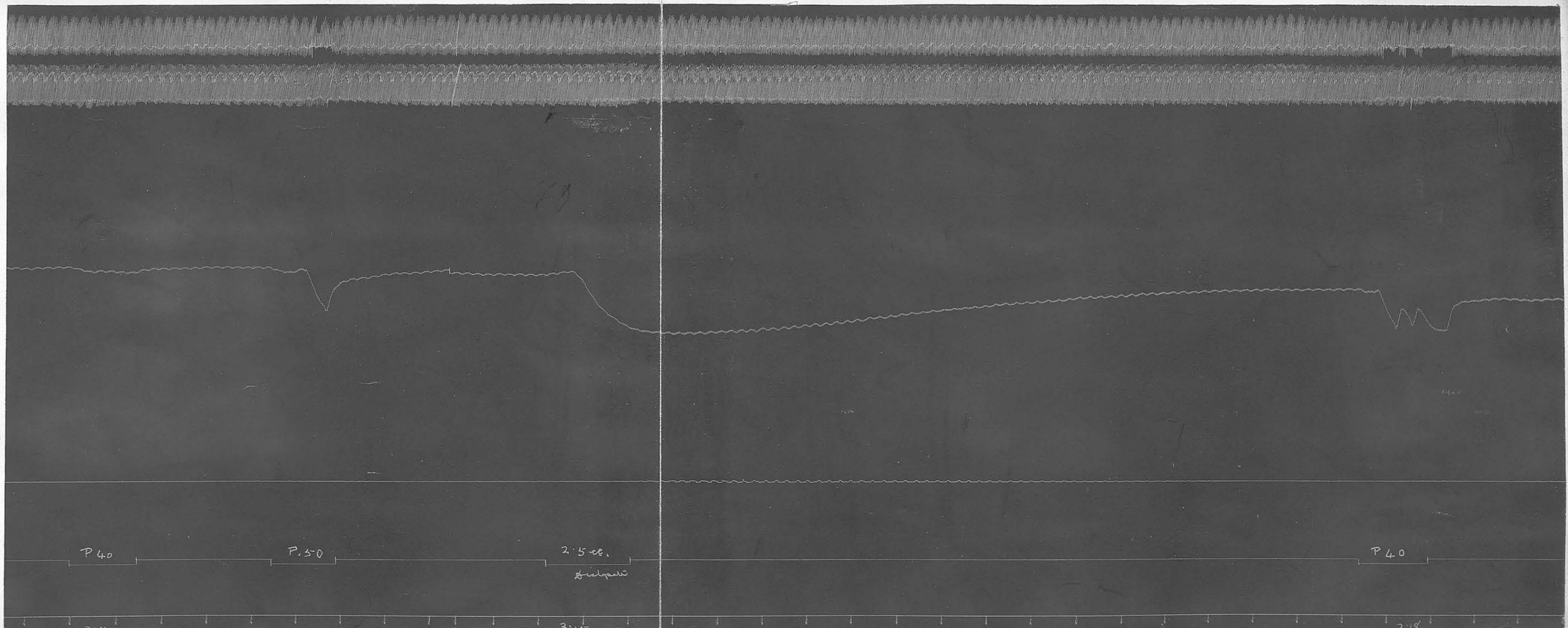
In three cats and one dog, stimulation of the cardiac vagus gave a greater response after the injection of the dialysate. In one cat a slight decrease in the response was observed.

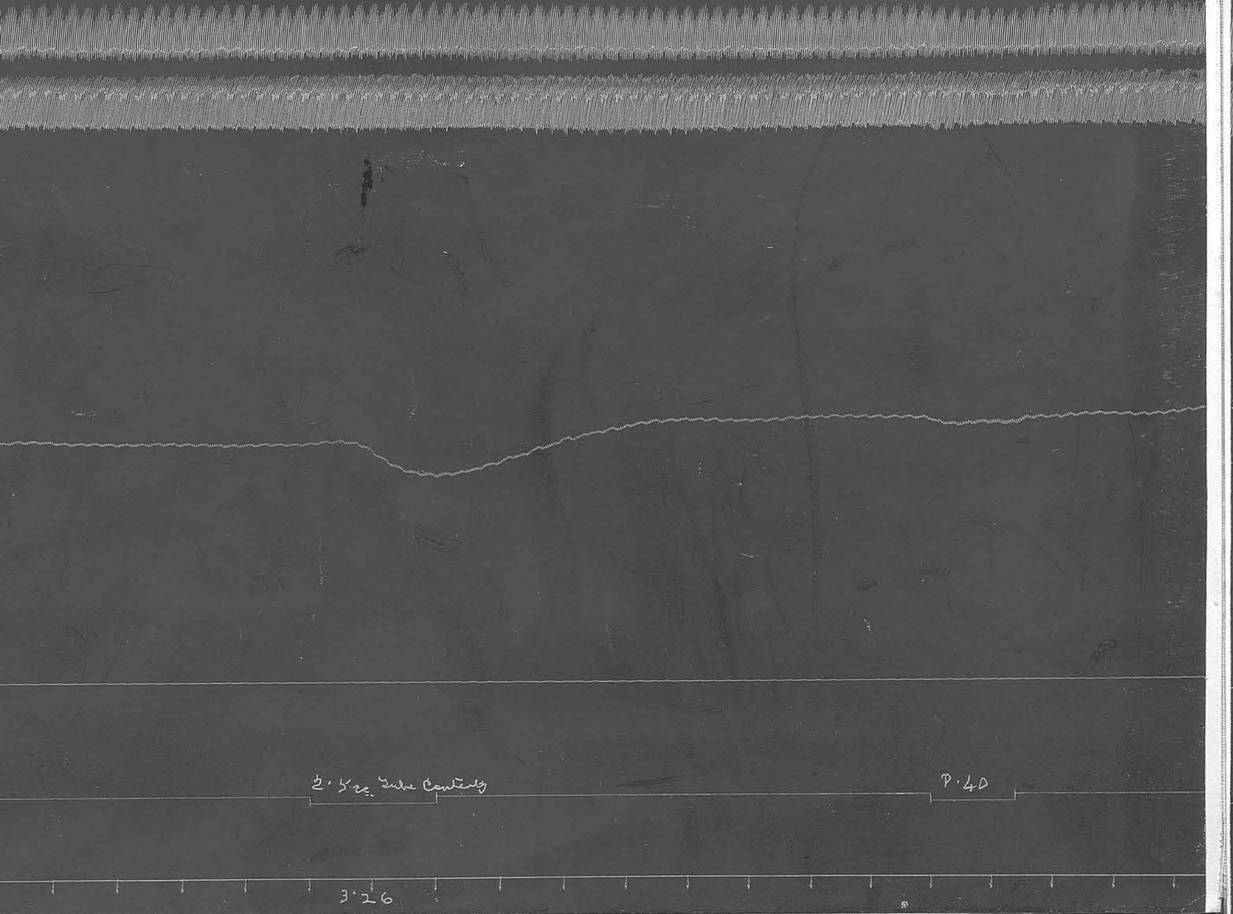
Effect of the dialysed extract.

Administration of the dialysed extract (the solution remaining within the collodion thimble) had a different effect from that of the dialysate. In two cats and one dog the sensitiveness of the vagus


P. 40

TRACING No. 25.




2.5 Sec. Time Constant
P.40
3.26


2.5 sec Time Constant
P.40

TRACING No. 25.

was decreased after such an injection; in one cat the response obtained was slightly increased.

In these experiments there is evidence of an antagonism between the ingredients present in the two solutions. Indeed, in one of the cats the increased response of the vagus to stimulation occurring after the injection of the dialysate, was brought back to normal by a subsequent injection of the dialysed extract, and the response was again increased after a further dose of the dialysate. TRACING No. 25, illustrates this "neutralising" effect of the dialysed extract upon the change of sensitiveness following an injection of the dialysate.

Effect of solution of sediment.

The effect of the injection of the solution of the sediment appearing in the collodion tube was tried on two cats. In one animal no effect was apparent, in the other a decrease in the response was obtained. It may be worth observation that the latter cat gave a decreased response after each of the three injections. The number of animals treated with this solution of the sediment however is too few to warrant any definite conclusions being formed.

Variation in the order of the injections did not seem to have much effect upon the results. There were indications that the effect, if any,

attributable to the dialysed extract was more apparent when this was administered after the dialysate. Quantitative measurements however of this nature are not of much value, owing to the varying degree of response obtained from different animals.

DELAYED EFFECTS OF THYROID.

It has been suggested by various workers, that in an "acute" experiment, the time elapsing between the injection of thyroid extract and the observation of its effects, is too short. Some observers have stated that the effects are not apparent until two hours have elapsed after the administration of the thyroid. In many of my experiments this interval has been exceeded, with results that did not differ materially from those observed over a more limited interval.

In those experiments of other workers where it was found that thyroid injections increased the sensitiveness of the vagus, the postulating of the long time interval as a necessary condition for the appearance of this effect strengthens the suspicion that the increased response was due to an alteration in the physical condition of the exposed nerve.

It is possible however that changes might

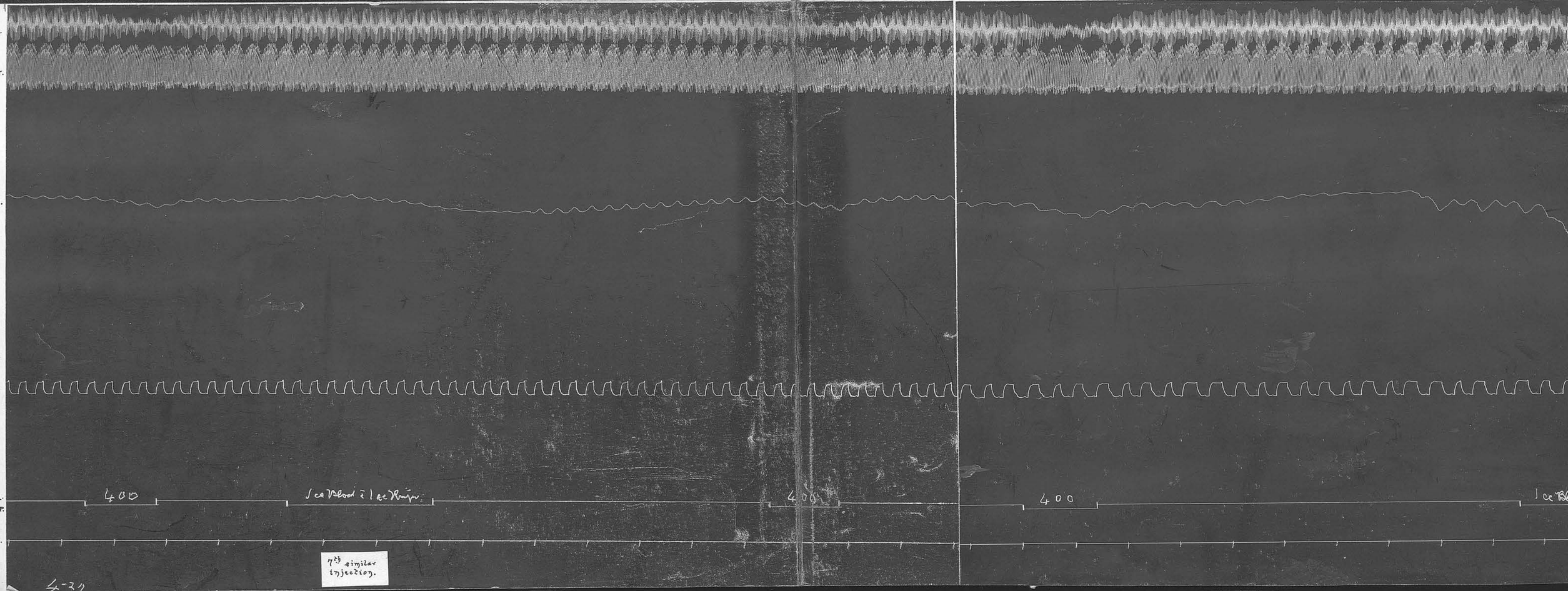
occur in the thyroid substance as a result of the prolonged contact with the blood or tissues. Alternatively some ingredient in the blood or tissues might be altered by the action of the thyroid, and such product might be able to affect the sensitiveness of the nerves under examination. This could be investigated by the administration of thyroid spread over several days or weeks, to be concluded by an acute experiment. Difficulties however present themselves.

It is obvious that before the observer could decide whether or not an increased response was being obtained from the cardio-vagus fibres of animals so treated, it would be necessary to know the response given by normal animals. This latter is a matter of difficulty. From the results I have obtained by the investigation of over one hundred animals I find that there are wide differences between the strengths of the minimal stimuli necessary for the inhibition of the heart to an equivalent extent.

In an effort to meet some of these difficulties within the compass of an acute experiment, I utilised another expedient.

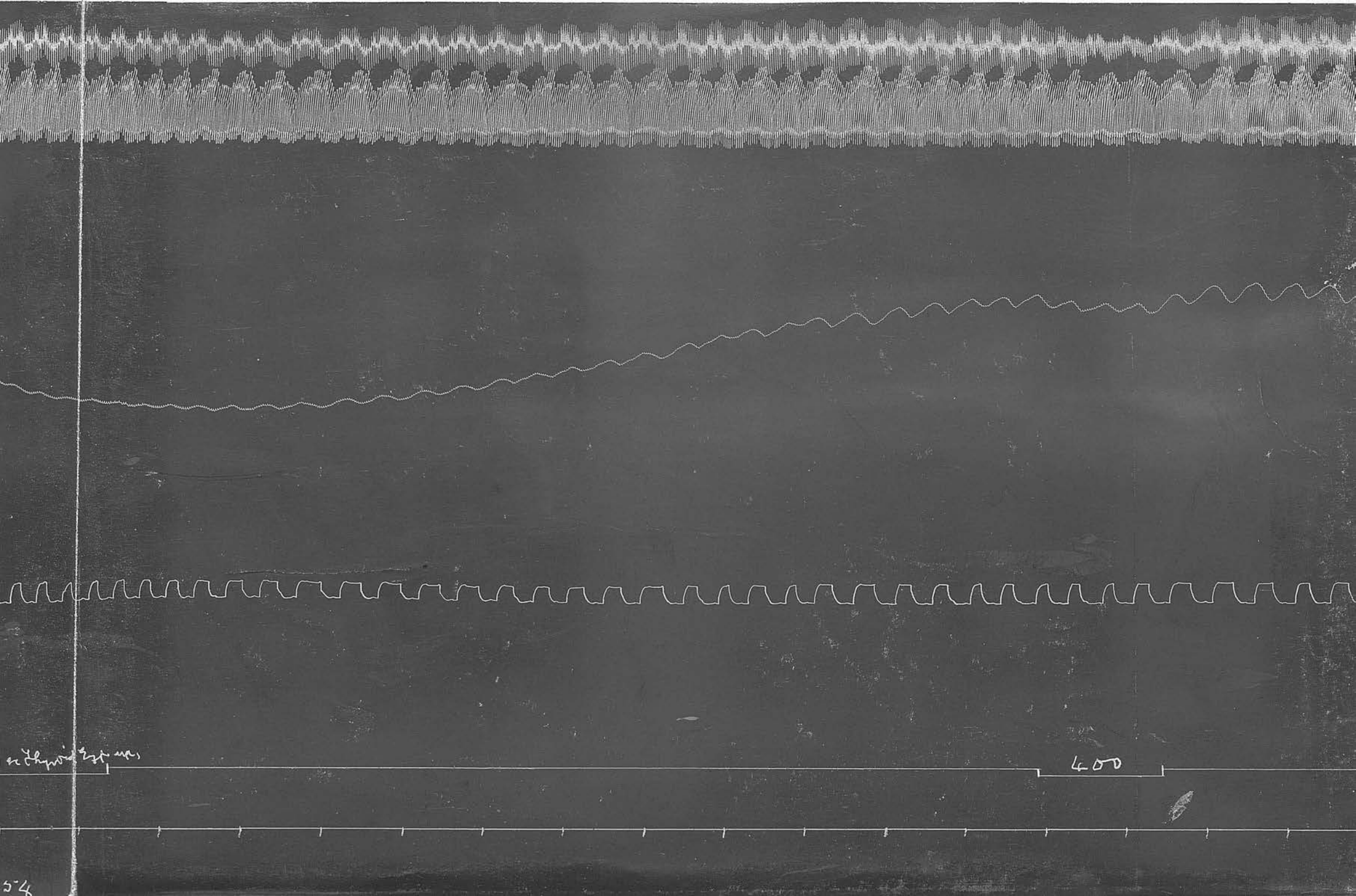
THYROID EXTRACT INCUBATED WITH BLOOD.

As it was possible that the thyroid extract underwent a change after contact with blood, some of



TRACING No. 26.

4-34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this was obtained from a cat. The blood was immediately defibrinated, and a portion of it mixed with an equal volume of thyroid extract. This was placed in an incubator at 37°, and kept there for six hours. The remainder of the blood was mixed with an equal volume of Ringer's solution and kept under similar conditions.

A fresh cat was obtained for the experiment, and in order to acclimatise it to the injection of the blood of the first cat, several injections were made of the mixture of blood and Ringer. The usual fall in bloodpressure occurred after each of these, and although this got progressively less with each subsequent injection, it was not abolished altogether.

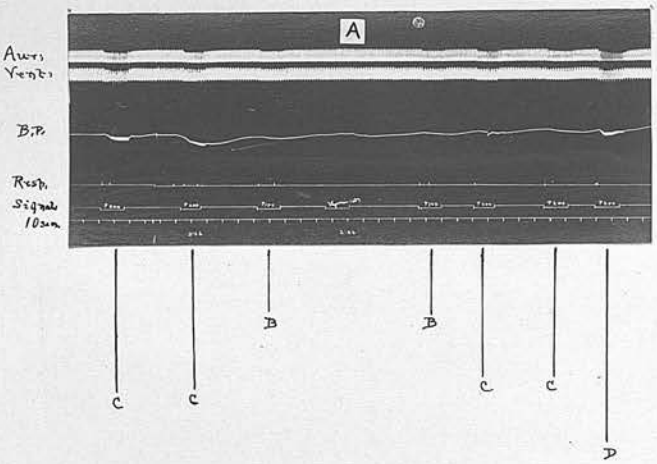
The sensitiveness of the vagus was tested after each of these injections, and found to remain constant.

After administering seven of these injections, an equivalent dose of the blood was then given but containing thyroid extract with which it had been incubated for six hours. The resulting fall in bloodpressure, as was to be expected, was greater than in the previous injections. On now testing the sensitiveness of the vagus, it was found to be unaltered, and a like condition succeeded a subsequent and similar injection of blood and thyroid extract.

TRACING No. 26, shows the essential part of this experiment, and the description thereunder gives

the details of the injections.

From these results there is no evidence to show that contact of thyroid extract with warm defibrinated blood for a period of six hours has any effect upon the extract or upon the blood, which will enable either readily to effect the sensitiveness of the cardiac fibres of the vagus. Whilst this single experiment is not conclusive, it is at least suggestive. It however still leaves undetermined the effects, if any, which prolonged contact of thyroid extract with the tissue cells may have upon the responses.



TRACING No. 27.

A. Injection 1 mg. thyroxin,

B. 150 Kroenecker units,

C. 200 do. do.

D. 250 do. do.

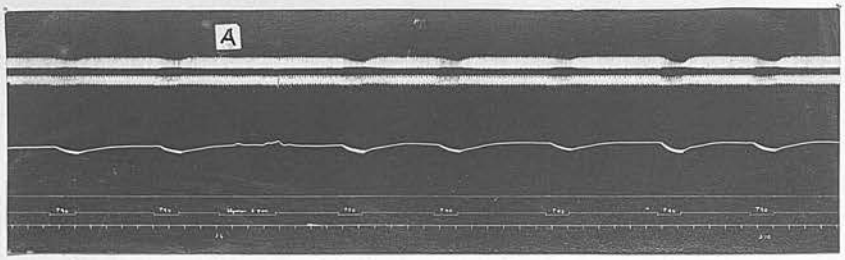
THE EFFECT OF THYROXIN UPON SUBSEQUENT STIMULATION OF THE CERVICAL VAGUS.

Investigations, upon similar lines to those used for the thyroid extracts, were undertaken regarding the effect of thyroxin injections upon the response of the cardio-vagus fibres to stimulation.

These injections also, gave variable results. The alterations from the normal however, when they occurred, were never so extreme as those obtained from the whole extract. The general tendency was to decrease the amount of response, and such an effect is seen in TRACING No.27.

It will be noticed that a stimulation of 250 units is required after the thyroxin to give a response equal to that obtained from 200 units before the injection.

An apparent increase in the sensitiveness of the cardiac fibres of the vagus was occasionally noticeable, and in illustration of this I give a



TRACING No. 28.

A. Injection thyroxin 0 8 mg.

All stimuli 90 Kroenecker units.

reproduction of TRACING No. 28, wherein the responses obtained from stimuli of unvarying strength may be observed.

Thyroxin incubated with blood.

A rabbit weighing 1930 gm. was injected with 0·2 mgm. of thyroxin which had been incubated with defibrinated rabbits' blood for six hours. As a control there were also injected equivalent amounts of blood which had been similarly incubated with NaOH solution equal in strength to the NaOH contained in the thyroxin solution.

The sensitiveness of the vagus and the depressor nerves to stimulation were investigated. No decided effect upon the responses obtainable were observed after the injection of the thyroxin and blood so treated. Any slight deviations from the normal were obtained equally readily after the blood+NaOH solutions.

EFFECT OF THYROID ON THE RESPONSE TO ADRENALIN.

Experimental evidence.

Asher and Flack in 1910 (37,38) showed that stimulation of the laryngeal nerves in rabbits with intact thyroid glands, increased the response to subsequent injections of adrenalin. These workers had assumed that such stimulation produced a secretion from the thyroid, and that the increased response to adrenalin was due to the sensitising effect of the thyroid secretion upon the tissues.

This work has met with much criticism, and in the light of more recent research (Cannon and Cattell, 1916)(39), it becomes still less convincing.

It is exceedingly difficult to prove the presence of the thyroid secretion in the blood.

Response to adrenalin.

Schafer (40) was unable to find any alteration in the period of metamorphosis of tadpoles (Gudernatsch)(41) which had been fed with normal blood, or the blood of patients suffering from exophthalmic goitre. Weak extracts of thyroid gland create a contractile response when applied to isolated strips of intestinal muscle, and Schafer utilising this observation has observed a greater contractile response after the application of the blood of exophthalmic patients than from that of normal individuals, but this test is not specific.

Oswald (1915)(42) found that although the injection of iodothyreoglobulin had no effect upon the bloodpressure or heart rate, that a subsequent dose of adrenalin gave an increased and a more prolonged effect upon the bloodpressure than that previously obtained. His statements are not supported by any tracings.

Albertoni (1916)(43) as a result of prolonged feeding of dogs with increasing, and large, doses of thyroid substance, found a less response than normal, to adrenalin injections. It is difficult however to form conclusions from his results because from the nature of these experiments he was unable to know the "normal" response of the individual dogs he was using.

Levy (1916)(44) in an investigation into

the secretory nerves of the thyroid, used an increased response to adrenalin as an indication of the activity of that gland. He found he obtained this result in cats after stimulation of the cervical sympathetic nerve. There was a latent period of about one hour, but when he injected thyroxin he found an almost immediate effect in increasing the response to adrenalin. He used decerebrated and pithed animals.

Ascoli (1920)(45) states that infinitesmal quantities of thyroid markedly strengthen the action of adrenalin.

Marine and Lenhart (1920)46) failed to get such results from thyroid preparations in the course of acute experiments. They used as their standard of measurement, the variations which occurred in the respiratory exchanges consequent on the injection of adrenalin.

Levy's observations could not be confirmed by Lieb and Hyman (1922)(47), and these workers lent support to the view expressed by Marine that there was no evidence of co-operation between thyroid and adrenalin. They were able to produce evidence which suggested that the increasing responses to successive adrenalin injections are due to a sensitisation of the sympathetic nerves, caused by these repeated doses.

Clinical evidence.

The evidence produced from clinical obser-

vations are equally divergent. Goetsch (1918)(48) finds that in a normal individual the subcutaneous injection of adrenalin chloride (0·5 cc. of 1:1000) gives a rise in bloodpressure of not more than 10 mm. In cases of hyperthyroidism he obtains a local and general reaction in excess of those obtained normally, the rise in bloodpressure being more than 10 mm.

Asher states (1920)(49) that if thyroid medication be incorrectly employed it may lead to so strong an activation of the accelerans cordis that the inconsiderable amount of adrenalin physiologically present in the blood may produce a powerful tachycardia.

Claude (1920)(50) observes that 1 cc. of 1:1000 adrenalin injected into a person with hyperthyroidism results in intense tachycardia of immediate or delayed onset. The increase in the pulse may be 40 or more per minute, and there may be an increase of 30-40 mm. in the systolic bloodpressure. In a normal individual the acceleration in the heart is very late and does not exceed 15 per minute, and the increase in the bloodpressure is within the limit of 10 mm.

Lewis and Davies (1921)(51) discussing cases of endocrine mal-functioning associated with mental diseases conclude that "------- a preliminary administration of thyroid enhances the action of ------ suprarenal ------ to be given later".

Doubt is cast however, upon this hypersen-

sitiveness being due to hyperthyroidism by the findings of Peabody, Sturgis, Tompkins and Wearn (1921)(52); and by those of Van Wagenen (1922)(53) who applied Goetsch's test to fifty normal individuals, and found a positive reaction in 20% of these cases.

ORIGINAL OBSERVATIONS.

Opportunity was taken during this research to reinvestigate this suggested synergism between thyroid and adrenalin.

I utilised for these experiments, cats and rabbits. All the animals were under general anaesthesia, the anaesthetics used being ether, urethane, and paraldehyde. The method adopted was to inject into an animal a dose of adrenalin sufficient to give a submaximal rise in the bloodpressure. This was then repeated several times in order to observe the normal response to repeated similar doses.

Precautions were taken to see that the volume of each injection was the same, and that the rate of administration was uniform throughout the series. The vein-tube was washed out with Ringer's solution between each injection, in order to prevent any remainder augmenting a subsequent dose.

After uniformity had been established, the

animal was given an intravenous injection of the thyroid preparation to be tested, and this was followed by further doses of adrenalin of the same strength and volume as before.

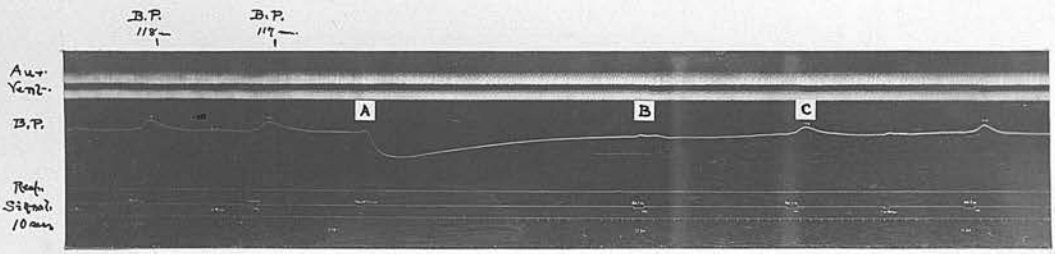
Thyroid extract was investigated in four cats, and the effect of thyroxin in eight cats and two rabbits. After the thyroid had been administered, the adrenalin injections were given intermittently over a length of time varying from 13 minutes, to 68 minutes.

The dose of the extract administered varied from 0·5 cc. (equal to 0·5 grm. of the fresh gland) to 1·2 cc. per kilo. The amount of the thyroxin for a similar weight of animal varied from 0·1 mg. to 0·6 mg.

R E S U L T S.

Of the four animals (all cats) which were treated with injections of thyroid extract, three gave a diminished response to adrenalin, and one showed no alteration. Of these three, two were under urethane anaesthesia, and one under ether. The remaining cat was anaesthetised with paraldehyde. In two of these the experiment was continued for 1 hr. 6 mins. after the injection of the extract.

Thyroxin was administered to the two rabbits. In one (under urethane anaesthesia) the adrenalin was



TRACING No. 29.

A. Injection of thyroid extract.

B. Adrenalin showing very little response.

C. Adrenalin, crest of B.P. 106 mm.; the next injection reached 110 mm..

Previous to "A" the adrenalin raised the bloodpressure to 118 mm. and 117 mm. as shown on tracing.

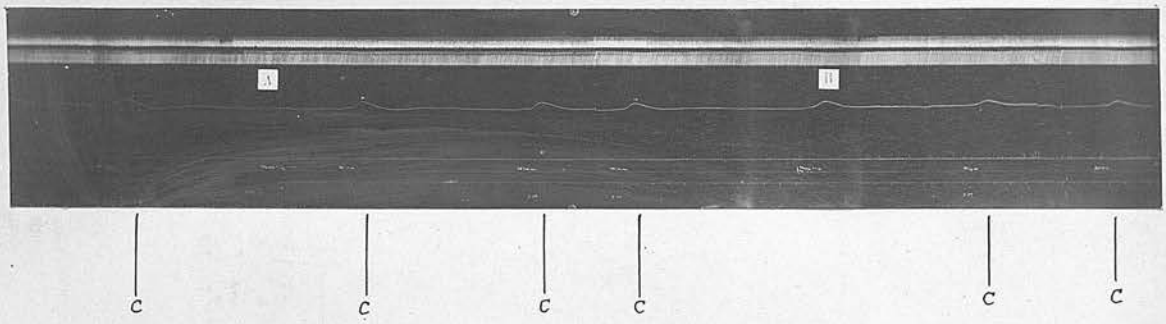
injected up to 23 min. after the thyroxin had been given, and a diminished response was obtained; in the other (under ether) no change was observed during a period of 1 hr. after thyroxin administration.

In the remaining eight cats, all treated with thyroxin, a diminution in the response was obtained in three, one showed no alteration, and four showed a slight increase in the pressor effect. In two of these latter animals, control injections were made with solutions of NaOH similar in strength to that contained in the thyroxin injections, and these gave increased responses to adrenalin injections similar in magnitude to those caused by the thyroxin. In these eight animals there was no apparent correlation between the anaesthetic used and the results obtained. We have the evidence of Collip (1921)(54) that a decrease in the H ion concentration of the blood gives an increased response to adrenalin injections, but it is difficult to conceive this effect being brought about as a result of the exceedingly small amount of alkali present in the thyroxin solution.

Photographs of part of the records of two such experiments are shown herewith. TRACING No. 29, being that of one using thyroid extract; and TRACING No.30, showing the effect of thyroxin upon the action of adrenalin.

Conclusion.

From the results of these experiments I



TRACING No. 30.

A. Thyroxin 0·1 mg.

B. Do. do. with adrenalin.

C. Injection of adrenalin.

All adrenalin injections were of same strength and volume.

Conclusion.

conclude that neither thyroid extracts nor thyroxin have any sensitising effect upon the action of adrenalin which is demonstrable in the limited duration of an "acute" experiment.

D I S C U S S I O N.

This research was directed towards an investigation of the statements of Asher and his co-workers that injection of thyroid extract into animals had a sensitising effect upon the cardio-inhibitory fibres of the vagus. The work was further extended to the examination of closely related problems.

An enquiry of this nature is open to the introduction of many fallacies which would invalidate the results. Those have been discussed, and the methods adopted to prevent them have been described.

The work resolved itself into the observation principally of two sets of phenomena:-

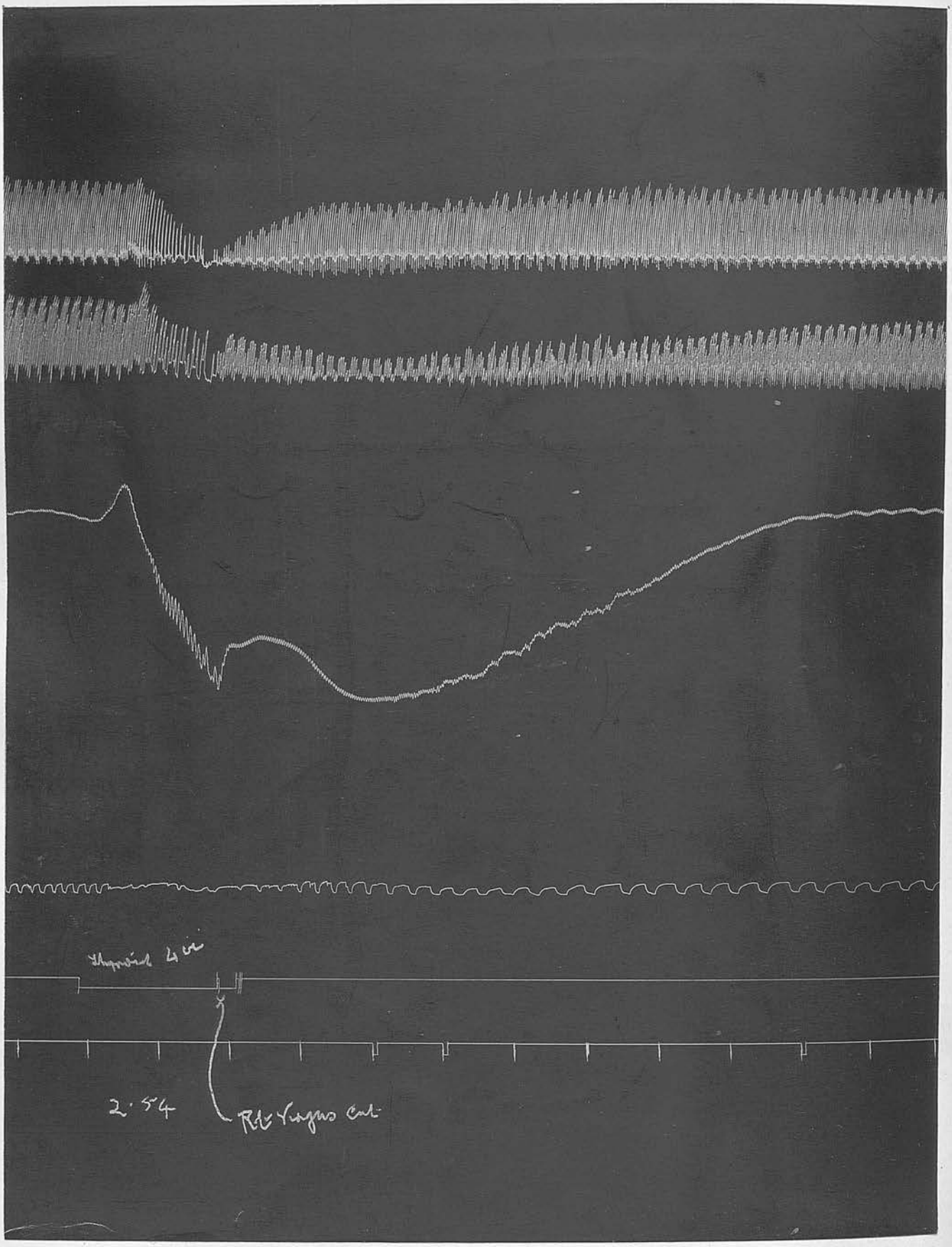
a. Those occurring during the administration of the injections,

b. Those arising subsequent to the injections.

EFFECTS DURING INJECTION.

The effect upon the bloodpressure.

We have seen from the evidence produced



TRACING No. 31.

Showing effect of cutting Right Vagus during injection.
Left vagus cut previously.

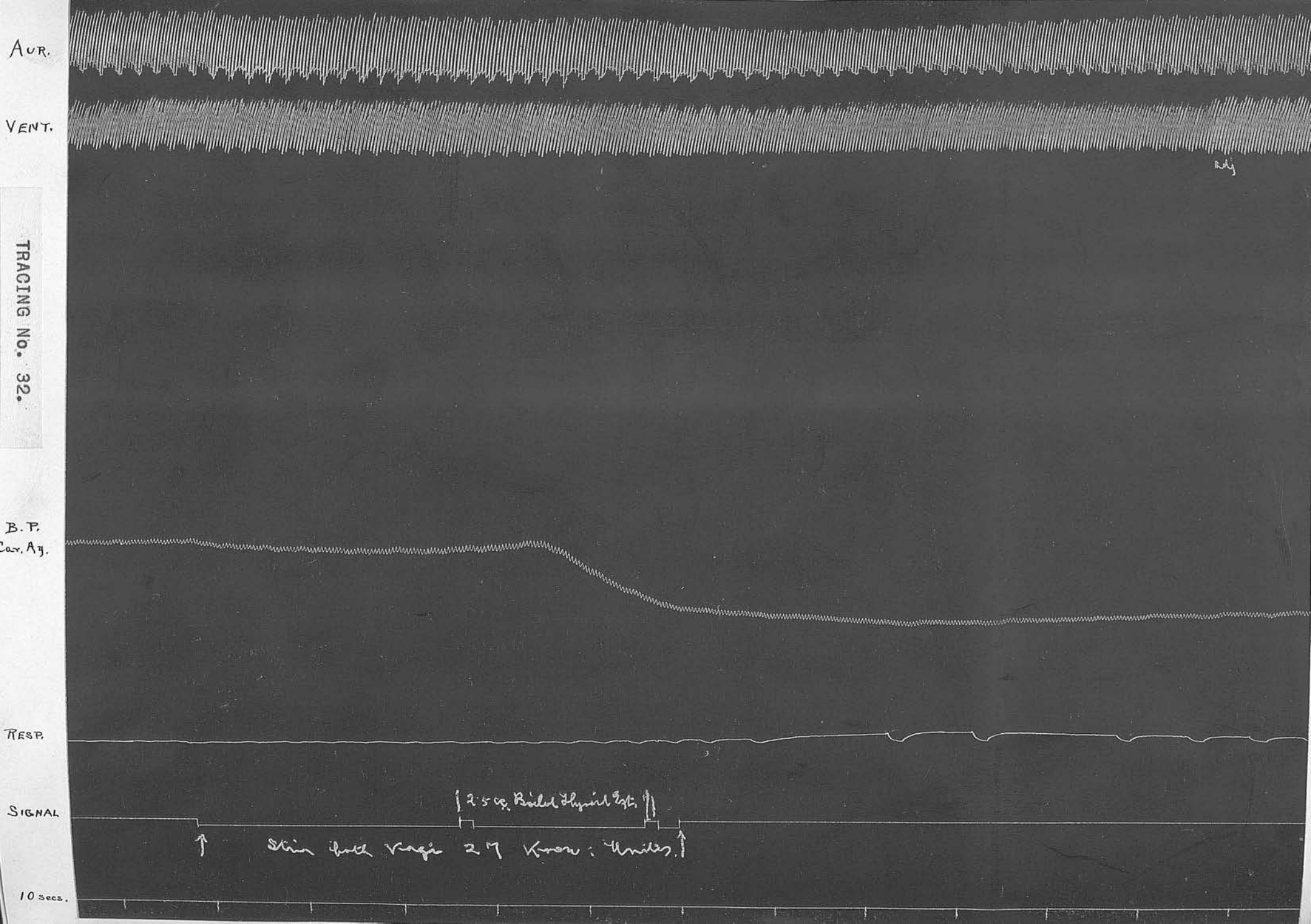
here, that the injection of thyroid extracts into cats is always associated with a fall in the bloodpressure. In 65% of the animals this was not accompanied by any alteration in the heart rate, and in these therefore the effect was purely a vascular one.

In the remainder of the animals, there was an alteration in the pulse rate, more or less marked, which might be attributable to a direct action of the drug upon the cardiac nerves or their centres, or which might be a secondary effect due to the anaemia of these centres, consequent upon the fall in the bloodpressure.

The effect upon the heart.

An examination of my records shows that where a slowing of the heart did occur during the injection, the alteration in the rate always succeeded, and never preceded the fall, although their incidence was frequently very close. This does not preclude an effect of the thyroid upon the vagus centre or its endings, but if such an effect is produced, the latent period is comparatively long compared with that upon the vascular control.

It became of interest to see if any light could be thrown on this, by severing the remaining vagus nerve during the injection, and at a time when the pulse rate was reduced. This was tried, and the effect was an immediate recovery in the heart rate. This is shown in TRACING No. 31, which is a record of


AUR.
VENT.
TRACING No. 32.
B. P.
Cor. Aj.
RESP.
SIGNAL
2·5 cc. Boiled Thyroid Ext.
Skin both Thighs 2·7 Krom: Units.
10 Secs.

such an experiment.

From this it might be inferred that either stimuli of increased intensity passed down the vagus as a result of the injection, or the vagal endings were made more sensitive to the impulses normally passing. This point was investigated by cutting both the vagi in a cat, and subjecting the peripheral portions to "continuous"stimulation by an induced current, during which period the drug was injected. By this procedure any variations in the tonicity of the vagus centres would have no effect, and only such changes as might occur in the sensitiveness of the nerves or their endings would be apparent.

An examination of TRACING No. 32, taken from an experiment of this nature shows that the injection has no effect whatever upon the rate of the heart beat. In this particular record, the stimulation of the vagi was discontinued almost at the bottom of the fall, and it will be observed that their cessation was followed immediately by a diminution in the amplitude of the heart movements, more particularly evident in the auricle.

From these supplementary experiments I conclude that when any alteration occurs in the heart rate, as a consequence of the injection of thyroid extract, that it is due to an effect upon the cardio-inhibitory centre. Whether this is primary or secondary still remains to be determined, but the evidence

obtained from my experiments tends to the latter alternative.

Evidence has been produced to show that this alteration in the heart rate is not a property belonging specifically to thyroid extracts, but may be observed during the injection of other tissue extracts.

The depressor principles.

The nature of the depressor principles present in the thyroid extracts has been examined, and some of their properties compared with the depressor substances present in extracts of liver and of tonsil.

The bulk of the bloodpressure lowering substances present in thyroid were found to be dialysable, the dialysate having a much more powerful depressor effect than an equivalent dose of the dialysed extract. That this property of the dialysate is not due principally to choline is shown by the negligible effect that a previous dose of atropine has upon it.

Independently however it has been shown that choline is readily dialysable, and this being so, it may be assumed that any choline present in the extract will have passed into the dialysate. The comparative ineffectiveness of atropine to alter the depressor property of this solution indicates therefore that relatively little choline must be present in these extracts.

Depressor principles.

Evidence is produced to show that even after prolonged dialysis with frequent and voluminous changes in the surrounding water, an appreciable depressor property may still be retained by the "dialysed extract". This property however is lessened somewhat by atropine, and may even be turned into a slight pressor effect. As it is scarcely conceivable that choline could still remain in this solution, it is inferred that at least two depressor substances are present, one of which is affected by atropine and the other not.

It is shown that these substances have their properties materially effected by alkaline hydrolysis being reduced to a substance having properties similar to those of trimethylamine; and further that they are destroyed by incineration.

From these observations it is concluded that the depressor properties of thyroid extract depend upon more than one substance, and there are indications of at least four. Of these the more effective are substances other than choline, which are dialysable.

All the depressor substances are of an organic nature, and in view of their ready hydrolysis by alkali it is inferred that they have a relatively large molecule.

Depressor principles having similar properties to these are shown to be present in extracts of

tonsil; but from the evidence produced there are indications that the bloodpressure lowering substances in liver preparations are not entirely similar to those of thyroid.

The variations in the bloodpressure consequent upon the injection of thyroxin departed so little from the normal, that the conclusion is formed this substance has no definite and unmistakable effect demonstrable in a limited period of observation.

EFFECTS SUBSEQUENT TO THE INJECTIONS.

Cardio-inhibitory fibres.

The effects subsequent to the injection of thyroid extracts have been investigated by determining the variations, if any, occurring in the response of the cardio-inhibitory fibres of the vagus to electrical stimulation; and also by noting any modification in the responses to subsequent injections of adrenalin.

The difficulties associated with the control of the electrical stimuli have been discussed, and the means adopted to overcome these are enumerated.

From the records obtained it is seen that injections of the whole thyroid extract may be followed by an increased, or by a decreased response to stimulation of the vagus, or it may be that no alteration may be manifest.

It is possible that such diversity of results may be due to the presence in the whole extract of substances having antagonistic physiological properties. In several of the animals evidence was incidentally obtained to show that choline had a depressing effect upon the sensitiveness of the cardiac vagus to stimulation. An attempt was therefore made to separate these substances by dialysing the extract, and using separately the solutions so obtained. By this means indications were given of a greater specificity and consistency in the effects. The number of observations however are not sufficient to warrant any conclusions being formed.

The incubation of thyroid extract with blood, and its subsequent injection, left the sensitiveness of the vagus to stimulation unaltered.

It is shown from the records herein, that whatever variations may be obtained in the sensitiveness of the vagus as a result of injections of thyroid extract, they are not specific. Similar results may be obtained after the injection of extracts of liver and of tonsil; of Locke's solution, and of glucose.

It may be argued that from the complex nature of thyroid extracts, indefiniteness in the results was to be expected. Evidence is produced however to show that when a chemically pure active principle of thyroid-thyroxin, is administered, there are no specific and unmistakable effects upon the

sensitiveness of the cardiac fibres of the vagus to electrical stimulation.

Response to adrenalin.

Thyroid extract was injected into some animals, and into others, thyroxin. After varying intervals of time, which have been detailed, adrenalin was then injected. The rise of bloodpressure so obtained was compared with those from similar doses of adrenalin administered previous to the thyroid preparation.

No evidence was obtained of a synergism between these two autacoid substances.

C O N C L U S I O N.

Reviewing the whole series of experiments, it might be suggested in explanation of such variations as occurred that they were incidental to factors other than those being specifically investigated.

Such criticism can be legitimately applied to a research of this nature, and it may be of note to record here what precautions were taken to prevent undue variations in the factors under my control, in addition to those already discussed.

The condition of the animal. All the animals used had received no food on the day of the experiment. No record was utilised from an animal which showed obvious signs of distemper or "snuffles".

Degree of anaesthesia. When urethane was used each animal received 1·6 gm. per kilo injected subcutaneously, one hour previous to the preliminary dissection. These injections were invariably made under ether anaesthesia. At the end of the hour the animal received a further dose of ether sufficient to induce complete anaesthesia, and the ether was then withdrawn, no further application being necessary. When ether alone was used, the animal was kept under just sufficient to lose the conjunctival reflex.

Temperature of the room. This was regulated by electric radiators or by fans, so as to be reasonably consistent.

Conclusion.

Temperature of the injections. These were brought to the body-heat of the animals, and the syringes were warmed to a like temperature.

Of the factors outwith my control, the following were noted on every record obtained:-

Weight,

Colour,

Sex, (if female-whether pregnant or not),

Any unusual feature,

Date.

Finally, in surveying the records of all the experiments, amounting to more than one hundred, there have been taken into account the season of the year, and the nature of the anaesthetic utilised, together with the relative dose of the drug employed.

A systematic classification of all these data together with the results obtained, indicates that no relationship exists between any of these factors and the variations observed in the responses.

As the same batch of thyroid gland was used throughout, and as frequently the same batch of extract, preserved aseptically, was used for succeeding experiments, with divergent results, the conclusion is inevitably formed that these varying effects upon the sensitiveness of the vagus are due to some factor undetermined, which might be conveniently referred to as an "idiosyncrasy" in the animal; or they may be

merely of an incidental nature.

SUMMARY.

1. A historical survey of the subject is presented.

2. Possible explanations of the divergent results of previous workers are given.

3. A new type of electrode which I have evolved for this class of work is described.

4. Original observations on the action of thyroid preparations are presented, from which the following conclusions may be drawn:-

    a. Injection of thyroid extract invariably produces a fall in the bloodpressure in cats and dogs. This may or may not be accompanied by an alteration in the heart rate.

    b. More than one substance is responsible/

responsible for this depressor effect, all of which are readily hydrolysed by alkali, to substances having physiological properties similar to those of trimethylamine.

c. The injection of thyroid extract has no definite and specific effect which is demonstrable in the course of an "acute" experiment, upon the response to subsequent stimulation of the cardiac fibres of the vagus in the cat.

d. Injections of extract of liver or of tonsil may be followed by effects upon the cardio-vascular nerves, similar to those succeeding thyroid extract.

e. These phenomena may also occur after the injection of Locke's solution, or may appear apparently spontaneously.

f. Thyroxin has no definite and unmistakable effect upon the cardio-vascular system, demonstrable in an "acute" experiment.

g. No support can be given to the statement that the injection of thyroid extract, or of thyroxin, increases the response to subsequent injections of adrenalin.

5. There are indications throughout these experiments which suggest that the minimal stimulus to be applied to the vagus in order to produce cardiac inhibition in the cat, varies in strength between wide limits.

6. Further investigation into the conditions affecting the excitability of, and the onset of fatigue in, the cardio-inhibitory fibres of the vagus, is indicated.

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