THE IMMEDIATE ACTION OF AN INTRAVENOUS INJECTION OF BLOOD-SERUM.

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In carrying out some experiments with antitoxic serum I was surprised to find that the intravenous injection of diluted blood-serum into a cat produced a marked effect upon the heart and respiration.

As serum is now being extensively used for therapeutic purposes I thought it would be well to follow this observation further. At the outset I must mention, that of the animals tested, dog, cat and rabbit, the effect is only to be observed in the cat. A second important point is that it is not a particular property of antitoxic serum for it can be obtained by injecting serum from any source, including even the serum obtained from the particular animal experimented upon. It was also found that several other substances, *e.g.* egg-white and the bromine compounds of proteids, could produce the same effect but in the experiments described in this paper serum has been chiefly employed. It may be mentioned that milk has a slight effect, but gelatine and peptone have none.

That serum is by no means an innocuous substance when injected subcutaneously or intravenously into an animal is already well known, but I need only refer to the most recent papers upon this subject. Weiss[1] describes a long series of experiments dealing with the toxicity of serum. The serum from all species of animal tested was invariably found to be toxic, though possessing different degrees of toxicity, and caused death if a sufficient quantity was injected. The symptoms

[1] Weiss. *Pflüger's Archiv*, LXV. p. 215. 1896. In this paper will be found full references to previous work. See also *Pflüger's Archiv*, LXVIII. p. 348. 1897.

produced as described by Weiss and other observers are briefly, diminution in the amount of urine and albuminuria, increased rapidity of the respiration and of the heart, and a rise in temperature. These symptoms might persist several days. If serum was continuously but slowly injected until it caused death, the respiration first became very rapid, then laboured, and finally the animal became convulsed. The rate of heart-beat was increased and at first the beat was stronger but gradually failed, though it persisted after the respiration had ceased. The pupils dilate and exophthalmos is produced. There is increased peristalsis of the small intestines. Death is due to paralysis of the respiratory and vaso-motor centres. Weiss chiefly employed rabbits for his experiments, but he obtained similar results with the cat and the dog. The degree of toxicity of different forms of serum for the rabbit has also been determined by Guinard and Dumarest[1]. They determine the quantities sufficient to kill within a few days. The most toxic serums are those of the ox, dog and cat; the least that of the horse. Another paper dealing with the same side of the subject is that of Friedenthal and Lewandowsky[2], which confirms and extends the previous work in this direction. They point out one further very important fact, viz., that if the serum be heated to 58°—60° C. for an hour it can then be injected without producing any symptoms of a toxic action[3].

In Fig. 1 is reproduced a typical tracing showing the effect produced by an injection of blood-serum upon the blood-pressure and respiratory tracings. The injection was made into the external jugular vein and it is seen that it is immediately followed by marked inhibition of the heart, the blood-pressure nearly falling to zero. Recovery of the heart sets in in about 10 seconds and then the blood-pressure begins to rise, at first rapidly as the rate of beat increases and then more slowly. After about 10 minutes the heart had regained its original rhythm and the blood-pressure had returned to its former height. The effect upon respiration is just as striking. Simultaneous with the arrest of the heart the respiration ceases in the expiratory phase, a prolonged and very slow inspiration follows and as the heart recovers a few irregular respirations occur, many of them being very deep, and then a second standstill occurs until 15 secs. later the normal rhythm once more returns. This is the ordinary effect produced by a fairly large injection

[1] Guinard and Dumarest. *Comp. Rend. Soc. de Biol.* XLIX. pp. 414, 416, 495. 1897.

[2] Friedenthal and Lewandowsky. *Archiv für (Anat. u.) Physiol.* 1899, p. 531. See also Munk and Lewandowsky. *Ibid.* 1899, supp. p. 73.

[3] *l.c.* p. 539.

—in this instance 5 c.c. of normal horse serum—but several variations from this type are met with. The inhibition is commonly not so

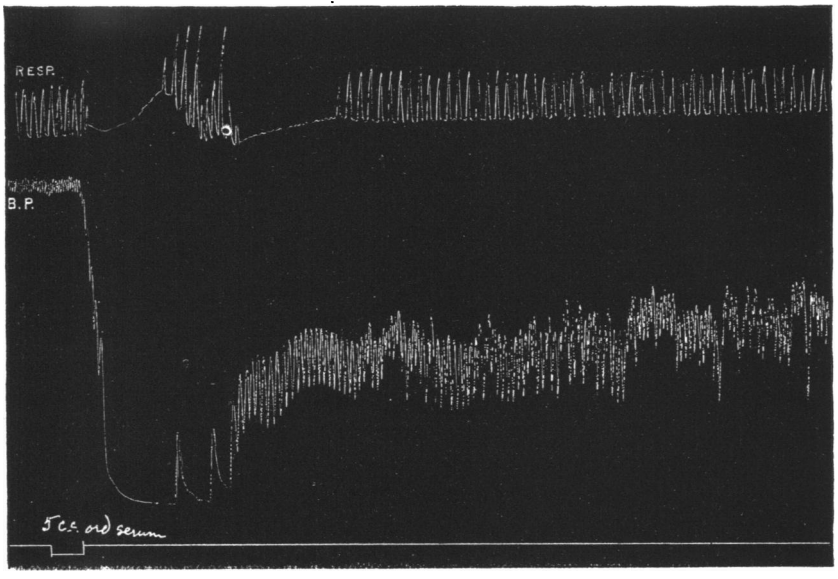

Fig. 1. Cat under urethane[1]. The upper tracing is that of respiration. Rise of the lever indicates inspiration. The second tracing is that of the blood-pressure by a mercury manometer connected to the carotid. The lower line is the abscissa line of zero-pressure. At the time indicated by a break in this abscissa line 5 c.c. of normal horse-serum was injected.

complete as in the case recorded, and recovery is then as a rule quicker. Moreover the effect on respiration is often different. Frequently the first change observed is the occurrence of two or three shallow and rapid respirations followed by arrest in expiration, and usually the tracing at this time is quite horizontal. On a few occasions I have observed as the first effect the occurrence of two or three deeper and slower respirations before the arrest.

In other instances the respiration simply becomes shallower (Fig. 11) and this is followed by quicker respirations which are also frequently deeper.

The recovery of respiration is, as a rule, not so sudden as in Fig. 1, but on returning the rate and depth gradually increase and may for a time exceed the normal. (See Fig. 15.) Occasionally animals have

[1] All the figures in this paper are to be read from left to right.

been met with which show no effect whatever upon the respiration although the cardiac effect is typical.

In addition to the two well-marked results already described a third effect, viz. one upon the vaso-motor centre, is also produced. The experiments proving this will be described later.

One of the most striking features of the reaction is the great suddenness of the changes in the heart and respiration and the fact that both are identical in point of time.

My experiments upon this result of a serum injection have been chiefly concerned with four main points, and it will therefore be most convenient to describe them in four sections. In the first of these the mode of production of the arrest of the heart and respiration is discussed; in the second the effect upon the vaso-motor system; in the third the production of immunity to a serum injection, and in the fourth the constituent of serum which produces the reaction.

Throughout the experiments the animals were anæsthetised with the ordinary A.C.E. mixture, though in a few instances urethane was employed.

The amount of serum required to produce the result varies considerably with the animal, often as little as 0·1 c.c. produced a typical reaction, whilst at times as much as 10 c.c. had to be injected. On a few occasions the animals produced no reaction at all. The time of year has also seemed to have some influence, for the animals appeared to be more sensitive in the winter months than in the summer.

As a rule the injections have all been made into the jugular vein, but the result is exactly the same when the injection is made into the femoral vein or into a branch of the mesenteric vein.

I. THE MODE OF PRODUCTION OF THE ARREST OF HEART AND RESPIRATION.

The fact that the action on the heart and respiration occurred simultaneously seemed to indicate that the action was one on the medulla, but this is at once negatived by cutting both vagi in the neck after which a serum injection produces very little effect. This is seen in Fig. 2 taken from an animal which had given a well-marked reaction to an injection of 3 c.c. of cat's serum before the vagi were cut in the neck. In the experiment given as much as 10 c.c. were now injected and the only result observable is a small and transient fall in blood-pressure. There is no slowing of the heart and practically no

alteration of respiration. The cause of this fall in blood-pressure will be discussed later. The conclusion to be drawn from these two results

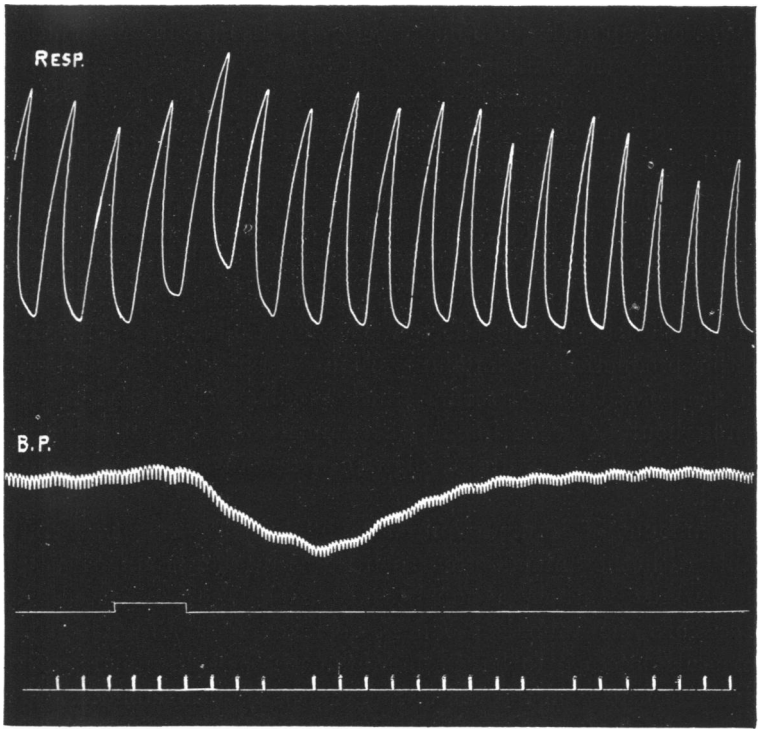

Fig. 2. Cat. Respiration and blood-pressure. The vagi have been previously divided in the neck. The zero-abscissa line has been raised 55 mm. At the time signalled on this line 10 c.c. of cat's defibrinated blood was injected. Time record = seconds.

is that some substance in the serum excites the terminals of some set of afferent fibres of the vagus, and these impulses act upon the medullary centres and produce, as their chief effect, slowing of the heart and arrest of respiration.

The stimulation of these fibres lasts a considerable time after the serum has been injected. This is well seen from Fig. 3, in which experiment the vagi were cut in the neck during the effect upon the heart and respiration. Immediate recovery follows. In one experiment the section of the vagi led to failure of the heart and death of the animal. I have repeated this experiment many times but have never, with this single exception, obtained any result differing from that recorded in Fig. 3.

In some cases the excitation of the vagal fibres lasts so long as to cause the death of the animal. This I have often observed with doses as large as 10 c.c. and on one occasion with 2 c.c. of serum.

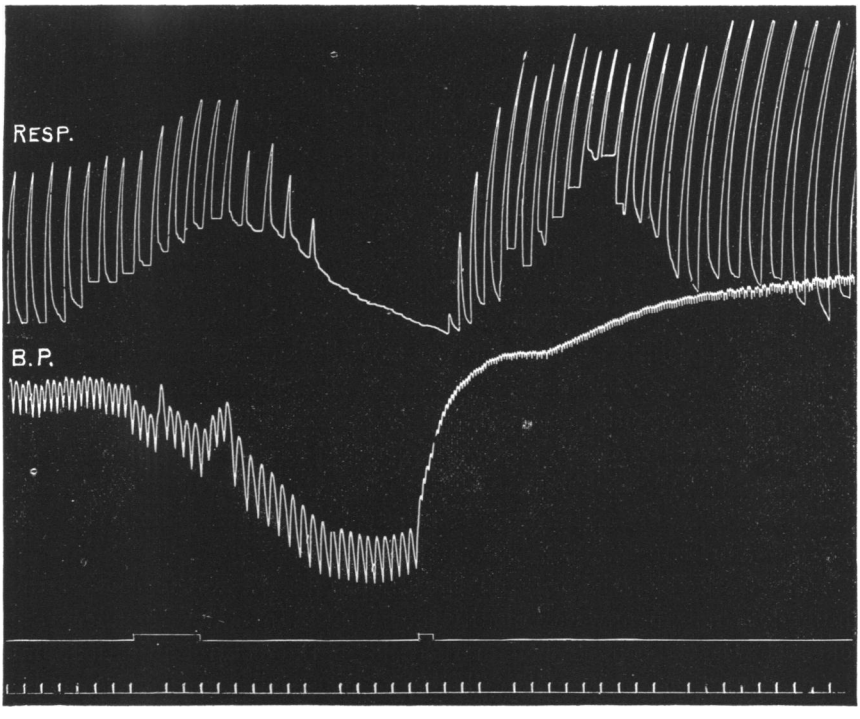

Fig. 3. Cat. Respiration and blood-pressure. Zero-abscissa line has been raised 15 mm. At the first signal on this line 10 c.c. cat's defibrinated blood was injected. At the second the two vagi were divided in the neck. Time record = seconds.

That the action of the serum is upon the periphery and not on the medulla is further shown by injecting the serum into the peripheral end of the carotid artery, when the reaction should occur more quickly and in greater intensity if the action were one upon the medulla. This is, however, not the result, but on the contrary there is a considerable latent period before the typical reaction occurs. During this latent period no alteration in the blood-pressure or respiratory rhythm was, as a rule, observed. In other instances, the injection was immediately followed by a rise of blood-pressure which suddenly fell later as the usual reaction was produced. In these injections into the carotid less effect is, as a rule, observed upon the respiratory rhythm.

It being evident that the effect is a reflex one the afferent impulses being carried along the vagus, I performed several experiments to determine which were the vagal fibres stimulated. The general plan of these experiments was to first insert cannulæ in one carotid, one external jugular and the trachea and then to expose the vagus in the chest. In the first instance the two vagi were obtained on the œsophagus. For this purpose the thorax was opened by resection of three ribs, artificial respiration being established. The two vagi were isolated and loops of thread passed round them. The carotid was then connected to the manometer and a dose of serum injected. The vagi were next gently lifted up by the threads and divided. This was followed by a second injection of serum.

In other experiments the pulmonary branches and the cardiac branches were similarly treated. These fibres were obtained by removing the upper half of the sternum and the anterior portions of the upper three or four ribs. The fibres were then isolated and the experiment completed as above.

In another set of experiments the left vagus was cut in the neck and then the various branches of the right vagus were isolated and the action of serum tested before and after their section. Both methods yielded similar results, which were as follows:

(i) Section of the vagi on the œsophagus below the root of the lung does not in any way modify the result. In a few instances the effect has not been so well marked as in the control injection, but this is due to a cause explained later (see pp. 56 et seq.). Hence we can conclude that the afferent fibres, stimulation of which produces the reaction, are not contained in any of the vagal branches below the pulmonary.

(ii) Section of all the cardiac branches of both vagi only produces a modification of the reaction due to the absence of the reflex cardiac effect (Fig. 4). The result obtained here is directly comparable to that seen in an atropinised animal (Fig. 12). There is still in both cases a marked fall in pressure. As artificial respiration had to be employed in the experiments when the nerves were divided it was very difficult to determine any alterations in the respiratory rhythm. This difficulty was overcome in the following manner. When everything was ready for the serum injection the amount of artificial respiration was gradually decreased until the animal made voluntary respiratory movements and the serum was then injected. The movements at once ceased. The cardiac branches were next cut and serum a second time injected

under similar conditions. Again the respiratory movements ceased. Hence it follows that the cardiac fibres of the vagus do not contain any

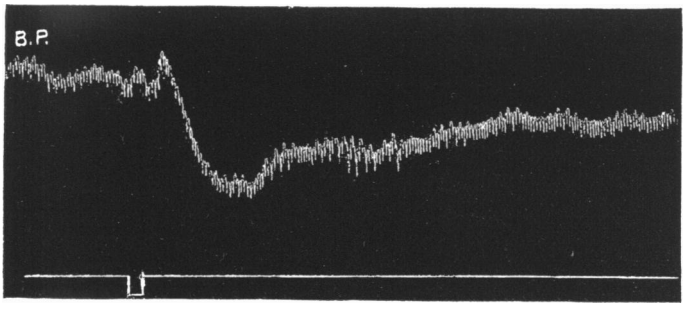

Fig. 4. Cat. Thorax opened. Blood-pressure tracing. The zero-abscissa line raised 30 mm. The cardiac branches have been previously divided in the thorax. At the time signalled 3·5 c.c. horse-serum was injected.

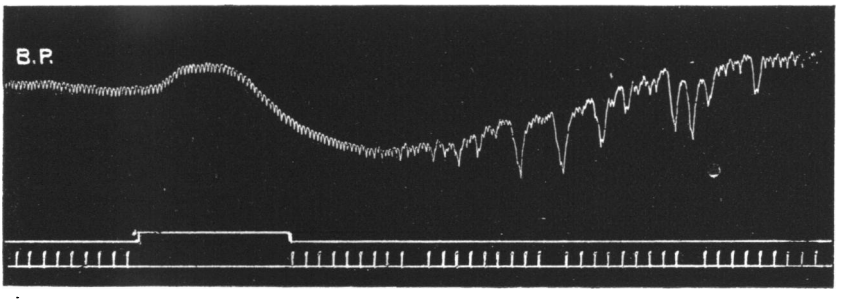

Fig. 5. Cat. Thorax opened. Blood-pressure tracing. The zero-abscissa line has been raised 40 mm. The pulmonary branches of the vagi previously divided where they enter the root of the lung. Result of injecting 10 c.c. horse-serum. Time record = seconds.

afferent fibres, stimulation of which by serum can cause the arrest of respiration or depression of the vaso-motor centre. Moreover, from the results recorded in the next paragraph it is certain that the reflex arrest of the heart is not due to excitation of any afferent fibres in the cardiac branches of the vagus. In all cases care was taken that all the cardiac branches were cut. This was tested by stimulating the two vagi in the neck after the division of the branches in question and observing that no inhibition resulted.

(iii) Section of the pulmonary branches completely cuts out the reaction (Fig. 5). The behaviour of the blood-pressure curve is exactly the same as after the division of the vagi in the neck. (Compare Fig. 5

with Fig. 2.) A subsequent injection after the vagi have been divided in the neck shows no further modification. The action on the respiratory rhythm was tested as described under (ii) p. 54, and the respiratory reflex also found to be abolished.

Hence it is the pulmonary afferent fibres stimulation of which by serum produces in the cat reflex arrest of the heart and respiration.

This result is of further interest when taken in conjunction with the results obtained by Russell and myself[1] upon reflex cardiac inhibition.

During the course of the experiments in which the thorax was opened it frequently happened that the reaction to a serum injection failed. This often occurred even with animals in which a typical reaction had been obtained before the major part of the operation had been commenced, thus proving that it was not due to any idiosyncrasy on the part of the animal. I at first thought it might be due to the production of some degree of immunity due to the absorption of some of the serum from the blood escaping during the operation. Great care was therefore taken to prevent all bleeding during the operation but still the same results were obtained. For a long time this proved a great hindrance to the completion of the experiments and the cause and the way in which it was to be avoided was only discovered when the work was nearly completed. After I had found that the whole reaction was due to the excitation of the pulmonary nerves and after Russell and I had shown how readily cardiac inhibition could be produced by electrical stimulation of these same branches it struck me that the failures just mentioned might be due to the artificial inflation of the lungs. I argued that the forcible inflation might be exciting these fibres so much that they became insensitive to the serum injection. In favour of this idea, we know that if in an animal, with the thorax intact, artificial respiration is established the animal soon ceases to make voluntary respiratory efforts unless the degree of inflation is slight. In my experiments the force of the air-pump used for the lung inflation was kept low and the lungs only distended so far as to nearly fill the thorax. Still the repeated distension of the lungs might be producing so much mechanical excitation of the pulmonary fibres that they became insensitive to the serum. On the other hand the pulmonary fibres might be carrying up such strong impulses to the bulbar centres that the impulses started by the serum could produce no effect. For the purpose of testing these views the following experiments were performed.

[1] See this *Journal*, *infra*.

The chest was opened as if for an operation upon the cardiac nerves, artificial respiration being carried out. Then, while the blood-pressure was being recorded the extent of inflation of the lung was considerably decreased. When this had been carried out for some time and the rate of heart-beat had become steady the amount of air pumped in at each distension of the lung was suddenly increased. This caused considerable inhibition. The amount of air was next decreased, when the rate of beat gradually quickened. Complete and sudden cessation of artificial respiration caused as a primary result acceleration of the heart. These results upon the heart at once showed that its rate could be materially modified by impulses started from the pulmonary fibres of the vagus. Hence as both respiratory and cardiac rhythm can be modified by the same excitation of the pulmonary nerves, it seemed likely that that excitation would materially modify the action of serum when injected, since as we have just seen it acts upon the same nerve terminals. The following experiments were therefore tried. In the first the animal was prepared as for an ordinary injection and a cannula tied in the trachea. A dose of serum was now injected to show that the animal gave a typical reaction. The tracheal cannula was now connected to the respiration pump and, the thorax still being intact,

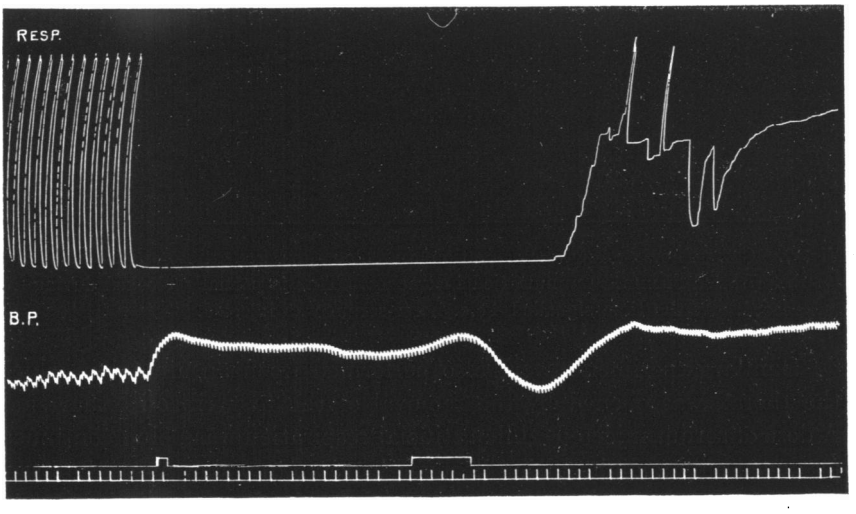

Fig. 6. Cat. Thorax intact. Upper tracing, respiration. Lower = carotid blood-pressure. Zero-abscissa line raised 40 mm. The thorax is being artificially inflated up to the first signal. At the second and during the apnœa 4 c.c. horse-serum was injected. Time record = seconds.

artificial respiration was started, using a degree of distension a little greater than normal. The animal's respiratory movements soon ceased and the heart beat somewhat slower. After a time the artificial respiration was stopped and, during the period of apnœa which followed, a dose of serum injected. It was found to give only a slight fall in blood-pressure and no inhibition of the heart. The effect upon respiration was to produce inspiratory tetanus (Fig. 6). In another experiment the mechanical excitation of the pulmonary fibres was brought about by partially blocking the tracheal cannula, so as to cause marked dyspnœa. The reaction was markedly altered (Fig. 7). Respiration was a little slowed, the rate of the heart was not altered, and there was only a moderate fall in blood-pressure.

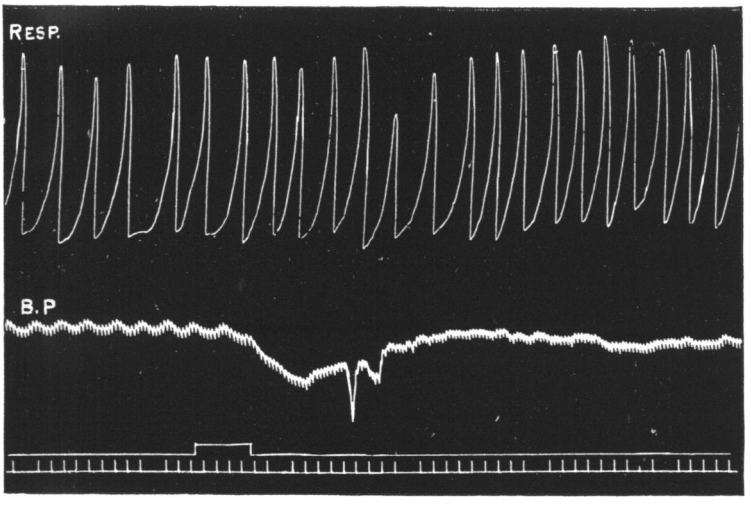

Fig. 7. Cat. Thorax intact. Respiration and blood-pressure. Zero-abscissa line has been raised 35 mm. Cannula tied in trachea. This is partially blocked during the record. At the time signalled 4 c.c. horse-serum was injected.

Similar experiments were also performed upon an animal in which the thorax was opened by the removal of the first rib on either side. A dose of serum injected while artificial respiration was being continued produced only a slight fall in blood-pressure and no slowing of the heart (Fig. 8). In the next place the artificial respiration was diminished in extent until spontaneous respiratory movements appeared. The artificial respiration was then completely stopped, and 30 seconds later serum injected (Fig. 9). The effect produced was a good fall in blood-pressure

and marked slowing of the heart. The effect on respiration was first to accelerate and shorten expiration, and then to produce a tendency

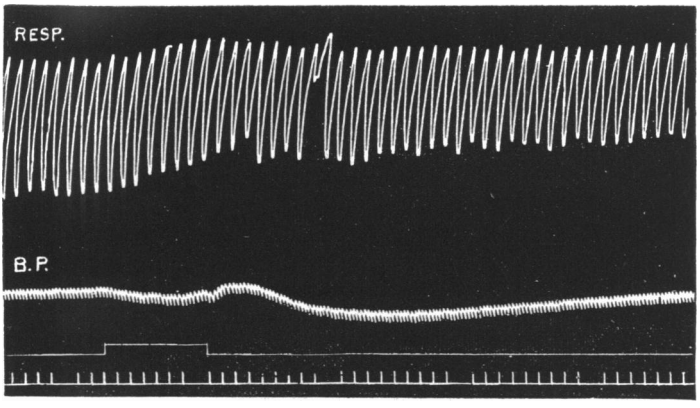

Fig. 8. Cat. Thorax opened at upper end. Respiration and blood-pressure. Zero-abscissa line has been raised 50 mm. The force of inflation of the lungs was only slight and carried out so as to cause the lungs to nearly fill the thorax with each inflation. At the time signalled 5 c.c. horse-serum was injected.

towards expiratory standstill. Artificial respiration then had to be restarted when gradual recovery took place.

These experiments also explained the result which I had usually obtained upon curarised animals. Here again a similar result is produced if the serum be injected while the artificial inflation of the lungs is continued. There is only a fall in blood-pressure, but no effect upon the rate of beat of the heart. If on the other hand the respiration is stopped, and serum injected early in the course of the asphyxia which follows, a quite typical reaction upon the heart is recorded.

II. THE ACTION OF SERUM UPON THE VASO-MOTOR SYSTEM.

In addition to the effect the afferent vagal impulses produce upon the cardiac and respiratory centres, they also act upon the vaso-motor centre inhibiting its tonic action. A serum injection also acts upon the blood vessels apart from this action through the bulbar vaso-motor centre, as is seen for instance in the tracings obtained after both vagi have been divided in the neck.

That there is a reflex action upon the blood vessels through the bulbar vaso-motor centre follows from a study of the following points. In the first place the great fall in blood-pressure produced by an

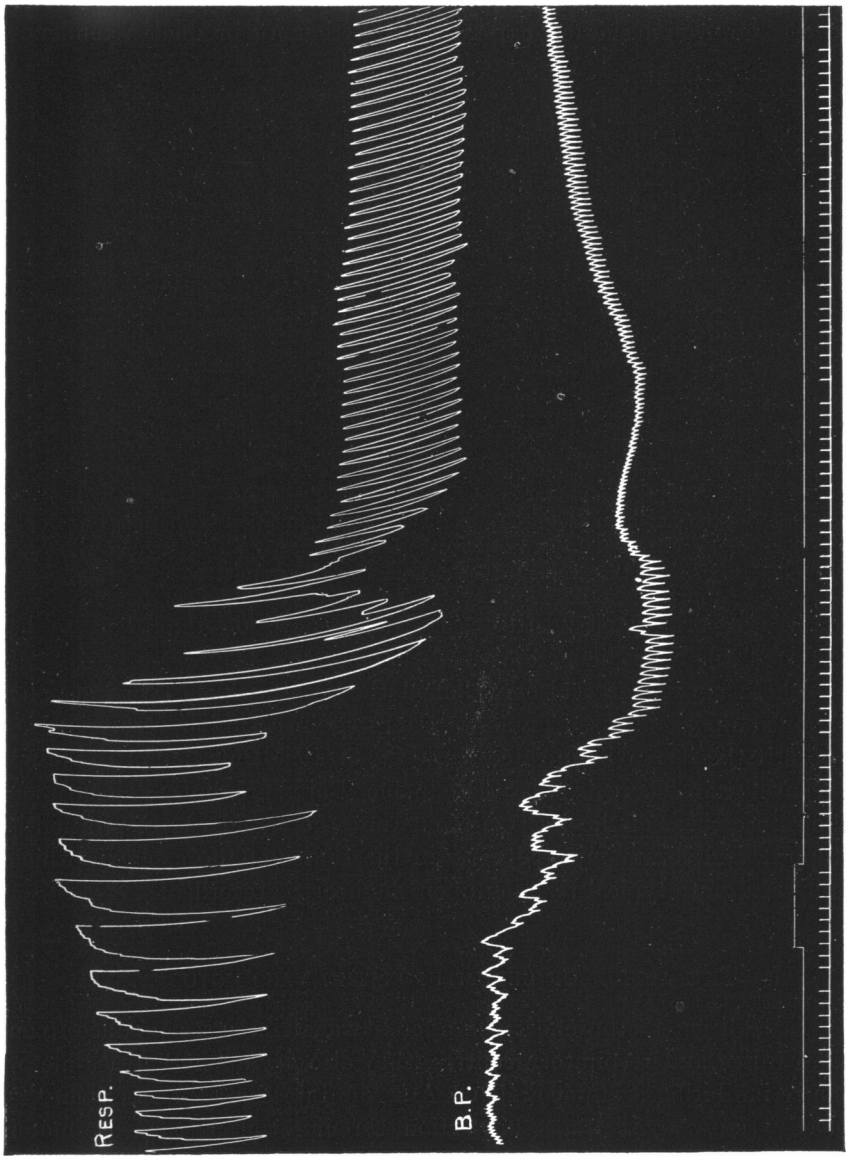

Fig. 9. Cat. Same animal as in Fig. 8. The artificial respiration was stopped 20 secs. before the commencement of the tracing and the animal was making respiratory efforts as indicated by the upper record which gives the abdominal movements. The thorax open. At the first signal 5 c.c. horse-serum was injected. At the second artificial respiration restarted.

injection cannot always be explained by the amount of cardiac inhibition produced. In some animals only slight, and in others no slowing of the heart occurs (Fig. 10), although the blood-pressure falls considerably,

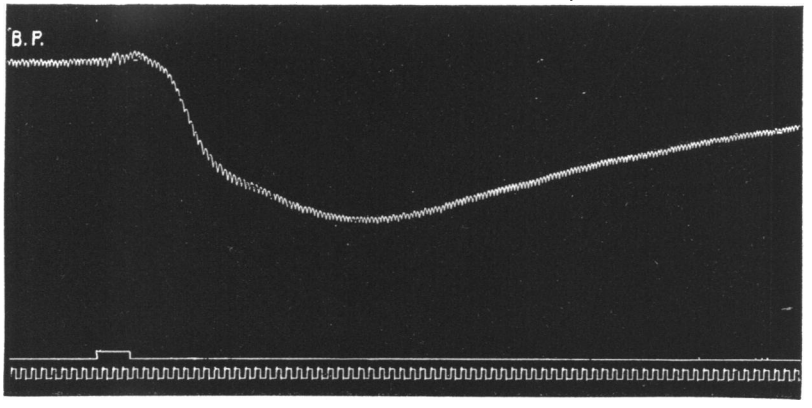

Fig. 10. Cat. Blood-pressure. Injection of 10 c.c. horse-serum. Time-tracing = seconds.

a result which is commonly seen in animals into which several injections of serum have been previously made. In the next place a marked fall in pressure follows an injection made after all the cardiac branches of both vagi have been divided, thus eliminating the effect due to inhibition (Fig. 4).

Again, in animals in which inhibition has been prevented by a preliminary injection of a small dose of atropine, serum still produces a marked fall in pressure (Fig. 12). The fall in pressure in these two instances might be due to a direct action of the serum upon the heart muscle. This influence is however practically negligeable, for oncometric records of the heart-beat show but very slight changes following an injection. Moreover, in either of the two cases just mentioned, in which cardiac inhibition is avoided, if the vagi be divided in the neck, a serum injection now produces a much smaller fall in pressure than that occurring before the division of the nerves.

I have also studied the action upon the blood vessels directly by means of the plethysmographic method. The intestinal plethysmograph shows a dilatation occurring with the fall in blood-pressure (Fig. 11). The fall of pressure, some of which is of cardiac origin, would in itself cause a diminution in volume of the intestine, but the dilatation of the vessels is sufficient to more than counterbalance the effect due to the heart.

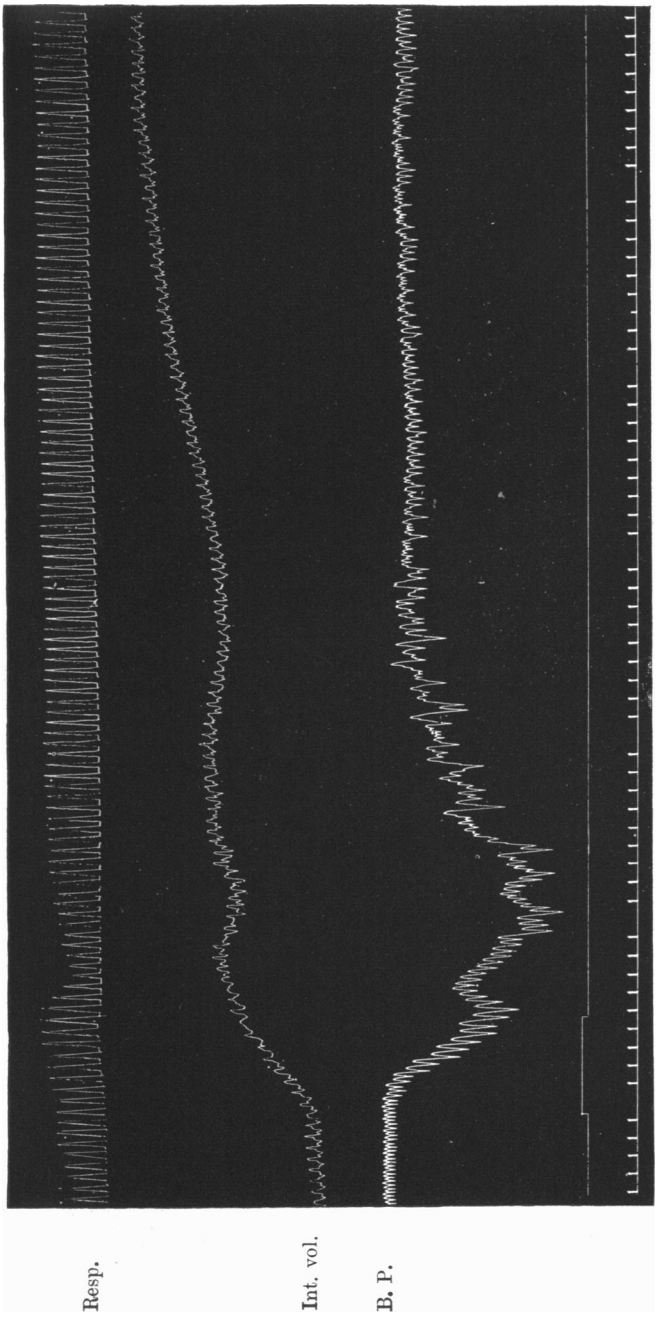

Fig. 11. Cat. Upper tracing = respiration. Second = volume changes of small intestine. Lower = blood-pressure. Injection of 5 c.c. dog's-serum. Time-tracing = seconds.

After the heart has regained its initial rate, the intestinal vessels still show a very decided dilatation. Plethysmographic records of the heart prove that at this stage the output of the heart is quite at its original value. In a few experiments I have found that the intestinal tracings record no alteration in volume, *i.e.* the volume decrease due to the fall in pressure is exactly counterbalanced by the increase due to vaso-dilatation.

If the record be taken upon an atropinised animal, the action on the blood vessels stands out still more clearly. Thus Fig. 11 shows a very marked dilatation of the intestinal vessels which commences a little earlier than the fall in blood-pressure. This tracing is of further interest, for it records an effect upon respiration which is to be frequently observed in atropinised animals. Instead of the respiration becoming arrested, it is quickened and becomes a little shallower. To obtain arrest a much larger dose of serum must be injected.

That most of the vaso-dilatation is due to an action upon the vaso-motor centre follows from the record of the effect produced by a larger dose of serum after both vagi had been divided in the neck.

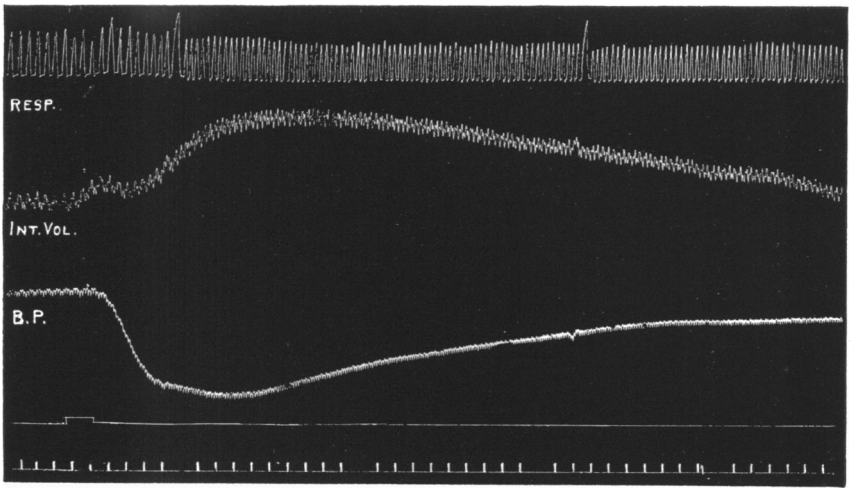

Fig. 12. Same animal as in Fig. 11, but 1·5 c.c. of a 0·5 % solution of atropine has now been injected. At the signal 5 c.c. of dog's-serum was injected.

Most of the effect upon the intestine is in this way abolished, but there is still some fall in blood-pressure. The fall in pressure rarely exceeds that seen in Fig. 2, p. 52, and the effect upon the intestinal plethysmograph is in proportion.

From plethysmographic records, taken after division of the vagi in the neck, one can also show that the fall in pressure which now occurs is almost entirely due to vaso-dilatation. This is the case in atropinised animals as well as in those which have not received a dose of the drug. I have been able to exclude the possibility of the effect being due to a direct action of the serum upon the heart by recording the volume changes of the heart during an injection. The volume remains nearly unaltered, only a slight diminution in output per beat, and no alteration in rate taking place.

As to how the serum acts when both vagi are divided, I have not been able to decide. It is not a direct action of the serum upon the vaso-motor centre in the medulla, for an injection into the carotid does not increase the effect, it only delays its onset.

In studying the behaviour of the vaso-motor system after an injection of serum, other organs, *e.g.* the limbs, the lung, the testis, have been examined plethysmographically but they all, with one exception, only confirm the results that have been described above. This exception is the kidney, which behaves in a totally different way. Instead of dilating as a result of serum injection the kidney diminishes in volume. This decrease in volume is due to an active constriction of the kidney vessels, for if the tracings are examined it is seen that the waves due to the heart-beat are considerably diminished in extent and in most cases completely obliterated. Moreover, as a rule, the decrease in volume does not correspond to the variation in blood-pressure, for the constriction usually persists much longer than the fall in pressure, in some instances it continues to increase more than a minute after the blood-pressure has quite recovered. This prolonged constriction is followed by a slow relaxation which may extend beyond that recorded before the injection was made. If the kidney is watched during these changes the constriction of its vessels is easily followed, for marked pallor is produced. The result of an injection into an atropinised animal also shows this change well (Fig. 13). From this tracing it will be seen that the volume changes do not coincide with the fall and rise of blood-pressure though in this particular instance this is not so marked as is frequently the case. The heart-beats on the kidney tracing are also seen to greatly diminish with the decrease in volume. Later in the same experiment an injection, after both vagi had been cut in the neck, caused only a slight fall in blood-pressure and the kidney volume scarcely changed. The result is not always so marked but all lead to the conclusion that the constriction of the

kidney is a reflex and is due to an excitation of the vaso-motor centre in the medulla.

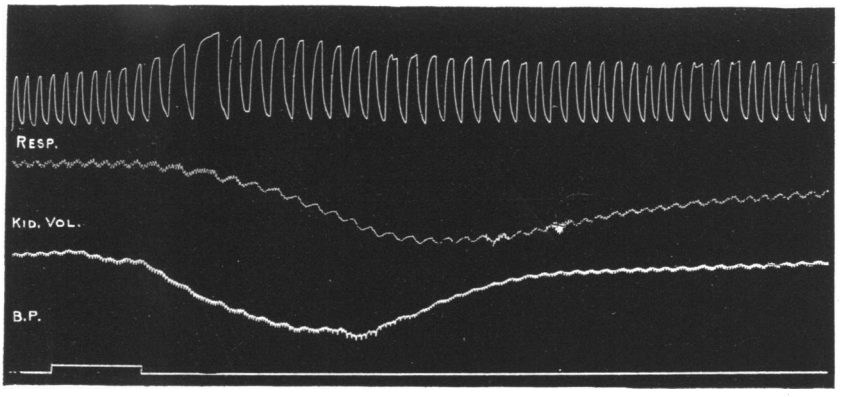

Fig. 13. Cat. Respiration. Volume changes of R. kidney. Blood-pressure. The animal has been previously injected with 2·0 c.c. of a 0·5 % solution of atropine. Injection of 5 c.c. horse-serum.

That the kidney vessels should behave in the opposite sense to the intestinal vessels is a fact of considerable importance. It has been observed among others by Halliburton and Mott[1] for cholin and neurin and by Dixon[2] for spermine, though it was not shown to be due to an action on the medullary centre. The result I have obtained modifies the general conclusion drawn by Porter and Beyer[3] as to the action of the bulbar vaso-motor centre. They consider that all afferent impulses which act upon the bulbar vaso-motor cells influence them all alike and that this centre therefore plays no part in the distribution of the blood to the several organs and regions of the body. My experiments indicate that this is not always true and that the kidney may prove an exception. Further experiments upon this point are now in progress.

III. Effect of repeated injections. Production of a condition of immunity.

Early in the course of these experiments I found that, if several injections were made into the same animal, the later injections produced a much less marked effect than the first. The more frequent

[1] *Phil. Trans.* B. CXCI. p. 211. 1899.
[2] This *Journal*, XXV. p. 356. 1900.
[3] *Amer. Journal of Physiol.* IV. p. 283. 1900.

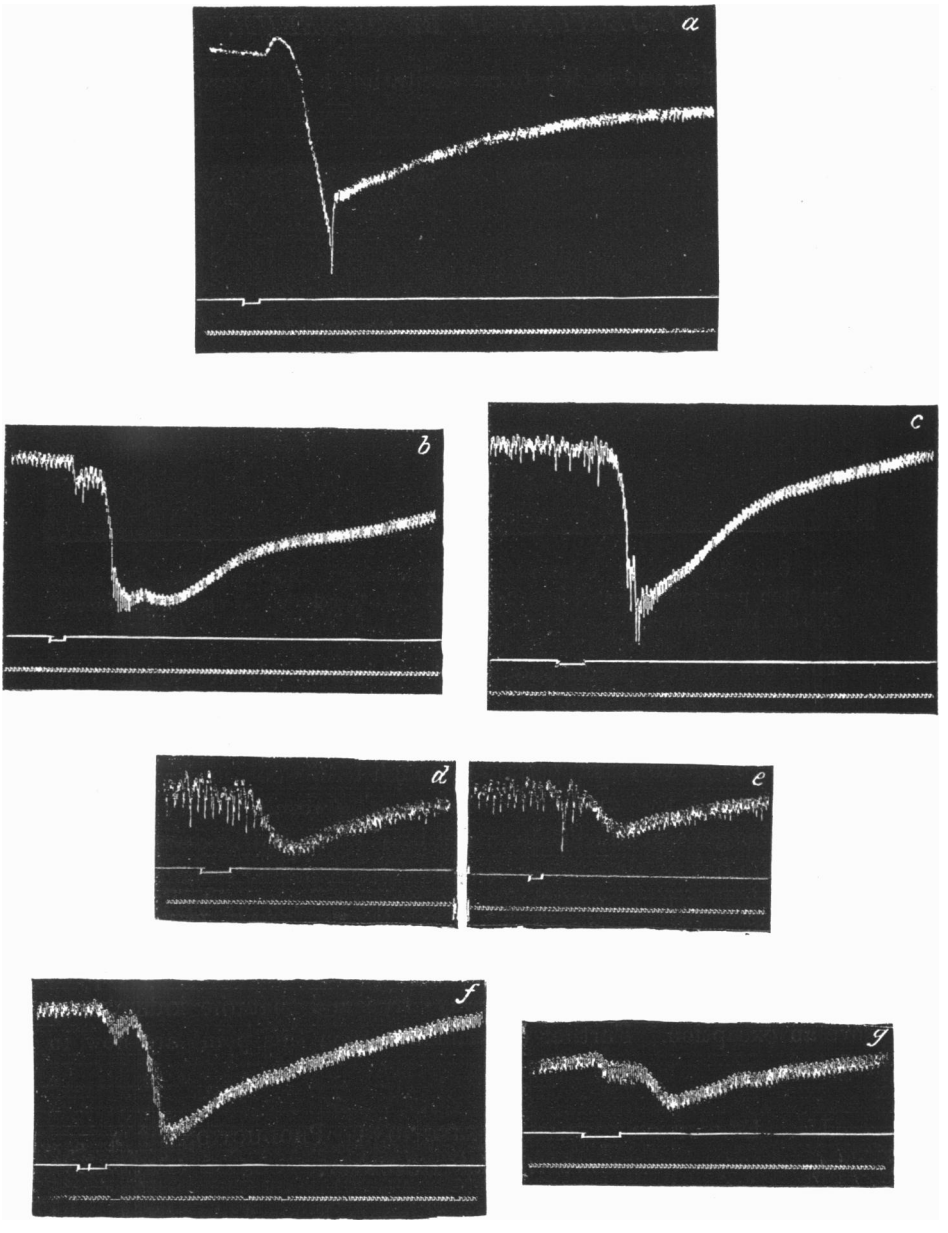

Fig. 14. Cat. Blood-pressure records. A series of successive injections of 5 c.c. horse-serum. In each case the blood-pressure started at approximately the same level. The first injection at 3 hrs. 0 min. p.m. Tracing (*a*). The respiration was at once arrested. The second injection (*b*) at 3.7 p.m. The respiration was arrested. The third (*c*) at 3.9 p.m. The respiration became slower and deeper but was not arrested. The fourth (*d*) at 3.12 p.m. The respiration was slightly slowed. The fifth (*e*) at 3.13 p.m. Respiration not affected. The animal was now kept under the anæsthetic for 40 min. and then a sixth injection made (*f*) at 3.53 p.m. Respiration was slowed but not arrested. A seventh injection (*g*) was made at 3.55 p.m. Respiration unaffected.

the injections the more pronounced was this result. The reaction which first disappeared was nearly always that upon the respiration. Either the respiration was only slowed and diminished in depth, or in other cases no effect was produced. Later the effect upon the heart became modified, the inhibition being less marked and lasting a shorter time, until in still later injections no action upon the heart could be distinguished. Even in these cases however some of the effect upon the vaso-motor centre was still observable, though this too was of smaller extent.

The tracings (Fig. 14 *a*—*e*) show the result upon blood-pressure of a series of five successive injections. These were made at short intervals and it is seen from the tracings that the reaction gradually disappears. There was then an interval of 40 minutes, at the end of which an injection produced the result seen in Fig. 14 *f*. Thus, during this period of rest the animal had to a considerable extent regained its first condition as to this reaction, but it is seen from the next tracing (Fig. 14 *g*) in which a further injection of serum was made two minutes later that it very soon returns to its non-reactive state.

Injection of serum therefore sets up a condition of immunity. This immune stage lasts for some time and is followed by a second in which a single injection at once causes a return to the immune condition. The length of this second period extends, so far as my present observations have shown, at least two hours. I have not been able to observe any alterations in the blood during this period either with respect to its rate of coagulation, or in its corpuscles. The condition of immunity is apparently due to an alteration in excitability of the pulmonary nerve endings.

As previously mentioned there are several other bodies, chiefly of a proteid nature, which produce the same result as serum when injected into the veins of a cat, and I have similarly found that they also are able to produce an analogous immune condition. Further, I have found that if immunity be produced by one substance, that animal also gives, as a rule, no reaction to the other substances which act similarly.

The other two animals I have tested, viz. the dog and the rabbit, are naturally immune. The reaction given by a dog for instance very closely resembles that obtained in the cat when both vagi have been divided in the neck (Fig. 2). An injection of serum into a dog causes a slight rise of blood-pressure lasting about 10 seconds and followed by a more marked but still slight fall which lasts about 25 seconds[1]. There

[1] The variations are exactly like those reproduced in Fig. 5.

is no alteration in the rate of heart-beat nor in the respiration unless very large doses are injected. Section of both vagi in the neck in no way modifies this reaction.

IV. Which constituent of serum is it which produces the reaction?

The second series of experiments in connection with this investigation were devised with the object of ascertaining whether there was only one constituent in serum which was the active body, and if so what that constituent was.

In attempting to determine this the following points were proved:

(*a*) *The active substance is a proteid.* It was found that, if serum was boiled and the resulting coagulum filtered off, the filtrate gave no reaction. It was thought that possibly the active substance was the nucleo-proteid of serum. To test this, serum was diluted five-fold and weak acetic acid added. The resulting precipitate of nucleo-proteid and globulin was collected, dissolved in a small bulk of 1 % sodium chloride, and the solution injected. It was found to be quite inactive, while the filtrate from the original precipitate was as active as before precipitation.

(*b*) *The active substance is a proteid of the albumin class.* This was ascertained by half-saturating serum with ammonium sulphate and filtering off the precipitated globulin, which was then dissolved and again precipitated. This second precipitate was collected, dissolved and injected with negative result. In a control experiment, the injection of ammonium sulphate of corresponding strength was proved to produce no result. Some of the filtrate from the globulin precipitate was diluted, in order to lower the percentage amount of ammonium sulphate, and then injected. It gave the usual reaction. The rest of the filtrate was therefore saturated with ammonium sulphate and the precipitated albumins collected, and dissolved. This solution was found to be active, whereas the filtrate was quite inactive.

The active substance is therefore to be found among the albumins. A further difficulty was however met with here. For the purification of the albumins, solution and precipitation was repeated four times. The final solution was found to be nearly inactive. The filtrates obtained in the process of purification were therefore dialysed but were found to be quite inactive. Dialysed serum is however just as active as the original serum. The addition of salts to the purified albumin

solution produced no effect on the general result. Hence it seems that the active material is some more complex combination of the albumin, and that it is broken down by repeated precipitation with ammonium sulphate.

(*c*) *The active substance is coagulated at a temperature of* 78° C. In order to determine the temperature at which the active substance is destroyed, serum was diluted with an equal volume of water, rendered just acid with acetic acid and heated. It was kept at a definite temperature two minutes, filtered if necessary and injected. These filtrates were always active until those were reached which had been heated to or above 78° C.

A further experiment of this class was carried out for the following reason. As previously mentioned (p. 49) Friedenthal and Lewandowsky found that if serum be heated for an hour to 58° C.—60° C. it loses its toxic properties. In order to test whether the substance producing the reaction described in this paper is the same as that which

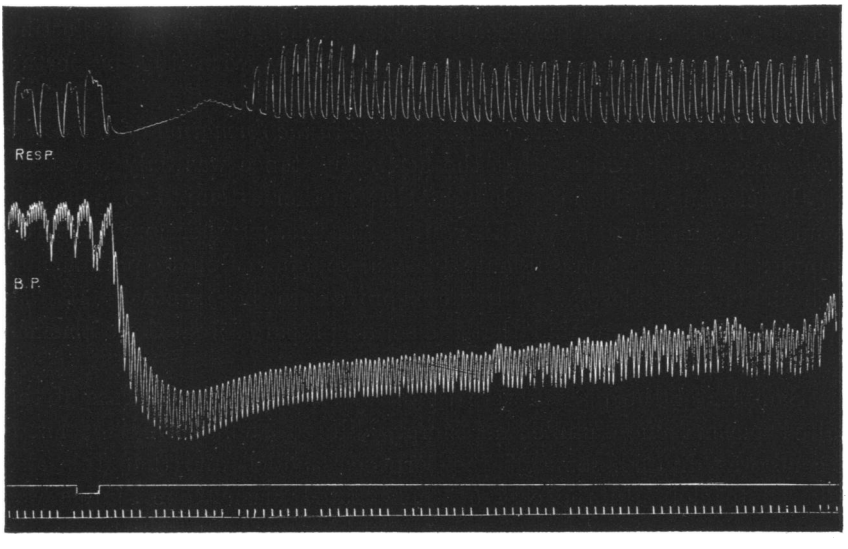

Fig. 15. Cat under urethane. Respiration and blood-pressure. The prolonged inspiration seen at the beginning of the record is due to the urethane. At the signal 4 c.c. of cat's-serum, which had been previously heated to 59° C. for one hour, was injected.

kills an animal when subcutaneously injected I treated serum in the manner they describe and then injected it. A typical reaction was however produced (Fig. 15), so that the two substances are apparently different.

(*d*) *The active substance is produced during the process of clotting.* Having found that serum from any animal, including the cat, was active I determined to try whether whipped blood taken from the same animal would also prove active. The animal being therefore prepared in the usual way, a sample of blood was collected from the carotid. This was whipped and immediately injected. It gave a perfectly typical reaction (Fig. 3). The time elapsing between the collection of the blood and its injection into the vein never exceeded two minutes. Having obtained this rather surprising result I then prepared the opposite carotid, tied in a cannula and connected it to the cannula in the jugular vein. The blood from the artery was then allowed to flow directly into the vein with completely negative results. Hence it follows that the active substance is only produced during the process of clotting. This fact was further corroborated by many other experiments as will be presently described. The result suggested that the active substance might be the proteid split off from fibrinogen during fibrin formation. The one body at present known is Hammarsten's fibrino-globulin. This however is a body of the globulin class, clotting at 64° C. and therefore disagreeing markedly with the properties previously found for the active substance.

(*e*) *The corpuscles take an active part in the formation of the active substance.* After finding that the active substance was only produced on the clotting of blood, I thought that further attempts to isolate it would be simplified by working upon plasma. I therefore prepared samples of magnesium sulphate plasma, sodium sulphate plasma and sodium citrate plasma. All these proved to be inactive. It was surprising however to find that the serum prepared from these plasmas was, in nearly all instances, also inactive.

Samples of the plasma were therefore taken and in the case of the salted plasma one sample was simply diluted and allowed to clot, a second was diluted, some of the corpuscles added and then this also allowed to clot. In the case of the citrated plasma one sample was treated with calcium phosphate and brought to body temperature, a second sample being mixed with some of the corpuscles, and then, by similar treatment, made to clot. In the majority of such experiments it was now found that the serum from the pure plasma was inactive, whereas the serum from the plasma admixed with corpuscles was as a rule active. In a few experiments when adding the corpuscles those were chosen which formed the upper layer of the sediment of corpuscles and contrasted with a similar sample taken from the lowest layers.

I was not however able to make out any difference in the behaviour of the serum obtained, although microscopic examination proved that the upper layers were much richer in white corpuscles and in blood-platelets. Although from the many experiments of this kind that I have made, I feel sure that the statements just given are in the main correct, yet I have met with several exceptions which have proved very puzzling. Thus at times a serum prepared from the plasma above has proved active, and on the other hand a serum formed from a plasma mixed with corpuscles has on a few occasions proved inactive. I have never found a serum or a defibrinated blood obtained in the ordinary way which has proved inactive. I have also tested hydrocele fluid. The sample I obtained had clotted before I received it. The clot was quite colourless, there was no sign of any admixture with blood, yet the serum when injected proved typically active. Unfortunately neither the clot nor the serum was examined for white-corpuscles and platelets.

In regard to these latter points the experiments are incomplete and though I contemplate continuing them in this direction, other work prevents their renewal now, and I have therefore thought it best to publish the results as they at present stand.

Conclusions.

1. Intravenous injection of blood-serum from any source into a cat causes arrest of respiration, inhibition of the heart and vaso-dilatation. The effects persist for some time.

2. These effects are reflex, and are almost entirely absent if the vagi be previously divided in the neck.

3. The effect is due to excitation of the pulmonary nerves. It is cut out by division of the pulmonary branches of the vagus, not by division of the cardiac or lower branches.

4. Repetition of the injection leads to the production of an immune state.

5. The active substance in the serum is a proteid. It is of the albumin class and is coagulated on being heated to 86° C.

6. The active substance is only produced when the blood clots.

7. The interaction of the blood corpuscles is a necessary condition for its formation. Serum obtained from plasma is inactive.

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