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THE ACTION AND FATE OF INJECTED POSTERIOR PITUITARY EXTRACTS IN THE DECAPITATED CAT

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Since their discovery the effects produced on the blood pressure by injections of extracts of the posterior lobe of the pituitary body have been recognized as remarkable for their long duration. Not only does the blood pressure remain raised for a considerable period, but the animal acquires a tolerance, so that a second injection may have a smaller effect; this phenomenon was first described by Howell [1898]. Since then it has come under review by a number of authors, more particularly as an obstacle to making use of the pressor response for standardizing extracts. Thus Dale and Laidlaw [1912] were induced by its existence to turn their attention to the oxytocic activity, and they devised the method of assay with the isolated uterus of the virgin guinea-pig, and this is now probably the method most extensively employed. Hogben, Schlapp and Macdonald [1924], in elaborating their method of pressor assay on the spinal cat, were able to show that for small doses up to 2 mg. of dried posterior pituitary substance, the period of tolerance did not exceed 1 hour.

Little is known concerning the factors responsible for the tolerance, but Dale and Laidlaw [1912] surmized that it might be the consequence of a slow disappearance of the active principles from the blood. On the other hand, it seemed possible that an effect on the blood volume was to some extent concerned. Underhill and Pack [1923], giving "pituitrin" intravenously to dogs, found a 20 p.c. reduction in hæmoglobin occurring both with and without the simultaneous administration of water.

In the experiments to be described here, the disappearance of the active principles from the blood has been followed, its relationship to the tolerance investigated and the effect of the principles on the blood volume is examined. The excretion of the pressor principle in the urine is studied quantitatively, and the destructive action of tissue extracts on the oxytocic and pressor activities demonstrated.

Experimental

The experiments were performed on cats. Under ether anæsthesia the carotid arteries were tied in the neck, and the vagi cut; the spinal cord was divided through the occipito-atlantal space after the application of the vertebral clamp described by McDowall [1930]. The brain was destroyed. The anæsthetic was then discontinued and artificial respiration supplied from a Palmer "Ideal" pump, by means of a tube tied into the trachea. The blood was rendered incoagulable by the injection into the femoral vein of 200 mg. of chlorazol-fast pink dissolved in the minimum quantity of water. This procedure has been found to be without effect on the phenomena with which this paper deals. Moreover, in experiments in which a record of the blood pressure was taken it was possible to substitute saline for the anti-coagulant solution usually employed in connecting the artery to the mercury manometer, a procedure which obviates the passage of foreign substances into the circulation during a fall of blood pressure.

The posterior pituitary extracts were prepared from fresh beef glands placed in acetone at the slaughter house. They were immediately transported to the laboratory where the posterior lobes were dissected out, cut up and placed in fresh acetone for a few hours; they were next transferred to ether for 24 hours and dried at 37° C. overnight. After grinding up, the powder was extracted in a Soxhlet apparatus with absolute ethyl alcohol which had been distilled, with a reflux condenser, over metallic calcium. The powder was again dried at 37° C. and finally ground up so that it would pass through gauze; it was preserved in a vacuum desiccator over calcium chloride. 200 mg. of this powder in 15–20 c.c. of distilled water with three or four drops of 5 p.c. acetic acid in a boiling tube were placed in a boiling water bath for 2 min. The extract was filtered and made up to 20 c.c. with distilled water. In all the experiments the extract used was of this strength (unless it is stated to be otherwise). It was kept in sealed ampoules each containing 5 c.c. and could, provided it was sterile, be preserved for a considerable period without loss of activity.

The assays of oxytocic activity were made by the guinea-pig uterus method of Burn and Dale [1922], but the size of the organ bath was reduced. The pressor assays were made by the method of Hogben, Schlapp and Macdonald [1924].

The disappearance from the circulating plasma

In preliminary experiments it had been noticed that the plasma of blood withdrawn at varying times after the injection of posterior pituitary extract possessed the less oxytocic power the longer after the injection it was withdrawn. In order to determine the rate of disappearance the amount present at various times after the injection was assayed. As the blood volume could not be determined experimentally for each cat the precise initial concentration of the active substances in the blood was unknown. It was therefore necessary to make assays at various times, comparing the amount present at each time with a standard. This standard was withdrawn 30 sec. after the injection into the femoral vein of 1 c.c. of extract per kg. body weight. As blood volume varies with body weight the initial concentration would be approximately the same in all cases, mixing being assumed to be complete in the 30 sec. The withdrawal of the 20 c.c. of blood involved was effected through a tube tied into the stump of a carotid artery. The cat was later exsanguinated through the abdominal aorta. The blood thus obtained and the standard sample were centrifuged at high speed and the incoagulable plasmata were compared for oxytocic and pressor activity.

The small amounts of the samples available made simultaneous assays of pressor and oxytocic activities difficult, and the majority of the estimations were carried out in separate animals. It may be seen from the results which are given in the form of a graph (Fig. 1) that the pressor and oxytocic activities disappear from the plasma at the same rate. It might seem at first sight that at the longer time intervals the pressor activity lags behind the oxytocic as regards disappearance; but this is due to the large bulk of plasma which has to be injected in these assays. For the plasma itself in bulk produced a perceptible pressor effect. In two cases in which it proved possible to perform pressor and oxytocic assays on the same sample, and in which the amount of plasma injected in the pressor assays was rigidly controlled, the correspondence was satisfactory, as may be seen from the points marked *C* on Fig. 1.

A number of experiments were undertaken in order to follow the last traces of the active substance in the plasma. Thus after 1 hour it was found that there might be from 3 to 5 p.c. of the oxytocic activity of the standard sample remaining in the plasma; after 2 hours there remained no oxytocic effect. It proved impossible to repeat these experiments with the pressor activity, as the effect in the assay was completely masked by the pressor effect of the bulk of plasma which had to be injected. In view

of the fact that 85 p.c. of the activities disappear simultaneously it would be unreasonable to suppose that the last 15 p.c. do otherwise unless positive evidence to that effect were forthcoming.

Before finally accepting the view that the pressor and oxytocic activities disappear simultaneously from the plasma, it was necessary to show that the procedure of injecting the pituitary extract and the subsequent withdrawal of the 20 c.c. standard sample did not produce a degree of dilution of the plasma which would account for any significant

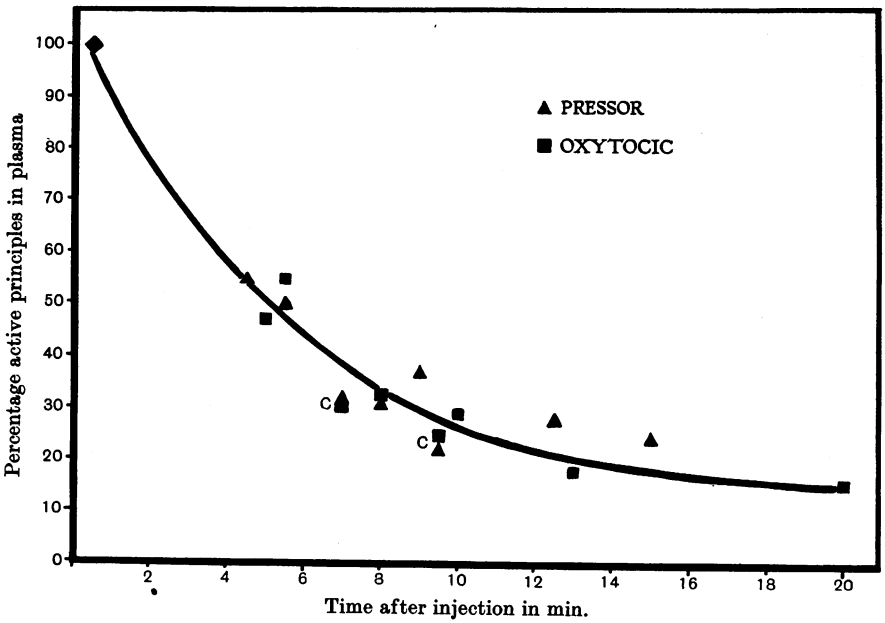

Fig. 1.

part of the results. In a series of experiments with the hæmatocrite undertaken to investigate this possibility, it was found that the average dilution occurring in the time involved was 5 p.c. with a maximum of 8·5 p.c. But even this maximum degree of dilution is insufficient to account for any significant part of the disappearance and in any case it lies within the error of the methods of assay employed.

The period of tolerance

Having determined the time during which the active substances circulate in the plasma it was obviously of interest to find out whether this time corresponded to the duration of the tolerance. But certain

difficulties arise in the estimation of the period of tolerance. There is no test for the sensitivity of an animal to the pressor principle other than the injection of an extract intravenously. If the sensitivity is tested in this way, and the response has not returned to normal, no further observation can be made on that animal with that dose, as the tolerance due to the second will obscure that due to the first. On the other hand, if the response has returned to the original level then this condition may have existed for any time before the second injection. Further, the return of

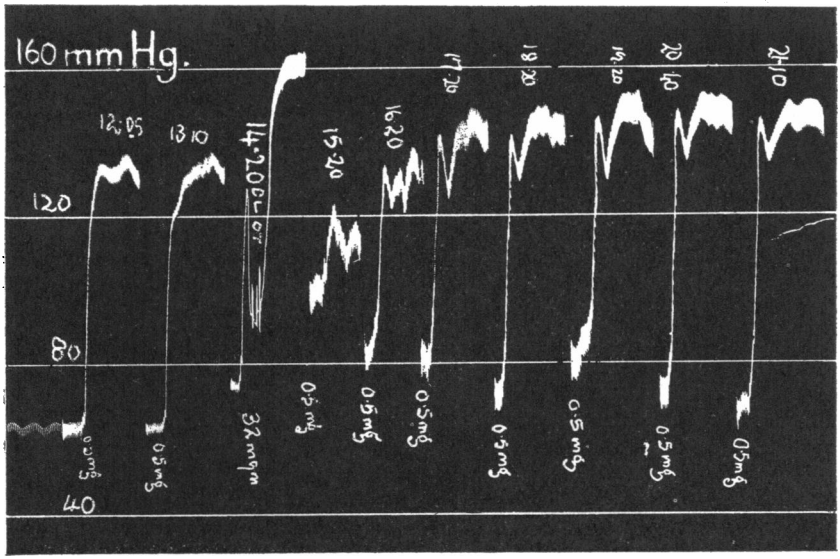

Fig. 2. Successive hourly injections of 0·5 mg. of dried gland. The response is constant before the injection of the "standard" dose and returns to normal 3 or 4 hours later.

the response to the original level is not necessarily indicative of the complete recovery of the animal, for it is not unusual for the sensitivity to rise above the original level after a dose of the size used. In our experiments the period of tolerance has been taken to be the time which elapses after the injection before a constant response is obtained to small doses injected at hourly intervals, for recovery from the latter would certainly be complete in 1 hour as has been shown by Hogben, Schlapp and Macdonald [1924].

The difficulties outlined above indicate that only an approximate estimate of the duration of the period of tolerance can be made. The details of the method are as follows. Very small doses of the extract

(0·05 c.c. suitably diluted) were injected at hourly intervals for 2 or 3 hours, and then a large dose of 1 c.c. per kg. This was followed by the hourly injections of the original small doses, starting 1 hour after the injection and continuing until the response had reached a constant level. This occurs 3 to 4 hours after the large injection (Fig. 2). The small

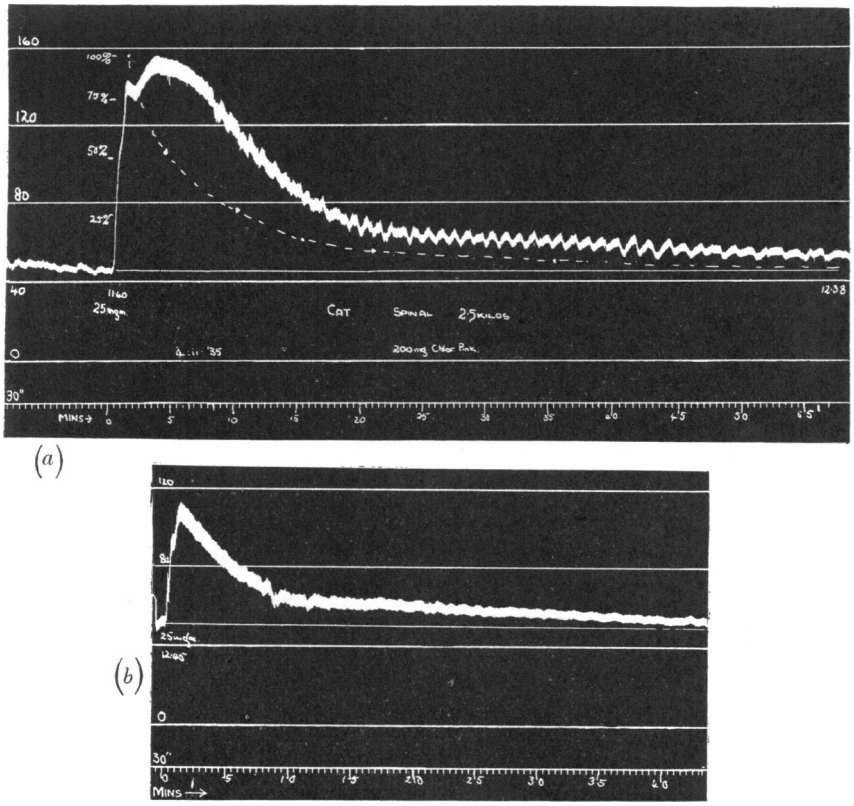

Fig. 3. Successive injections of the "standard" dose at 1-hour intervals. A considerable degree of tolerance is shown in (*b*).

injections 1 hour and 2 hours after the large injection must have contributed to the period of tolerance, but it is clear that the response had returned to normal in less than 4 hours. The experiment was therefore repeated omitting the small injections 1 hour after the large one. The response had returned to a constant level in 2 hours, so that the duration of the period of tolerance cannot exceed this; the tolerance after 1 hour has however been found to be considerable (Fig. 3). These experiments

show that the period of tolerance to the dose used is between 1 and 2 hours, and suggest that it corresponds to the time during which the active substances can be detected in the circulating plasma.

It is interesting to observe that the time during which the blood pressure remains raised above the normal corresponds roughly to the period of tolerance and to the period during which the active principles

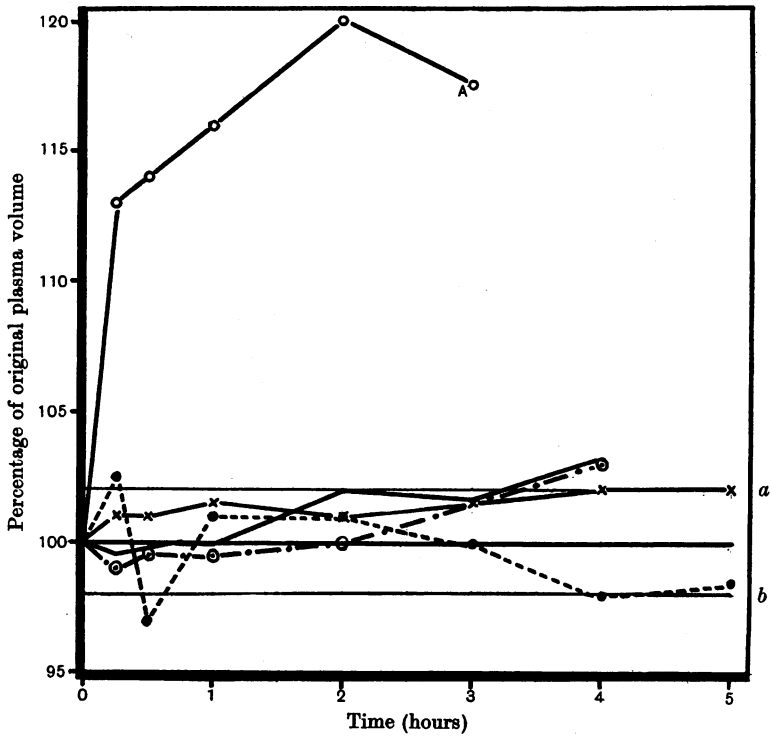

Fig. 4. The lines *a* and *b* give the limits of normal variation.

are circulating. This can be seen in Fig. 3 where the dotted line shows the rate of disappearance of the active substances from the plasma. But the blood pressure during the period of tolerance is always less than it would be were the amount of the active substances circulating in the plasma at the time injected into a fresh animal. It would seem, therefore, that the tolerance may be regarded as a mechanism of adaptation to the slow rate of disappearance of the active substances from the plasma. The phenomena of tolerance apply equally to the original dose and to subsequent doses injected during the time the active principles are circulating.

The dilution of the blood, which has been stated by Underhill and Pack [1923] to occur both with and without the simultaneous administration of water, seemed to have a duration corresponding to that of the probable period of tolerance and, as it might have turned out to be a factor in the production of the latter, it seemed desirable to undertake some experiments to investigate this phenomenon.

In a number of cats, prepared as has been described, standard doses of 1 c.c. of pituitary extract per kg. were injected, and the relative volumes of plasma and corpuscles determined by means of a hæmatocrite over a period of from 3 to 4 hours. The results of four experiments are given graphically in Fig. 4, and they show that no appreciable dilution takes place. Such a result cannot however be attained without taking the greatest care to avoid procedures of a kind which would of themselves tend to produce dilution. Among these are the injection of the extract in a dilute solution, and the withdrawal of unnecessarily large quantities of blood for the hæmatocrite determinations. If, as was the case on one occasion, an attempt is in addition made to follow the tolerance by hourly injections of extract and a simultaneous recording of blood pressure, further means of producing dilution of the blood come into operation, and such a dilution may easily take place as can be seen from curve *A* in Fig. 4. It is not disputed, of course, that dilution of the blood takes place if water be given *per os*, and an explanation of this has been advanced by Smirk [1933]. But it is evident that the injection of posterior pituitary extract alone does not produce a degree of dilution which would account either for the raised blood pressure or for the tolerance.

The fate of the active substances

The disappearance of the active principles from the plasma of the circulating blood may be due (1) to their destruction there, (2) to their entrance into the corpuscles, or (3) to their passage into the tissues.

The first of these possibilities is definitely excluded by the fact that the incoagulable plasma may be incubated at 37° C. for 2 hours with the active principles without any appreciable destruction of the activities. The plasma with the extract added to it possesses the same activity whether the addition is made before or after the incubation.

On the other hand, the second possibility cannot be entirely excluded. It has been found that when whole blood, rendered incoagulable as has been described above, is incubated for 2 hours at 37° C., with the extracts, and when care is taken to ensure mixing by frequent stirring, there is a small diminution of the activity of the plasma as compared with

that of blood to which the extract was added after incubation. The results hold equally for pressor and oxytocic activities and the loss of activity may amount to between 20 and 30 p.c. It is not intended to deal here with the mechanism of this disappearance. But it may be said that in preliminary experiments it has been shown that laked blood corpuscles possess the power of destroying the active principles. The disappearance from the circulating plasma may thus in part be due to their passage into, and destruction in, the blood corpuscles.

It is impossible to say to what extent the phenomenon takes place *in vivo*. The concentration throughout the *in vitro* experiments was considerably higher than the initial concentration *in vivo*, where the rate of loss of activity has been shown to be dependent on the concentration. In view of the small loss of activity on incubation with whole blood, and the fact that there is evidence that the greater part of the active principles leave the plasma quite rapidly it is unlikely that the corpuscles are of paramount importance as a factor in the disappearance.

The fact that at least a portion of the pressor principle leaves the blood stream in an active state was discovered by Dale [1909] when he showed that the urine acquires a pressor activity after the injection of posterior pituitary extract. There are, however, no quantitative data available, and in the experiments now to be described an attempt has been made to determine to what extent urinary excretion plays a part in the elimination of the active principles.

Excretion of pressor principle in the urine

Decapitated cats were used and chlorazol-fast pink was injected in about half of them; this dye appears to have no effect on the rate of excretion. The urine was collected by means of a tube in the urethra. All tubes, vessels and instruments were sterilized before use. The bladder was emptied before the injection of the standard dose, and the collected urine was used as a control. The extract was then injected and the bladder again emptied after the desired time. The second sample was assayed against the control urine to which known amounts of the extract were added.

The assay of the oxytocic power of the urine was found to be impracticable. The control urine possessed an oxytocic activity equivalent to from 30 to 80γ of dried gland per c.c., and it was not possible to separate this activity from that due to the oxytocic principle. In addition, this content varies from time to time so that in some cases it would be impossible to carry out even an approximate assay.

The same difficulties did not arise with the pressor assay. Normal cat's urine contains both pressor and depressor substances, the nett effect being slightly depressor. But the effect is a negligible one compared with the effect of the excreted pressor principle (Fig. 5). The urine containing the excreted pressor principle was assayed against a urine with added posterior pituitary extract. The former was in all cases withdrawn 2 hours after the injection of the extract, by which time all the active substances are known to have left the blood.

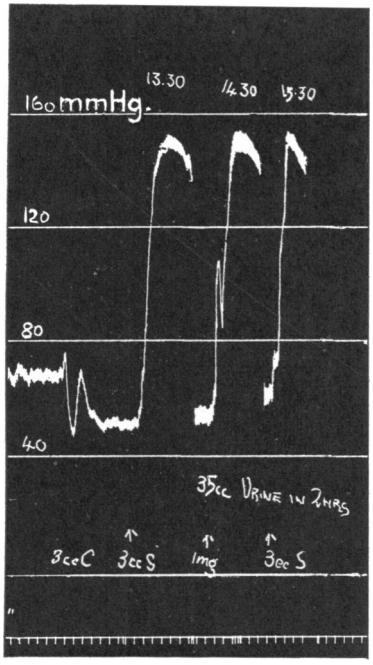

Fig. 5. *C* = control urine. *S* = urine excreted in 2 hours following the injection of the "standard" dose. The assay is made against 3 c.c. of control urine made up to contain 1 mg. of dried gland.

On the average, 28 p.c. of the pressor activity appeared in the urine in nine experiments (Fig. 6). The highest figure obtained (Fig. 6) was 39 p.c. It is concluded that approximately one-third of the pressor activity appears in the urine.

It seemed worth while to determine whether the urine itself had a destructive action on the pressor principle. For it was possible that more than one-third was thus excreted into the bladder, but that some

of it was destroyed in this viscus during the 2 hours following the injection. No disappearance of pressor activity could be detected however when normal urine was incubated with pituitary extract for 2 hours.

While destruction in the corpuscles and excretion in the urine might account for the disappearance of up to 50 p.c. of the active substances from the plasma, the remainder must pass into the tissues and is probably destroyed there. The destructive properties of extracts of certain tissues were next examined.

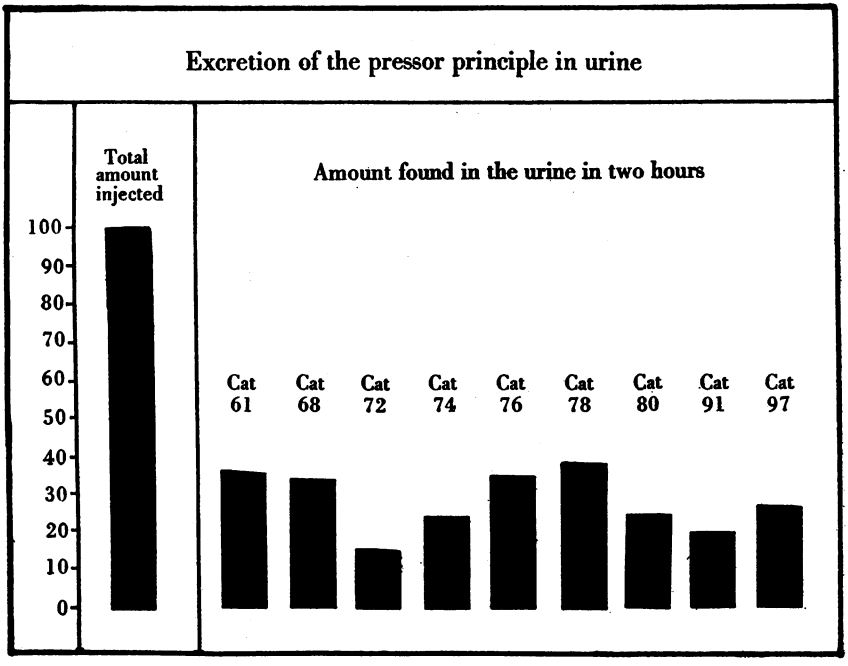

Fig. 6.

Action of tissue extracts

The extracts used in our experiments were made from the livers, kidneys and spleens of cats which had been injected with chlorazol-fast pink. This was done to ensure uniformity, as it had been employed in all the previous experiments. The organ was cut into small pieces and ground up with sand in a mortar, with the addition of 100 c.c. of 70 p.c. glycerol for every 60 g. of tissue. The mixture was filtered through glass wool and

the filtrate centrifuged at a high speed. The supernatant fluid was used. Three tubes were made up as follows:

I. 9 c.c. saline and 1 c.c. tissue extract.
II. 8 c.c. saline and 1 c.c. tissue extract.
III. 8 c.c. saline and 1 c.c. tissue extract and 1 c.c. pituitary extract (made up in saline).

The three tubes together with a quantity of pituitary extract were incubated at 37° C. for 2 hours. After this the extract was added to tube II. Thus tube II differed from tube III only in that pituitary extract was added to it after instead of before incubation. The contents of tube I were used to control the effect of the extract alone. It is clear (Fig. 7) that the pressor activity in the incubated specimens has been destroyed by the extracts of kidney, liver and spleen. It was also shown that the tissue extracts become inactive on boiling. It is impossible to say on the basis of these experiments what is the nature of the active agent in the extracts. Since the work described here was carried out, Heller and Urban [1935] have adduced evidence to show that the antidiuretic substance is destroyed by liver extracts. They suggest that destruction occurs in two stages. First there is adsorption, for the activity may be recovered by boiling, but finally the change becomes irreversible in this way, and appears to be a true destruction. It has not been possible to obtain any evidence of the adsorption phenomena with the pressor and oxytocic principles. Liver extract with added pituitary extract has exactly the activity of the same quantities of saline and pituitary extract respectively and boiling does not of course increase that activity. When the activity is lost by incubation it cannot be recovered by boiling.

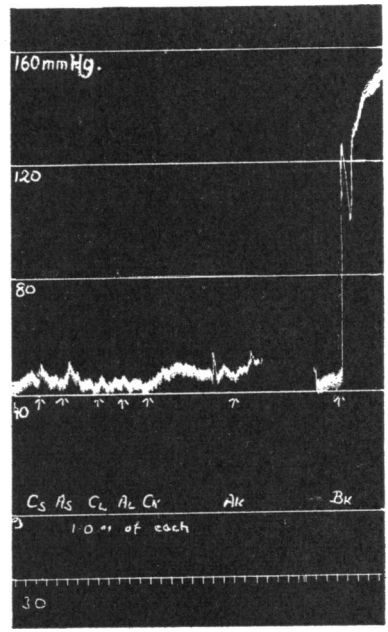

Fig. 7. C_S = pituitary extract incubated with spleen extract. C_L = pituitary extract incubated with liver extract. C_K = pituitary extract incubated with kidney extract. A_S, A_L, A_K, control tissue extracts as above. B_K = pituitary extract added to kidney extract after incubation of the latter.

Discussion

It should be pointed out that there is no method at present of detecting the presence of the pressor and oxytocic principles other than by their biological activity. Thus if they undergo any change in the body which destroys their activity they are completely lost. On the other hand, if they undergo any change which does not affect their activity it cannot be detected.

Although the pressor and oxytocic principles of posterior pituitary extracts are obtainable in separate fractions, there is a considerable and increasing body of evidence showing that they possess strikingly similar properties in many respects. The fact that both are readily dialysable and that the dialysis of both occurs at the same rate [Smith and M'Closky, 1924; Kamm, 1928] may possibly be of significance as an explanation of the simultaneous disappearance of the pressor and oxytocic activities from the circulating blood.

The tolerance which follows the injection of posterior pituitary extracts has been studied by previous workers because it modifies the action of a subsequent dose. As has already been pointed out, it also modifies the effect of the initial dose while that is circulating in the plasma. And this is a point of some interest, for it means that the blood pressure will be lowered much more quickly than it would be were the pressor effect proportional to the amount present in the circulation. It may therefore be regarded as a mechanism which minimises the effect of a large amount of a foreign pressor substance suddenly injected into the circulating blood. When all that substance has been removed the sensitivity returns to normal. It modifies the effect of a subsequent injection only incidentally, and only if that injection is made before all the previous one has left the circulating blood.

The ultimate fate of the active substances is accurately known only as regards the proportion which is excreted in the urine. There can be little doubt but that the remainder is destroyed in the body and it is clear that the cellular elements of the blood and extracts of liver, kidney and spleen possess this power. It would however be unwise to attempt to draw any conclusions as to the part played by these organs *in vivo*. The results of experiments with tissue extracts can scarcely be expected to do more than provide indications for further investigation.

SUMMARY

1. After intravenous injection into decapitated cats of posterior pituitary extract (10 mg. of dried gland per kg. body weight) the pressor and oxytocic activities of the circulating plasma disappear at the same rate: 85 p.c. is lost in 20 min. and none remains 2 hours after the injection.

2. The tolerance which follows the injection lasts for approximately the time during which the active substances circulate in the plasma. It is regarded as an adaptation to the slow passage of the active substances out of the blood stream.

3. No perceptible blood dilution, such as has been described by other workers, follows the injection of posterior pituitary extract alone. Dilution of the blood cannot therefore play any part in the effects of the injection.

4. The activity of the principles is unaffected by incubation with incoagulable plasma, but there may be a slow loss of both activities on incubation with whole blood.

5. About 30 p.c. of the injected pressor principle appears in the urine during the time the active principle is leaving the blood stream.

6. Glycerol extracts of the liver, kidney and spleen are able to destroy the oxytocic and pressor activities of posterior pituitary extracts on incubation. The extracts lose this property on boiling.

7. Wherever it has been possible to obtain comparative data it has been shown that the pressor and oxytocic principles behave identically.

We wish to express our thanks to Miss Mary Fleure for help given in certain of the experiments with tissue extracts.

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