PITUITARY SECRETION. By W. E. DIXON.

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This communication deals with the conditions which cause secretion of the pituitary gland to meet the needs of the animal economy. Cushing and Goetsch(1) examined the cerebro-spinal fluid of patients for the presence of "pituitrin": they used the physiological method and claimed to obtain effects corresponding with those produced by the posterior lobe of the pituitary. Carlson and Martin(2) failed to obtain a pressor effect by injecting the cerebro-spinal fluid of normal dogs into other animals; Cow confirmed Cushing, and Herring(3) found no evidence of pituitrin in the cerebro-spinal fluid of thyroid-fed or thyroidectomised cats.

In the present experiments dogs only were used: they were anæsthetised first with ether and then by injections of morphine and urethane. Tracheotomy was performed. The blood-pressure was taken from the right femoral artery and all injections were made into the right femoral vein. The cerebro-spinal fluid was collected from the sub-cerebellar cisterna by the method used by Dixon and Halliburton(4) and samples were collected for analysis at fixed intervals generally of fifteen minutes. It was essential in some experiments to obtain all the cerebro-spinal fluid available immediately after an injection, and in these instances a slight negative pressure was used.

Normal cerebro-spinal fluid. Samples of normal cerebro-spinal fluid of the dog show every known chemical and physiological action of pituitrin. The active substance is insoluble in absolute alcohol and in ether but is soluble in water. It is destroyed by digestion with trypsin but not with pepsin. It contracts the uterus and blood vessels, raises blood-pressure, increases the urinary flow and under suitable conditions causes a secretion of milk.

The amount of pituitrin in normal cerebro-spinal fluid varies greatly, and it is necessary to adopt some standard for comparison. After several trials I used the pituitrin of Parke Davis & Co., in sealed ampoules, different samples of which vary only slightly in activity. It was found that ten drops of normal cerebro-spinal fluid contain from 1 to 10 mgms. of this "pituitrin."

The quickest and most convenient method for determining the

quantity of pituitrin in cerebro-spinal fluid is by comparing the action of the fluid on the isolated uterus of the virgin guinea-pig with that of the standard pituitrin. One horn is attached to a suitably weighted lever and immersed in a bath of Ringer's fluid (Locke's modification) at 39° C. The bath contained 80 c.c. of fluid which could be readily replaced by fresh Ringer in 5 or 6 seconds. Each uterus requires to be standardised first by determining the minimal amount of standard pituitrin which will evoke a maximal contraction. This is essential since a sensitive uterus may enter into maximal contraction when the amount of pituitrin in the bath does not exceed 1 in 100,000, whilst occasionally a uterus is found which requires 1 in 5000 to produce a like effect. Cerebro-spinal fluid may be added in drops to the bath directly, since it is practically a Ringer's solution, and thus a comparative effect is easily obtained. After each dose of either pituitrin or cerebro-spinal fluid the Ringer must be changed and a sufficient time given, generally about 10 minutes, for complete relaxation. In this way a number of samples can be tested on the same horn. Occasionally I have seen as little as 10 drops of normal cerebro-spinal fluid produce a decided contraction of the uterus but the usual amount required to elicit an effect is 3 or 4 c.c., and occasionally even larger amounts are inactive. This is not remarkable since it is reasonable to suppose that pituitrin is secreted in variable amounts to meet the needs of the body.

Experimental. The present communication is characterised by the very large number of negative experiments. No form of nerve stimulation, sensory nerves, vagus and sympathetic in the neck, caused any change in the secretion of the pituitary. The inhalation of oxygen and carbon dioxide is likewise without effect. Certain drugs were injected intravenously or given by inhalation but all with negative results. Thus alcohol and chloroform were given by inhalation because they increase the flow of cerebro-spinal fluid; urea and caffeine because of their diuretic or cerebral stimulant action; histamine, 20 mgms., because of its marked action on the uterus; adrenalin, and β telluronium dichloride because the latter specifically excites the suprarenal glands; several alkaloids and glucose were inactive. One typical experiment will explain the procedure.

PROTOCOL 1. Dog, male, 20 kilos cerebro-spinal fluid collected as follows:

A. Normal fluid.

At 11.50, 3 c.c. 1 % $Te(CH_3)_2Cl_2$ given.

B. Fluid collected from 11.50 to 11.55
C. „ „ „ 12.20 „ 12.30
D. „ „ „ 12.50 „ 12.60
E. „ „ „ 2 „ 2.10

These samples were then tested on the uterus.

5 drops	pituitrin (1 in 100)	= maximum contraction.
5 „	„ („)	= „ „
50 „	cerebro-spinal fluid A	= nil.
70 „	„ B	= „
50 „	„ C	= „
70 „	„ D	= „
80 „	„ E	= „
2 „	pituitrin (1 in 100)	= maximal contraction.
200 „	cerebro-spinal fluid A	= nil.
1 drop	pituitrin	= maximal contraction.

(The normal cerebro-spinal fluid in this dog contained hardly any pituitrin.)

Each of the other drugs mentioned were tried on at least two occasions and gave negative results.

Preparations of the posterior lobe of the pituitary injected into the general circulation cause pituitrin to appear immediately in the cerebro-spinal fluid. It is present in excess in about a minute after the injection and the cerebro-spinal fluid is again free from such excess in five minutes. This effect can be repeated an indefinite number of times.

PROTOCOL 2. Dog, male. Cerebro-spinal fluid collected as follows:

1. Normal.
2. Immediately after intravenous injection of choroid extract.
3. 20 minutes „ „ „ „ „ „
4. 60 „ „ „ „ „ „ „
5. Immediately „ „ „ „ 2 c.c. pituitrin.
6. 20 minutes „ „ „ „ 2 „ „

The cerebro-spinal fluid was tested for pituitary on the uterus.

No effect produced by samples 1, 2, 3, 4 and 6 in 30-drop doses.
3 drops of 5 = slight contraction.
6 „ „ 5 = maximal „

(This corresponded with pituitrin in cerebro-spinal fluid, 1 in 100.)

Certain crystalline drugs are secreted into the cerebro-spinal fluid but always in such minute amounts that they are difficult to detect even by the most delicate chemical reagents: traces of salicylates and urotropine are so excreted. Halliburton and I showed that the current flows in the opposite direction, foreign crystalloids injected into the cerebro-spinal fluid pass into the general circulation with ease and great rapidity. The facts do not suggest that the presence of pituitrin in the cerebro-spinal fluid under the conditions named, is through the circulation. First because the amount of pituitrin is so large: if an injection of 10 c.c. 1 in 4 pituitrin is made into a vein, the concentration of pituitrin in the cerebro-spinal fluid reaches a strength of from 1 in 100 to 1 in 500. Moreover other uterine stimulants such as histamine, which has a relatively small molecule, when injected in 20 or 30 mgm. doses is not excreted by the cerebro-spinal fluid in amounts detectable by the physiological method. For these reasons I conclude that the secretion of

pituitrin under these circumstances is specific. That is, either the pituitary gland picks up the excess of pituitrin from the blood just as the

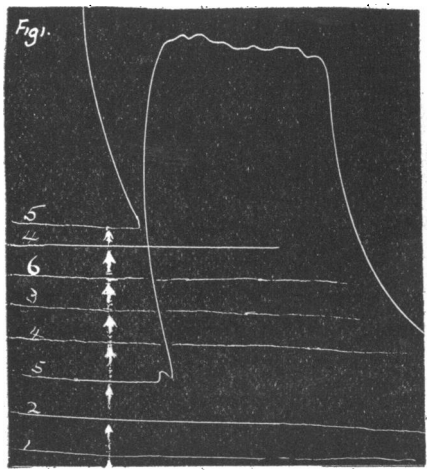

Fig. 1. For details see Protocol 2.

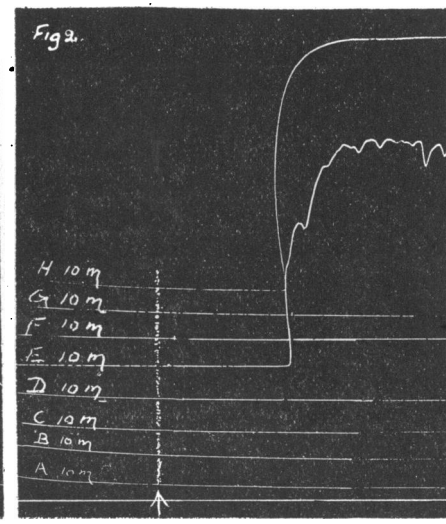

Fig. 2. For details see Protocol 5.

liver picks up bile salts, the process in each case causing secretion—or conceivably pituitrin acts as a pure stimulant to the gland.

The view that pituitrin might reach the cerebro-spinal fluid by the circulation was made improbable by three experiments, one on a dog and two on cats in which the pituitary gland was destroyed by the cautery from the ventral surface. Pituitrin was injected into the circulation before and after destruction and it was found that injections after destruction did not alter the amount of pituitrin in the cerebro-spinal fluid. The following protocol is typical.

PROTOCOL 3. Dog, male, 16 kilos. Normal cerebro-spinal fluid (A). 2 c.c. pituitrin in 10 c.c. Ringer's solution injected and the fluid collected during the next ten minutes (B). The spinal cannula was then removed and the pituitary destroyed by the cautery. (This was confirmed in the post-mortem examination.) After an interval of half-an-hour the spinal cannula was reinserted and a sample of cerebro-spinal fluid collected (C). A second injection of pituitrin identical with the first was given and the fluid collected in the next ten minutes (D).

The blood-pressure of the dog remained high throughout the experiment.

A, C and D had no action on the uterus in 20-drop doses though in 30-drop doses each caused a similar though sub-maximal contraction. B caused maximal contraction in 15-drop doses.

Pituitrin therefore stimulates the pituitary gland just as bile salts stimulate the liver. This experiment shows that the cerebro-spinal fluid

after extirpation of the pituitary still contains some pituitrin, and I have no explanation to offer of this phenomenon. What purpose the pituitrin serves in the cerebro-spinal fluid is not clear, but any excess rapidly disappears. Halliburton and I(5) showed that when it is introduced into the cerebro-spinal fluid it rapidly reaches the circulation and exerts its ordinary specific effects on plain muscle throughout the body. A balance appears to be struck between the amount in the blood and in the cerebro-spinal fluid. An excess in the blood is absorbed by the gland and some of it reaches the cerebro-spinal fluid: an excess in the cerebro-spinal fluid is excreted rapidly into the blood.

Animal extracts. Extracts of different tissues were prepared by pounding the fresh organ with sand adding about double the weight of saline, boiling and filtering. The extracts were injected slowly into the femoral vein and the cerebro-spinal fluid examined at intervals for excess of pituitrin. The following extracts gave negative results: liver, brain, choroid extract of brain, testis, epididymis, anterior lobe of pituitary, thyroid and pancreas. One protocol of these experiments is given which exemplifies all.

PROTOCOL 4. Dog, male. Cerebro-spinal fluid collected as follows.

1. Normal cerebro-spinal fluid.
2. Immediately after injection 2 c.c. pituitrin.
3. 15 minutes „ „ 2 „ „
4. 15–45 „ „ „ liver extract of rabbit.
5. 45–60 „ „ „ „ „ „ „
6. 1, 2 hours „ „ „ „ „ „
7. Immediately after a further injection 2 c.c. pituitrin.

Numbers 2 and 7 caused maximal contraction of the uterus in 15-drop doses. The others were inactive in 20-drop doses.

Ovarian extract is the only animal extract apart from pituitary so far examined which produces an immediate secretion of the pituitary gland. The earlier experiments were made from fresh tissue. After such an injection pituitrin appears in the cerebro-spinal fluid in a minute or two and the action is over in ten minutes. The fluid takes a little time to fill and drop from the cannula which may appear to increase the latent period; to avoid this as far as possible a little gentle suction was used.

PROTOCOL 5. Dog, female. One ovary removed between ligatures and a saline extract made. Cerebro-spinal fluid collected as follows.

A. Normal fluid.
B. Cerebro-spinal fluid collected during asphyxia.
C. „ „ „ 30 minutes after.
D. „ „ „ up to 5 minutes after ovarian extract.
E. „ „ „ „ 15 „ „ „ „
F. „ „ „ immediately after pilocarpine.
G. „ „ „ „ „ 2 c.c. pituitrin.
H. „ „ „ up to 5 minutes „ „

All the samples were inactive except D and H, which caused maximal contraction in 10-drop doses. This effect corresponds with a normal cerebro-spinal fluid, 1 c.c. of which contains 1 drop of pituitrin.

In a further series of experiments the sterile extracts of Parke Davis were used. These were of three kinds. (1) The whole ovary. (2) The ovary from which the corpus luteum had been removed. (3) Corpus luteum. The corpus luteum was without any action on the pituitary secretion but the other two extracts immediately excited the pituitary to secrete: the secretion of pituitrin commenced about 20 seconds after injection and was present in excess for a minute or two when the cerebro-spinal fluid became normal. The effect was repeated on the same animal several times. The record of one experiment is shown in Fig. 3. The increase of tone with pituitrin is abrupt; with the cerebro-spinal fluid it is more gradual. Perhaps the secretion in the cerebro-spinal fluid is not quite in the same form as that of "pituitrin." Iscovesco(6), Aschner(7), Hermann(8) and others have shown that extracts of corpus luteum induce hyperæmia and hyperplasia of the muscle and mucous membrane of the uterus. Observations have also been recorded suggesting that pituitary extracts exert a like action, but more extended observations have disproved this view.

The facts seem clear that ovarian conditions determine the secretion of the pituitary and thus react indirectly on the uterine tonus. Pituitrin is very largely employed in medicine to contract the uterus: its employment would seem to be so far rational that it is the drug manufactured by the body for this specific purpose.

The effect of alimentary extracts. Cow(9) working in this laboratory on diuresis believed that water taken by the mouth was a better diuretic than water injected subcutaneously, because in its passage through the alimentary canal it absorbed some substance which excited the pituitary. The secretion of the pituitary is influenced by injecting boiled and filtered extracts of mucous membrane but the effect is of a different nature from that caused by ovarian injections. With ovarian extract the latent period is in seconds and the effect is rapidly over. With the intestinal mucous membrane the effect is delayed and the augmented secretion may not be noted for an hour; the percentage increase of pituitrin in the cerebro-spinal fluid is not so great as after ovarian extract but it remains in the fluid longer. I repeated Cow's observations on seven animals and obtained a positive result in five though one of these was not very decided. After such a long latent period the flow of fluid may be small and find a more ready absorption by the vessels than by outflow through the cannula and this may account for the failures.

The effect is not protein shock since practically no protein is present in the injections. Nor does the long latent period suggest that it is a

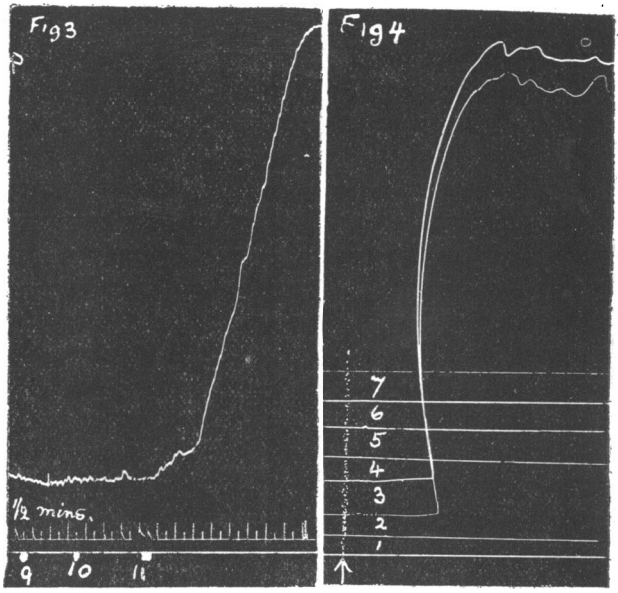

Fig. 3. Record of isolated uterus. Fig. 4. For details see Protocol 6.

At 9. 24 drops normal cerebro-spinal fluid added.

At 10. 15 drops cerebro-spinal fluid ten minutes after injection of ovarian extract without corpus luteum.

At 11. 15 drops cerebro-spinal fluid 1 minute after injection of ovarian extract without corpus luteum.

direct effect on the pituitary. To test this point I injected the extract into the central end of the carotid artery in two further experiments, and these gave completely negative results within the next hour. However this effect is produced we only know the beginning and end points. The following protocol is typical.

PROTOCOL 6. Dog, male. Duodenum removed between ligatures and the mucous membrane extracted in saline, boiled and filtered. Cerebro-spinal fluid collected as follows:

7.	10	drops	normal	cerebro-spinal	fluid.					
6.	10	„	„	„	„	15 minutes	after	injection.		
5.	10	„	„	„	„	35	„	„	„	
4.	10	„	„	„	„	60–120	„	„	„	Diges. 37° C. 18 hours.
3.	5	„	„	„	„	60–120	„	„	„	
2.	10	„	„	„	„	60–120	„	„	„	
1.	10	„	„	„	„	60–120	„	„	„	„ 37° C. 18 „

1 drop of trypsin to 25 drops cerebro-spinal fluid incubated for 18 hours at 37° C. Controls without trypsin.

2 and 3 produced maximal contraction of the uterus. No other sample produced any effect.

The experiments appear to me conclusive that some constituent of the intestinal mucous membrane so affects metabolism that about one hour or more after its liberation, the pituitary is induced to secrete.

If the pituitary is a remnant of a gland once secreting into the alimentary canal, as Gaskell believed, it is perhaps not extraordinary that its secretion should be associated in some way with the functions of the alimentary canal. The prolonged latent period which injections of mucous membrane require before the pituitary secretes makes it certain that the pituitary takes no active part in the immediate processes of digestion. I was therefore led to investigate its action on absorption. Definite quantities of tap water were placed in 6-in. loops of intestine chosen from various parts of the alimentary canal in anæsthetised cats and dogs. Every care was taken to ensure an efficient circulation. The normal slow absorption which occurs was entirely uninfluenced by injections of pituitary. The experiments were repeated with the same results on surviving intestines.

During these observations another phenomenon was observed. Pituitary extract exerts a very decided effect in increasing the tone of the small intestine without effect on peristalsis. This effect is, of course, well recognised and the modern treatment of post-operative atony of the gut is by pituitary injections. This action is peculiar to the small intestine. The large intestine behaves differently: the first effect of injection is to increase the tone for from 10 to 20 seconds; this is followed by a marked relaxation of tone at a time when the effect is maximal in the small intestine. This effect is shown graphically in Fig. 5, which is a record of two experiments on two cats. A loop of six inches of intestine was tied at one end and fitted with a cannula at the other. This cannula communicated with a Mariotte's bottle and the records in the lower curve represent bubbles of air replaced from the upper part of the bottle as more fluid passes into the intestine. The water or saline was at a pressure of 10 cms. The upper tracing is from the small intestine, the normal rate of absorption can be gauged from the fact that seven bubbles correspond with 1 c.c. The effect of injection of pituitary is to diminish the number of bubbles, that is to increase the tone since the rate of absorption remains constant. The lower tracing (B) is from the large intestine; the first effect is to increase the tone to such an extent that the water rises in the tube. The effect is followed in a little over a minute by profound

relaxation, an effect which I have never observed in the small intestine. This phenomenon may be the result of the direct action of the pituitary

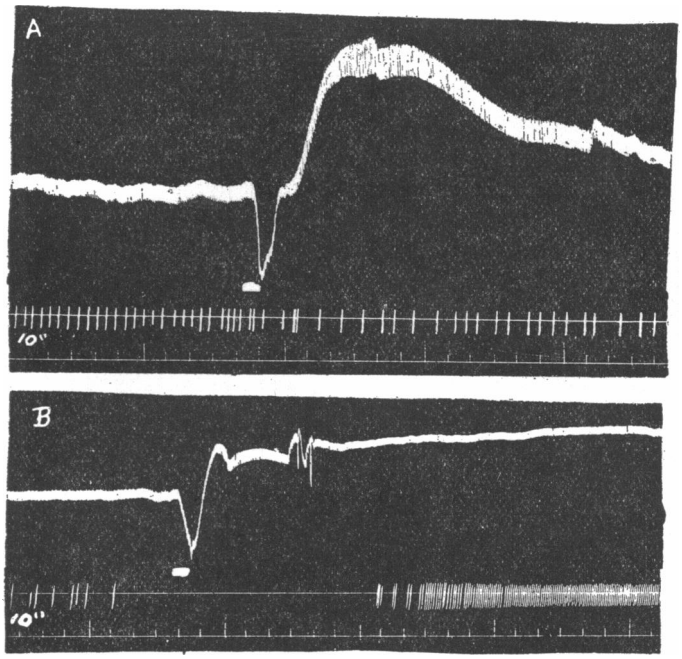

Fig. 5. Record of blood-pressure in two cats, the middle curve in each experiment is described in text, A in the case of the small intestine and B the large intestine.

on muscle. Such a highly specific effect seems inherently improbable but it cannot be dismissed off-hand since we know that on isolated and surviving arteries the effect of pituitrin may be different according to the situation of the excised portion. On the other hand it may be a reflex phenomenon, that conditions which throw the small intestine into tone may relax the large intestine: this is teleologically understandable.

The cardiac sphincter is relaxed as food moves towards it in the œsophagus: the duodenum is inhibited during peristalsis of the pyloric end of the stomach (10) and Cannon (11) states that "as food is nearing the ileo-colic valve the large intestine is usually quiet and relaxed." The passage of food through the ileo-colic valve causes relaxation of the colon which occurs in the absence of nervous connections with the spinal cord (12). The pituitary reaction is I think different from these: the intestine is isolated and the tube for recording was always inserted at

least an inch from the ileo-colic sphincter. The result is receiving further consideration.

The pituitary exerts another function on digestive processes. By some means it regulates the amount of sugar in the blood: it depresses hyperglycæmia whether produced by anæsthetics or adrenaline, and the evidence points to the fact that it bears some relation to glycogenesis in the liver. It has been suggested that one cause of the diabetic condition may be pituitary insufficiency. This is, also, a property of the posterior lobe and I have mentioned that extracts of the anterior lobe, anæsthetics, adrenaline, and excess of sugar in the blood do not influence the secretion of pituitrin.

Conclusions.

1. The pituitary gland secretes into the cerebro-spinal fluid.

2. Pituitary extract injected into the circulation causes the gland to secrete. Pituitary extract injected into the cerebro-spinal fluid rapidly causes the ordinary systemic effects by passing into the general circulation. A balance is struck between the amount in the blood and cerebro-spinal fluid.

3. Ovarian extract specifically excites the gland to secrete. The effect is immediate and lasts only two or three minutes. The active substance is not in the corpus luteum.

4. Duodenal extract causes a secretion after one hour, but neither so much nor so constant as that caused by ovarian extract, the effect however is more prolonged. Some suggestions as to its significance are offered.

5. Pituitary extract increases tone in the small intestine, and diminishes tone in the large intestine.

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