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COUNTRY Poland

REPORT

SUBJECT Research Project - Secretion of Anterior Pituitary Hormones and Ovulation in Small Ruminants (conducted at Institute of Animal Physiology & Nutrition at Jablonna, near Warsaw)

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an annual report (16 pages, English) of a Polish research project entitled "Secretions of Anterior Pituitary Hormones and Ovulation in Small Ruminants" which is being conducted at the Institute of Animal Physiology and Nutrition at Warsaw, under the direction of Eugeniusz Domanski. CONFIDENTIAL

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Institute of Animal Physiology and Nutrition
(Polish Academy of Science)

Jabłonna near Warsaw
Poland

Chief Investigator : Eugeniusz DOMAŃSKI
Title of problem : "Secretion of anterior pituitary hormones and ovulation in small ruminants."

Report covers the period: January 1.- December 31.1963.

I. Summary.

The aim of our work is to investigate the role of the hypothalamus and the process of transmission, from the hypothalamus to the hypophysis, of the impulses which regulate the secretion and release of gonadotropins by adenohypophysis in sheep. In previous our experiments on this problem we investigated the role of the pituitary stalk in the process of impuls transmission using the method of the stalk section. In these experiments we were able to evidence in sheep after stalk section the disappearence of ovulation as well as the atrophic changes in adenohypophysis and in sexual organs. We showed also that the development of the mammary gland for lactation as well as the secretion of milk were strictly bound with the integrity of the hypothalamo-hypophyseal junction. Thus on the basis of these results we were able to conclude that the hypothalamus and the hypothalamo-hypophyseal junction in sheep plays a fundamental and controlling role in the secretion and release of gonadotropic hormones (FSH, LH, LTH).

Starting from the thesis that this controlling role is exerted by neurosecretory materials elaborated in central nervous system (CNS) and transmitted on the vascular way of the pituitary stalk, we have undertaken in 1963 the search for these neurosecretory substances responsible for the release of gonadotropic hormones by adenohypophysis. For these purposes we prepared extracts from various parts of the hypothalamus and other parts of CNS on the chemical way and we tested their activity by biological methods. Namely, we injected these extracts immediately into the hypophyses in rabbits and sheep (the method in vitro) or we added extracts to the incubated slices of adeno-

hypophyses and after incubation we titrated the content of gonadotropic hormones in incubated medium (the method in vitro).

By using the method of injections of extracts immediately into the adenohypophysis in rabbits we induced ovulation in these animals.

By the method in vitro we were able also to show the stimulation of release of gonadotropins by the slices of adenohypophysis.

Thus, our experiments seem to show the presence in neural tissue of median eminence of some substance(s) being able to promote the release of gonadotropic hormones by adenohypophysis. These results raised the question as to whether this substance, obtained by extraction from nervous tissue, releases FSH and LH together or LH only. The experiments concerning this question are currently being performed.

In 1963 we continued also our work on vascularization of the pituitary gland. For in the physiology of this gland and especially in investigating the process of impuls transmission from the hypothalamus to the hypophysis an understanding of the structure and architecture of the vascular system of the hypophysis is indispensable. As important achievement of these investigations and worthwile to note there was a finding of hypothalamopetal flow of venous blood from adenohypophysis. This concept of hypothalamopetal flow of venous blood from adenohypophysis, gives rise to the idea of the feed-back regulation possibly existing between CNS and adenohypophysis. And this idea has not been yet a subject of any investigations.

Moreover in 1963 the technical preparatory work to investigations on the function of the hypothalamus in sheep was being performed. For these purposes we prepared histological slices of the hypothalamo-hypophyseal area of the brain of sheep to design the stereotaxical map of this area. By means of coordinates of stereotaxical map we will be able to place lesions at particular areas in hypothalamus and on this way to localize the function of these areas.

II. Detailed report.

I n t r o d u c t i o n .

As presented in our first and second annual report, the main aim of our work was to investigate the role of the hypothalamus and the process of transmission, from the hypothalamus to the hypophysis, of the impulses which regulate the secretion and release of pituitary gonadotropins, and, especially, of FSH and LTH in sheep. Starting from the generally accepted thesis that the nervous regulation of adenohypophysis is exerted by neurosecretory materials transmitted from the median eminence to this gland on the vascular way of the pituitary stalk, we investigated first the role of the pituitary stalk in the process of this transmission using the method of stalk section. By this method we were able to evidence that the ewes after pituitary stalk section showed no sexual cycles, ovulation had disappeared and the autopsy of these animals revealed atrophy of ovaries and uteruses. Histological examinations of these pituitaries, stained with heamatoxylin - eosin, and according to PAS method, showed in all animals with the stalk section an atrophy of the glandular tissue and a hyperplasia of conneo-

tive tissue. The dimensions of cell cytoplasm and nuclei were reduced, delta cells showed no granulation (i.e. were PAS negative).

Thus on the basis of the above cited findings we were able to conclude that the disappearance of ovulation as well as the changes in pituitaries and in sexual organs were caused by the lack of impulses from the hypothalamus to the adenohypophysis following the transection of the pituitary stalk.

Moreover we investigated also the role of the hypothalamo-hypophyseal junction on the process of lactation. And we were able to show that the development of the mammary gland for lactation as well as the secretion of milk were strictly bound with the integrity of the hypothalamo-hypophyseal junction. When this junction was damaged, the mammogenic and lactogenic function of the pituitary gland was abolished. After these facts had been stated the following experiments were continued and undertaken in the third year of our investigations:

1. Experiments searching for the substance(s) in the hypothalamus responsible for the release of pituitary hormones (LH and FSH).

2. Histological investigations upon the vascularization of the pituitary gland in sheep.

3. Technical preparatory work to investigations on the function of hypothalamus by the method of lesions.

Progress Report.

1. Experiments searching for the substance(s) in the hypothalamus responsible for the release of pituitary gonadotropins.

To investigate this problem we prepared extracts from various parts of the hypothalamus and from the cortex, we infused them immediately into the adenohypophysis of rabbits and sheep and observed the reaction of the animals after these infusions.

These extracts were tested also by the method in vitro; namely, they were added to the incubated slices of adenohypophyses and then the released gonadotropins in incubated medium were titrated. The extracts from nerve tissue were prepared in the following way: The whole brains were removed and deep-frozen immediately after the slaughter of the animals. Then all the isolated median eminences were put together and all the anterior parts of the hypothalamuses were also put together. Isolated tissues were extracted with 0,1 N HCl. This crude extract was lyophilized, stored in the refrigerator, and immediately before testing it was dissolved in saline. Extracts of cerebral cortex (from the frontal lobe) were prepared in a similar way and used for control infusions. The lyophilized crude extracts were dissolved immediately before tests in various concentrations in physiological saline and slowly infused into the adenohypophysis of rabbits and sheep through chronically implanted cannulae. In rabbits the dose level amounted 25 μl and contained as low as 0.175 mg equivalent (concentration I) and as

high as 1.4 mg equivalent (concentration II) of lyophilized tissue per one animal. Ovaries in rabbits were examined by laparatomy after 48 hrs and on the 5th day after infusion.

The results of these infusions were as follows:

Table 4.

Part of the brain and concentr. of the extract	No. of rabbits used for test	Changes in ovaries after infusion		
		No of rabbits with bloody spots in ovaries 48 hrs after infusion	No of rabbits with corpora lutea, 5 days after infusion	No of corpora lutea per one animal
median emin. rabbit conc. I.	5	0	0	-
median emin. sheep, conc. I.	8	0	0	-
median emin. rabbit conc. II.	9	8	6	8
median emin. sheep conc. II.	3	3	2	7
hypothalamus rabbit, conc. II.	5	2	0	-
cerebral cortex rabbit conc. II.	6	1	1	9
Pitocin /oxytocin/ 0,4 I.U. /Parke Davis/.	3	0	0	-
Pitressin /vasopressin/ 2.0 I.U. /Parke Davis/	3	0	0	-
acetyl choline 0,2 µg	3	0	0	-

In sheep, we have to date been able to carry out only a few experiments with injections of extracts. We injected these extracts into two sheep during the sexual cycles, in one on

the 10th and into the second on the 12th day of the cycle. The dose level amounted 200 µl and contained as much as 14 mg of lyophilized median eminence of sheep. The effect of the injections in sheep was tested by vaginal smears and by the running of the ewe with the ram. To date we received positive reaction i.e. ovulation in one sheep only, namely in the sheep which was injected on the 12th day of its sexual cycle.

2. Investigations upon the vascularization of the pituitary gland in sheep.

An understanding of the structure and architecture of the vascular system of the hypophysis is indispensable in investigating the physiology of this gland and especially in investigating the process of impuls transmission from hypothalamus to the adenohypophysis. This basal problem of blood circulation in hypophysis and especially vascular relations between hypothalamus and hypophysis in sheep is known rather very little, therefore we undertook this.

To this aim the arteries and the veins of the heads of sheep were under pressure injected with indian ink or prussian blue, then after their fixation in formalin, the hypothalamo-hypophyseal areas of the brain were embedded in celluloidin and sectioned in slices fifty micron thick. All slices were examined in the stereoscopic microscope and on the basis of these examinations the vascularization of the pituitary gland and the blood circulation-relation between hypothalamus and hypo-

physis were reconstructed. The pictures Nr 3 and 4 represent these reconstructions. According to these pictures the anterior and posterior lobes receive separate arterial supplies. Namely, the anterior lobe (adenohypophysis) is supplied by the arteries running from circulus of Willis and approaching to the median aminence. Branches of these arteries encircle the median aminence like a ring. Numerous small descending branches spring from this ring and form the capillary bed of the stalk. Capillaries around the stalk were seen to empty into the dilated channels of the anterior lobe. The effluent blood drains from adenohypophysis through two venous systems. The first one drains the blood directly into the venous sinus which surrounds the pituitary gland (in pituitary fossa) and the second one conveys the venous blood upward to the floor of infundibular recess, where the veins break up into a system of fine channels, a secondary distributing net.

According to the anatomical sizes of veins of these two systems, we suppose that the majority of the blood is drained from the adenohypophysis upward hypothalamopetal and smaller part of its is conveyed directly into the perihypophyseal sinus.

The posterior part of the pituitary gland is supplied by the arteries originating from the so called rete situated in pituitary fossa and formed by the internal carotid arteries.

Connections between these two arterial systems of anterior and posterior part of the hypophysis, exist in special anatomical places of the gland; they are, however, not numerous (see picture Nr. 4).

The effluent blood from posterior part of the hypothalamus is drained also by two ways, namely by the immediate way into perihypophyseal sinus, and by the veins running upward hypothalamopetal.

3. Technical preparatory work to investigations on the function of hypothalamus by the method of lesions.

This work was carried out to prepare stereotaxical map of the hypothalamo-hypophyseal area of the brain in sheep. This map is indispensable for the method of lesions and other methods applied in electrophysiological investigations of CNS. For these purposes the hypothalamo-hypophyseal areas of the brain of sheep were embedded in celloidin and sectioned at fifty micron thickness. Every fifth section was mounted. The sections of two brains were stained for cells and those of two other brains were stained for fibers. On the basis of these histological sections, coordinates for particular nuclei of the hypothalamus will be drawn. This last cited work is currently being performed.

1. The problem of substances transmitting impulses from hypothalamus to hypophysis.

The ability to induce ovulation in rabbits by the infusion of extracts from the hypothalamus directly into the adenohypophysis and the difference in the action between the extracts from particular parts of the hypothalamus and the cortex seem to show the presence in median eminence of some transmitting substance being able to promote the release of gonadotropins by the adenohypophysis. This phenomenon raises the question as to whether, in induced ovulation by infusion of the extracts, FSH and LH are released together, or LH only. Our preliminary unsuccessful attempts to induce ovulation in sheep with these extracts seem to show that the releasing of FSH does not occur; at same time, however, the results of extracts tested in vitro show that even the FSH is released by slices of pituitary gland. These results I was able to discuss with the scientists most advanced in this field, namely, with Dr.C. Sawyer[x] and Dr.S.M.McCann[xx], during my stay in the U.S.A. (October and November 1963).

The result of discussions with the above cited Scientists was a conclusion that to elucidate this problem further experiments in this area are needed.

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x) Dr.C.Sawyer, Department of Anatomy, School of Medicine, UCLA (California).

xx) Dr. S.M.McCann, Department of Physiology, School of Medicine, University of Pennsylvannia, Philadelphia.

2. Hypophyseal vascularization.

The most important problem in the hypophyseal vascularisation is the relation in the blood circulation between hypophysis and hypothalamus. Our findings of arterial blood supply of adenohypophysis in sheep by the capillary bed of the stalk is in a whole agreement with the generally accepted concept on the hypophyseal portal system. The direction of the flow of arterial blood in this system (from infundibulum to adenohypophysis) supports the thesis of neurohormones transport from hypothalamus to the adenohypophysis on the vascular way.

On the opposite, the views on the draining of venous blood from adenohypophysis are not conclusive. Popa[x], as first, in his theory of a portal circulation (dated back to 1930) suggested the concept of hypothalamopetal venous blood flow from adenohypophysis. This concept, however, was not supported by reliable anatomic data by Popa himself and rejected later by several other authors[xx]. Newer work concerning this con-

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x) Popa, G.A. and U.Fielding: A portal circulation from the pituitary to the hypothalamus region.- J.Anat. 65, 1930.

xx) Wisłocki G.B., 1937. The vascular supply of hypophysis celebri in cat.-

Yewell, D.A., 1956. The vascular link between the posterior and anterior lobes of the pituitary gland.- J.Endocrinol., 14: 24.

Barnett R.I. and R.O.Greep, 1951. The direction of flow in blood vessels of the infundibular stalk.- Science, 113:185.

cept give also little information[x]. Our findings in sheep support the concept of hypothalamopetal flow of venous blood from adenohypophysis and demonstrate the breaking up of the veins running hypothalamopetal into a secondary distributing net in the area of infundibulum. This concept of hypothalamopetal flow of venous blood from adenohypophysis gives rise to the idea of the feed-back regulation possibly existing between CNS and adenohypophysis, idea being not yet a subject of any investigations. For these reasons our investigations upon vascularization of hypophysis aroused a great interest and vivid discussion with several scientists[+] during my above cited stay in the United States.

This discussion, however, revealed that to support the concept of hypothalamopetal flow of venous blood from adenohypophysis, further experiments are needed.

Moreover there is another problem, very important, connected with the vascularization of the adenohypophysis; namely, the problem of arterial blood supply to adenohypophysis after the stalk section. According to the investigations of Daniel and

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x) Landsmeer J.F.M., 1963. A survey of the analysis of hypophyseal vascularity.- Advances in Neuroendocrinology, University of Illinois Press - Urbana - 1963.

+) Dr. H.T.Clegg, Animal Husbandry Department, University of California, Davis.-

Dr. Ganong; Department of Physiology, School of Medicine, University of San Francisco.-

Dr. de Groot and Dr. B.Donovan, Department of Anatomy, Medical School, University of San Francisco.-

Prichard[x] and supported by our (described above) the adenohypophysis in sheep is supplied mainly by arterial vessels of the stalk. Daniel and Prichard[xx] were able to find massive infarctions, necrosis and regeneration in adenohypophysis after the stalk section in sheep. These changes in adenohypophysis after stalk section were interpreted by Daniel and Prichard as the result of the deprivation of the blood supply to the gland. In our experiments none of the 6 ewes operated on and slaughtered about one year after operation showed no traces of necrosis in adenohypophyses. The proces of necrosis was also not found in 2 ewes, of which one was slaughtered 2 weeks, and the second one 4 weeks after the operation. Thus, on the basis of our observed histological changes, it seemed us that the atrophy of glandular tissue in adenohypophysis and particularly the degeneration of delta cells after stalk section were caused not by disturbances in the blood circulation but rather by lack of functional correlation following the absence of substances transmitting the impulses from the hypothalamus to the adenohypophysis regulating its function. This view can be supported also by our investigations on the blood circulation in hypophysis and statement of vascular connections between its anterior and posterior part. For these vascular connections give possibilities of compensation in blood circulation in the adenohypophysis after the operation of stalk section.

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x) Daniel P.M. and M.M.L. Prichard, 1957. The vascular arrangements of the pituitary gland of sheep.- Quart.J. Expl.Physiol. 42:237.

xx) Daniel, P.M. and M.M.L. Prichard, 1957. Anterior pituitary necrosis in sheep produced by section of the pituitary stalk.- Quart.J.Expl.Physiol. 42:433.

Prospectus for investigations in the next year.

The ability to induce ovulation in rabbits by the infusion of extracts from the median eminence shows the presence in this part of the brain of some substance (factor) being able to promote the release of gonadotropins by the adenohypophysis. This phenomenon raises the question as to whether, in the process of ovulation there is one only factor of hypothalamic origin responsible for the release of both FSH and LH, or there are two different factors responsible for the release of each of these hormones. To elucidate this question we plan:

1. to continue our experiments with injections of crude extracts in order to induce ovulation in animals with spontaneous ovulation (chiefly in sheep and also in guinea pigs),
2. to fractionate our active crude extracts from median eminence by chemical methods and to try to induce ovulation by the use of purified fractions.

Moreover we will damage some neural centers in the hypothalamus by electrocoagulation in sheep and we will observe the effect of these injuries upon the functioning of the adenohypophysis. Therefore, as it is noted above, we are now working on an histological map (atlas) of the hypothalamus of sheep to be used in connection with these studies.

In addition, according to this report, we will continue our work on the vascularization of the pituitary gland in sheep.

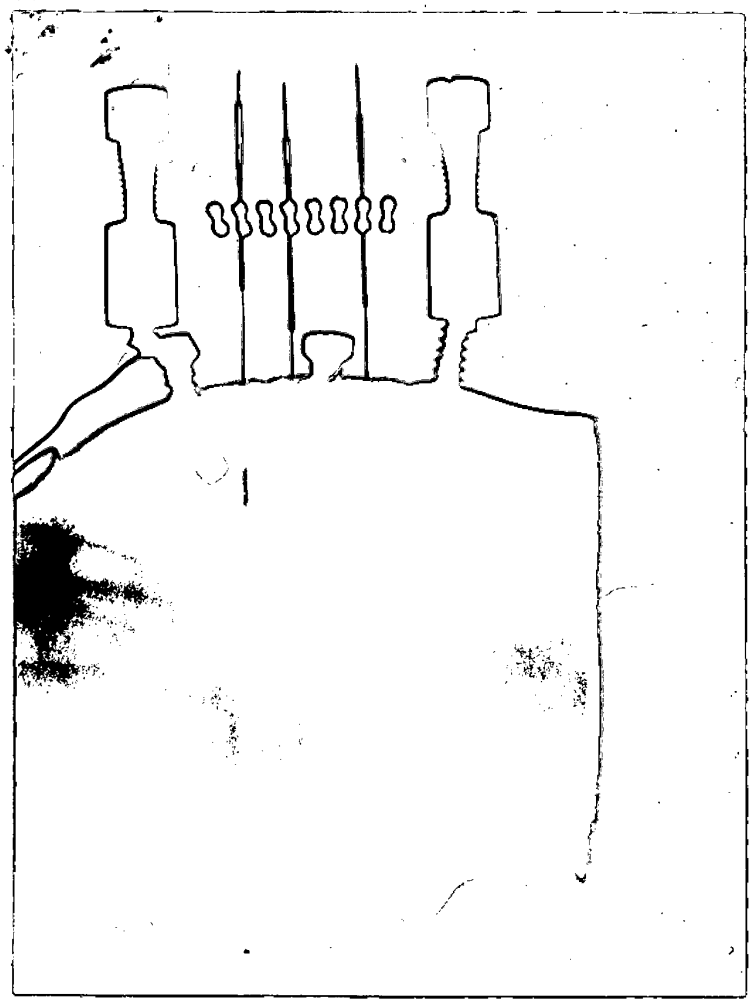

Fig. 1. X-ray pictu
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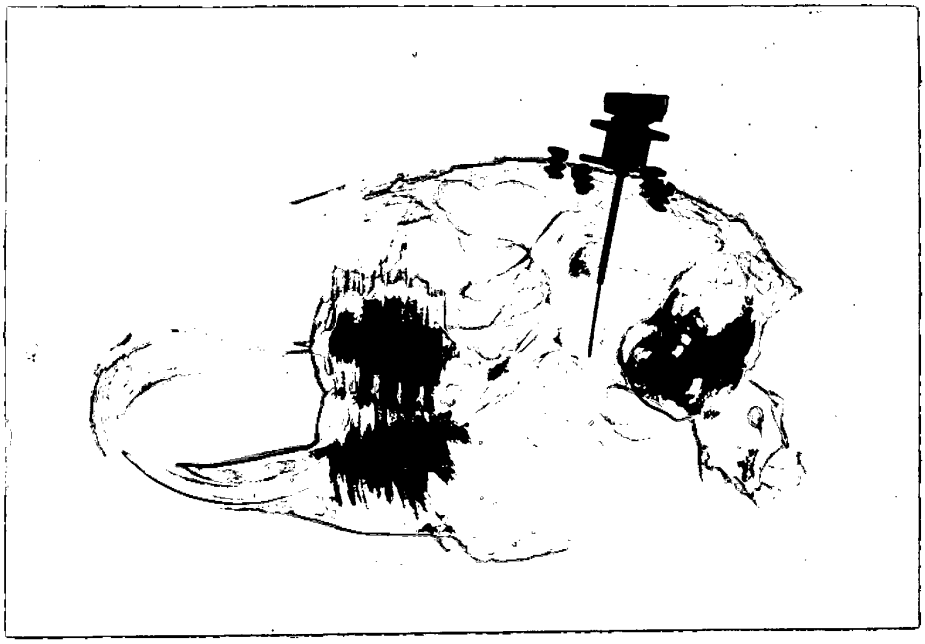

Fig. 2. X-ray pic
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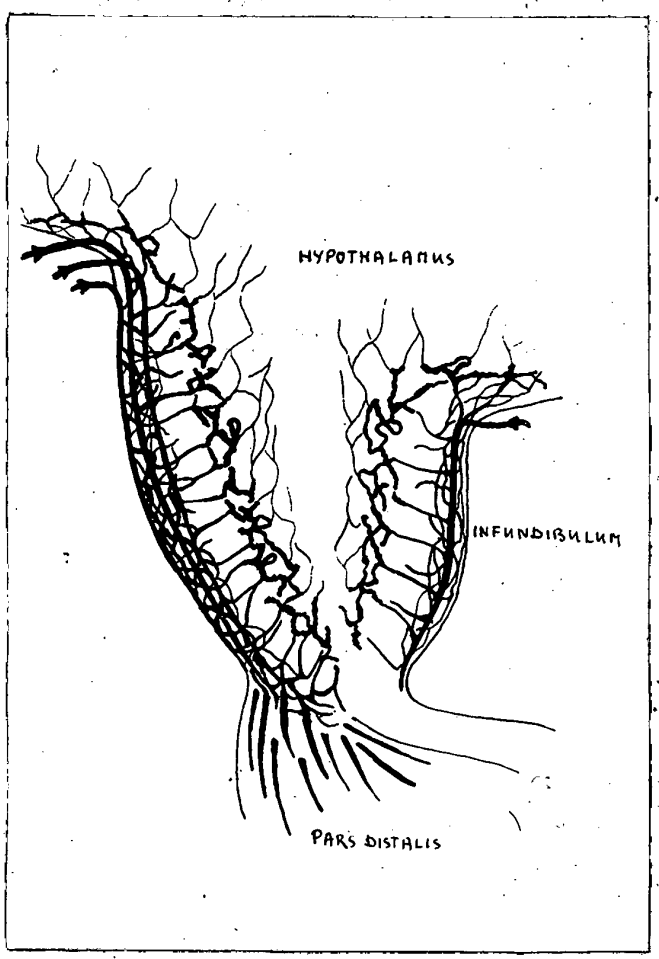

Fig. 3. Reconstruction of arterial vascularization and direction of arterial blood flow in pituitary stalk in sheep.

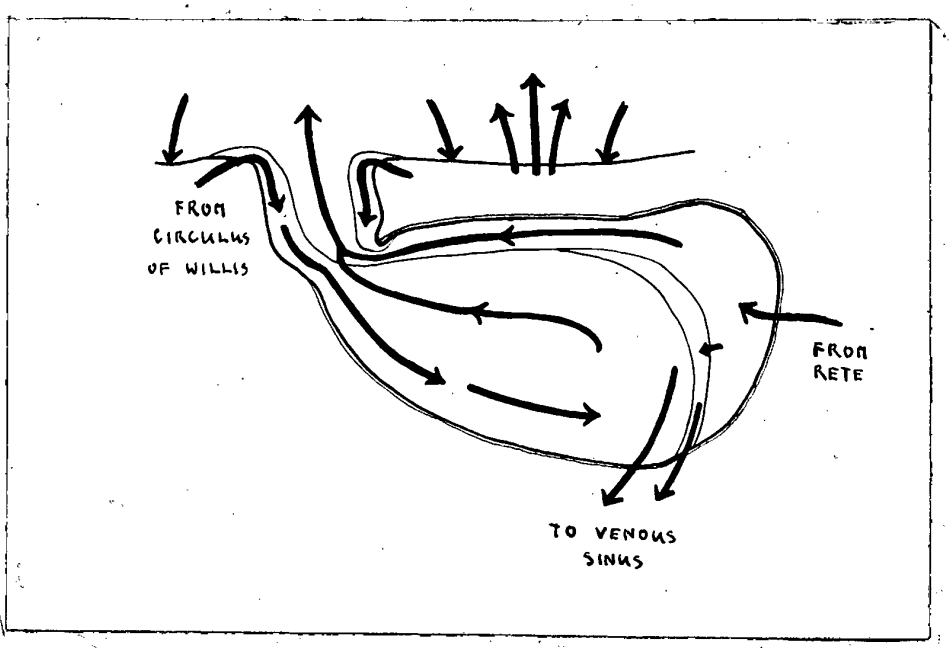

Fig. 4.- Reconstruction of arterial blood supply and direction of venous blood drainage of pituitary gland in sheep.-