NERVE FIBRES IN THE PITUITARY OF A RABBIT.

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Comparatively little is definitely known about the nerves in the three different parts of the pituitary. It is indeed stated by Bailey(1) that there is no proof of the existence of any other than sympathetic fibres to the blood vessels.

Rival theories are held as to the origin of such conditions as diabetes insipidus: (1) that it is due to an affection of the pituitary, either of the pars intermedia or pars nervosa, (2) that the pituitary plays no part in the production of symptoms, the condition being caused by lesions in the nervous system, more especially in the tuber cinereum. It seemed therefore of interest to ascertain if there is any innervation of the pituitary other than that to the vessels.

Berkley(3) was probably the first to use special methods for the demonstration of nervous elements in the pituitary. He worked with dogs and mice, and used chiefly the chrome-silver method of Golgi. He described in the posterior lobe both nerve cells and nerve fibres, and in the anterior lobe sympathetic fibres ending in knobs between the cells. It seems probable that the cells described in the posterior lobe by Berkley are really neuroglia. The present writer finds that they can easily be demonstrated in the human pituitary and in that of the rabbit by the Bielschowsky silver method, and that they resemble neuroglia more closely than nerve cells.

Herring(5), who discusses in some detail the views of the older anatomists on the subject, was not able to satisfy himself as to the nervous character of the cells and fibres in the pars nervosa, and considers them to be neuroglia. In the pars anterior of the cat's pituitary he did not succeed in demonstrating nerve fibres either by the Cajal or the Golgi methods.

Dandy(4) studied the anatomical but not the histological distribution of nerves. He states that the supply to the posterior lobe in the dog and kitten is poor in comparison with that of the anterior, and also that it is often possible to trace a single nerve fibre with its branches passing down the sheath and spreading out over the pars intermedia. He regards the nerves as secretory in function rather than vasomotor.

Tello(8) traced fibres from the pars nervosa passing into the pars intermedia and ending in the epithelium.

Pines(6), using a modification of the Cajal silver method, found that all parts of the pituitary—anterior, intermedia and nervosa—were richly innervated.

The whole question is still regarded by most people as unsettled.

Methods.

A number of methods were tried—many of them being entirely unsuccessful. In view of the capricious nature of both silver and gold methods it obviously may be fallacious to draw definite conclusions from negative results. A method, such, for example, as Löwit's, gives beautiful preparations with muscle, but is totally unsuited for the pituitary, as the latter dissolves in strong formic acid and is considerably damaged by weak.

As it was thought that the nerve supply to the pituitary would be mainly unmyelinated, it was decided to try various modifications of Ranson's silver-pyridine technique. The use of pyridine renders the staining of unmyelinated fibres more intense(7).

The following methods were used:

(1) Golgi methods.
   (*a*) Rapid modification.
   (*b*) Cox-Golgi modification.

(2) Sihler's method, used on frozen sections.

(3) Gold chloride.
   (*a*) The pituitary was placed in 2 p.c. gold chloride (brown variety of Merck) for 4 hr. One half was reduced by ultra-violet rays and one half by sunlight, the pieces being placed in water acidulated with acetic acid.
   (*b*) Löwit's method.

Of these three methods those of Golgi and Sihler were unsuccessful, while the gold chloride method gave deep purple sections in which no nerve fibres could be distinguished.

(4) Bielschowsky's silver method.

This proved unreliable, as connective tissue, blood and cell nuclei were stained. Comparing the sections later with successful specimens by the Ranson method, black nerve fibres could be identified in the pars intermedia. This method gave beautiful pictures of neuroglia in the posterior lobe.

(5) Modifications of Ranson's method.
  (*a*) Variations in the time in silver nitrate.
  (*b*) Variations in the time in pyrogallic acid.
  (*c*) Alterations in the fixing fluid.
    (i) The addition of 2 p.c. veronal in alcohol for 24 hr. instead of ammonia alcohol for 48 hr.
    (ii) 10 p.c. aqueous chloral hydrate for 24 hr. before using ammonia alcohol.
  (*d*) Alteration in the method of fixation by injecting the fixing fluid into the circulation of the animal before fixing the pituitary.

Ranson's method never failed to give results with the pars intermedia, but it seemed possible that negative results in the pars anterior might be due to failure of penetration either of the silver nitrate or of the fixing fluid, as the cells are very closely packed together. In all cases the pituitaries were cut in half before fixing. To test if a longer time in silver were necessary, pituitaries were left in the silver solution for periods varying from three to nineteen days. In no case did the increased time in silver appear to improve the results. Lengthening the time in the reducing fluid also seemed to have no effect. In the case of two other modifications, one, in which veronal in absolute alcohol was used for fixing, gave very poor results; the other, in which chloral hydrate was used before ammonia alcohol, was more successful, several sections showing black fibres in the pars anterior.

The most successful of the methods used was as follows: the blood vessels of a rabbit were washed out with Ringer's solution, which was injected into the aorta after all the lower vessels had been tied off. 160 c.c. of ammonia alcohol (100 p.c. alcohol with 1 p.c. ammonia) were then injected. The pituitary was removed, and cut in half; the anterior lobe was cut through again and the pieces fixed for 24 hr. in ammonia alcohol. After washing in distilled water, they were transferred to pyridine for 24 hr. and washed for another 24 hr. in many changes of distilled water. The pieces were then placed in the dark in a large quantity of 2 p.c. silver nitrate at 35° C. for seven days, rinsed in distilled water and reduced for 60 hr. in a 4 p.c. solution of pyrogallic acid in 5 p.c. formalin. They were washed, taken quickly through the alcohols, embedded rapidly in paraffin and cut at 18μ and in one or two cases counter-stained with safranin or methylene blue. This modification gave extremely good results in the case of the pars intermedia, and in some cases parts of the anterior lobe also showed black fibres.

A pituitary in which the animal had been injected with ammonia alcohol with 10 p.c. paraldehyde before using Ranson's method, gave sections which were much too dark to distinguish anything with certainty.

It is of course possible that some of the modifications would have proved successful had more variations of them been tried.

The pars intermedia is richly supplied with small non-myelinated nerve fibres, which often end in knob-like structures in the cells. These fibres are especially numerous at the junction of the intermedia and nervosa (Fig. 1), but are also found to spread all through the intermedia

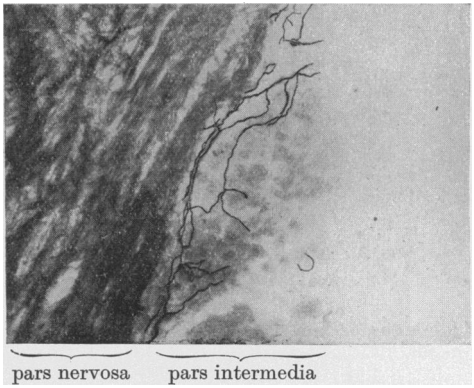

Fig. 1. Pars nervosa and pars intermedia (rabbit). ×500, reduced ⅔. A modification of Ranson's silver-pyridine technique. Non-myelinated nerve fibres seen as thin black lines at the junction of the two lobes and extending into the intermedia. One fibre can be seen ending in a knob, apparently on a cell.

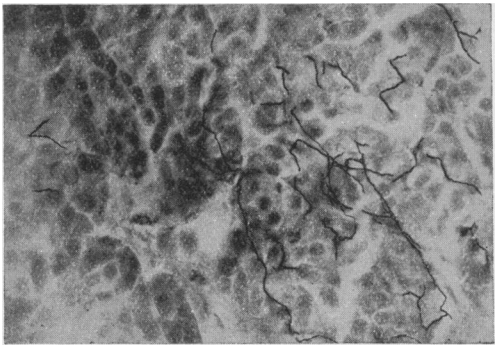

Fig. 2. Pars intermedia of rabbit. ×500, reduced ⅔. A modification of Ranson's silver-pyridine technique, counter-stained with safranin. Non-myelinated fibres seen as black lines.

towards the anterior lobe (Fig. 2). In deciding if these are simply vasomotor fibres, it is interesting to note that in the rabbit, while the pars anterior where nerve fibres are rare is extremely vascular, in the intermedia itself where there are numerous nerve fibres there are very few capillaries to be seen (Fig. 3). It would seem unlikely that the fibres are vasomotor in character.

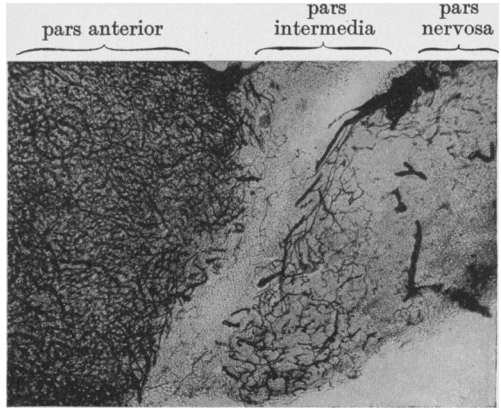

Fig. 3. Pituitary of rabbit showing portions of the three lobes. ×65, reduced ⅔. Injected carmine-gelatine. The pars intermedia is comparatively non-vascular.

The possibility that these fibres are part of the connective tissues has been considered; but it does not seem to carry much weight. On comparing sections showing these fibres with others stained by Van Gieson or by Benian's method (2), it is obvious that the connective tissue is small in amount and does not resemble either in position or form the black fibres stained by Ranson's method. This method is considered to be specific for non-myelinated nerves, staining them black, whereas it usually stains connective tissue brown, if at all.

In the case of the pars anterior it is more difficult to be definite. Black fibres, running between the cells, and ending occasionally in small black knobs, are seen in parts of the sections (Fig. 4). That they do not occur constantly in all parts of the anterior lobe may be due to the capricious nature of the silver methods and to the resistance offered by the closely packed cells of this region of the pituitary. From the sinus-like nature of the blood vessels, it is hardly probable that any nerve fibres which are present are vasomotor.

The picture given by the posterior lobe is somewhat obscured by the number of neuroglial fibres. Black nerve endings in the walls of the

blood vessels resembling sympathetic endings are clearly shown and there are also thin black fibres extending for some distance round the vessels. These are perhaps rather numerous to be connected only with

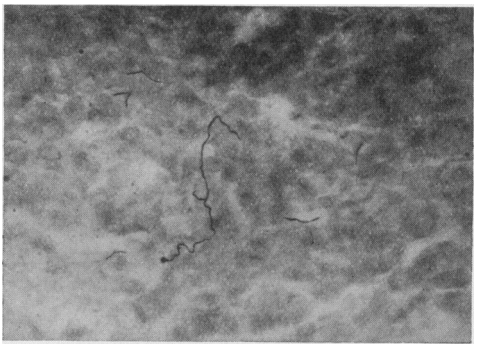

Fig. 4. Pars anterior of rabbit. ×500, reduced ⅔. A modification of Ranson's method. The cells are out of focus in order to enable a long length of fibre to be seen.

the vessels. Fibres resembling those seen in the intermedia are seen near the junction of the two and often pass into the intermedia from the nervosa.

Some attempts have been made to demonstrate the nerve supply in the human pituitary. Perhaps owing to the difficulty of obtaining human material in sufficiently fresh condition and to the larger size of the human pituitary, the methods have not so far been successful. The work will be continued later.

Summary.

(1) Modifications of Ranson's silver-pyridine technique are most successful for demonstrating nerve fibres in the pituitary.

(2) The pars intermedia shows numerous thin black fibres running between the cells and sometimes ending in knobs. They are considered to be non-myelinated nerve fibres which are not vasomotor in function, as the intermedia in the rabbit is very poorly supplied with blood vessels. They do not resemble connective tissue, and appear constantly in sections stained by Ranson's method.

(3) Non-medullated nerve fibres are present in the pars anterior. They do not occur in such number nor so regularly as in the intermedia.

(4) Owing to the great wealth of neuroglial fibres in the pars nervosa the identification of nerve fibres in many areas is difficult. They are, however, abundant in the vicinity of the vessels and at the junction of the nervosa and intermedia.

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