REMARKS ON THE PERFORMANCE AND CONTROL OF HYPOPHYSECTOMY IN THE RAT

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During the last few decades hypophysectomized rats have become of increasing importance in biological and biochemical research. The term hypophysectomy refers to the operative intervention. When the experimental results are described, however, the meaning of this term is often changed, denoting a complete removal of the hypophysis. This paper is concerned with the possibilities of obtaining a satisfactory and uniform result with respect to a) the operative technique, b) the assessment of the result of the operation. Finally, the consequences of an erroneous selection of ectomized animals are exemplified.

OPERATIVE TECHNIQUE

Hypophysectomy in the strict sense of the word, i.e. the anatomical removal of all hypophyseal structures, can hardly be carried out. Thus, it is impossible to remove a part of the stalk, i.e. a part of processus infundibuli and the glandular part called pars tuberalis. These minor remnants of the hypophysis are abandoned if the influences of hypophyseal factors are going to be regarded as eliminated in an experiment.

Good results can be achieved, however, with the technique originally devised by Smith (1927). Other technical descriptions have been published by e.g. Möller-Christensen (1935), Büllring, cited by Burn et al. (1950), Freud (1938), Faber (1945), Selye (1947). The hypophysis is exposed by the parapharyngeal approach and is sucked out with a glass cannula. Tracheotomy is usually carried out as the upper respiratory tract is compressed. A common drawback of this procedure, however, is the development of a subcutaneous emphysema which may kill the animal when it is breathing the air of the subcutaneous pouch. We have found intubation of the trachea with a polythene tube useful, in order to avoid this complication and to provide satisfactory aeration and ether anaesthesia during the operation. A polythene tube of 7 cm. length and...
1.40 × 1.90 mm. of inside × outside diameters will suit rats in the weight range of between 100 and 150 gm. The tip is cut at an angle of about 45 degrees. The passage through the inlet of the larynx is facilitated by deepening the narcosis at that moment and by inspecting the tracheolaryngeal region after having made the usual midline incision in the skin of the neck. It is advisable to suck out the mucous from the trachea carefully before removing the tube at the end of the operation. By this modification of the technique, time is gained for the untrained as well as for the more advanced operations, e.g. transection of the stalk. In some places the removal is made very rapidly in order to make a tracheotomy unnecessary. Such a procedure is, however, incompatible with careful work under a dissecting microscope. The best results with respect to the completeness of the hypophysectomy are obtained, if the opening in the dural membrane is made large enough to expose the hypophysis properly and to make it possible to inspect the gland with a dissecting microscope with 10 or 20 times magnification. Too «careful» preparation may sometimes increase the mortality. This may be due to a faulty technique resulting in hypothalamic lesions as it is often associated with pulmonary edema during the first 24 hours after the operation. The edema is often hard to detect in the still living rat but sometimes it develops so suddenly that much frothy fluid emerges from the nose and throat prior to death. Pressure applied to the hypothalamic region rostral to the anterior part of the hypophysis has been found to be a common cause of a fatal pulmonary edema. This part has to be exposed when the aim is a transection of the stalk. In ordinary hypophysectomy such an exposure ought to be avoided, as well as any undue pressure upon the gland. Another reason is the increased risk of bleeding if the bore hole is placed too rostrally (Fig. 1 a). The mortality is on the other hand not influenced by reserving more time for taking care of a bleeding from the bore hole before the thinning of the inner compact bone lamella is carried out with the drill. There is no risk of causing pulmonary edema by complete removal of residual bone fragments and making a wide opening of the dura by careful traction with a small hook provided that no pressure is applied.

It is difficult to discern the neurological pattern by which pulmonary edema is produced. It has been experimentally induced by circumscribed lesions in the rostral hypothalamus (Gamble & Patton, 1953, Maire & Patton, 1954). Pressure on the hypophysis may possibly affect these centres or their pathways.

**Assessment of the Result of the Operation**

The only safe way of testing the absence of hypophyseal residues is a histological control of the operation region post mortem. The use of such signs as retardation of body growth, thyroid inactivity and atrophy of the adrenals or
the gonads, is insufficient for testing the completeness of the hypophysectomy, although they may suffice for checking a reduced hypophyseal secretory activity.

The microscopic investigation ought to be carried out on serial sections of the operation region. After opening the skull, the brain is elevated with a small curved pair of scissors, the curvature of which is kept against the base of the brain, the *fasciculi optici* are divided and the hypophyseal stalk first stretched and thereafter transected together with a good portion of the hypothalamus attached to it. After having removed the other part of the brain the hypophyseal region of the base of the skull is cut out with a suitable pair of scissors with enough bone left around the bore hole in order to avoid fracture of the bone from the edge to the hole. The specimen is thereafter inspected from below and is freed from muscles and other soft parts. Decalcification is performed in 10 per cent trichloroacetic acid for 5–6 days. The central part surrounding the

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*Fig. 1.*

Microscopic pictures of the hypophyseal region after sham-operation, complete and incomplete hypophysectomy. The specimens are fixed in Heidenhain’s Susa and the sections stained according to his Azan method. All rats were killed 18 days after the operation.

a) Survey of the operation field after sham-operation. The hypophysis with its three lobes is seen undamaged with the stalk rostrally to the right. The anterior lobe is displaced to the bony defect of the bore hole and lies upon granulation tissue, adjacent below the pharyngeal mucous membrane. In the extreme right part of the bone there is seen a wide interlaminar venous sinus, the constant occurrence of which ought to be remembered at the operations. The bore hole in this case might have been situated nearer this sinus than shown by the picture, as new bone ought to have been formed after the operation.

b) The rostral part of the operation field after complete hypophysectomy. The stalk is seen in the right part. No hypophyseal remnants are seen in the richly vascularized granulation tissue.

c) Detail of an operation field. In the bore hole, the edges of which are not seen in this picture, scanty granulation tissue is found together with glands from the pharyngeal mucous membrane. To the right and tapering against the centre of the picture the neural part of the stalk is seen. Downwards at its tip a small group of cells from the *pars tuberalis* appear. The small number of these as well as the absence of other residues in the serial sections has justified the acceptance of this removal. The picture exemplifies the maximal appearance of the *pars tuberalis* cells allowable.

d) Incomplete hypophysectomy. The rostral part of the operation field is located to the right as in the other pictures, but the stalk has been bent 180° backwards in the removal of the brain which happened to cover the operation field *post mortem*. Some distinct rows of cells from the *pars tuberalis* are seen to originate from the artificial bending of the stalk and going downwards in the granulation tissue. A possible explanation for the failure of hypophysectomy is a lateral displacement of the bore hole. The bone defect is rather small considering that the section through the stalk is situated near the median plane.
bore hole is then cut out with a razor blade and serially sectioned in the sagittal plane.

Fig. 1 illustrates the histological examinations in the case of sham-operation, incomplete and complete hypophysectomy. Even in cases where the removal ought to be classified as complete, the existence of a greater or smaller number of cells from the pars tuberalis cannot be avoided. An example of a too large number of such cells is shown in Fig. 1 d, while a more abundant appearance of them than in Fig. 1 c can hardly be accepted. The judgement of lateral and posterior remnants presents no problem as they are not allowable, and no example is therefore included.

**THE RISK OF BIASED SELECTION**

The requirements of careful histological control ought not to be reduced. This is especially important if the experiments have a high mortality which means a selection of the surviving animals. This outcome may easily happen as is shown by the following example. We wished to investigate whether diabetes could be provoked with alloxan in the absence of the hypophysis. As the mortality after alloxan treatment was very high the histological control was extended to the rats which had succumbed. In these, the frequency of acceptable hypophysectomy was fully satisfactory while all surviving animals showed at least small hypophyseal remnants. Thus, it was impossible to draw any conclusion except that a biased selection had taken place.

**SUMMARY**

The term hypophysectomy is discussed, as it has been shown to be incorrectly defined in several publications.

A uniform and acceptable result denotes a satisfactory operative technique as well as the exclusion of animals with hypophyseal remnants as determined by histological control.

With regard to the operative technique the procedure of exposing the hypophysis without producing pulmonary edema is discussed. Intubation is preferred to tracheotomy as the postoperative development of a fatal subcutaneous emphysema is thus avoided.

The essential points of the histological control are stated and illustrated. If these criteria are not fulfilled a serious selection of the material may take place. This is exemplified by an experiment involving the production of alloxan diabetes in hypophysectomized rats.

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REFERENCES

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