

# A HISTOLOGICAL STUDY OF THE OX PITUITARY GLAND AFTER FREEZING AND EXPOSURE

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### I. INTRODUCTION.

THE invariable contamination of commercial preparations of the anterior lobe of the pituitary by autacoids (normally confined to the posterior lobe) suggested that an enquiry should be made into the nature of the processes occurring in the gland subsequent to death. The results of such an investigation showed that post-mortem diffusion between the two lobes took place in addition to autolysis, and that the extent of these processes depends upon the duration of post-mortem exposure and temperature (Spaul, 1925, 1927). Further experiments showed that if the glands are frozen immediately after excision from the skull these changes are inhibited, although there was invariably a small amount of posterior lobe principle present in the anterior lobe; apparently diffusion took place in the interval between death and complete dissection of the gland from the skull. It has, therefore, been impossible to obtain preparations free from posterior lobe principle and, with glands which have been exposed for several hours before freezing, preparations of the anterior lobe have been found to be inactive. These results were obtained by Spaul (1925, 1927) by exposing glands for different times to various temperatures and estimating the amounts of the principles present in extracts prepared from them at the completion of these periods. By these means a graded increase was demonstrated in the amount of melanophore principle in the anterior lobe and a corresponding decrease in the posterior lobe; at the same time a corresponding decrease occurred in the amount of metamorphic principle in the anterior lobe. These effects continued for about 4 hours (depending upon the temperature) after which a decrease in the amount of both principles occurred, presumably owing to the onset of autolytic action. These results have lately received further confirmation from the chemical investigations (unpublished) of Spaul and Myddleton (1929).

In view, therefore, of this sequence, it was of interest to examine similar material histologically, to determine whether a sequence of correlative cytoplasmic changes could be detected. With this object in view the present work was undertaken.

## 2. METHODS.

It was realised that, in order to obtain reliable results, as large a number of stains and fixatives as possible should be used. Further, sections from each of the series of glands must be treated, as far as possible, in exactly the same manner and with the same solutions. Only progressive stains, not liable to be removed during clearing and mounting, were therefore used. Different times of staining and concentrations of stain were also employed, as it was felt that by so doing, optimum conditions for the demonstration of the finer cytoplasmic changes might be obtained.

For these experiments fresh and frozen ox pituitary glands were obtained at the abattoir and rapidly transferred to the laboratory, where they were exposed in a hot air oven, maintained at constant temperature, for the required length of time.

The following series were used (the times given are those which lapsed between the placing of the gland in the oven and its withdrawal):

*A.* Unfrozen glands fixed fresh and after an exposure of 3 and 6 hours to 40° C.

*B.* (1) Frozen glands, fixed after an exposure of 0 and 3½ hours to 30° C.

(2) Frozen glands, fixed after an exposure of 0, 2, 4 and 6 hours to 40° C.

(3) Frozen glands, fixed after an exposure of 0, 2½ and 5 hours to 45° C.

(4) Frozen anterior lobes, separated before exposure and fixed after 0, 1½, 2½, 3½ and 5 hours at 45° C.

(5) Frozen posterior lobes, separated before exposure and fixed after 2½ hours at 45° C.

The glands were cut into three pieces in sagittal, horizontal and transverse planes and fixed in Bouin, Carnoy, Flemming without acetic, Gilson and formalin bichromate. Fresh and frozen glands were also fixed in Cajal's uranium formol and Da Fano's cobalt formol. After taking up through the alcohols, the material was cleared in cedar-wood oil, embedded, and sections cut throughout the depth of each piece.

The following stains were used: haematoxylin and Biebrich scarlet, Mallory's connective tissue stain, Weigert's iron haematoxylin and Van Gieson, Westphal's and Ehrlich's mixtures for granules and various mixtures of magenta and light green, but all except the first two gave no differentiation between the basophil and oxyphil cells, and were therefore discarded. An attempt to fix methyl green and methyl violet in the tissues with iodine was made, but the strongly iodophil reaction of certain cells thwarted any efforts to obtain good preparations. Leishman, Hastings-Romanowsky and the iodine leucobase technique (Spaul and Howes, 1929, unpublished) were also used, but a constant depth of stain could not be obtained owing to their solubility in alcohol and, accordingly, their use was confined solely to the checking of the other results.

The series eventually used throughout this work was:

Haematoxylin	Biebrich scarlet	Mallory's connective tissue stain
2½ min.	15 min.	Solution 1, 5 min.
5 "	10 "	" 2, 2 "
5 "	15 "	" 3, 10 "
5 "	20 "	
10 "	15 "	
15 "	15 "	

Since it was found that only in this range of the first series were basiphil and oxyphil differentiated at all, times beyond these limits were not used except in preliminary experiments. Similarly, no times other than those given above were used for Mallory, as these were the most effective in giving a marked differentiation between the various types of cells present in fresh glands. With this stain the most consistent results were obtained with Gilson material.

Slides were stained in pairs in 40 c.c. of stain in specimen tubes. Sections from alternate members of each series of glands were taken in each pair, so that any adventitious error, due to alterations in the concentration of the stain, or to different rates of dehydration, was more or less neutralised and tended to destroy, rather than enhance, any continuity that might be present in the series. Large stocks of stain were made up at the beginning of the experiment and the stain in the tubes frequently renewed from stock, in order to avoid errors due to dilution or concentration.

### 3. OBSERVATIONS.

An understanding of the changes described below involves some knowledge of the distribution of the cells in the anterior lobe of the ox pituitary. Briefly, there is a central axis, with a cone-shaped expansion at the anterior end, which is marked off from the more peripheral region by the coarse white fibrous tissue trabeculae which it contains, and by the fact that the cells present are basiphil in their staining reaction; these latter are of two kinds: (a) weak basiphils, of oval or often irregular outline, and (b) strong basiphils, oval, and generally regular in shape (Smith and Smith, 1925; Spaul and Howes, 1929). In the surrounding areas, the connective tissue trabeculae are much finer and the cells either weakly or strongly oxyphil, the latter, large, oval, and regular, predominating, particularly where this area is in contact with the basiphil area; the former cells are frequently irregular in shape and are found in greatest numbers towards the periphery.

#### *Low power examination.*

Only detailed descriptions of the changes found in series A and B 2 (*i.e.* unfrozen glands exposed for 3 and 6 hours to 40° C. and frozen glands exposed for 2, 4 and 7½ hours to 40° C.) are given, as the most pronounced results were obtained at this temperature. Results obtained in other series are referred to these for comparison. The colours observed with Mallory's stain are used as a basis for description because, although similar results were obtained with haematoxylin and Biebrich scarlet, the bright colours and strong differentiation given by this technique enabled a more detailed study to be made with low power objectives.

With Mallory's method the cells in the gland are stained as described in Table I.

Table I.

Tissue	Staining reaction	Tissue	Staining reaction
Weak basiphils Weak oxyphils Pars intermedia Connective tissue	Faintly blue Faintly blue Fawn Blue	Strong basiphils Strong oxyphils Pars nervosa Nuclei	Deep purplish red Bright orange red Slate grey Yellow or unstained

The unfrozen glands were not greatly affected by exposure, but it was observed that there was a progressive invasion of the basiphil area by acid dye, accompanied, towards the end of exposure, by the formation of a rind of reddish orange protoplasm round the anterior lobe; the original slate grey coloration of the pars nervosa was gradually replaced by blue.

The glands fixed while still frozen showed a vacuolated appearance, but the distribution of the basiphil area and the extent of its demarcation from the oxyphil area were unaltered. It was found that upon exposure the frozen gland lost its vacuolated appearance and that the acid dye became evenly distributed throughout the oxyphil area, whereas in the unexposed gland it was more concentrated in the neighbourhood of the basiphil area. After the first exposure the line of demarcation between the basiphil and oxyphil areas was still distinct, but a gradual invasion of the former by acid dye took place and eventually the whole pars anterior showed an oxyphil reaction, although the staining capacity throughout the whole gland had decreased. A rind of reddish orange protoplasm, differing slightly in colour, and in its solid non-granular consistency, from the rest of the oxyphil area, was gradually formed. Concurrently with these processes the original slate grey of the pars nervosa was replaced irregularly by blue.

Corresponding, but much slower, changes were observed in the glands exposed to 30° C. and 45° C.; the latter showed a very thick oxyphil rind.

#### *High power examination.*

The effect of exposure on the unfrozen glands was to cause the appearance of numerous oxyphil granules in the cytoplasm of the cells of the basiphil area. The oxyphil cells themselves tended to be less discrete and many showed degenerative changes; a rind, presumably of coalescent oxyphil cells and of clear non-granular protoplasm, was formed.

The glands fixed while still frozen showed good fixation and the staining seemed unaffected. Large needle-shaped spaces, particularly in the pars nervosa, and also the displacement of cells towards one side in the spaces between the connective tissue trabeculae—themselves scarcely deformed—indicated the presence of ice crystals at the time of fixation. An interesting phenomenon was the complete disappearance of the weakly staining cells, all the cytoplasm being either strongly basiphil or oxyphil, yet a number of intact strongly basiphil and oxyphil cells were still to be found. Golgi bodies were not observed in frozen glands, although noted in similarly treated fresh glands, but a large number of cells containing numerous medium sized black granules were found. Further, intact oxyphil cells remained in exposed frozen glands even after 4½ hours' exposure. The cells of the basiphil area first showed an oxyphil reaction in isolated granules; these gradually increased in number until the whole of their cytoplasm became oxyphil. The junction between the pars anterior and the pars intermedia became progressively less well defined as the length of exposure increased.

*Summary of results.*

The unfrozen glands show, with exposure, a tendency for the basiphil area to be invaded by acid dye, accompanied by the formation of a broadening band of oxyphil staining, coalescent cells round the periphery of the gland. This effect is dependent upon temperature and length of exposure.

The frozen glands show a much greater tendency to equalisation in the two staining areas, at the same time the depth of staining decreases and a movement toward an intermediate reaction is evident. The rind formation is more marked, more rapidly developed, and its colour brighter, affecting proportionately more cells.

The demarcation between the pars anterior and the pars intermedia becomes less distinct.

The development of the bluish patch in the pars nervosa is more marked in the frozen than in the unfrozen gland.

4. DISCUSSION.

These observations show a distinct and interesting sequence of histological change, the significance of which becomes apparent when it is compared with the results of investigations upon the biological activity, and also of chemical studies, of glands similarly exposed (Spaul, 1925, 1927; Spaul and Myddleton, unpublished). As already indicated, these studies showed a loss of activity of both lobes due to post-mortem diffusion between them and, later, also to autolysis. These effects were accentuated as exposure increased and by rise of temperature. This agrees very closely with the histological sequence noted, particularly in the frozen glands.

Fischer (1899), and later Mann (1902), performed experiments with different concentrations of stains and times of staining upon films of precipitated albumen and gelatine, but no record of any attempt to apply these methods to the study of post-mortem changes affecting specific secretions has been found. The application of this method has been successful here, since its delicacy has made it possible to follow the course and direction of certain cytoplasmic changes by means of alterations in staining capacity. The general effect produced is indicative of the changes in progress but, on the other hand, it must not be inferred that any specific secretion is selectively stained.

The effects of freezing and exposure upon the microscopic structure of the gland were not surprising since alternate freezing and thawing is an orthodox method for obtaining tissue juice. The most striking feature in this case is the destruction of cells, especially of the weakly staining basiphils and oxyphils. This destruction both facilitates the preparation of extracts and makes for the more rapid diffusion and destruction of the active principles, as demonstrated above.

The manner in which the acid dye spreads into the basiphil area, and the eventual approach to homogeneity of staining reaction throughout the anterior lobe after exposure, are parallel to the results of the biological and chemical investigations upon the distribution of the activity before and after exposure of the gland (Spaul, 1925, 1927; Spaul and Myddleton, 1929, unpublished). This work showed that, with

exposure, the higher concentration of metamorphic activity at the inside of the lobe becomes more evenly distributed after exposure.

A further point is the unaltered relationship of the basiphil area after freezing; this is of interest in view of the unaltered activity of extracts, when prepared from glands kept in the frozen condition for several days (Spaul, 1925).

The changes in the anterior lobe, apart from those of freezing, thus seem to indicate the diffusion of the principles; but a demonstration of similar diffusion between the lobes was not nearly so definitely established. The only sign which can be justly so interpreted is the increasing vagueness in the delimitation between the pars intermedia and the pars anterior at their junction around the cleft. There is thus a tendency for the pars intermedia to merge into the pars anterior.

The peripheral changes noted, the cloudy appearance of the cells after long exposure, and the altered staining reaction as uniformity is approached in the anterior lobe, are undoubted signs of the autolytic actions shown by Spaul in the decrease in activity of extracts made after similar exposures.

It will be noticed that the graded sequence with temperature became less constant at 45° C.; at this temperature, which is far above body heat, autolytic and probably coagulative changes occur, which would explain this inconsistency.

In conclusion, it is of interest to record some results of the examination of glands used for commercial preparations. The material consisted of three groups of ox pituitaries, each collected from a single breed of animals, and imported frozen from the Argentine. Sample glands, taken from each group, were examined histologically. By comparing these with the sequence obtained in this work, their probable biological activity was judged. Actual biological tests showed that this estimate was accurate within its obvious limitations. Hence, it is possible that the suitability, or otherwise, of glands for the preparation of extracts, could be judged by this means.

## 5. SUMMARY.

1. A histological method is described for a study of post-mortem cytoplasmic changes; the method depends upon observed variations in staining capacity.

2. By the application of this method, a series of cytoplasmic changes were found in ox pituitary glands which had been exposed, after the death of the animal, for varying times to different temperatures. These changes correspond to alterations in the biological activity and chemical content previously investigated.

3. These methods showed that freezing apparently causes no alteration in the distribution of biological activity.

4. It is possible that the histological appearance can be used to determine the suitability of ox pituitary glands for the preparation of active extracts of the anterior lobe.

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#### 6. REFERENCES.

- FISCHER, A. (1899). *Fixirung, Färbung u. Bau d. Protoplasms*, p. 122.  
MANN, G. (1902). *Methods and Theory of Physiological Histology*, p. 208.  
SMITH, P. E. and SMITH, I. P. (1925). *Proc. Amer. Assoc. Anat. Rec.* **25**, 150.  
SPAUL, E. A. (1925). *Brit. Journ. Exp. Biol.* **2**, 427.  
—— (1927). *Brit. Journ. Exp. Biol.* **5**, No. 2, 166.  
—— (1929). *Brit. Journ. Exp. Biol.* (In press.)  
SPAUL, E. A. and HOWES, N. H. (1929). *Brit. Journ. Exp. Biol.* (In press.)  
SPAUL, E. A. and MYDDLETON, W. (1929). Unpublished.