- (4) Rinse in 2% lead nitrate for 5~10 minutes.
- (5) Wash in water.
- (6) Rinse in diluted yellow ammonium sulfide solution for 2~3 minutes.
- (7) Wash in water.
- (8) Mount in gum syrup of glycerin-jelly.

Results and Discussion

Moderately positive reactions were recognized in pancreatic cells. However, no reaction was seen Langerhans' islet cells. Slightly positive reactions were seen in proximal convolution of kidney. However, no distinctly positive reactions were seen in liver cells, stomach and intestine.

The present histochemical method is a preliminary one and will be improved by the further experiments, because the author thinks that other methods for getting much better emulsified incubation medium may be developed. The substrate specificity of the enzyme activity should be considered in connection with lipase. In control tests using heated tissue sections there were not recognized any positive reactions.

Histochemical Studies on the Iron Hematoxylin Stained Granules Found in the Adrenal Cortex and the Anterior Pituitary of Rats

Ichiro YAMAGATA, Tatsuji HAGIHARA, Jun KATADA,
Reiko TAKADA, and Ryuei MAEDA

Department of Pathology, Kansai Medical School, Moriguchi City, Osaka

An assumption has been made in our previous report¹⁾ that the sudanophilic granules in paraffin sections of the adrenal cortex fixed with Tellyesniczky's fluid contain corticoids from a result of a successful corticoid determination made of the extracts from sudanophilic components of bovine adrenals fixed with the above fixative.

In 1963, at 52nd Annual Meeting of the Japan Society of Pathology, a subsequent report was made in which the authors have proved that the iron hematoxylin stained granules could be found in increased amount in the state of hyperfunction of the adrenal cortex induced by administration of ACTH.

Present study is to investigate the nature of iron hematoxylin blue stained granules from the histochemical approach, and pursue further on the character

of these granules of the adrenal cortex and anterior pituitary gland when stimulated under an influence of various hormones.

Materials and Methods

The experimental animals used were the rats of Wistar and Sprague-Dowley strains numbering 106 of which 45 were males and the remaining 61 were females.

A group of animals of respective sex were administered of testosterone hypodermically daily for a period of two weeks, dosage ranging from 0.2 mg. to 2.0 mg. A group of animals given with estrone was injected daily for a period of two weeks, the dosage was 0.2 mg. for males and 0.001 mg. and 1.0 mg. for females.

Another group of animals were orally administered of thyroid powder daily for a period of two weeks in the dosage of 5.0 mg. and 50 mg. in suspended fluid by means of a catheter. The room temperature was kept around 25°C. The accompanied tables show numbers of the animals, average weight of adrenals and pituitaries.

Table

Treatment (mg.)		Testosterone		Estrone		Thyroid powder		Control
		2.0	0.2	1.0	0.001	50.0	5.0	
No. of rats	♂	14	10	4*		9	8	10
	♀	12	9	14	8	10	8	10
Mean body weight (g.)	♂	177.4	213.0	187.5*		176.2	215.6	153.0
	♀	156.6	169.3	132.3	155.2	138.2	148.1	138.7
Mean pituitary weight (mg.)	♂	6.0	6.4	11.4*		6.2	6.3	5.1
	♀	6.8	7.0	11.1	6.1	5.8	6.9	5.9
Mean adrenal weight (mg.)	♂	14.2	15.7	25.4*		20.1	19.8	15.2
	♀	16.3	25.3	24.7	26.2	29.0	25.1	18.6

* Estrone 0.2mg.

For the fixation of adrenal and pituitary glands, the following fluid methods can be enumerated: i.e., Telleyesniczky's fluid containing 3% potassium dichromate dissolved in 5% glacial acetic acid and Elftman's fixative⁶⁾ containing 2.5% potassium dichromate dissolved in 5% mercuric chloride solution. For the staining, besides our devised iron hematoxylin-Sudan III stain¹¹⁾, there have been in use such methods as Wilson-Ezrin's PAS-methyl blue method¹²⁾, Chiffelle Putt's propylene glycol-Sudan black B method²⁾ in paraffin sections, Klüber-Barrera's Luxol Fast blue B method¹⁰⁾ for phospholipids, and Barnett-Seligman's alkaline blue tetrazolium (B.T.) reaction¹⁾ for disulfide and sulfhydroxyl groups.

Results

In the differentiation of the three cellular characteristics of the acidophils, basophils and chromophils of the anterior pituitary body fixed with Elftman's fluid, Wilson-Ezrin's PAS-methyl blue method seems to offer superior staining quality. The acidophils could be well stained with iron hematoxylin after fixed in either Elftman's or Telleyeniczky's fluid, alkaline B. T. method for disulfhydroxy groups also having offered positive result; such staining quality of the acidophils appears to be different from that of the adrenocortical cells which usually show negative test with Heidenhain's iron hematoxylin staining when Elftman's fixative is employed.

The cytoplasmic granules equivalent to the iron hematoxylin stained granules in the adrenocortical cells or acidophils of the anterior pituitary gland fixed in Elftman's fluid could readily be stained with Luxol Fast blue B or propyleneglycol-Sudan black B method, but these granules were negative with Sudan III stain in frozen sections.

With a group administered of testosterone, the hormonal sensitivity seemed to be more marked with the males than females, so with the sudanophilic granules which became less fine and definitely decreased in the zona fasciculata. In some instances, the major portion of protoplasm of the zona fasciculata cell was replaced with the sudanophilic granules with lesser iron hematoxylin stained granules; on the contrary the latter was increased in the anterior pituitary body along with increased acidophils containing these granules.

In those groups of animals which were given thyroid powder 50 mg. or estrone 1 mg., the zona fasciculata cells have undergone hyperplastic changes, presenting a wider boundary. The sudanophilic granules became minutely fine and decreased though with the increased iron hematoxylin stained granules. The zona reticularis also presented picture of hyperplasia. The other prominent changes of the above two zones of reticularis and fasciculata frequently observed were the appearance of the fine sudanophilic granules or iron hematoxylin stained granules in the sinusoids. These changes would be presumably attributable to the presence of the granules which hold corticoids released from the cytoplasm of the adrenocortical cells into the sinusoids. Accordingly it would be reasonable to assume that an increased amount of the iron hematoxylin stained granules implies the elevated function of adrenocortical cells. Reduction in percentage of acidophils in the anterior pituitary body has occurred only in the groups in which thyroid powder has been given but not in the group which received estrone.

Discussion

As Parse¹²⁾ had already pointed out Luxol Fast blue B method has proved to be a reliable test for phospholipid other than the sphingomyelin. Propylene glycol-Sudan black B method²⁾ used in the present study to test phospholipid has been based on principles of a modified Elftman's controlled chromatin⁵⁾.

While Rennels has demonstrated presence of phospholipid in acidophils of the anterior pituitary using Bakers method but made no reference as to its possible combination with protein, he believed that this phospholipid was to be identified as the cytoplasmic secretory granules rather than mitochondria of acidophils, in conformity with the authors opinion. Therefore, it seems reasonable to presume that a chief constituent of the iron hematoxylin stained granules found in adrenocortical cells or acidophils of the anterior pituitary has been derived from insoluble phospholipids (masked phospholipids) which have been combined with certain proteins, considering a fact that the frozen section has failed to present positive result with Sudan III stain.

Barnett and Seligman have made a detail report on the distribution of protein bound disulfides and sulfhydroxyl groups: the same result been obtained with our experiment.

Deane⁴⁾ et al and Wallach¹⁵⁾ have already demonstrated hyperplastic changes of the zona fasciculata following thyroxin administration, Greep and

Jones⁸⁾ having mentioned the similar phenomenon in connection with oestrogen, a well demonstrated fact in the present study.

Considering the increased iron hematoxylin stained granules in the adrenocortex of the groups receiving estrone and thyroxin powder, the authors have inclined to support the view of Symington et al^{3,7)} emphasizing that the synthesis of corticoids is carried out in the zona reticularis, the zona fasciculata taking a role of storing steroid precursor in resting condition and that the synthesis and secretion of corticoids occur markedly in the zona fasciculata in conditions of stress.

As for the hypophysis, the remarkably increased weight of the anterior pituitary in females following oestrogen administration seems to agree with what Wolfe et al^{16,17)} have reported. Halmi¹⁹⁾ has reported on the reduced size of acidophils of the anterior pituitary, while in the present study a reduction in the levels of acidophils has been found. The high rate of acidophils of the anterior pituitary, as Waiman¹⁴⁾ and Wolfe¹⁸⁾ have already stated, was caused under the influence of testosterone in our investigation.

Summary

It has been proved that the chief component of the iron hematoxylin stained granules found in rat adrenocortical cells as well as acidophils of rat anterior pituitary may be considered to be protein bound phospholipid.

Further conclusion has been obtained that the increased iron hematoxylin stained granules should be regarded as the bodies intimately concerned in the corticoid formation.

Explanation of Figures

The adrenals and pituitaries were fixed in Telleyesniczky's fluid or Elftman's Fluid and cut in paraffin sections.

- Fig. 1 The adrenal cortex of a female rat, weighing 130 g. injected with testosterone 2 mg daily for 2 weeks. Photomicrograph shows the area of fatty metaplasia in the zona fasciculata. The sudanophilic granules appear to be roughened and iron hematoxylin staining quality seems to be reduced. Iron hematoxylin-Sudan III stain.
- Fig. 2 The adrenal cortex of a female rat, weighing 180 g, administered of thyroid powder 50 mg, daily for 2 weeks. The iron hematoxylin stained granules in the zona fasciculata are increased remarkably while the sudanophilic granules are decreased and extremely fine in appearance. Dark blue coloration of the zona fasciculata is almost indistinguishable with color of the zona reticularis. Iron hematoxylin-Sudan III stain.
- Fig. 3 The adrenal cortex of a normal female rat, weighing 150 g. Iron hematoxylin-Sudan III stain.
- Fig. 4 The adrenal cortex of a female rat, weighing 140 g, injected with estrone 1 mg daily for 2 weeks. Numerous fine sudanophilic granules presumably released from the cellular cytoplasm of the zona reticularis could be found in the sinusoids as indicated by arrows. The finding presents the secretory picture of the eccrine gland. Iron hematoxylin-Sudan III stain.
- Fig. 5 Paraffin section of the adrenal cortex of a normal female rat, weighing 150 g, fixed in Elftmans fluid. Note a greenish blue coloration of the cytoplasmic granules. Luxol Fast blue B staining.
- Fig. 6 Paraffin section of the anterior pituitary of a normal female rat, weighing 150 g, fixed in Elftmans fluid. Luxol Fast blue B staining.

- 1) Barnett, R. J., and Seligman, A. M. : Nat. Canc. Int., 14, 769, 1954.
- 2) Chiffelle, T. L., and Putt, F. A. : Stain Technol., 26, 51, 1951.
- 3) Currie, A. R., Symington, T., and Grant, J. K. : The Human Adrenal Cortex, Livingstone, Edinburgh & London, 1962.
- 4) Deane, H. W., and Greep, R. O. : Endocrinology, 41, 243, 1947.
- 5) Elftman, H. : J. Histochem. & Cytochem., 2, 1, 1954.
- 6) Elftman, H. : Stain Technol., 32, 29, 1957.
- 7) Grant, J. K., Symington, T., and Duguid, W. P. : J. Clin. Endocrinol. & Metab., 17, 933, 1957.
- 8) Greep, R. O., and Jones, I. C. : Recent Progress in Hormone Res., 5, 197, 1950.
- 9) Halmi, N. S. : Anat. Rec., 112, 17, 1952.
- 10) Klüber, H., and Barrera, E. : J. Neuro-path. & Exper. Neurol., 124, 400, 1953.
- 11) Maeda, R., Takada, R., and Yamagata, I. : Proc. Jap. Histochem. Ass., The Second Annual General Meeting, p. 211, 1961.
- 12) Pearse, A. G. E. : Histochemistry, Theoretical and Applied, 2 Ed., Churchill, London, 1961.
- 13) Rennels, E. G., Anat. Rec., 115, 659, 1953.
- 14) Waiman, P., Reese, J. D., and Koneff, A. A. : Endocrinology, 31, 302, 1942.
- 15) Wallach, D. P., and Reineke, E. P. : Endocrinology, 45, 75, 1949.
- 16) Wolfe, J. M., and Chadwick, C. S. : Endocrinology, 20, 503, 1936.
- 17) Wolfe, J. M., and Wright, A. M. : Endocrinology, 23, 200, 1938.
- 18) Wolfe, J. M. : Endocrinology, 29, 969, 1941.

Studies on Some Properties of Protein Granules in Parathyroid Cells of the Japanese Quail (*Coturnix Coturnix Japonica*)

Kazuyori YAMADA

Department of Anatomy, Nagoya University School of Medicine, Nagoya, Japan

In the course of our current attempts to cytologically demonstrate particular inclusions associated with the functional activity of parathyroid cells, three types of protein granules have been found which exhibit morphological features suggestive of their importance in the cytophysiology of the cells (Hara and Yamada, 1962 and 1963; Yamada, 1963). These granules were shown to react positively to coupled tetrazonium, 2,2'-dihydroxy-6,6'-dinaphthyl disulfide (DDD) diazo blue B and 2-hydroxy-3-naphthoic acid hydrazide (HNAH) diazo blue B stains respectively and conceived to be concerned with either secretory or metabolic activity of the parathyroid cells. Although morphological features and chemocytological properties of the protein granules have been studied previously in the mammalian and amphibian parathyroid cells, precise examination still remains to be made on the granules occurring in the avian cells.

It is the purpose of this paper to study morphological features and chemocytological properties of the three types of protein granules in the parathyroid cells of the Japanese quail (*Coturnix coturnix japonica*) and to discuss the cytophysiological significance of the granules on the basis of the data obtained.