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Localization of Adrenal Cortical Hormones in the Adrenal Cortex of the Cat.

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Recently Reichstein and his collaborators (Reichstein,[1, 2] Reichstein and von Euw[3]) have isolated 3 ketone steroid compounds—corticosterone, desoxycorticosterone, and dehydrocorticosterone, any one of which will, in pure form, maintain the life of adrenalectomized animals. They have shown that all the biological activity in an alcoholic extract of beef adrenals is confined to the ketone fraction of the extract, whereas the ketone-free fraction is without specific activity in adrenalectomized animals (Reichstein[4]). All the active compounds so far isolated have been shown by Reichstein to possess 2 or 3 keto groups. They can be separated, with other keto compounds, from a crude extract by means of their reaction with semicarbazide, and they will reduce ammoniacal silver solution. This latter reaction is due to the presence in all three compounds of a side chain bearing an α-hydroxy keto grouping.

In the present study these reactions, with others, have been applied to frozen sections of fresh or formol-fixed cat adrenals in an attempt to localize the hormones in the cortex. The ascorbic acid was removed from the sections by treating with iodine, indophenol, or oxygenated glycine-sodium carbonate buffer at pH 8.4. The sections were then placed in a solution of phenylhydrazine hydrochloride in de-oxygenated sodium acetate-acetic acid buffer at pH 6.5-6 for a few hours. A distinct yellow band appeared in the outer portion of the fasciculata in the zone occupied by the large lipoid-rich cells called "spongiocytes" by Guieysse.[5] This yellow band appears to be due to the formation of phenylhydrazones with the carbonyl groups in the cortex. The phenylhydrazone formation was prevented by extracting the sections with acetone or alcohol, or by treating the sections with semicarbazide before immersing in phenylhydrazine. This zone

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1 Reichstein, T., *Helv. Chim. Acta*, 1937, **20**, 953.

2 Reichstein, T., *Helv. Chim. Acta*, 1937, **20**, 978.

3 Reichstein, T., and von Euw, J., *Helv. Chim. Acta*, 1938, **21**, 1197.

4 Reichstein, T., *Helv. Chim. Acta*, 1936, **19**, 29.

5 Guieysse, A., *J. de l'anat. et de physiol.*, 1901, **37**, 312.

of cells was further characterized by reducing an ammoniacal silver solution. This reduction was also prevented by the previous treatment of the sections with acetone or alcohol or with semicarbazide. In cortical tissue outside this zone there was no detectable phenylhydrazone formation, or any reduction of ammoniacal silver solution which could be prevented by semicarbazide or extraction with acetone or alcohol.

It follows from these observations that the distribution of acetone or alcohol soluble keto compounds—and hence of the adrenal cortical hormones—must be confined to the zone of spongiocytes in the outer fasciculata, and that such compounds are absent in detectable quantities from other zones of the cat's adrenal cortex. Moreover, desoxycorticosterone acetate has been found to blacken osmic acid. From this it might be inferred that the hormones in the cells would also blacken osmic acid, and if this is so, the hormones must be present only in the osmophile portions of the cells showing the histochemical reactions consistent with those of the cortical hormones. This would localize the hormone more precisely in the osmophile lipoid vacuoles of the spongiocytes.

The zone of spongiocytes is further characterized by showing numerous birefringent crystals in frozen sections of glands treated with aqueous or alcoholic digitonin solution. These crystals are scanty or lacking in other zones of the cortex, and are probably for the most part due to the presence of cholesterol, which is of interest since Fieser[6] regards steroid hormones as probably being formed by the oxidation of cholesterol. Although Reichstein[2] regards this as highly questionable and thinks of cholesterol as perhaps an end-product of synthesis rather than a precursor of the hormone, the presence of cholesterol in the zone showing evidence of containing the hormone makes it seem likely that the adrenal secretion is actually formed in the spongiocytes and not merely stored there.

For these reasons the zone of spongiocytes, in which the adrenal cortical hormones and cholesterol are present, should be regarded as the "secretory zone" of the adrenal cortex of the cat.

Since it has been shown that the cells of the adrenal cortex form in the sub-capsular region and migrate toward the medulla, the zone of cells between the secretory zone and the capsule in which no phenylhydrazones form, and where osmophile vacuoles are scanty, could conveniently be termed the "presecretory zone," whereas the area between the secretory zone and the medulla could be regarded as

[6] Fieser, L. F., *The Chemistry of Natural Products Related to Phenanthrene*, Reinhold Publishing Co., 1936, p. 255.

the "postsecretory zone." The latter is in part characterized by the presence of numerous degenerating and senescent cells.

A complete account of the methods used and a correlation of the histochemical findings with histological and cytological data will appear in the *American Journal of Anatomy.*

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Determination of Prothrombin.

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The author[1] developed a quantitative method for the determination of prothrombin based on the principle that the clotting time of blood or plasma is a quantitative measure of the prothrombin concentration provided an excess of thrombin and a constant concentration of calcium are present. For convenience and accuracy, the blood is oxalated and the test done on the plasma. It was demonstrated, however, that the test can be applied to whole blood.[2] In this determina-

TABLE I.
Comparison of Clotting Times of Unoxalated Plasma and of Recalcified Oxalated Plasma in Presence of Excess Thromboplastin.

	cc		cc
Chicken plasma unoxalated	0.1	Chicken plasma oxalated	0.1
Saline (0.85%)	0.1	Calcium chloride 0.025 M	0.1
Thromboplastin*	0.1	Thromboplastin*	0.1
Clotting time in seconds, 10 to 12		Clotting time in seconds, 10 to 11	
Goose plasma unoxalated	0.1	Goose plasma oxalated	0.1
Saline (0.85%)	0.1	Calcium chloride 0.025 M	0.1
Thromboplastin*	0.1	Thromboplastin*	0.1
Clotting time in seconds, 12		Clotting time in seconds, 11	
Human blood	0.9	Human plasma oxalated	0.1
Thromboplastin†	0.1	Calcium chloride 0.025 M	0.1
		Thromboplastin†	0.01
Clotting time in seconds, 12		Clotting time in seconds, 12½	

* From chicken brain.
† From rabbit brain.

[1] Quick, A. J., *J. Biol. Chem.*, 1935, **109**, lxxiii.

[2] Quick, A. J., Stanley-Brown, M., and Bancroft, F. W., *Am. J. Med. Sci.*, 1935, **190**, 501.