

After 25-30 days on the diet, a marked darkening of the fur amounting practically to a cure (only "stippling" remaining) was noted in the group supplemented with calcium pantothenate alone and with calcium pantothenate plus p-amino benzoic acid. The animals receiving p-amino benzoic acid alone were not altered in appearance and were indistinguishable from the controls. There was an evident stimulus to growth as well as cure of the graying in the animals supplemented with the calcium pantothenate alone or the pantothenate and p-amino benzoic acid. The p-amino benzoic acid alone, which had no influence on the graying, also evoked no growth response.

In spite of the negative curative efforts just enumerated, there remained the possibility that graying could be produced with the exact Ansbacher diet regardless of the fact that it was supplemented with 500 μ g of calcium pantothenate daily and that such graying could be prevented or cured with p-amino benzoic acid.

Accordingly 30 animals were divided into 3 groups of 10 each, as follows: (1) This group received the Ansbacher supplements. (2) Same as above; it was planned to give p-amino benzoic acid when graying developed. (3) This group received prophylactically the Ansbacher diet plus 3 mg p-amino benzoic acid.

After more than 2 months on this diet, no evidence of graying has appeared in any of these animals. In our experience animals deficient in pantothenic acid invariably show marked graying before the expiration of this time interval.

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Cellular Response to Insulin in Suprarenals of Pigeons.

RICHARD A. MILLER AND OSCAR RIDDLE.

From the Carnegie Institution of Washington, Department of Genetics, Cold Spring Harbor, N. Y.

Several years ago this laboratory reported that the suprarenals of pigeons and doves respond by enlargement to single large doses or to repeated smaller doses of insulin.¹ It was found further that delayed or secondary effects of heavy doses of insulin sometimes included the production of abnormally high blood sugars. Histological examination of the suprarenals indicated that the enlarge-

¹ Riddle, O., Honeywell, H. E., and Fisher, W. S., *Am. J. Physiol.*, 1924, **67**, 333.

ment was chiefly or wholly in the cortical tissue. Poll² confirmed this enlargement of the cortical tissue of pigeons (and rats) following insulin; in rabbits Schenk and Langecker³ reported like results, while Thatcher⁴ noted no effect. Clear and definite results with pigeons is doubtless associated with the fact that this species survives enormous doses of insulin.⁵ In all the earlier studies on pigeons, adult birds of various races with varying weight of body and adrenals were used. We have now repeated and extended those observations on a uniform stock of young White Carneau pigeons (1.9 months from hatching), utilizing modern criteria of cellular activity in adrenal tissue, and also measured the influence of various levels of the reduced food-intake which accompanies all treatment of pigeons with insulin.

Results. Both before and during the present study it was known that the weights of adrenals of uninjected birds of this age and race are fairly constant. Even the fact that birds killed in autumn and winter are heavier than those of spring and summer involves a mean difference of only 9% in adrenal weight in the two groups. Thus for autumn-winter the mean weight of 62 pairs of adrenals is $31.63 \pm .62$ mg ($\sigma = 4.96 \pm .44$); for spring-summer (25 pairs of adrenals), $28.8 \pm .89$ mg ($\sigma = 4.45 \pm .63$). Though correction to uniform body weight would still further reduce this variability (and these standard errors) it does not seem advisable to make this correction for either the control or injected birds used in this study. At 10 days after hypophysectomy (anterior lobe only) the adrenals of 22 uninjected control pigeons (eating about 9 g food per bird per day) had a mean weight of $24.00 \pm .88$ mg ($\sigma = 4.12 \pm .62$). The effects on adrenal weight of insulin injection and of various degrees of underfeeding may be appraised by reference to the data just given and by the data of Table 1.

The tabulated data show that adrenal enlargement is a very regular response to single or repeated injections of insulin within a wide range of dosage. The increase in weight is sometimes small but that it is significant is attested by results of the cytological study. We have previously reported⁶ briefly on the cytological criteria of cortical stimulation and a complete report is now in preparation. In column 6 of Table 1 the plus (+) signs indicate cortical stimulation and the minus (—) signs no stimulation. We emphasize the

² Poll, H., *Med. Klin.*, 1925, **46**, 1.

³ Schenk, F., and Langecker, H., *Endocrin.*, 1935, **16**, 305.

⁴ Thatcher, H. S., *J. Exp. Med.*, 1926, **43**, 357.

⁵ Riddle, O., *Proc. Soc. Exp. Biol. and Med.*, 1923, **20**, 244.

⁶ Miller, R. A., and Riddle, O., *Anat. Rec.*, 1939, **75**, 103.

TABLE I.
Effect of Various Doses of Insulin (Lilly) on Suprarenal Weight and Activity in Young (1.9 mo.) Carneau Pigeons.

Test	Dosage			Adrenal			Food given or eaten
	No. of birds	No. of days	Units per day	Wt, mg	Cytology		
					Cortex	Medulla	
I	4	2	1	35.4	+	—	Fed 15 g/bird/day
II	3H*	10	1†	47.6	+	+	Ate 0.2 " " "
III	1H	2	2‡	31.7	+‡	—	Fed 10 " " "
	1H	4		37.3	+	—	
	1H	8		27.2	+	—	
IV	5H	8	4‡	45.7	+	+	" 10 " " "
	1	2	6‡	38.5	+	—	" 10 " " "
V	1	4		46.5	+	—	
	1	8		41.8	+	+	
	5	10	6‡	37.2	—+§	—	" 20 " " "
VII	4	2	10	32.9	+	+	" 15 " " "
VIII	3H	2	10	35.1	+	+	" 15 " " "
IX	2H	5	15	47.8	+	+	" 15 " " "
X	2	1½ hr	30	31.9	—	—	" 15 g 24 hr earlier
XI	4	10 hr	30	33.1	+	+	" 15 " " "
XII	7	1	30	37.8	+	+	" 15 " " "
XIII	8	2	30	39.6	+	+	" 15 g/bird/day
XIV	3	3	30	40.2	+	+	" 15 " " "
XV	1	4	60	68.2	+	+	Ate no food
				Uninjected Controls.			
A	63	(Aut.-Win.)		31.6	—	—	Ate about 35 g/bird/day
B	25	(Spr.-Sum.)		28.8	—	—	" " 30 " " "
C	5			30.7	—	—	Fed 9 g/bird/day
D	26			31.0	—	—	Complete fast, 10 days
E	22H*	(Oper. 10 days)		24.0	—	—	Ate about 9 g/bird/day
F	20H	" " "		23.8	—	—	Complete fast, 10 days

*Hypophysectomized; anterior lobe only removed.

†Protamine zinc insulin used here.

‡The daily dose divided and given in two injections.

§Three unstimulated weighed 33.5 mg; 2 stimulated weighed 42.7 mg.

observation that the cytological changes in the cortex following insulin injection are entirely similar to those which follow the injection of a whole anterior lobe extract or an adrenotrophic fraction from pituitary tissue.

Cytological evidences of medullary activity are represented similarly in column 7 of Table I. Adrenals of control birds, after fixation in Champy and staining in anilin acid fuchsin and methyl green (our usual procedure), regularly show medullary cells which stain deep yellowish-green and are filled with closely packed chromaffin granules. A much smaller number of cells show only a light granulation and are blue-green in appearance. In some of the injected animals these lightly granulated medullary cells are more numerous and the heavily granulated cells relatively infrequent. It is also notable that the mitochondria are increased in number and the Golgi apparatus is hypertrophied, a condition which we believe

indicates increased medullary activity. Mitoses were also observed in the medullary cells following insulin. These are usually few in number, sometimes only one or two per section, but since we have never seen more than a total of one or two mitoses in hundreds of sections of adrenals from control animals this finding in insulin-treated animals is significant. Certainly, however, hyperplasia and hypertrophy are far less prominent in medullary cells than in cortical cells.

It is well known that fasting increases the sensitivity of the animal to insulin, and our data suggest that the response of the adrenal to insulin may also be somewhat modified by the amount of food consumed (Tests V, VI). Six units daily, given in 2 doses of 3 units each, effectively enlarged the adrenals at 2, 4, and 8 days in pigeons fed only 10 g of food daily; another group similarly injected for 10 days, but fed 20 g food per day, showed weight increase and cytological evidence of stimulation in only 2 birds of the group of 5. Fasting in itself has no observable effect on the weight or cytology of the adrenal (Tests D, F). Similarly the adrenals of hypophysectomized pigeons are more sensitive to insulin than are those of normals; they respond to a lower dose (Tests III, V; IV, VI) and show greater enlargement when the dosage is uniform (VII, VIII).

Discussion. Response of the adrenal to insulin has bearing on the question of the interpretation of the response of the adrenal to other agents. The work of others has shown that the mammalian adrenal reacts to toxic substances and various operative procedures by enlarging, one part of a syndrome which Selye has called the "alarm reaction." On Selye's view such adrenal enlargement is a secondary result of the action of the "alarming" stimulus upon the pituitary. He obtained no adrenal enlargement in hypophysectomized animals subjected to "alarming" stimuli.⁷ In our studies cortical enlargement and loss of chromaffin granules in the medulla, duplicate changes associated with the alarm syndrome. It is entirely certain, however, that following insulin these particular changes occur in pigeons deprived of their anterior pituitary gland with the same facility as in the intact animals.

Our published⁸ and unpublished data show that thyroxin and estrogens are effective in stimulating the adrenal in both normal and hypophysectomized pigeons. Insulin, however, stands apart from those 2 hormones in its marked ability to enlarge the adrenal, notably in the hypophysectomized pigeon. Since the adrenal is

⁷ Selye, H., and Collip, J. B., *Endocrinology*, 1936, **20**, 667.

⁸ Miller, R. A., and Riddle, O., *Proc. Soc. Exp. Biol. and Med.*, 1939, **41**, 518.

clearly involved in carbohydrate metabolism it seems more than coincidence that this gland—particularly the cortex—responds especially well to injections of insulin. The present data, and also the observation that repeated injections of huge doses of insulin progressively lose their ability to depress the blood sugar and usually soon lead to a marked hyperglycemia,^{1, 9} suggest that both cortical and medullary parts of the adrenal actively oppose the ability of insulin to decrease the blood sugar; and that this occurs in pigeons deprived of their anterior pituitary glands.⁹ In a recent review of pituitary-adrenal cortex relationships Swann¹⁰ concludes that in carbohydrate regulation the adrenal cortex is probably completely dependent upon pituitary stimulation. The present results seem to provide an exception.

Summary. In tests made on 41 normal young White Carneau pigeons daily injections of 1-60 units of insulin during 10 hours to 10 days enlarged the adrenals, produced mitosis and cellular activity. These cytological effects were more pronounced in cortical cells than in medullary cells. Wholly comparable effects were produced in the adrenals of 16 additional birds previously deprived of their anterior pituitary glands. The cytological changes in the cortex following insulin are identical with those induced by adrenotrophic pituitary extracts. Food consumption of the test animals and of 73 controls was regulated at different and definite levels. The relation of these results to the alarm reaction and to the rôle of the adrenals in carbohydrate regulation is discussed.

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Growth of the Lobule-Alveolar System of the Mammary Gland With Pregneninolone.*†

JOHN P. MIXNER AND CHARLES W. TURNER.

From the Department of Dairy Husbandry, University of Missouri, Columbia, Mo.

Turner and Frank¹ have shown that the growth of the lobule-alveolar system of the mammary gland which normally occurs dur-

⁹ Riddle, O., and Opdyke, D. F., unpublished data.

¹⁰ Swann, H. G., *Physiol. Rev.*, 1940, **20**, 493.

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¹ Turner, C. W., and Frank, A. H., *Missouri Agr. Exp. Sta. Res. Bul.* 174, 1932.