10731

Stimulation of Adrenal Cortex of Pigeons by Ant. Pituitary Hormones and by Their Secondary Products.

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Earlier work indicated that intact pigeons are very resistant to the toxic action of insulin and that under heavy dosage with insulin a rapid increase of adrenal cortical tissue occurs.[1,2] It is well known that thyroxine induces cortical hypertrophy in various mammals where this action is believed to be mediated through the anterior pituitary gland. The ability of both male and female sex hormone to stimulate one or another type of cortical cell has been reported.[3,4,5] A variety of toxic substances and conditions of stress also give rise to cortical hyperplasia.[6,7] These considerations, along with relatively difficult or insufficiently tried methods for the assay of "adrenotropin" (or corticotropin), still lead some investigators to reserve judgment concerning the conclusiveness of present evidence for the production of a distinct (and single) adrenotropic hormone by the hypophysis. Gonads and thyroids do not seem similarly susceptible of having their size and function *augmented* by such a variety of agents. Though it is established that the pituitary supports cortical function in one or another way, and though the earlier evidence for the existence of adrenotropin[8,9] has since been notably supported by proofs that prolactin does not sustain or repair the adrenal cortex,[10,11,12] further data concerning the basis or bases of cortical repair and hypertrophy seem useful.

In a more comprehensive study we are trying to identify the cytological criteria of activity and rest in individual cells of the

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suprarenal cortex of the pigeon, and to identify those hormones which increase the number and secretory activity of these cells. Results of that study will be published elsewhere, but in the present paper we unite certain determinations made in that investigation with another series of studies (assays) showing the character (hormonal content) of a variety of extracts which did, and also others which did not, stimulate cortical tissue in the pigeon. In effect, we here bring together the results of 3 methods of assay of "adrenotropin." In the first we use increased weight of the whole adrenal of normal pigeons; and, alternatively, support (or increase) of adrenal weight in the hypophysectomized pigeon (during 10 days following the operation). In the second we utilize the cytological evidence of stimulation or non-stimulation in these pigeon adrenals. Only White Carneau pigeons 2.5 mo. old (1.9 mo. after hatching) were employed. As a third method of assay we use the method of Moon[13] (20 mg/day for 3 days in 21-day rats), and the percentage increase in adrenal weight is used directly in our tabulation. In addition to these assays for adrenotropin all the pituitary preparations were assayed on immature doves and pigeons for prolactin, FSH and thyrotropin. Values for the 2 latter hormones are expressed in terms of percentage increase produced in testes and thyroids (over their control weights) per mg of the preparation used; in some cases (parentheses enclose these items) the assays were inadequate for determination of true values. The results are shown in Table I.

In general the adrenals of the pigeon show a rather limited capacity for enlargement by hormones in the dosages here used; but gravimetric increases exceeding 10% are here generally significant because of the uniformity of adrenal size (barring temperature effects) in these birds. Further, the cytological criteria of the presence or absence of stimulation were unequivocal except in the case of 2 groups (parentheses there used) in which the adrenals of some but not of all the tested individuals were stimulated. In both of those groups adrenal weight had been increased (normals) or partly maintained and stimulated (hypophysectomized), thus showing essential agreement for these 2 types of assay. Indeed, these 2 methods of assay gave equivalent results in all cases, unless a possible enlargement (12%) of the adrenals of normal birds under thyroxine with no cytological evidence of stimulation constitutes an exception. Moreover, these 2 methods of assay are in

[13] Moon, H. D., PROC. SOC. EXP. BIOL. AND MED., 1937, **35**, 649.

TABLE I.

Effects on Suprarenal Weight and Activity in Young (1.9 mo.) Carneau Pigeons of Various Anterior Pituitary (Also Thyroxine and Estrone) Preparations Otherwise Assayed for Their Hormonal Content.

Pigeons used			Dosage		Change in body wt during dosage, %	Effect on adrenals		Assays of preparations used				
								Adrenotropin	Prolactin	FSH	Thyrotropin	
Normal or Hypophysectomized	No. of birds	Preparation injected	No. of days	Mg per day		Wt mg	Stimulated (+) or not (—)	% increase in wt (rat)	No. units per mg	% increase in testis wt per mg	% increase in thyroid wt per mg	Source of material injected
Normal	50	—	10	—	+ 3	31.7	—	—	—	—	—	—
	3	669	10	10	+17	40.3	+	56	7	24	1.2	Beef
	3	682	5	5	—10	36.8*	+	74	.7	(0)	(.4)	"
	10	680	10	3.3	+18	39.2	(+)	5	11	0	0.5	"
	3	643	5	5	— 9	38.9*	+	10	.0	11	1.1	Pork
	2	632	10	25	—18	39.3	+	66	.0	24	.9	Beef
	8	Intermedin	10	1 cc	+ 4	32.2	—	0	.0	0	(0)	" (?)
	3	Gamone	9	1.5	—21	38.8	+	7[1]	.0	(2)	0	Human
	5	Thyroxine	10	0.05	— 3	35.4	—	25[2]	.0	0	deer.	Synthetic
	5	Estrone[3]	16	(2)	—15	38.3	+	35[4]	.0	0	(0)	"
Hypophysectomized	24	—	10	—	—17	25.3	—	—	—	—	—	—
	7	Muscle ext.	10	10	—24[5]	26.25	—	8	.0	0	0	Beef
	12	582	10	10	+15	33.0	+	81	1	14	1.0	Sheep
	6	437H	10	3.1	+10	38.9	+	98	5.5	0	0	Beef
	8	394H	10	5	—22[5]	25.35	—	0	8	0	0	"
	5	626	10	12	+17	27.6	—	25	6	(1)	1.1	"
	10	632	10	2	— 9	25.1	—	66	.0	24	.9	"
	6	600	10	10	—13	(40.9)[6]	+	42	.0	14	.5	"
	10	Gonadogen[7]	10	2 u	—15	26.1	—	?	.0	(30)	0	Horse
	8	Gamone	10	3	—19	56.5	+	7[1]	.0	(2)	0	Human
	10	Prolan	10	1	—20	26.7	—	8	.0	(.6)	0	"
	10	Thyroxine+[8]	10	0.05	—21	29.3	(+)	25[2]	.0	0	deer.	Synthetic

* Fasted during last 48 hours.

1 As assayed at one-fourth usual level (15 mg instead of 60 mg).

2 Assayed at 0.05 mg/day thyroxine.

3 Pellet implant in addition to injection.

4 Assayed at 0.1 mg/day.

5 Fasted 10 days.

6 Glands not completely dissected.

7 A preparation of pregnant mare serum.

8 In addition dosage included parathormone, Lilly (2 u./day), and supplements of vitamins A, B, D, G, E.

very substantial agreement with the method of Moon. We regard this common response of cortical tissue of rat and bird to a common component(s) of the anterior pituitary as a significant new fact. Among the true pituitary preparations discrepancy is observed only with No. 680; this preparation increased adrenal weights in normal pigeons and gave cytological stimulation in most of them, though it failed (apparently a 5% increase, but muscle extract gave an increase of 8%) to indicate the presence of adrenotropin by the Moon test.

Seven of the preparations used contained prolactin, but it is clear that their support of the adrenal was not at all related to the amount of prolactin injected. Birds dosed with No. 437H received much less prolactin than those treated with No. 626, but the three types of assay show that the adrenals of rats and birds were supported far better by No. 437H. It is to be noted that No. 437H tested free of FSH and thyrotropin. Gonadogen, rich in FSH and effective in maintaining the gonads of hypophysectomized pigeons, showed no ability to sustain or stimulate the adrenals of such birds. Preparations Nos. 600, 632, 643 contained no prolactin but, when given at moderate or high dose levels, they all induced marked cortical stimulation. Intermedin showed no ability to stimulate the adrenals of either rat or pigeon.

Gamone (from post-menopausal urine) produced peculiar and notable effects. This preparation stimulates the gonads of birds much less than those of rats; but at least in immature individuals of these two species it stimulates the bird adrenal far more than that of the rat; here the Moon test and the two pigeon tests give unlike or contrary results. It is further notable that under gamone dosage both medulla and cortex of the bird suprarenal are stimulated. When given at a high dosage level estrone clearly stimulates the adrenals of normal immature pigeons (and rats). The apparent ability of thyroxine (plus supplements) to stimulate and largely sustain the adrenals of hypophysectomized pigeons further suggests that similar actions of extra-pituitary hormones on cortical tissue have not been sufficiently explored.*

Summary. Increase of weight in the adrenals of the normal immature (1.9 mo.) pigeon, and degree of maintenance of adrenal

* Gamone used in this study was obtained through the courtesy of E. R. Squibb and Son; intermedin, from I. G. Farbenindustrie, through the Winthrop Chemical Co.; estrone, from the Schering Corporation; gonadogen, from the Upjohn Co. The various anterior pituitary preparations were made by Dr. R. W. Bates in this laboratory.

weight during 10 days following hypophysectomy in the pigeon, and also cytological evidence of stimulation in their cortical cells, are found to agree satisfactorily with Moon's method of assay of adrenocorticotropin in 21-day rats. As tested by these 3 methods the ability of pituitary extracts to stimulate cortical tissue is independent of their prolactin, FSH and thyrotropin potencies. Gamone stimulates both cortical and medullary tissue in both normal and hypophysectomized pigeons. Estrone stimulates cortical tissue at least in intact birds. Thyroxine, plus vitamin supplements, gave evidence of ability to stimulate cortical tissue in hypophysectomized pigeons.

10732

Metabolic Interdependence of Vitamin B_1 and Manganese. Reciprocal Neutralization of Their Toxic Effects.*

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We observed that rats fed our standard adequate, varied diet, supplemented with 100 gamma of vitamin B_1 daily, either in the form of yeast, or as synthetic vitamin B_1 (parenterally administered), demonstrated after one generation interference with lactation, loss of the maternal instinct, cannibalism and progressive loss of fertility.[1] With reduction in the amount of vitamin B_1 to 40 gamma or the elimination of the supplements of vitamin B_1 for short periods, normal lactation and normal interest in the young was restored. When the vitamin B_1 content was again increased the same toxic effects were observed.† Further study completely confirmed our earlier findings. With daily supplements of 60 gamma of vitamin B_1, progressive decrease in fertility also occurred, with a moderate incidence of loss of litters due to cannibalism. After four generations breeding decreased.

* A preliminary note of this work appeared in *Science*, 1939, **89**, 2302. Read before the American Society of Experimental Pathology, April, 1939, at Toronto.

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† Interference with lactation and reproduction could not have been due to the absence of vitamins L_1 and L_2 recently postulated by Nakahara Inukai and Ugami (*Science*, 1938, **87**, 372) since yeast is a rich source of these factors.