TABLE II. DNC in Chicken Tissues after the Feeding of Nicarbazin in the Diet.

Days after medication discontinued	0	1	2	4
Tissue	DNC, γ/g wet tissue or γ/ml blood plasma			
	0.015% nicarbazin in feed			
Thigh muscle				
Radio.	1.8	.5	.35	.32
Chem.	4.1	.0	.0	—
Breast muscle				
Radio.	2.1	.6	.0	.03
Chem.	1.7	.0	.0	—
Kidney				
Radio.	9.0	.2	.05	.0
Chem.	5.5	.0	.0	—
Liver				
Radio.	14.5	3.8	.54	.01
Chem.	8.0	1.0	.0	—
Blood				
Radio.	2.7	.5	.03	.05
Chem.	1.8	.1	.0	—
	0.06% nicarbazin in feed			
Thigh muscle				
Radio.	12.1	3.3	2.0	.19
Chem.	14.3	1.9	.4	—
Breast muscle				
Radio.	7.4	2.4	.4	.24
Chem.	7.2	2.0	.0	—
Kidney				
Radio.	31.5	1.1	.17	.0
Chem.	25.7	2.9	.0	—
Liver				
Radio.	25.7	11.3	3.6	.24
Chem.	15.6	9.6	.4	—
Blood				
Radio.	9.6	2.8	.75	.14
Chem.	7.0	1.4	.1	—

and 4 days later. Tissues and blood were assayed as in Exp. 1.

Results. Radioactivity in blood of chickens fed 0.015% nicarbazin (Fig. 1) approached a plateau in the 7-day feeding period. The slope of the curve relating radioactivity to time for birds fed 0.06% nicarbazin, decreased with time. If a longer feeding period had been used, it is probable that this curve also would have approached a plateau. Withdrawal of medication was followed by a precipitous fall in radioactivity to zero in 2 to 4 days.

Radioactive counts and chemical analyses of the tissues (Table II) agreed in demonstrating that two days after withdrawal of 0.015% medication, and 4 days after withdrawal of 0.06% medication, only traces of DNC or its metabolites were present in the tissues.

Summary. Tissues of chickens fed diets containing 0.015% and 0.06% radioactive nicarbazin for 7 days contained DNC and minimal quantities of its metabolites. Two and four days, respectively, after withdrawal of 0.015% and 0.06% medication, only traces of DNC and its metabolites remained in the tissues.

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Hormone Content of Anterior Pituitary of Monkey (*Macaca mulatta*)* with Special Reference to Gonadotrophins. (22154)

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Pituitaries from monkeys, of both sexes and of different ages, have been assayed in hypophysectomized rats for their content of gonadotrophic hormones. These studies were undertaken to determine any peculiarities either in quantity or proportion of the gonadotrophic hormones, preliminary to treatment of macaques with preparations from homolo-

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gous anterior pituitaries. Estimates of levels of other pituitary trophic hormones were made concurrently.

Methods. Pituitaries were removed from adult female monkeys at representative stages of the menstrual cycle, and from adult and immature males, for assay of hormone content of individual glands.† Pituitaries were also collected from large numbers of males and females of variable ages, and pooled for assay and future chemical fractionation. The anterior lobes were dissected free from dura and from posterior and intermediate lobes. Anterior pituitaries from individual animals were frozen at once and lyophilized; pooled anterior lobes were frozen within 2 hours after sacrifice. Wet and dry weights of individually collected anterior pituitaries were determined, and average weight in pooled lots could be calculated from the collective wet or dry weight and the number of glands. Assays were performed in immature female rats, hypophysectomized at 26-28 days of age, beginning 6 to 8 days after operation. Groups of 3 rats were used for each dose level. No data from incompletely hypophysectomized animals have been included. The postoperative period of one week used in most experiments was selected as fulfilling a condition defined in assay of gonadotrophins, follicle stimulating hormone (FSH) and interstitial cell stimulating hormone (ICSH). The approximate titer of the other trophic hormones of the pituitary, growth hormone (GH), adrenocorticotrophic hormone (ACTH), and thyrotrophic hormone

† Pituitaries of adult female monkeys in known stages of cycle were obtained from the Yale Obstetrics Colony. Pituitaries of immature male monkeys used to make implants of individual glands were obtained from the Virus Laboratory, University of California, through the courtesy of Dr. Mary McClain. The large numbers of glands which were pooled, frozen and dried, were obtained from the Cutter Laboratories through the kindness of Dr. Walter E. Ward, Dr. Donald Trotter and Mr. Donald H. Wonder. Pituitaries from estrinized monkeys (450 μg estradiol benzoate over a period of one week previous to autopsy) were obtained through the courtesy of Dr. H. R. Catchpole, University of Illinois School of Medicine.

TABLE I. Hormone Assay of Anterior Pituitaries of the Monkey. MED expressed as total dose in dry weight (mg).

Donors				Wt (mg) anterior lobe		Assay in hypophysectomized rats (mg)				
Sex	Age, Cycle day	Wt, kg	Test conditions	Wet	Dry	FSH	ICSH	ACTH	TSH	GH
Females	Adult 1	5.4	Subcut.: FSH, ACTH, TSH	59	13.2	1.0	.25	1.5	1.0	.25
	1	5.0	IP: ICSH, GH	58	12.5	1.5	.75	2.5	1.0	.08
	9	4.2	Daily, 3 da; 72 hr	43	9.8	.25	.1	>1.5	.18	.08
	9	6.8		48	11.9	.5	.15	1.0	>1.5	.05
	11	6.4		57	12.8	1.0	.1	>1.5	>1.5	.08
	15	4.5		46	10.0	2.5	.25	2.0	.25	.30
	22	4.3		42	9.6	1.5	.5	1.6	.3	.08
	Immature (estrin)	2.1	As above		6.7	>1.0	>1.0	>1.0	>1.0	.10
		2.1			6.3	>1.0	>1.0	.5	.5	.10
Males	Adult	8.2	As above	36	9.7	.75	.3	>2.0	1.5	.08
	Immature	3.0	One implant; 96 hr	24	6.0*	>3.0	>3.0	1.5	<1.5	<1.5
		3.4	*Idem*	20	5.0*	>2.5	>2.5	≥2.5	1.2	<1.2
♂ & ♀ pooled	Variable ages		Subcut. 4 da; 96 hr	13.9	3.2	3.2	3.2	6.4	.8	< .8

* Implanted fresh; approximate dry wt derived by factor of 4.

TABLE II. Hormone Content of Anterior Pituitaries of the Monkey.

Donors			Total units per anterior lobe				
Sex	Age	Cycle day	FSH	ICSH	ACTH	TSH	GH
Females	Adult	1	13	53	9	13	53
		1	8	17	5	13	150
		9	40	100	<7	54	120
		9	24	80	12	<8	240
		11	13	130	<9	<9	160
		15	4	40	5	40	30
		22	6	19	6	30	120
	Immature (estrin)		<7	<7	<7	<7	67
			<7	<7	13	13	63
Males	Adult		13	32	<5	5	120
	Immature		<2	<2	4	>4	>4
			<2	<2	≤2	4	>5
♂ & ♀ pooled	Variable ages		≥1	≥1	.5	>4	4

(TSH), could also be determined even though the postoperative period was too short to allow for an entirely satisfactory state of regression of the target organs.

Results. Potency of anterior pituitaries from various types of monkeys is expressed in Table I as the minimal effective dose (MED) or unit value for each of the 5 principal trophic hormones.‡ Total hormone content expressed in units per anterior lobe is given in Table II.

Pituitaries of Normal Adult Females. Anterior lobes from adult female monkeys sacrificed at known stages of menstrual cycle, were pulverized, suspended in saline and injected daily for 3 days. The MED for FSH ranged from 2.5 to 0.25 mg dry weight of gland; thus the total hormone per anterior lobe varied between 4 and 40 units. The titer of FSH was highest on ninth day of cycle, the MED being 0.5 to 0.25 mg; 24 to 40 units were therefore present per gland. In early and late periods of the cycle the MED varied from 2.5 to 1.0 mg, with 4 to 13 units present per gland.

Unitage of ICSH was higher than that of FSH. The MED varied from 0.75 to 0.1 mg and total content ranged from 17 to 130 units per gland. Potency was highest between days 9 and 11, MED being at this time about 0.1 mg with 80 to 130 units present per gland. The content of ICSH increased relatively more than that of FSH during middle of the cycle, so that the ratio of ICSH to FSH increased above its usual value of 3 to 1, and reached a value of 10 to 1 on days 11 and 15. Evidence from the response of hypophysectomized recipients is not conclusive that monkey pituitary at any specific period in the cycle contained the optimum ratio for corpus luteum formation. It is to be noted, however, that corpus luteum formation with ovulation was induced by a total dose of 1.5 mg of pituitary tissue taken during first half of menstrual cycle (days 1, 9 and 11), whereas even higher doses of pituitaries, removed during

‡ MED for *follicle stimulating hormone* is defined as the minimal total dose which will cause microscopically detectable growth of ovarian follicles beyond control level, specifically to the stage of beginning antrum formation. The MED for *interstitial cell stimulating hormone* is the total dose which will cause microscopic evidence of beginning repair of the "deficient" type of interstitial tissue, including enlargement of nuclei, lighter nuclear stain and reappearance of a rim of eosinophilic cytoplasm. The MED for *thyrotrophic hormone* is the total dose which causes increase in height of epithelium, from squamous to low cuboidal, with beginning vacuolation of colloid. MED for *growth hormone* is total dose which will cause an increase of 30 to 50 micra in width of uncalcified portion of proximal epiphyseal cartilage of tibia stained with silver nitrate. MED for *adrenocorticotrophic hormone* is total dose which causes beginning repair of adrenal cortex as seen in frozen sections stained with Sudan black; the criteria include replacement of coarse by fine lipid droplets, infiltration of lipid into the subglomerular lipid-free zone, increase in cell size and width of cortex, and regeneration of the zona reticularis.

the last half of the cycle (days 15 and 22), resulted only in minimal follicular stimulation in hypophysectomized rats. Growth hormone was present in considerable unitage in the adult female pituitaries, demonstrable at 0.3 to 0.05 mg total dose. There was no significant change in minimal effective dose or in total pituitary content during the cycle, more than 100 units per pituitary usually being demonstrable. The adrenocorticotrophic hormone content was consistently low, the hormone frequently detectable by change in lipid pattern in the adrenal of the recipient only at the highest levels tested, 2.5 or 1.5 mg. At most, 5 to 10 units were present per anterior pituitary. The minimal effective dose for thyrotrophic hormone was usually between 1.0 and 0.2 mg, total unitage ranging between 10 to 50 units per pituitary, with no cyclic pattern.

Pituitary of adult male. The anterior pituitary from an adult male macaque was assayed as above. The weight of anterior pituitary was about the same as the smallest from the normal adult females. Unitages of various hormones were roughly within the same range as in normal adult female pituitaries, though the content of FSH did not equal that reached in the female pituitary in the middle of the cycle. The total amount of ICSH present per anterior pituitary was within the lower range found in the normal female. The ratio between total content of ICSH and FSH (2 to 1) was as low as any encountered in the female pituitary, either at beginning or end of the cycle. Ovulation and corpus luteum formation in hypophysectomized rats resulted from total doses of 2 mg. The content of GH was comparable to that in the adult female, that of TSH and ACTH was even lower than in normal females.

Pituitaries of immature males. Assays of pituitaries of immature males were not strictly comparable to those of the normal adult male pituitary just cited, inasmuch as repeated injections were not given but fresh anterior lobe tissue was implanted. The content of all hormones, notably that of gonadotrophic hormones, appeared to be lower than in adult pituitaries.

Pooled pituitaries of monkeys. These pituitaries were collected from male and female monkeys of unknown age, though predominantly from young animals. The frozen-dried pituitaries were injected in saline suspension daily for 4 days. The hormone content per pituitary was lower than that in the individual adult female or male pituitaries but was within the same range as in individual immature male pituitaries.

Pituitaries of estrinized immature females. Pituitaries removed from 2 females which had received estradiol benzoate were lyophilized and assayed individually as above. The pituitaries were small. Neither FSH nor ICSH could be detected, even after injection of much higher doses than were required for normal adult female or for pooled pituitaries. The content of ACTH, GH and TSH was in the low normal range.

Discussion. Very few accounts have been found in the literature pertaining to the content of gonadotrophic or other trophic hormones in monkey pituitary. Salhanick, Hisaw and Zarrow(1) examined gonadotrophic potency of pituitaries of castrate macaques and of castrates treated with estradiol. Gonadotrophic potency of the castrate pituitary was high and was depleted by estrogen therapy; no value was given for hormone content in the normal animal. As stated in the present paper, gonadotrophins could not be detected, even at high doses, after estradiol treatment of immature females.

The hormone content of human pituitaries has received more attention. Some studies which should be mentioned are those of Witschi and Riley(2), Bruner(3), Bahn, Lorenz, Bennett and Albert(4-7), Leathem(8), and Burt and Velardo(9). Assays conducted by Witschi and Riley, and by Bahn *et al.*, have probably been the most comprehensive, including assays of pituitaries of normal adult men and women, of postmenopausal and pregnant women, of old men and of children. Those of Bahn and collaborators are particularly significant for comparison with our results for monkey pituitaries, because the same type of assay animal (the hypophysectomized rat) and similar morphological criteria were

used in establishing unitage. Furthermore, these authors assayed anterior lobe tissue free of posterior lobe so that their estimates of hormone content can be compared directly with those given here. A total dose of 3 mg of dry anterior pituitary tissue from women was the minimal amount necessary for detection of either FSH or ICSH. In the adult female monkey the minimal amount needed for demonstration of FSH ranged from 2.5 to 0.25 mg, and of ICSH from 0.75 to 0.1 mg, both varying with the menstrual cycle. The ratio between minimal effective doses for ICSH and FSH was 2 or 3 to 1 at beginning and end of the cycle and rose to 10 to 1 on days 11 and 15, whereas a ratio of unity was reported for the human pituitary. Bahn *et al.* found that the MED for gonadotrophic hormones was the same in men as in women, the ratio of ICSH to FSH again being unity. The values given here for minimal effective doses of adult male monkey pituitary were lower; ICSH 0.3 mg, FSH 0.75 mg, and the ratio was 2.5 to 1. Both Bahn *et al.*,(6) and Witschi and Riley(2) have found a low gonadotrophic content in pituitaries of children, the content being 1/5 to 1/10 that in adult pituitaries. The gonadotrophic hormone content of pituitaries of immature monkeys recorded here was correspondingly low.

The assays of monkey pituitaries were made to determine whether their gonadotrophins were peculiar either qualitatively or quantitatively, in the hope that such data might guide efforts to induce sexual maturation and ovulation in monkeys. All pituitary preparations hitherto injected in such experiments have been from heterologous pituitaries (chiefly from pig and sheep). The gonadotrophic potency of rhesus pituitaries was higher than that of sheep pituitaries. The MED for both FSH and for ICSH in adult female monkey pituitaries at the time of highest hormone content was 1/10 that for sheep pituitary(10). The two gonadotrophic hormones were present, however, in the same proportion in sheep and monkey pituitaries. In acetone dried sheep pituitaries the MED for ICSH was 1 mg, for FSH 2.5 mg; in lyophilized monkey anterior lobe, the MED for ICSH was 0.1 mg, for FSH 0.25 mg.

In the monkey anterior pituitary the content of FSH was highest on 9th day of menstrual cycle, compatible with the supposed preovulatory spurt of follicular growth at about this time. The content of ICSH was highest in preovulatory and ovulatory period, consistent with the synergistic action between ICSH and FSH, and its probable role in final follicular enlargement, and also with the importance of ICSH in corpus luteum formation. The ratio of ICSH to FSH was highest in ovulatory and early postovulatory period. However, pituitaries from female monkeys in the first half of the cycle, as well as those from the adult male, caused ovulation in hypophysectomized recipients, even though in both types of pituitaries the ratio of ICSH to FSH was low. Failure of pituitaries from female monkeys in the last half of the cycle to induce ovulation must have been due to low unitage, particularly of FSH.

Summary. Individual anterior pituitaries as well as pooled glands of *Macaca mulatta* have been assayed in hypophysectomized immature female rats for their content of trophic hormones, particularly gonadotrophic hormones. 1. In the adult female macaque, the content of follicle stimulating hormone was highest between the ninth and eleventh days of the cycle. The content of interstitial cell stimulating hormone was also maximum at this time of cycle. The ratio of ICSH to FSH was highest (10 to 1) on days 11 and 15. 2. In adult male anterior pituitary the total content of ICSH and FSH and the ratio between these hormones was approximately the same as in the adult female pituitaries at beginning and end of menstrual cycle. The gonadotrophic hormone content of pituitaries of immature animals was lower than in adults. 3. Other trophic hormones in the adult female pituitary, thyrotrophic, adrenocorticotrophic and growth hormones, did not change in amount during the menstrual cycle. In adult male, as well as in the female pituitary, thyrotrophic and adrenocorticotrophic hormones were present in low concentration, but growth hormone was present in high unitage.

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Determination of Evans Blue Dye in Blood and Tissues.* (22155)

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To our knowledge there is only one report in the literature of a method for determining Evans blue dye in whole blood(1) and only 3 for determining the dye in tissues(2-6). The present paper describes a new procedure for its estimation in tissues, of which blood may be considered a special case. In addition, by employing this method, data have been obtained on the fate of the dye for up to 140 hours after its injection into the rat.

Method. Description of method. The method is based upon 3 fundamental operations: (1) homogenization of tissue in a concentrated solution of urea, (2) splitting of the Evans blue-protein complex and partial precipitation of chromogens with acetone, and (3) complete precipitation of chromogens by addition of Somogyi reagents. Details of the method as employed for tissues are as follows: (1) A 5-g sample of the dye-containing tissue to be analyzed and 2 additional 5-g dye-free samples of similar tissue obtained from another animal were weighed. To one of the latter was added a known amount of dye in approximately the amount to be found in the unknown tissue sample. The 3 samples (tissue plus unknown dye, tissue plus known dye, tissue without dye) were minced. (2) Concentrated urea solution (equal weights of A.R. urea and distilled water) was added to each tissue sample until the total volume was 100 ml and the mixture was homogenized in a Waring Blendor for 10 minutes. The homogenate was poured into a flask in a cold tap water bath and allowed to cool for 5 minutes. (3) Two hundred ml of A.R. acetone added to each sample and each was shaken briefly. Ten ml of Somogyi reagent I (10% zinc sulfate • $7H_2O$) was added to each sample which was then mixed well by shaking. After further addition of 10 ml of Somogyi reagent II (0.5 N NaOH) to samples, the latter were mechanically shaken for 10 minutes and allowed to stand 15 minutes. (The 2 Somogyi reagents should be standardized by titration against each other to the end point of phenolphthalein). (4) The samples were filtered through Whatman No. 42 paper. Optical density of filtrates from the 2 dye-containing samples was determined at 620 mmu in colorimeter which has a 20 cm cell length. The tube containing dye-free tissue was used for the I_0 setting. *Procedure for analysis of blood.* The method was applied to blood as follows: (1) one-half to 2 ml of whole blood was taken and 0.9% NaCl was added where necessary to bring the final volume to 2 ml. Two ml of the urea solution used for tissue analyses was added followed by 8 ml of A.R. acetone and 0.5 ml each of the 2 Somogyi reagents. The samples were shaken between each addition. (2) After centrifugation at high speed for 15 minutes the supernatant liquid was taken for colorimetry at 620 mmu.

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