from the Q phase prototype virus A-Japan-305-57.

1. Meyer, H. M., Jr., Hilleman, M. R., Miesse, M. L., Crawford, I. P., Bankhead, A. S., PROC. SOC. EXP. BIOL. AND MED., 1957, v95, 609.
2. Salk, J. E., *J. Immun.*, 1944, v49, 87.
3. van der Veen, J., Mulder, J., *Studies on Antigenic Composition of Human Influenza Virus A Strains*, Stenfert Kroose, Leyden, 1950.
4. Isaacs, A., Andrewes, C. H., *Brit. Med. J.*, 1951, v2, 921.
5. Isaacs, A., Gledhill, A. W., Andrewes, C. H., *Bull. World Hlth. Org.*, 1952, v6, 287.

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Pineal and Hypophyseal Phosphate Uptake After Castration and Administration of Sex Hormones.* (24044)

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Physiological roles attributed to the pineal organ in terrestrial vertebrates have usually involved gonads and hypophysis, but relationships to the control of blood pressure and general body activity have been suggested also (1-10). Unfortunately, many results are contradictory and are not supported by quantitative or statistical documentation. The suggestive and significant studies of Borell and Orström(11-15) on pineal phosphate uptake or turnover merit reexamination. Their finding that rat pineal has a turnover rate 3 to 4 times that of "adenohypophysis" and much greater than that of various parts of brain has been confirmed by Reiss, Badrick and Halkerston(16). Although incorporation of P^{32}-labeled phosphate into pineal phospholipids and proteins as well as into carbohydrate phosphate esters occurred within 40 minutes of intraperitoneal injection(12), the major portion of P32 uptake can justifiably still be considered as an indicator of metabolic rate, as it is generally in other organs. Among interesting results obtained by Borell and Orström (11-13) with female rats are: (1) increase in phosphate turnover in hypophysis and tuber cinereum and decrease in ovary and uterus after pinealectomy (operated at 50-100 g; sacrificed 5 weeks to 9 months later), and (2) increase in hypophyseal and pineal phosphate turnover 6 weeks after castration (6 ♂'s and 6 ♀'s). Apparently, tissue samples were combined to facilitate P determinations and no standard errors are provided. They postulated that pineal activates ovaries and conversely is inhibited by them(13). This conclusion contradicts the view held by other workers that the pineal is primarily antigonadal or antihypophysis(10).

Materials and methods. Female rats of Long-Evans and 2 mixed strains were used. In most experiments, litter-mate experimental and control animals were used in pairs and handled in identical manners. All animals received Diablo Double Check Labration pellets and tap water *ad lib.* Animal room temperature was 75-80° F. and artificial illumination was provided in single daily photo-periods 12-14 hours. Bilaterally ovariectomized rats and their sham-operated controls were maintained 3 weeks to 2 months (Table I) before autopsy. Daily subcutaneous injections of 17β-estradiol (Schering) were administered in 0.2 ml sesame oil during 4 days preceding autopsy. Injections of progesterone ("Proluton," Schering) were administered similarly in daily doses of 0.1 ml (5 mg) during 3 days preceding autopsy. Control animals were injected similarly with equivalent volumes of

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TABLE I. Influence of Castration and Sex Hormones on Relative (P^{32}) Specific Activity in Pineal and Ant. and Post. Hypophysis in Female Rats (Long-Evans *(LE)*, *Mixed*, and Dept. of Genetics Hybrid *(Gen)* Strains). Autopsy 30 min. after intraper. inj. of P^{32} phosphate.

Treatment (strains)	Age at operation (days)	Age at autopsy (days)	P^{32} dose, μc	No. of animals	Ant. hypophysis $x \pm$ S.E.	Post. hypophysis $x \pm$ S.E.	Pineal $x \pm$ S.E.	Ratios P/AP	Ratios P/PP
Ovariectomy:	30–53	87–98	75	14–15	6.78 .89	12.53 1.79	37.10 5.45	5.5	3.0
sham operated *(LE)*				13–14	6.91 1.14	10.07 1.64	33.08 5.48	4.8	3.3
OVARIECTOMY + OIL:									
Ovariectomy +									
2 μg estradiol	25–36	50–61	75	6	*5.13 .78	9.53 1.30	34.86 7.24	6.8	3.7
(LE)				5	*7.94 .63	9.41 .54	36.55 5.18	4.6	3.9
2 μg estradiol	23–38	62–76	87	5	7.50 1.40	14.49 3.33	54.72 14.60	7.3	3.8
(Gen)				5	8.77 1.40	15.00 2.13	62.32 8.07	7.1	4.1
Totals of above				11	6.32 .85	12.01 1.88	44.79 8.36	7.1	3.7
				10	8.32 .69	11.95 1.30	48.26 5.97	5.8	4.0
Ovariectomy +									
5 mg progesterone	18–23	58–63	75	8–9	4.99 .47	10.08 1.14	37.35 4.40	7.5	3.7
(LE)				6–7	5.21 .86	10.89 1.61	46.33 9.95	8.9	4.3
Ovariectomy +									
250 μg estradiol	32, 43–51	56, 79–93	75–95	11	†4.01 .51	9.02 .90	33.26 3.28	8.3	3.7
(mixed)				11	†9.03 .95	11.53 1.26	40.59 4.06	4.5	3.5
INTACT + OIL:									
Intact +									
250 μg estradiol	59–71		75	12	7.29 1.35	13.20 2.56	42.75 8.53	5.9	3.2
(mixed)				11	9.77 .99	12.61 1.56	44.73 7.46	4.6	3.5
Intact +									
250 μg estradiol	200–230		93	5–6	11.60 1.32	18.33 1.88	66.16 4.97	5.7	3.6
(Gen)				6	16.63 2.08	23.00 2.50	81.55 5.31	4.9	3.5
250 μg estradiol	old		75	6	6.14 .86	10.38 1.59	28.13 2.91	4.6	2.7
(LE)				6	5.33 .74	7.16 .93	24.75 2.75	4.6	3.5

x = mean; S.E. = stand. error of mean.

* $P < 0.02$ } (Student-Fisher t test, for significance of differences).
† $P < 0.001$ }

sesame oil. During injection periods, control and experimental animals were kept in separate cages, usually in pairs. Methods of measuring phosphorus turnover are essentially those employed by Borell and Orström(11-15) but we made determinations for individual animals rather than grouped samples. Thirty to 40 minutes before autopsy each animal was injected intraperitoneally with 0.5 ml of isotonic saline containing 20-95 μc P^{32} as sodium phosphate. Autopsy was performed following ether anesthesia. Body weights and vaginal smears were recorded for each animal. The pineal, anterior lobe, posterior lobe (pars intermedia and pars nervosa) of the hypophysis, anterior part of cerebellar vermis, and a small drop of blood were placed separately on small pieces of ash-free filter paper, squashed and dried overnight at 37°C. Tissues were individually counted about 24 hours after autopsy in a flow counter. About a month later, total phosphate determinations were made utilizing King's modification of the Fiske-Subbarow technic(17) with "Elon" (methyl P-amino-phenol sulfate) as reducing agent and heating of samples (Gomori, 18). Readings were made on Beckman Model DU spectrophotometer. In the first experiment, counts/min/mg dry weight were obtained for each tissue sample. In other experiments "specific activity" (counts/min/total μg P) and "relative specific activity" (specific activity of tissue/specific activity of cerebellum of same animal) were obtained.

Results. The effect of bilateral ovariectomy on phosphorus turnover in the adult female was examined first, using tissue weight, and comparing relative specific activity with respect to blood and cerebellum. No differences were found. From data of other experiments (Table I) it may be seen that in relation to ovariectomy and treatment with sex hormones: (1) post. hypophysis is unaffected, (2) ant. hypophysis shows increased uptake after treatment with estradiol, especially in castrate and after pharmacological doses, (3) pineal, while in general slightly more active after steroid hormone treatment, does not seem to be *significantly* modified by any one treatment, (4) relative specific activity of the pineal is 4.5 to 8.9 times that of the ant. hypophysis and 2.7 to 4.3 times that of post. hypophysis.

Discussion. According to Borell, Westman and Orström(19) relative specific activities of the rat "adenohypophysis" and tuber cinereum are greatest during proestrus. In later studies(20) it was shown that only 5000 I.B.U. or more estradiol injected daily for 3 days was followed in 48 hours by increased relative specific activity in the adenohypophysis of intact female rats (80-145 g). In our experiments also, increased ant. hypophyseal phosphate uptake occurred most clearly after pharmacological doses of estradiol. Relative specific activity of "adenohypophysis" in rabbits was found by Borell, Westman and Orström(21) to be similar at estrus and anestrus and was increased 3 to 5 weeks after castration and following coitus. Likewise, increase in "adenohypophyseal" phosphate uptake was noted by Borell and Orström(13) in the rat (6 ♂, 6 ♀) "at least 6 weeks" after castration. But we have been unsuccessful in producing an unequivocal increase in ant. pituitary activity after castration (Table I). The variables of age and laboratory strain of rat may possibly be responsible for this discrepancy.

Mechanisms for regulation of pituitary metabolism and gonadotropic activity and their responses to castration and sex hormones have been extensively studied in the past and are closely dependent on age of animals, dosage and timing. It must be thought, moreover, that physiological modification of hypophyseal gonadotropic activity need not be necessarily reflected in a detectable change in metabolic rate or phosphate turnover of ant. hypophysis as a whole.

We have utilized recent work on effects of ovariectomy, estradiol and progesterone on gonadotropin secretion in the rat to provide procedures that are capable of modifying FSH and LH secretion in treated animals. If a major portion of pineal parenchyma is metabolically responsive to female sex hormones or gonadotropin levels, changes in rate of pineal phosphate uptake would be expected. However, in our experiments such changes marked enough to be regarded as significant, did not occur.

The ratio of pineal relative specific activity to relative specific activity of ant. hypophysis (Table I) shows wide fluctuation without apparent correlation with experimental treatment, whereas the post. hypophysis ratio varies less.

Summary. Uptake of radiophosphate (P^{32}) in the pineal and ant. and post. hypophysis was studied in female rats after ovariectomy and daily treatment with 17β-estradiol (2 or 250 μg) or progesterone (5 mg). Significant change (increase) in uptake occurred only in the ant. hypophysis after estradiol. Relative specific activity (in relation to cerebellar vermis) of the pineal is 4.5-8.9 times that of the anterior lobe and 2.7-4.3 times that of the posterior lobe of the pituitary gland and remains high in adult and old animals.

1. Alcozer, G., Giordano, G., *Arch. "E. Maragliano" pat. e clin.,* 1954, v9, 433.

2. Engel, P., Bergmann, W., *Z. f. Vit.-, Hormon- und Fermentforschung,* 1952, v4, 564.

3. Giordano, G., Alcozer, G., *Arch. "E. Maragliano" pat. e. clin.,* 1954, v9, 655.

4. Kitay, J. I., Altschule, M. D., *The Pineal Gland, Review of Physiologic Literature,* Harvard Univ. Press, Cambridge, Mass., 1954.

5. Lenti, G., Molinatti, G. M., Pizzini, A., *Folia Endocrinol.,* (Pisa), 1957, v10, 11.

6. Pflugfelder, O., *Zool. Anz.,* 1957 suppl. 20, 53.

7. Simonnet, H., Thiéblot, L., Melik, T., Segal, V., *Acta endocrinol.,* 1954, v17, 402.

8. Stebbins, R. C., Eakin, R. M., *Am. Mus. Novitates,* 1958, No. 1870.

9. Thiéblot, L., Simonnet, H., Bataille, L., Melik, T., *J. Physiol.* (Paris), 1954, v46, 539.

10. Thiéblot, L., LeBars, H., *La Glande Pinéale ou Épiphyse,* Librairie Maloine S. A., Paris, 1955.

11. Borell, U., Örström, Å., *Acta physiol. scandinav.,* 1945, v10, 231.

12. ———, *Biochem. J.,* 1947, v41, 398.

13. ———, *Acta physiol. scandinav.,* 1947, v13, 62.

14. ———, *Acta psychiatrica e. neur.,* 1947, v47 (suppl.), 144.

15. Borell, U., *Nord. medicin* (Helsingfors), 1947, v36, 2137.

16. Reiss, M., Badrick, F. E., Halkerston, J. M., *Biochem. J.,* 1949, v44, 257.

17. Lindberg, O., Ernster, L., *Methods of Biochemical Analysis* (ed. Glick, D.), *Interscience,* 1956, v3, 1.

18. Gomori, G., *J. Lab. Clin. Med.,* 1941-42, v27, 955.

19. Borell, U., Westman, A., Örström, Å., *Acta physiol. scandinav.,* 1948, v15, 245.

20. Borell, U., Westman, A., *Acta endocrinol.,* 1949, v3, 111.

21. Borell, U., Westman, A., Örström, Å., *Gynaecologica,* 1947, v123, 186.

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Effect of Antioxidants on Muscle and Plasma Lipids of Vit. E-deficient Guinea Pigs.*† (24045)

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Evidence has been accumulating showing that addition of certain antioxidants to Vit. E-deficient diets either delays or prevents occurrence of Vit. E-deficiency lesions in skeletal muscle of animals. DPPD (N, N'-diphenyl-p-phenylenediamine) delays the onset of muscular dystrophy in lambs fed a Vit. E-deficient diet(1) and also prevents muscular degeneration in chicks fed diets low in both Vit. E and sulfur(2). DPPD, DBH (2,5-di-*t* - butylhydroquinone) and Santoquin (6-ethoxy-2,2,4-trimethyl - 1,2-dihydroquinoline) delay but do not prevent occurrence of muscular dystrophy in guinea pigs fed a highly purified diet deficient in Vit. E(3). Accumulation of cholesterol and total lipid in the skeletal muscle in animals deficient in Vit. E (4-9) and the hypercholesteremia observed in Vit. E-deficient rabbits(4) suggests that this vitamin may have a role in controlling lipid metabolism of skeletal muscle and plasma. Since certain antioxidants can replace Vit. E in one of its functions, it seemed desirable to determine whether the ability of Vit. E to function as antioxidant is responsible for its role in regulation of cholesterol and lipid metabolism. Accordingly an investigation was undertaken in which groups of guinea pigs were fed diets free of Vit. E and diets supplemented with either α-tocopherol acetate or various other antioxidants. Progress of guinea pigs was closely followed to determine incidence and frequency of dystrophy.

Procedure. Female guinea pigs were fed a purified diet consisting of casein, 30 g; cornstarch, 19.5 g; dextrose, 11 g; sucrose, 10 g; cellulose, 10 g; Wesson salt mix, 6 g; stripped lard, 5 g; agar, 5 g; potassium acetate, 2.5 g; magnesium oxide, 0.5 g; inositol, 0.2 g; choline chloride, 0.2 g; niacin, 20 mg; p-amino benzoic acid, 10 mg; calcium pantothenate, 4 mg; thiamine chloride, 1.6 mg; riboflavin, 1.6 mg; pyridoxine hydrochloride, 1.6 mg; folic acid, 1 mg; 2-methyl-1,4-naphthoquinone, 0.5 mg; biotin, 0.1 mg; Vit. B_{12}, 10 μg; Vit. A, 500 U.S.P. units and Vit. D_2, 50 U.S.P. units; the tocopherol content of this diet is less than 25 μg. In addition, each animal received daily (6 times/week) an oral

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