TABLE I. Thyroxine Secretion Rate of Mice.

	Group	Age, days	No. of mice	TSR (μg/100 g body wt)	Distribution of TSR .5 μg	1 μg	1.5 μg	2 μg	2.5 μg
♂	Intact	140	14	1.20 ± .15*	3	6	3	1	1
	Castrate	140	12	.83 ± .06	6	5		1	
♀	Intact	120	26	1.26 ± .07		16	6	4	
	"	140	10	1.20 ± .11		7	2	1	
	"	180	9	1.33 ± .14	1	2	5	1	
	"	230	13	1.19 ± .01	2	5	5	1	
	"	All age groups	66	1.25 ± .05	3	30	18	7	
	Castrate	180	7	.92 ± .06	1	6			

* Mean ± S.E.

day. Gonadectomy produced a consistent, but not significant, reduction in TSR in both sexes. In the female no significant change in TSR was noted with increasing age between 120 and 230 days of age. The mean TSR of 66 female mice maintained at a temperature of 78 ± 1°F was 1.25 ± 0.05 μg 1-thyroxine/100 g/day, with a range between 0.5 and 2 μg.

1. Hurst, Victor, Turner, C. W., *Mo. Agr. Exp. Sta. Res. Bull.* 417, 1948.

2. Amin, A., Chai, C. K., Reineke, E. P., *Am. J. Physiol.*, 1956, v187, No. 3; *Am. J. Physiol.*, 1957, v191, 34.

3. Grosvenor, C. E., Turner, C. W., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 517.

Received August 6, 1959. P.S.E.B.M., 1959, v102.

Preferential Concentration of C^{14} in Posterior Pituitary Following Hydrocortisone C^{14} Injection.* (25334)

SHAWN SCHAPIRO† AND JOSEPH KATZ‡ (Introduced by A. L. Sellers)
Inst. for Medical Research, Cedars of Lebanon Hospital, Los Angeles, Calif.

Preferential uptake of some hormones by the pituitary gland has been reported by several investigators. Riegel *et al.*(1) have found the concentration of C^{14} progesterone to be much higher in the pituitary and adrenals of rats than in plasma or other tissues. Joliot *et al.*(2) and other investigators(3-5) noted a preferential accumulation of thyroxine I^{131} in the posterior pituitary. Courrier and Zizine (6) observed that 2 hours after administration of C^{14} cortisone to rabbits, the kidneys and posterior pituitary had slightly higher tissue plasma ratios than other organs. In view of the great interest in the regulation of steroid hormone levels by the pituitary and hypothalamus, we undertook to confirm and extend these observations.

Methods. Hydrocortisone 4-C^{14} (1.47 μc/mg) was injected intravenously into 4 rats, 5 guinea pigs and 2 rabbits. The amounts of hydrocortisone injected approximately doubled the hormone content in the body.§ The animals were anesthetized with ether, and the hormone in 1 ml of aqueous alcohol was injected into the foot vein. After 15 to 30 minutes, the vena cava was severed and the animal perfused through the aorta with 0.9% sodium chloride solution. The tissues were then excised, weighed, homogenized in 75% methanol and centrifuged. Precise separation

* Aided by Grants from National Heart and Cancer Inst., U.S.P.H.S.

† Present address: Neuropsychiatric Hosp., Veterans Admin. Center, Los Angeles, Calif.

‡ Advanced Research Fellow, Am. Heart Assn.

§ Hydrocortisone is the normal adrenal secretory product of the guinea pig, while the rat and rabbit primarily secrete corticosterone(7). Apparently, corticosterone and hydrocortisone are handled similarly by the rat(8).

TABLE I. Distribution of C^{14} after Hydrocortisone C^{14} Injection.*

	Posterior pituitary		Anterior pituitary		Liver		Kidney		Adrenal		Muscle§		Brain‖		Thyroid¶	
	cpm/mg	t/p†	cpm/mg	t/p	cpm/mg	t/p	cpm/mg	t/p	cpm/mg	t/p	cpm/mg	t/p	cpm/mg	t/p	cpm/mg	t/p
Rat	8.5 ± 1.1‡	8.6			3.0	3.3	1.0	1.1	1.5	1.6	.50	.5	.61	.6		
"	21 ± 9.0	58	2.0 ±1.3	5.8	.95	2.6	.65	1.8	2.0	5.8	.48	1.3	.67	1.5		
"	2.7 ± 2.5	4.5	.36 ± .32	.6	.41	.7	1.3	2.0					.05	.07		
Adrex rat	4.4 ± 2.2	14	1.8 ± .50	5.1	1.4	4.2	1.3	3.9					.12	.3		
Guinea pig	3.7 ± 2.2	16	.8 ± .8	4.2	.58	2.6	1.5	6.7	.46	2.0	.25	1.0	.78	3.5		
"	341 ±21.3	555	15.3 ±1.4	25	.80	1.3	1.0	1.6	.50	.9	.50	.8	.70	1.1	2.4	3.8
"	1.9 ± 1.3	6.6	.69 ± .45	2.2	.77	2.7	.73	2.5	.49	1.9	.26	.9	.09	.32	.16	.6
"	1.6 ± 1.1	4.0	.33 ± .39	.9	.86	2.6	1.3	4.0	1.2	3.6	.11	.3	.56	1.6	.25	.8
"	21 ± 1	2.2	.66 ± .42	.7	2.6	2.8	2.0	2.2	1.5	1.6	.40	.5	.72	.7	.70	.8
Rabbit	2.8 ± .40	4.6			.87	1.4	1.4	2.0	.21	.35	.25	.4	.15	.2		
"	38.5 ± 3.0	39	1.8 ± .23	1.7	2.1	2.1	2.3	2.3	.39	.39	.80	.8	.18	.1	1.0	1.0

* Radioactivity in methanol soluble fraction 30 min. after intrav. inj. of hydrocortisone 4-C^{14}. Dose: rats and guinea pigs were inj. with .16-.25 mg; rabbits, 1.2 mg.

† Tissue plasma ratio.

‡ Represents 99% error $= \pm 2.58 \sqrt{\left(\frac{N_s}{T_s}\right)^2 + \left(\frac{N_b}{T_b}\right)^2}$.

N and T represent numbers of counts and time of counting for samples and blanks respectively.

§ Heart and skeletal muscle showed no difference.

‖ Anterior hypothalamus, posterior hypothalamus, cortex, and cerebellum showed no difference.

¶ Activities of spleen, lung, thymus, and testis were approximately of the same order of magnitude and intermediate between that of adrenal and muscle.

of the hypothalamus, median eminence and the pituitary lobes was not always possible.‖ The supernatant solution, or in the case of large organs, extracts from 100 to 200 mg of tissue were evaporated to dryness. In 3 instances, the residue was fractionated between 75% methanol and 4 volumes of chloroform to separate the free steroids (chloroform soluble) from the conjugated compounds (soluble in aqueous methanol). After the solvents were evaporated, the residues were extracted with 1 ml absolute ethanol. Plasma was deproteinized with 3 volumes methanol, and aliquots of the supernatant were used for C^{14} assay. For radioactivity assay, the alcohol extracts were evaporated on 1.5″ flat aluminum discs and assayed with a plastic end window gas flow Geiger tube (efficiency about 600,000 cts/min/μc C^{14}). The weight of material on the discs was less than 1 mg and no self absorption corrections were used. In most cases, background counts were determined for each individual disc.

Results. We have observed, in agreement with others(8-10), that 2 minutes after injection only 20 to 30% of the injected counts could be accounted for in the plasma. Apparently the hormone is rapidly distributed throughout the body for within 15 to 30 minutes, radioactivity was found in all tissues examined (Table I). Results are presented as cts/min/mg of tissue and tissue/plasma radioactivity concentration ratio. In the case of the pituitary lobes, the 99% counting error has also been given. The 99% error equals 2.58 times the standard error and indicates that in 99 out of 100 determinations the values would fall within the indicated range. In general, liver and kidney had greater activity than muscle, brain, thyroid, spleen, lung, testis, and thymus. The striking observation is the high concentration of activity in the posterior pituitary. In 3 of 11 experiments, the activity (cts/min/mg) was more than 10 times that of the other tissues and in 6 experiments was more than 3 times the activity of the other tissues. In 2 experiments, however, the "specific activity" of the posterior pituitary was of the same order of magnitude as kidney or liver. In all cases, the posterior pituitary had at least 3 times the activity of the anterior lobe. In a single experiment, Courrier and Zizine(6) observed the highest activity in the kidney and the next highest in the posterior pituitary.

Since the posterior pituitary was the smallest organ counted, the absolute number of counts in the gland was often small. We, therefore, felt it advisable to use the 99% error rather than the more commonly used standard error.

Discussion. These experiments were performed in an attempt to determine whether receptor sites for circulating adrenal corticoids were present in hypothalamic or pituitary regions. Our results are compatible with the hypothesis that the posterior pituitary is relatively richer in such sites. Several regions of the hypothalamus were tested and no preferential concentration was observed.

The amount of C^{14} found in the posterior pituitary is of the order of magnitude of 0.003-0.1% of the injected dose. Despite this variability, our results indicate a preferential accumulation of C^{14} by the posterior pituitary of rats, rabbits, and guinea pigs following administration of hydrocortisone 4-C^{14}. However, it is not known what fraction of the C^{14} bound in the posterior pituitary is unaltered hydrocortisone. The turnover of the hormone is known to be rapid and a substantial portion of the C^{14} in the body may no longer be present as hydrocortisone. In 3 experiments, the C^{14} activity was separated into a water soluble and chloroform soluble fraction (presumably conjugated and free steroids). In 2 of these, the aqueous extract contained less than 15% of the total tissue activity, while in one case about 50% was in the aqueous phase. Both fractions were preferentially concentrated in the posterior pituitary. Due to the low specific activity and limited amount of hormone, an identification of radioactive compounds was not attempted. To evaluate the physiological significance of our findings, further clarification of the nature of the activity in the pituitary will be essential.

Summary. Hydrocortisone 4-C^{14} was in-

‖ Anterior lobe weights were: rat, 4.6-8.6 mg; guinea pig, 8.0-16.6 mg; rabbit, 9.1-17 mg. Posterior lobe weights: rats, 1.1-4.0 mg; guinea pig, 2.0-4.7 mg; rabbit, 3.2-6.0 mg.

jected into rats, guinea pigs, and rabbits, and distribution of C^{14}, 30 minutes after injection was studied. Activity was widely distributed and the results were variable; but in most experiments, activity (cts/min/mg tissue) was higher in the posterior pituitary gland than in any other tissue. Activity in the anterior pituitary, liver, and kidney was higher than that in muscle and brain.

We wish to thank the Endocrinology Study Section, U.S.P.H.S., for radioactive hydrocortisone.

1. Riegel, B., Hartop, W. H., Kittinger, G. W., *Endocrinology,* 1950, v47, 311.

2. Joliot, F., Courrier, R., Horeau, A., Sue, P., *C. R. Acad. Sc.,* 1944, v218, 769.

3. Courrier, R., Horeau, A., Marois, M., Morel, F., *ibid.,* 1951, v232, 776.

4. Jensen, J. M., Clark, D. F., *J. Lab. Clin. Med.,* 1951, v38, 663.

5. Taurog, A., Harris, G. W., Tong, W., Chaikoff, I. L., *Endocrinology,* 1956, 59, 34.

6. Courrier, R., Zizine, L., *C. R. Acad. Sc.,* 1956, v242, 315.

7. Bush, I. E., *Schweiz. Med. Woch.,* 1955, v27, 645.

8. Ulrich, F., Long, C. N. H., *Endocrinology,* 1956, v59, 170.

9. Firschein, H. E., DeVenuto, F., Fitch, M. M., Pearce, E. M., Westphal, U., *ibid.,* 1957, v60, 347.

10. Peterson, R. S., Wyngaarden, M. B., Guerra, S. C., Brodie, B. B., Bumin, J. J., *J. Clin. Invest.,* 1955, v34, 1779.

Received August 7, 1959. P.S.E.B.M., 1959, v102.

Use of Human Kidney Cultures in the Study of Enteroviruses.* (25335)

G. D. HSIUNG (Introduced by R. H. Green)

Section of Epidemiology and Preventive Medicine, Yale University, School of Medicine, New Haven, Conn.

Use of human kidney tissue in suspended cell cultures for propagation of polioviruses was reported by Weller *et al.*(1); however, studies on Coxsackie and ECHO viruses have been conducted almost entirely in other tissue culture systems, chiefly rhesus monkey kidney. It was previously reported that kidney tissue cultures from relatively few monkey species have proven satisfactory for studies of the entire family of enteroviruses(2,3), and cultures prepared from non-primate kidney tissues have consistently proved unsatisfactory for propagation of these viruses(4). For these reasons the susceptibility spectrum of primary human kidney cell cultures to the 3 groups of enteroviruses (poliomyelitis, Coxsackie and ECHO viruses) was investigated.

Materials and methods. Source of material. Human kidney specimens were obtained following surgical removal or at autopsy. In the latter case only those kidneys were used which had been removed within a few hours of death, with the capsule intact and, in so far as possible, with aseptic precautions, to avoid gross contamination. After examination by the pathologist, kidneys were delivered to our tissue culture laboratory in a sterile jar which contained Hanks balanced salt solution and antibiotics. *Preparation of human kidney (HK) cultures.* The human kidney tissues were trypsinized in the same manner used for preparation of monkey kidney cultures(5). The trypsinized cell suspensions were centrifuged and the packed cells resuspended in growth medium (Hanks salt solution, 0.5% lactalbumin hydrolysate, 10% medium #199, and 20% calf serum) at a final cell concentration of 3-5 x 10^5 cells/ml. Three-ounce prescription bottles and tubes (16 x 150 mm) were seeded with 10 ml and 1 ml, respectively and incubated at 37°C. When a confluent cell sheet was obtained, usually within 3-5 days, the growth medium was removed and replaced with maintenance medium containing Earles salt solution, 0.5% lactalbumin hydrolysate, and 2% calf serum. In cases where cell growth appeared to be poor, the cultures were usually changed with fresh growth medium 3-5 days after seeding and

* Aided by grant from National Fn.