Distribution of Radioactivity in Different Tissues of Pigs After ^{3}H-Hydrocortisone Injection[1] (34381)

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(Introduced by E. V. Morse)

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Corticosteroids have a variety of physiological effects and are being used extensively for many therapeutic purposes; however, their basic sites of action are still unknown. Previous studies on the distribution of radioactivity in tissue following the administration of isotopically labeled glucocorticoids to rats have been reported (1–3). Radioactivity after an injection of labeled corticosteroids has been found in all tissues studied with selective accumulation in some tissues. The supernatant fraction from all tissues studied has been shown to contain significantly more radioactivity than any of the other fractions (2).

Studies on the binding of the radioactive hormone to macromolecules in tissues highly responsive to glucocorticoids have been reported (4–7). Litwack *et al.* (5) and Fiala and Litwack (6) presented evidence of hydrocortisone binding to proteins in rat liver supernatant fraction. Munck and Brinck-Johnson (7) recently reported binding of glucocorticoids to rat thymus cell *in vitro*.

The purpose of this study was to determine the uptake and association of ^{3}H-hydrocortisone to macromolecules in tissues of male Yorkshire pigs. Tissues highly responsive to glucocorticoids, *e.g.*, the liver and thymus as well as other tissues which do not respond greatly to glucocorticoid treatment were selected. Pigs were selected as experimental animals since larger tissue samples are available in such critical organs as the hypothalamus and pituitary. Also, the pig offers a closer analogy to man in almost every way than many other laboratory animals.

Methods and Materials. Six male Yorkshire pigs weighing approximately 3.5 kg were anesthetized with methoxyfluorine. A cannula was placed in the left recurrent tarsal vein and a tracer dose, 100 μCi/kg, of ^{3}H-hydrocortisone (sp. act 20–30 Ci/mmole) was injected through the cannula. Forty-five min later the jugular veins were isolated, a blood sample was collected and the veins were severed. This time was selected since ^{14}C-hydrocortisone reaches a maximum in rat tissues 45 min after injection (8). After exsanguination, the pigs were perfused with saline using a 3-in., 16-gauge needle to make a cardiac puncture. After perfusion, the brain, pituitary, thymus, heart, liver, spleen, skeletal muscle, and testes were removed. About 200 mg of each tissue were minced and placed in tared liquid scintillation vials. Two-tenths-ml of 70% perchloric acid was added to each vial and agitated. Four-tenths ml of 30% hydrogen peroxide was added to each vial and again agitated. The vials were capped tightly and placed in a 70° oven for 2–4 hr. Halfway through the digestion, the vial caps were removed to release pressure. After digestion, 10 ml of liquid scintillation fluid (toluene–2-ethoxyethanol, 1:1, and 6 g of PPO/liter) were added to each vial and the radioactivity was determined using a Beckman DPM-100 liquid scintillation counter. The amount of quenching in each vial was determined by the external standard–channels ratio technique.

Tissues from 2 other pigs treated as above were collected and homogenized in isotonic sucrose containing 0.01 *M* Tris buffer. The 105,000*g* supernatant was obtained and an

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TABLE I. Mean Tissue Activity and Tissue/Plasma Ratio 45 min after an iv Injection of 100 μCi of ^{3}H-Hydrocortisone. Each value represents the mean of 6 pigs ($\pm$ SE).

Tissue	(dpm/g of tissue)	Tissue/plasma
Blood plasma	16,068 $\pm$ 694	1.0
Left cortex	6899 $\pm$ 603[b]	0.42 $\pm$ 0.03
Right cortex	6666 $\pm$ 568[b]	0.41 $\pm$ 0.03
Cerebellum	6806 $\pm$ 646[b]	0.42 $\pm$ 0.04
Brain stem	7840 $\pm$ 844[b]	0.47 $\pm$ 0.03
Hypothalamus	7080 $\pm$ 614[b]	0.43 $\pm$ 0.03
Pituitary	16,786 $\pm$ 2701	1.01 $\pm$ 0.15
Thymus	9872 $\pm$ 1702[a]	0.59 $\pm$ 0.08
Heart	11,833 $\pm$ 1830[a]	0.82 $\pm$ 0.12
Liver	59,498 $\pm$ 7637[b]	3.58 $\pm$ 0.38
Spleen	17,801 $\pm$ 1147	1.05 $\pm$ 0.04
Skeletal muscle	16,483 $\pm$ 2575	0.99 $\pm$ 0.26
Testes	14,543 $\pm$ 717	0.98 $\pm$ 0.05

[a] $p < 0.05$; [b] $p < 0.01$; plasma vs. tissue.

aliquot was chromatographed on a column of Sephadex G-100 gel (2.5$\times$50 cm) using 5 m*M* KH_2PO_4 (pH 7.4) as the eluent. The void volume of the column was determined using dextran blue (Pharmacia). The absorbance at 280 mμ was determined on each effluent fraction and 1 ml of each fraction was counted.

Results. The radioactivity in the tissues 45 min after an iv injection of ^{3}H-hydrocortisone was expressed as dpm/g of tissue and as tissue/plasma ratio (Table I). Only the liver was capable of concentrating radioactivity from the blood. The concentration of radioactivity in the pituitary, spleen, skeletal muscle, and testes was the same as plasma. In the brain, thymus, and heart, radioactivity was less than in the plasma (Table I).

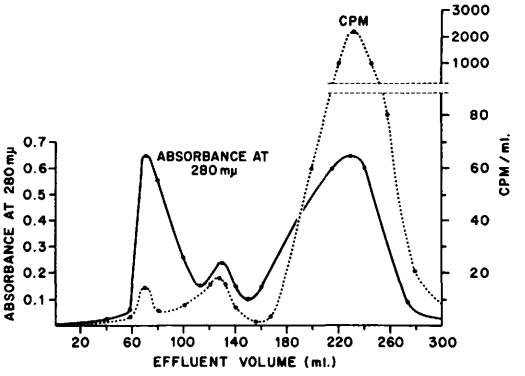

FIG. 2. Molecular sieve chromatography on Sephadex G-100 of the 105,000g spleen supernatant from 2 pigs, 45 min after an iv injection of ^{3}H-hydrocortisone (100 μC/kg).

Molecular-sieve chromatography on Sephadex G-100 of the supernatant fractions from liver, spleen, theymus, and heart is shown in Figs. 1, 2, 3, and 4. Three peaks of radioactivity were found in the liver and spleen supernatant, two associated with an early effluent, the high molecular weight fraction, and a third with a late effluent, the low molecular weight fraction (Fig. 1). The last peak of radioactivity probably consisted of unbound steroid and steroid metabolites. Two peaks of radioactivity were found in the thymus supernatant; one associated with the high molecular weight fraction and one with

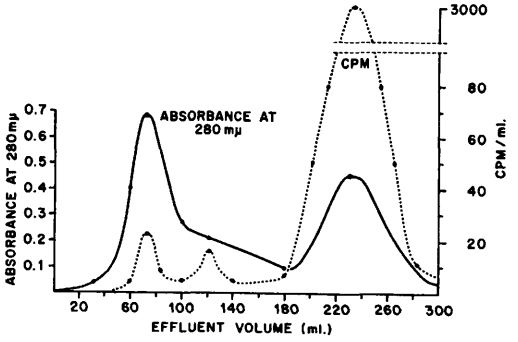

FIG. 1. Molecular-sieve chromatography on Sephadex G-100 of the 105,000g liver supernatant from 2 pigs, 45 min after an iv injection of ^{3}H-hydrocortisone (100 μCi/kg).

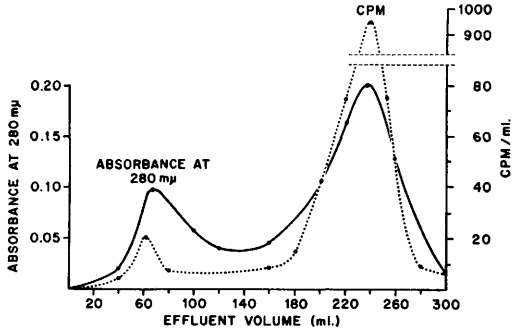

FIG. 3. Molecular-sieve chromatography on Sephadex G-100 of the 105,000g thymus supernatant from 2 pigs, 45 min after an iv injection of ^{3}H-hydrocortisone (100 μCi/kg).

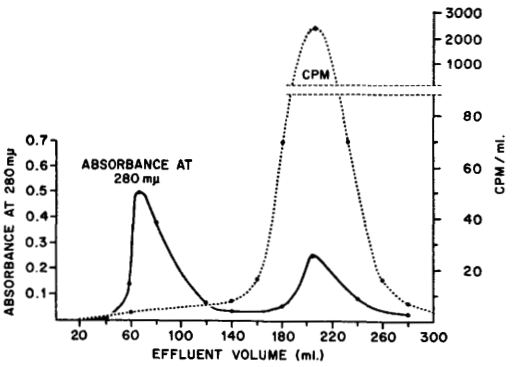

FIG. 4. Molecular-sieve chromatography on Sephadex G-100 of the 105,000g heart supernatant from 2 pigs, 45 min after an iv injection of ^{3}H-hydrocortisone (100 μCi/kg).

the low molecular weight fraction (Fig. 3). The fact that the early eluted fractions were completely excluded or almost completely excluded on Sephadex G-100, suggested that the molecular weight of the protein eluted with the first peak of radioactivity was approximately 100,000 or greater. The protein eluted with the second peak of radioactivity was partially retained and has a molecular weight less than 100,000. These molecules differ from plasma binding proteins in their retention time on the column.

Radioactivity was present only in the late eluted fraction from heart, cerebellum, cerebral cortex, and hypothalamus. The elution pattern for these tissues was similar to that observed in the heart (Fig. 4).

Discussion. The observation that hydrocortisone was concentrated only by the liver and not by other tissues known to respond to glucocorticoids indicated that concentration of the steroid to levels greater than present in plasma is not necessary for physiological action. Some binding of hydrocortisone to tissue proteins was observed in liver, spleen, and thymus (Figs. 1, 2, 3,). The high concentration of radioactivity in the liver (Table I) was probably due to the binding of hydrocortisone to tissue proteins (Fig. 1) which may represent action sites or sites of steroid metabolism.

Although previous evidence indicates that the site of feedback inhibition for ACTH is in the hypothalamus (9, 10), radioacivity in the hypothalamus was only about 43% of plasma concentration (Table I). The concentration in the thymus and heart was also less than that of plasma. This suggested that only the free unbound plasma hydrocortisone diffused into these tissues. In those tissues which contained few binding molecules, an equilibrium between the free hydrocortisone in the plasma and the tissue was quickly reached. Reports indicate that about 85% of hydrocortisone present in plasma is bound to plasma proteins (11, 12) and that plasma unbound cortisol level determines the extent of cortisol action on cellular functions (13). Therefore, it is possible that only a portion of the free hydrocortisone present in the plasma diffused into these tissues and since little or no binding occurred, equilibrium was quickly reached.

The concentration of radioactivity was greater ($p>0.01$) in the pituitary than in the hypothalamus or other parts of the brain. If there is any relationship between concentration and activity, these data suggest the possibility of a feedback control action of hydrocortisone directly on the pituitary in the pig. A concentration of radioactivity in the liver, pituitary, spleen, skeletal muscle, and testis equal to, or greater than, plasma (Table I) indicated a small amount of binding to macromolecules in these tissues. Molecular-sieve chromatography of the supernatant fractions indicated that binding did occur in the liver, spleen, and thymus (Figs. 1, 2, 3) since radioactivity was associated with the protein peaks. This probably represents firm binding since it withstood the endless dilution of gel filtration. Although there is no evidence to verify a relationship between actions of steroids and their selective uptake and binding, it seems possible that such a relationship may exist.

Summary. The concentration of radioactivity in different tissues of Yorkshire pigs 45 min after an iv injection of ^{3}H-hydrocortisone was determined. Also, chromatographic separation of the tissue supernatant fractions was conducted. Only the liver was capable of concentrating radioactivity from the blood. Radioactivity in the brain, thymus, and heart was less than in the plas-

ma, while activity in the spleen, skeletal muscle, testes, and pituitary was the same as plasma. Molecular-sieve chromatography of the supernatant fractions from the liver, spleen, and thymus on the Sephadex G-100 indicated part of the radioactivity was present in the macromolecular fractions excluded from the gel. It is possible that a relationship between the intracellular binding of the radioactive steroid and its physiological actions may exist.

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