Subcellular Distribution of the (^{3}H) Corticosterone Fraction in Brain, Thymus, Heart, and Liver of the Rat.* (31818)

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Several investigators have studied the subcellular distribution of labeled glucocorticoids *in vivo* and *in vitro* in rat tissues(2,3,4,6,7,9). Accumulation into most tissues with selective accumulation in such organs as the liver, kidney, intestine, bronchial mucosa, ductus epididymus, vas deferens, and interstitial cells of testes and ovaries has been reported(7). Activity from the administration of isotopically labeled cortisone and hydrocortisone has been reported in all subcellular fractions of the rat liver. In all cases much of the activity has been reported in the supernatant fraction, but a high percentage of the total homogenate activity has been reported in the microsomal(9) and mitochondrial(5) fractions as well. A study of the intracellular binding of hydrocortisone in the rat liver has shown that hydrocortisone did not bind with DNA(6).

The work reported here represents a study of the subcellular distribution of the (^{3}H) corticosterone fraction in the brain, thymus, heart and liver of the rat 30 minutes after an IV injection of 20 μc of (^{3}H) corticosterone. These tissues were selected so that the subcellular distribution of corticosterone in the cells of known target tissues of corticosterone, *e.g.*, liver and thymus, could be compared with the distribution in cells of tissues not recognized as primary target tissues of corticosterone, *e.g.*, brain and heart. Corticosterone was selected since it is the natural glucocorticoid in the rat. Attempts were made to extract and count only (^{3}H) corticosterone in each subcellular fraction.

Materials and methods. Adrenalectomized rats weighing about 150 g were anesthetized with sodium pentobarbital. The left femoral vein was exposed and 20 μc of (^{3}H) corticosterone with a specific activity of 0.7 C/millimole was injected intravenously.

Thirty minutes after the injection of (^{3}H) corticosterone, one ml of blood was collected by cardiac puncture. The animals were immediately sacrificed by decapitation and the liver, heart, thymus, and brain were removed and rinsed twice in a 0.3 M sucrose solution. The brain, thymus, and heart were each placed in a solution which contained 0.3 M sucrose, 0.005 M ethylenediaminetetraacetic acid (EDTA), and 0.003 M trishydroxymethylaminomethane and HCL (Trizma) buffer, pH 7.5. The liver was placed in a solution containing 0.25 M sucrose, 0.005 M EDTA and 0.003 M Trizma buffer, pH 7.5. Each tissue was homogenized in a Potter-Elvehjem homogenizer. One hundred μg of carrier corticosterone were added to each tissue homogenate and an aliquot of each homogenate was saved for extraction. The remaining homogenates were fractionated into nuclei, mitochondria, microsomes, and supernatant fractions. The nuclear and mitochondrial fractions of the brain were obtained by a method previously described by Baylazs(1). The thymus and heart were fractionated according to the procedure described by Cleland (4). Liver mitochondria were isolated according to the procedure described by Hogeboom(8). The supernatant for each tissue was centrifuged at 105,000 $\times$ *g* for 60 minutes in a Beckman model L-2 ultracentrifuge in order to obtain the microsomal and supernatant fractions.

Microscopic examination of the nuclear fraction indicated the absence of intact cells. Electron micrographs of the mitochondrial fraction showed a very high concentration of mitochondria and very few contaminants.

Each subcellular fraction was submitted to preliminary purification to remove fats and alkaline-soluble substances by extracting with

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TABLE I. Mean Subcellular Distribution of the (^{3}H) Corticosterone Fraction in Rat Brain, Thymus, Heart and Liver 30 Minutes After an IV Injection of 20 Microcuries of (^{3}H) Corticosterone.

	Brain		Thymus		Heart		Liver	
Number of rats	3		4		4		4	
Nuclei*	14.4†	2.4	13.9	3.5	15.2	5.1	23.8	10.2
Mitochondria	6.4	1.8	6.8	2.2	3.1	1.4	6.5	2.2
Microsomes	4.6	1.6	4.0	1.5	6.1	2.5	14.4	5.2
Supernatant	69.0	13.6	76.5	18.0	75.7	16.5	48.0	15.1
Recovery	94.4		101.1		100.1		92.7	

* The possibility of cross contamination cannot be completely excluded.
† Activity expressed as per cent of total activity contained in the homogenate.

15 volumes of hot acetone (54-60°C). After preliminary purification, the residue was reconstituted in 1 ml of chloroform-methanol (1:1). One-tenth ml of this mixture was spotted and developed on thin layer plates which were coated with silica gel. Spots corresponding to standard corticosterone were removed, extracted, and counted in a Packard Tri Carb Scintillation Counter (Model 314-DC). The samples were prepared to give a counting rate at least twice the background count, the total counts being at least 400 for the counting time.

Results. The activity in each subcellular fraction expressed as per cent of total disintegrations per minute contained in the homogenate is given in Table I. For each tissue the supernatant fraction contained signifinificantly more activity than any of the other fractions. The nuclear fraction of all tissues contained more activity than the microsomal fraction; however, this difference was not significant in the case of the liver. The nuclear fraction of each tissue contained significantly more activity than the mitochondrial fraction.

A comparison of the radioactivity of one tissue fraction with the corresponding fraction of other tissues indicated that the activity of the supernatant fraction of the thymus and heart was significantly greater ($P<0.05$) than that of the liver. A difference significant only at the 10% level was observed between the activity of the supernatant fraction of the brain and the activity of the supernatant fraction of the liver. The activity of the microsomal fraction of the liver was significantly greater ($P<0.05$) than the activity of the microsomal fraction of the brain, thymus, and heart.

The activity in one gram of blood collected by cardiac puncture just prior to sacrificing the rats and the activity in one gram of each tissue were used to determine tissue/blood activity ratios. The average results of these determinations are shown in Table II. Only the liver was capable of concentrating the (^{3}H) corticosterone fraction from the blood.

Discussion. This experiment was designed to localize (^{3}H) corticosterone within the cells of known target tissues of corticosterone as well as other tissues not recognized as target tissues of this hormone. Although it cannot be said that the activity represented only (^{3}H) corticosterone, purification procedures were used in order to minimize the presence of metabolites of corticosterone. The subcellular distribution of the (^{3}H) corticosterone fraction in the different tissues studied is shown in Table I. Corticosterone was present in all subcellular fractions and most of the activity was found in the supernatant fraction of the tissues studied; however, it cannot be definitely concluded that the activity was not concentrated in some particulate fraction since the relative volumes occupied by the nucleus, mitochondria, microsomes, and supernatant are considerably dif-

TABLE II. Mean Tissue/Blood Activity Ratio 30 Minutes After IV Injection of 20 Microcuries of (^{3}H) Corticosterone.

Tissue	Tissue/blood
Brain	.57*
Thymus	.42
Heart	.59
Liver	1.51

* Activity per minute per gram net tissue weight compared to activity per minute per gram of blood.

ferent within the cell. The volume occupied by the supernatant portion of the cell is probably greater than the volume occupied by the nucleus, mitochondria, or microsomes. Therefore, a particulate fraction may have had a much higher concentration of the steroid than the supernatant, yet when the percentage of total activity in all fractions was compared, the supernatant contained most of the activity.

By comparing the subcellular distribution of the (^{3}H) corticosterone fraction in the various tissues, it was found that the pattern of subcellular distribution within the brain, thymus, and heart was similar, whereas within the liver, a different pattern of subcellular distribution was observed. The percentage of activity in the microsomal fraction of the liver was greater than the activity in the microsomal fraction of the other tissues. These data suggest that corticosterone may have a relatively more active role in liver microsomes than in microsomes of other tissues.

Little difference in the activity due to the (^{3}H) corticosterone fraction was observed in the mitochondrial fractions of the different tissues. The relatively constant percentage of activity in this fraction of these tissues indicates that if there is a specific site within the mitochondrion whose function is altered by corticosterone it may be essentially the same in all tissues.

Although not significant, the nuclear fraction of the liver contained slightly more activity than the nuclear fraction of the other tissues. The high percentage of activity in the nuclear and microsomal fractions of the liver is compatible with the hypothesis that within the liver the glucocorticoids affect protein synthesis.

Tissue/blood activity ratios of less than one are reported for tissues other than the liver in Table II. Corticosterone was not concentrated by the thymus, brain, or heart. The low ratio for the thymus is somewhat surprising since it is known that the thymus is a major target organ of glucocorticoids. Only relatively low concentrations of the hormone may be necessary to initiate any direct action upon these tissues. At this time there is little convincing evidence that the glucocorticoids have a direct effect on the heart and brain but there is ample evidence indicating a direct action of these hormones on the thymus. Since the tissue/blood activity ratio for the thymus is about the same as for the heart and brain, it is possible that a concentrating ability by a tissue is not necessary for physiological actions. The steroid may be concentrated only in organs involved in the metabolism of the steroid, and concentration may not be related to physiological action.

The ability of the liver to concentrate corticosterone is not surprising in view of the fact that the liver it a target organ of the glucocorticoids and it is also the major site of conjugation and degradation of the steroids into normal excretory forms. Since cortisol is known to enter the liver by diffusion(3), a favorable diffusion gradient appears to be maintained by the intracellular absorption of free glucocorticoids. The data reported in Table I indicate that the nuclear and microsomal fractions of the liver may in some way bind more corticosterone than these fractions in other tissues and provide a favorable diffusion gradient.

The glucocorticoids may enter all body cells and exert some action in each cell. Specific subcellular sites may be vulnerable to corticosterone in some tissues and not in others. This may result in an overall effect in one tissue different from the overall effect in another tissue. The difference in subcellular distribution may be related to the known different effects of glucocorticoids in different tissues of the body.

Summary. A study was made of the subcellular distribution of the (^{3}H) corticosterone fraction in the brain, thymus, heart, and liver of the rat 30 minutes after IV injection of the steroid. Each tissue fraction was extracted and subjected to purification procedures to remove labeled metabolites. Corticosterone was present in all subcellular fractions of the tissues studied and the liver was the only organ capable of concentrating corticosterone. Most of the activity was found in the supernatant of each tissue. Some differences occurred in the subcellular distri-

bution of the (^{3}H) corticosterone fraction in different tissues. Distribution within the liver was different from that in other tissues. This difference is of interest because of the wide range of effects that result after glucocorticoid treatment. The subcellular distribution was similar in extrahepatic tissues whether they are recognized target tissues of corticosterone or not.

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Binding of Sulfobromophthalein Sodium (BSP) and Other Organic Anions By Isolated Hepatic Cell Plasma Membranes *in vitro*.* (31819)

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The movement of organic anions from blood to bile involves their transfer across the sinusoidal and bile canalicular portions of the plasma membrane of the parenchymal liver cell. Plasma membranes may be isolated from rat liver by differential centrifugation(1), and their lipid, protein, and enzymatic compositions have been investigated (2-5). Because the liver selectively and rapidly removes BSP and other organic anions from blood, the ability of isolated hepatic plasma membranes to bind BSP *in vitro* was investigated. Since several organic anions compete with BSP for hepatic uptake *in vivo*(6), their effect on BSP binding by isolated hepatic plasma membranes *in vitro* was also investigated.

Materials and methods. Cell membranes were isolated from rat liver according to the method of Neville(1). The final sucrose gradient centrifugation was deleted which permitted a better yield of membrane fraction for studies *in vitro*. Final membrane preparations were essentially free of nuclei, mitochondria, and microsomes when examined by phase and electron microscopy and cytochrome oxidase(7), glucose-6-phosphatase(8) and glucuronyl transferase activities (9) were absent or negligible. BSP binding by plasma membranes *in vitro* was investigated in an aqueous solution of 2.1 ml containing 1 mg BSP in 0.05 M Na_3PO_4 buffer at pH 7.4 and 100 λ of the membrane preparation. The mixture was incubated with constant shaking at 37°C for 10 minutes, immediately chilled to 3°C, and centrifuged for 10 minutes at 2000 $\times$ g. The supernatant was discarded and the membrane resuspended in phosphate buffer and gently mixed for 20 seconds. Following recentrifugation, the membrane was immediately harvested free of its supernatant and suspended in 0.1 M NaOH. Bound BSP was determined colorimetrically at 565 mμ.

Binding was expressed both as μg BSP bound per mg of membrane protein(10) and per unit of Co^{++}-stimulated cytidine monophosphatase (Co^{++}-CMPase) activity.‡ Because Co^{++}-CMPase was observed by Novikoff(11) to be concentrated in hepatic cell plasma membranes, this enzyme was used to estimate the quantity of membrane present

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‡ 1 Co^{++}-CMPase Unit = 1 μg P liberated per 15 min in incubation system(11).