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The Uptake of Cortisol by Rat Tissues

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Steroid hormones that are released into the blood pass through all the organs of the body but may exert their major effect by action on only one or two specific tissues. This raises the possibility of the selective uptake and concentration of steroids by special 'target' cells. Although the quantity of corticosteroids in vertebrate plasma is small the distribution of these hormones between blood and tissues may be conveniently investigated with radioactive-isotope techniques.

Some of the first experiments with radioactive steroids (see Samuels, Brown, Eik-Nes, Tyler & Dominguez, 1957) emphasized the non-uniform nature of the equilibration of exogenous hormones with the body fluids. For example, Bradlow, Dobriner & Gallagher (1954) clearly demonstrated that 5 min. after the injection of tritiated cortisone into mice most of the radioactivity was in the liver with only a trace in the blood. The presence of a steroid-concentrating mechanism in liver was apparent from the work of Szego (1953) on the uptake of oestrogens by liver slices, and a similar hepatic uptake of [^{14}C]corticosteroids has since been demonstrated *in vitro* (Sandberg, Slaunwhite & Antoniadis, 1957). This paper gives the results of experiments on the short-term distribution of

^{14}C derived from cortisol between plasma and various tissues of the rat, and, in particular, presents details of the mechanism by which cortisol is concentrated in liver slices.

MATERIAL AND METHODS

Distribution of radioactivity between plasma and tissues

Animals used were male albino rats (approx. 300 g.) from a colony maintained in the Department of Zoology, University of Sheffield. [$4\text{-}^{14}\text{C}$]Cortisol ($42\cdot4\mu\text{C}/\text{mg.}$) was dissolved in 1 ml. of fresh heparinized rat blood and injected into the right saphenous vein. A clamp was placed on the penis and the animal kept under ether anaesthesia for 30 min. About 8 ml. of blood was then taken from the junction of the iliac arteries with the aorta. Tissues were removed and frozen in beakers immersed in solid CO_2 -ethanol. Urine was collected from the bladder with a syringe.

Extraction of radioactive steroids

Tissues. The frozen tissue was cut into approx. 1 mm. cubes (tissue slices were used intact), CHCl_3 -methanol (2:1; 5 ml./100 mg. wet wt.), together with 50 $\mu\text{g.}$ of carrier cortisol, was added and the tissue was shaken for 2 hr. at 50° . Initial experiments showed that the radioactivity of the medium reached a maximum in about $1\frac{1}{2}$ hr. The super-

nant fluid was pipetted off and the flasks were washed with 10 ml. of CHCl_3 -methanol. The extract was evaporated to dryness in a rotary flash evaporator at 30–40° and the residue dissolved in 11 ml. of toluene containing 4 g. of 2,5-diphenyloxazole and 50 mg. of 1,4-di-[2-(5-phenyloxazolyl)]benzene/l.

Plasma, urine and incubation media. The aqueous solution (1 ml.) was evaporated to dryness on a rotary flash evaporator at 30–40° and the residue refluxed with 50 ml. of CHCl_3 -methanol containing 50 μg . of carrier cortisol. After 2 hr. the CHCl_3 -methanol extract was pipetted off, the flask washed out with 10 ml. of solvent and the combined solutions were evaporated to dryness and dissolved in 11 ml. of toluene-phosphor solution as described above.

The above methods extracted at least as much radioactivity as other published methods based on the use of acetone and ethanol homogenates (Forchielli, Rosenkrantz & Dorfman, 1955; Berliner, Keller & Dougherty, 1961). About 77% of cortisol added to the tissue before extracting with CHCl_3 -methanol was recovered in the toluene solution. To separate the radioactive steroids into water-soluble and CHCl_3 -soluble fractions the dry residue was dissolved in water (100 ml./g. of original tissue) and extracted three times with equal volumes of CHCl_3 .

Measurement of radioactivity

The radioactivity of 10 ml. of the toluene solution was measured with a T.M.C. model L.P. 2A Liquid Phosphor Coincidence Counter (Technical Measurement Corp., Conn., U.S.A.). It was possible to count ^{14}C with 40% efficiency. The background counting rate was 8 counts/min. and samples were prepared to give a counting rate at least twice that of background and counted for a period that reduced the counting error to less than 5%. After counting, each solution was tested for 'quenching' by the addition of a known quantity of [^{14}C]cortisol.

Chromatography

Purification of tissue extracts. The CHCl_3 -methanol extracts were purified by preliminary chromatography on a silica-gel column prepared according to Bush & Sandberg (1953). The dry extract was dissolved in 10 ml. of light petroleum (b.p. 60–80°)-ethyl acetate (1:1) containing 50 μg . each of cortisol, cortisone and corticosterone. The solution was passed through the silica-gel column, followed successively by 20 ml. of light petroleum-ethyl acetate (1:1), a mixture of 9.9 ml. of light petroleum-ethyl acetate and 0.1 ml. of methanol and finally a mixture of 6.0 ml. of light petroleum-ethyl acetate and 4 ml. of methanol. Tests showed that the last solution completely eluted cortisol, cortisone and corticosterone from the silica gel.

Paper chromatography. The eluate containing the corticosteroids was evaporated to dryness, dissolved in a known volume of toluene and a measured amount spotted on the bottom of a square of unwashed Whatman no. 1 chromatography paper. The paper was fitted to a chromatography frame (Datta, Dent & Harris, 1950), sprayed lightly with the lower phase of the B(5) chromatography system of Bush (1952) and the chromatogram developed with the upper phase ascending the paper (about 90 min.).

With the above method it was possible to separate cortisol from cortisone and corticosterone. However, the tissue extracts contained a relatively large amount of a

yellow substance, with a similar R_f to that of cortisol, and this limited the amount of extract that could be put on the paper.

The distribution of radioactivity on the chromatogram was determined as follows. The chromatography 'lane' was cut into 1 in. squares and each one was placed upright in a counting vial containing 10 ml. of toluene-phosphor solution. The radioactivity of the solution was measured with the paper aligned parallel with the two phototubes. The results were corrected for quenching as above.

Tissue preparations

Slices. Liver slices were cut free-hand, washed in 0.9% NaCl and incubated in a phosphate-saline solution containing [^{14}C]cortisol (medium II, Krebs, 1950; 10 ml./100 mg. of tissue). Gas was passed through the solution by means of a polythene capillary. After incubation, the slices were briefly washed in water, drained of surplus water on a glass surface and weighed in conical flasks.

Homogenates. Whole liver was cut into small pieces and homogenized in 10 vol. of phosphate solution, as above, in which the required amounts of 1.15% KCl and 0.9% NaCl were interchanged. The distribution of ^{14}C between the solid tissue and supernatant fluid was determined by the method of Werkheiser & Bartley (1957) after the homogenate was centrifuged at 125 000g-min. The radioactivity in the tissue pellet was extracted with 5 ml. of CHCl_3 -methanol/0.2 g. wet wt. at 45° for 2 hr. The concentration of ^{14}C in the supernatant fluid was determined as above.

RESULTS

Distribution of radioactivity between plasma and tissues after injection of [^{14}C]cortisol. It was difficult to decide upon a standard time at which to examine the distribution of ^{14}C because the plasma radioactivity began to fall almost immediately after injection. A 30 min. period was chosen arbitrarily. It was noted that after this time the tissue radioactivity also slowly decreased. This is in agreement with the results of Berliner *et al.* (1961), who found that ^{14}C derived from injected cortisol reached a maximum in mouse liver after about 20 min.

Thirty minutes after the injection of [^{14}C]cortisol into the saphenous vein all of the tissues examined were found to be radioactive (Table 1). It was calculated that the blood sample taken before dissection amounted to at least half of the total blood volume. This means that the tissue radioactivity was probably largely associated with the extracellular space and cells. The results indicate the presence in most of the tissues of uptake mechanisms for steroids. The amount of urine which could be collected after the 30 min. period varied between 0.1 and 0.7 ml. The concentration of ^{14}C in the urine was two to seven times that in plasma although equivalent to less than 1% of the cortisol injected.

The amount of radioactivity per unit weight of tissue was always higher in liver than in other tissues (a tissue/plasma concentration ratio of about 6). Spleen, kidney and intestine also had higher amounts of radioactivity than plasma (tissue/plasma ratios of 5, 3 and 3 respectively). The concentration of ^{14}C in the various salivary glands, adrenal glands, pituitary gland and skeletal muscle was about the same as or slightly higher than in plasma. Nerve tissue was exceptional in that the concentration of ^{14}C was always much lower than that of plasma. Thus the concentrations in whole brain and cerebral cortex were only one-tenth of the plasma concentration. The sum total of radioactivity in the tissues examined was equal to about 70% of that injected.

From these distribution studies it appears that the tissues of the rat differ greatly in their ability to concentrate or bind steroid molecules. As the concentration of injected cortisol was increased the radioactivity in the tissues increased in proportion, although there was some evidence that at the highest concentration the radioactivity of kidney and liver increased faster than in other tissues (Table 1). This may reflect both the greater affinity of these tissues for steroids and the disproportionate increase in the quantity of steroids which are not bound to plasma proteins and are therefore free to enter the cell (e.g. Samuels *et al.* 1957). In general the results of experiments on the whole animal were similar to those of previous workers (Bradlow *et al.* 1954; Sandberg *et al.* 1957 Schapiro & Katz, 1959; Berliner *et al.* 1961).

Uptake of [^{14}C]cortisol by liver slices. From previous work it is likely that the contribution made by cortisol to the total radioactivity of rat tissues would decrease rapidly from the time of injection (e.g. Samuels *et al.* 1957; Berliner *et al.* 1961). Indeed after 30 min., in the present experiments, nearly 50% of the total plasma radioactivity, and 76% of liver radioactivity, was found

in the water phase when partitioned between chloroform and water, indicating the rapid formation of more polar metabolites (see also Berliner *et al.* 1961). These findings make it difficult to examine the kinetics of cortisol uptake in the intact animal. In an effort to simplify the system the uptake of [^{14}C]cortisol was examined in liver slices incubated in a medium with a high cortisol/tissue ratio (10 ml. of soln./100 mg. of tissue).

Liver slices incubated at 38° in oxygen showed a similar rate of uptake of radioactivity to that observed in the liver of the intact animal (Fig. 1). The uptake occurred in three distinct phases. There was an initial rapid uptake during the first few minutes of incubation followed by a much slower linear rate for the next 30 min. Subsequently the uptake was further reduced, although it remained linear for at least 90 min. After incubation for 2 hr. the amount of radioactivity in the medium had dropped by about 25% and the tissue/medium distribution ratio was nearly 7. To investigate the possibility that uptake was coupled with oxidative metabolism, slices were incubated with nitrogen instead of oxygen as gas phase. As this procedure produced no changes in the rate of ^{14}C uptake all subsequent incubations were carried out with air as gas phase.

Effect of temperature on the uptake of [^{14}C]cortisol by liver slices. The results of previous experiments indicated that a considerable proportion of the cortisol that entered liver was converted into more polar metabolites. To dissociate uptake processes acting on cortisol from those involving the metabolism of cortisol it was necessary to reduce the activity of enzymes utilizing cortisol as substrate. This problem has been previously overcome for other transported molecules by working at low temperatures.

A temperature reduction from 38° to 10° inhibited the uptake of cortisol by 75% (Fig. 2). A further decrease in temperature from 10° to 4°

Table 1. *Radioactivity of plasma and tissues after the injection of [^{14}C]cortisol*

Cortisol was injected intravenously under ether anaesthesia. Thirty minutes later the distribution of ^{14}C between plasma and tissues was determined as described in the text.

$10^{-6} \times$ Total radioactivity injected (counts/min.)	2.04	1.02	0.5	0.25
Plasma radioactivity (counts/min./ml.)	464	265	85	76
Tissue radioactivity (counts/min./g. wet wt.)				
Liver	2814	1104	748	439
Kidney	1775	792	396	298
Submaxillary gland	692	416	165	99
Parotid gland	—	215	164	79
Skeletal muscle	417	215	108	91
Brain	—	27	16	19
Urine (counts/min./ml.)	606	621	356	505

had little effect on the uptake rate. There was no evidence for the typical 50% fall in reaction rate for each 10° drop in temperature that is a characteristic of most chemical reactions in the cell. In this respect the results are similar to those of Levin, Daughaday & Bremer (1955), on cortisol uptake at very high steroid concentrations. Calculations with the Arrhenius equation gave an activation energy of 340 cal. for uptake between 4° and 10°. The activation energy increased with temperature, reaching a maximum of about 8000 cal. between 20° and 30°. The results indicate that at low temperatures there is a physical binding of the cortisol molecule on to sites associated with tissue constituents, whereas at higher temperatures chemical reaction of the steroid may also be involved in uptake.

Kinetics of cortisol uptake by liver slices at 4°. At 4° all of the extractable radioactivity that accumulated in liver slices appeared in the cortisol area of paper chromatograms. Since the results indicated that the metabolism of cortisol was decreased to negligible proportions subsequent experiments were carried out at this temperature. When the cortisol concentration in the medium was varied

over a tenfold range, the uptake process was found to follow the kinetics of a single-step enzyme reaction. It was possible therefore to determine values of K_m and V_{max} from a Lineweaver & Burk (1934) plot of the uptake rates at different concentrations of [^{14}C]cortisol. The maximum velocity was calculated to be 33.4 $\mu\text{mg./min./g. wet wt.}$ with K_m 100 $\mu\text{mg./ml.}$ This affinity constant (about 0.3 μM) is of the same order of magnitude as that for the interaction of substrates with 3-hydroxy steroid dehydrogenases (Marcus & Talalay, 1955; Tomkins, 1956) and for the association of inhibitory steroids with reduced nicotinamide-adenine dinucleotide oxidase (Endahl & Kochakian, 1961).

Assuming that rat liver takes up all corticosteroids at a similar rate and that the rate of uptake balances the rate of secretion by the adrenal gland, it was calculated that the turnover time of the endogenous corticosteroids was just over 1 hr. This value is similar to those obtained by other methods for the turnover of the miscible pool of cortisol in man (e.g. Peterson, 1959). The calculated rate of cortisol uptake by rat liver (4.4 $\mu\text{mg./min./g.}$) is about 30% lower than the steady-state

Fig. 1. Uptake of [^{14}C]cortisol by liver slices at 38°. Liver slices (approx. 100 mg. each) were incubated at 38° in a phosphate-saline solution (medium II; Krebs, 1950; 10 ml./slice) containing [^{14}C]cortisol (600 counts/min./ml.) with O_2 as gas phase. At intervals three slices were removed and the ^{14}C content was determined as described in the text.

Fig. 2. Effect of temperature on the uptake of [^{14}C]cortisol by liver slices. Liver slices were incubated with [^{14}C]cortisol (700 counts/min./ml.) at different temperatures (○, 4°; ●, 10°; □, 20°; ■, 30°; ○, 38°) and the ^{14}C content was determined at intervals of time.

level of corticosterone secretion in the rat (Glenister & Yates, 1961). At least part of the latter discrepancy may be explained by experiments (D. Bellamy, unpublished work) which show that corticosterone is taken up by rat-liver slices faster than cortisol. However, the rate of plasma-steroid removal, as calculated above, is likely to be a minimum value because, although liver appears to be the main site of uptake and metabolism, appreciable amounts of radioactivity derived from cortisol were found in other tissues, notably kidney, spleen and intestine (Table 1). In this respect it has previously been observed that tissues other than liver are capable of metabolizing adrenocortical steroids to excretory products (Schneider & Horstmann, 1952; Ganis, Axelrod & Miller, 1958; Dutton, 1959; Berliner *et al.* 1961; Gold, 1961).

At 4° the uptake of cortisol by liver slices declined slowly with time so that after incubation for 6 hr. it was about 25% of the initial rate (Fig. 3). At this time the tissue concentration of ^{14}C was between four and six times that in the incubation medium irrespective of the concentration of medium cortisol. The relationship between the logarithm of the uptake rate (measured for separate 30 min. periods) and the time of incubation was

linear, with a slope that increased with the concentration of medium cortisol (Fig. 4). According to Reiner (1961) this relationship would be expected if cortisol diffused across the cell surface and was adsorbed on to intracellular sites. In this respect the process may be distinguished from adsorption to the cell surface and uptake involving a carrier mechanism (Reiner, 1961). The present experiments gave no indication of the total number of adsorption sites involved but previous work with higher cortisol concentrations suggests that the maximum binding capacity is at least 1.8 mg./g. of liver (Levin *et al.* 1955).

State of cortisol in liver slices. At 4° the uptake of cortisol by liver slices, particularly during the initial stages, may have been due in part to the penetration of cortisol into the extracellular space. When slices containing [^{14}C]cortisol were incubated in cortisol-free solution (Fig. 5) there was a rapid drop in the tissue radioactivity during the first 10 min.; subsequently the radioactivity decreased at a slower rate for a further 50 min. After 60 min. in the unlabelled medium the tissue medium concentration ratio for cortisol was about 15:1, whereas initially, during preincubation, it was about 2.5:1. If it is assumed that the initial loss represented cortisol that was present in the slices at the same concentration as in the medium used for

Fig. 3. Effect of [^{14}C]cortisol concentration on the uptake of ^{14}C by liver slices. Liver slices were incubated at 4° in media containing varying amounts of [^{14}C]cortisol (see Fig. 1): ●, 175 counts/min./ml.; ⊙, 350 counts/min./ml.; ○, 700 counts/min./ml. At intervals, slices were removed, weighed and the ^{14}C content was determined as described in the text.

Fig. 4. Effect of incubation time on the uptake of [^{14}C]cortisol at 4°. Results were taken from the experiments shown in Fig. 3. The logarithm of the uptake rate for separate 30 min. periods (du/dt) is plotted against the incubation time. For ●, ⊙, ○, see Fig. 3.

preincubation, it may be calculated that this cortisol was distributed in about 40% of the tissue water. Thus the initial loss of ^{14}C may well represent that in the extracellular space of the slices. Supporting evidence for this idea comes from experiments in which the penetration of inulin into the slices gave an inulin space of between 40 and 50% (D. Bellamy, unpublished work).

When allowance is made for extracellular penetration there was a possibility that the uptake of cortisol by liver slices was due to an exchange reaction with unlabelled endogenous steroid. The fresh slices could not be tested for the presence of endogenous cortisol because of the difficulties in concentrating the tissue extracts. However, the

Fig. 5. Uptake and release of [^{14}C]cortisol by liver slices at 4° . Liver slices were incubated at 4° as in Fig. 1 with [^{14}C]cortisol (648 counts/min./ml.) and the rate of uptake was determined as described in the text. After 60 min. the remaining slices were removed, quickly dipped in a large volume of 0.9% NaCl and placed in an incubation medium containing no cortisol. The ^{14}C content of the slices was determined at intervals of time.

Table 2. *Effect of various steroids on the uptake of [^{14}C]cortisol by liver slices*

The incubation medium was the same as in Fig. 1, containing $6.7\text{ }\mu\text{mg.}$ of [^{14}C]cortisol/ml. (280 counts/min./ml.). Various steroids dissolved in ethanol were added to give a concentration of $7\text{ }\mu\text{mg./ml.}$ and the slices were incubated for 60 min. The concentration of ethanol in the incubation medium was 1%.

Addition	Concn. of ^{14}C in tissue water (counts/min./g.)
Nil	3134
Corticosterone	3086
9 α -Fluorocortisone	2888
Aldosterone	2876
Prednisolone	2756
11-Dehydrocorticosterone	2528
11-Deoxy-17-hydroxycorticosterone	2438
Cortisone	2331
Deoxycorticosterone	1938

possibility of exchange was investigated by examining the effect of external unlabelled cortisol on the loss of radioactivity from slices preincubated with ^{14}C . The loss of radioactivity was measured in slices that had been preincubated in a cortisol-free medium for 10 min. to remove most of the extracellular cortisol. The slices initially contained 5954 counts/min./g. dry wt. After incubation for 50 min. in media with and without unlabelled cortisol the radioactivity had dropped to 3199 and 4111 counts/min./g. dry wt. respectively. In this experiment the rate of cortisol uptake during preincubation was 35 640 counts/min. (per hr./g. dry wt.) whereas the rate of loss of ^{14}C by exchange with unlabelled cortisol was 1376 counts/min. (per hr./g.) It is unlikely therefore that the initial uptake was due to exchange with endogenous cortisol.

Effect of other steroids on the uptake of [^{14}C]cortisol by liver slices. There was a possibility that access of steroid to the uptake sites in liver slices was limited and that other steroids of similar structure would compete with cortisol for adsorption. However, when a variety of unlabelled steroids were incubated with [^{14}C]cortisol in equal concentration, the amount of ^{14}C taken up was not markedly reduced. The greatest inhibition of cortisol uptake (about 40%) occurred with deoxycorticosterone (Table 2). Other steroids fell into two groups according to the degree of inhibition. Corticosterone, 9 α -fluorocortisone and prednisolone had little effect on cortisol uptake, whereas 11-dehydrocorticosterone, 11-deoxy-17-hydroxycorticosterone and cortisone had a more pronounced effect. In general it appeared that the molecules with the least number of substituent groups attached to the steroid nucleus, particularly those involving C-11, exerted the greatest effect on cortisol uptake. These results are similar to those obtained for the adsorption of cortisol to plasma proteins (Daughaday, 1958).

If there was a limited number of sites for cortisol entry into liver slices then the presence of an equal concentration of a steroid with the same affinity would reduce the rate of cortisol uptake by a half. The maximum inhibition by deoxycorticosterone was less than 50% and most of the steroids had little effect. If there are few uptake sites, this could mean that some of the sites are relatively specific for cortisol. However, the presence of a large number of non-specific sites that were not saturated with steroid during the experiment cannot be ruled out (see Levin *et al.* 1955). It has been previously demonstrated that the adsorption of oestrogens and corticosteroids to the surface of red cells may inhibit the penetration of several unrelated molecules such as phosphate, uric acid and glucose (Christensen, 1961). Thus the inhibitory effect of some of the steroids on cortisol

uptake by liver may occur because of the close proximity of surface-adsorption sites to uptake sites.

Distribution of [^{14}C]cortisol between liver homogenate and incubation medium. Liver homogenates were used to test for the presence of cellular adsorption sites for cortisol, which might account for the concentration of cortisol in slices. The homogenates were incubated with [^{14}C]cortisol and at various time-intervals a sample was removed,

Fig. 6. Distribution of [^{14}C]cortisol between centrifuged homogenates and the supernatant solution. A liver homogenate was prepared as described in the text. Samples of the homogenate were added to an equal volume of medium containing varying amounts of [^{14}C]cortisol. The diluted homogenate was immediately centrifuged at 25 000g for 5 min. and the distribution of radioactivity between the water of the tissue pellet and the supernatant solution was determined as described in the text.

Table 3. *Distribution of cortisol and metabolites between plasma and liver*

The amount of radioactive cortisol and polar metabolites in plasma and liver was determined after the injection of [^{14}C]cortisol as described in the text (Fig. 1). The plasma ratio of free to protein-bound steroids was calculated from the data of Bush (1957), Ceresa & Cravetto (1958) and Mills (1961).

	Concn. of steroid			
	Plasma ($\mu\text{m-moles/l.}$)		Cell ($\mu\text{m-moles/l. of tissue water}$)	
	Adsorbed	Free	Free	Adsorbed
Metabolites	9.09	1.32	18.0	179
Cortisol	13.5	1.51	1.40	9.15

all particulate matter was centrifuged down and the distribution of radioactivity between the supernatant solution and the water in the tissue pellet was determined (Werkheiser & Bartley, 1957). The results showed that the ^{14}C concentration in the water of the tissue pellet reached a higher value than that in the medium within 5 min. This was the time taken to add the cortisol and to sample and centrifuge the homogenate. The distribution ratio for several samples taken during a 60 min. incubation period was the same and was relatively constant over a tenfold variation in the concentration of supernatant cortisol. These results may be expressed as an adsorption isotherm (Fig. 6), and are similar to those obtained for the binding of cortisol to plasma proteins (Bush, 1957), the adsorption of corticosteroids to dialysed liver homogenates (Levin *et al.* 1955), to isolated mitochondria and reduced nicotinamide-adenine dinucleotide oxidase (Ulrich, 1959; Jensen & Neuhaard, 1961).

A general view of the situation in the intact animal after the administration of [^{14}C]cortisol is given in Table 3. Although the amount of cortisol was equal to 70% of the normal pool of extracellular corticosteroids, 30 min. after injection most of the radioactivity was accounted for by bound intracellular metabolites. The plasma concentration of radioactive steroids was only about 5% of the normal values. The distribution of cortisol between plasma proteins and the intracellular adsorption sites had apparently reached an equilibrium, with the concentration of free cortisol in the plasma equal to that in the cell.

DISCUSSION

The results obtained for the distribution of ^{14}C derived from cortisol in the rat indicate that a number of tissues had the ability to concentrate steroids from the blood. Although there appeared to be considerable differences in the affinity of the various tissues for radioactive steroids, these results might have been due, in part, to differences in the rate at which these substances were presented to the tissues by the blood. It has been demonstrated that liver is the most important organ in removing cortisol from plasma (e.g. Peterson & Wyngaarden, 1956) and that the rate of cortisol disappearance from plasma is proportional to blood flow through the liver (Samuels *et al.* 1957). This suggests that hepatic uptake is normally limited by the availability of plasma cortisol. From the present results on the uptake of cortisol by liver slices, assuming a blood flow through the liver of 1.0 ml./g./min. (Spector, 1956), it may be calculated that the maximum rate at which steroids are removed by the liver is 10% of the rate at which they pass

through the liver in the blood. When the rate of flow of unbound steroids only is considered, the rate of uptake is still less by a factor of about 2.5. This suggests that steroid flow through the liver does not normally limit uptake and this is in agreement with other experiments which show that an appreciable amount of cortisol is found in hepatic venous plasma (Hechter, Frank, Caspi & Frank, 1957).

It may be supposed that the rate of equilibration of plasma free cortisol with the extracellular fluid normally limits uptake. Thus uptake would be expected to depend on both the concentration of plasma cortisol and the difference in blood pressure available for the ultrafiltration of cortisol through the walls of the hepatic capillaries. Supporting evidence for the first of these relationships comes from the work of Peterson, Wyngaarden, Guerra, Brodie & Bunim (1955). Evidence for the second comes from experiments on dogs, in which the half-life of plasma cortisol was considerably reduced after the shunting of blood from the aorta into the portal vein (Samuels *et al.* 1957). Apparently the total steroid in liver may limit uptake but only at very low blood flows (Hechter *et al.* 1957).

In tissues with a low blood flow, such as muscle, it is not possible to say whether the low amounts of radioactivity were due to differences in rates of uptake or circulation. In brain, however, where the blood flow is half of that in liver (Spector, 1956) and the concentration of ^{14}C differed by a factor of 50, it is likely that a comparison of the amounts of radioactivity gives a true measure of the relative affinities of the two tissues for steroids. The concentration of ^{14}C in brain was always much lower than that in plasma and indicates the presence of a barrier to the diffusion of cortisol and its metabolites into the cells. These results are in agreement with those of Schapiro & Katz (1959). This barrier may explain the low concentrations of adrenocortical steroids found in cerebrospinal fluid (Abelson, Baron & Toakley, 1955). In similar experiments with the posterior pituitary gland, Schapiro & Katz (1959) found that the tissue plasma ratio of ^{14}C derived from cortisol ranged between 4.5 and 58. Assuming that the amount of radioactivity in the anterior pituitary gland was the same as that in brain, it can be calculated from the present results that the concentration in the posterior pituitary gland would be seven times that in plasma. Thus the available evidence suggests that the concentrating ability of the posterior pituitary gland is probably as high as, if not higher than, that of liver. A similar selective concentration of adrenocortical steroids in certain areas of brain may account for the known effects of steroids in inducing convulsions (Heuser & Eidelberg, 1961).

The advantages of a mechanism for concentrating steroids are less obvious for the posterior pituitary gland, kidney, spleen and intestine than for liver. The uptake process in the first-named tissues may simply reflect the presence of chemical structures that fortuitously have the property of adsorbing steroids. The high concentration of ^{14}C in liver, however, taken together with the importance of this tissue in steroid conjugation (Gold, 1961), suggests that the ^{14}C uptake is related to processes for regulating the amount of circulating adrenocortical hormones.

We found that liver slices incubated with ^{14}C developed tissue-medium gradients of radioactivity which were similar to those found in the intact animal. The occurrence of a gradient in favour of tissue accumulation could be due either to the operation of a specific carrier system requiring metabolic energy or to a non-specific binding or adsorption mechanism with a relatively long equilibration time. The fact that the uptake process followed Michaelis-Menten kinetics (Michaelis & Menten, 1913) does not restrict the mechanism to one that operates with a carrier system (e.g. Haldane, 1930).

Also an argument against the first possibility is that accumulation is independent of aerobic metabolism and the temperature-activity curve is unlike that of most metabolic processes. Indeed, the time-curves for uptake suggest that it occurs by adsorption to intracellular sites (Reiner, 1961). Strong evidence for intracellular adsorption was also obtained from the experiments which showed that the distribution of cortisol in homogenates could be expressed as an adsorption isotherm. In liver slices at 4° the uptake of cortisol appears to be limited by the rate at which the steroid can reach these intracellular adsorption sites by diffusion (see also Christensen, 1961, on red cells). Under these circumstances the uptake would be expected to cease when the tissue-medium gradient was the same as the intracellular adsorption isotherm (cf. Fig. 3). At higher temperatures it seems that enzymic reactions are also involved in the removal of cortisol from the extracellular space. The entry of cortisol into the cell is probably still a passive process and follows a gradient which is maintained as a balance between cortisol metabolism (probably involving reduction of the C-4 double bond; Peterson *et al.* 1955; Glenister & Yates, 1961), adsorption on intracellular sites and the rate of diffusion into the cell.

SUMMARY

1. The distribution ratio of ^{14}C between tissues and plasma was determined 30 min. after the intravenous injection of $[4\text{-}^{14}\text{C}]$ cortisol into male rats.

Liver had the highest ratio (about 6) and the ratios for kidney, spleen and intestine were considerably greater than 1. Brain was the only tissue in which the ratio was less than 1.

2. Cortisol was slowly concentrated by liver slices incubated at 4°. After a period of 6 hr. the cortisol concentration was about six times as high as that in the incubation medium. A large fraction of the tissue cortisol was not in equilibrium with that in the external solution.

3. The activation energy for uptake varied from 340 cal. between 4° and 10° to 8000 cal. between 20° and 30°. The process followed Michaelis-Menten kinetics and at 4° $V_{\max}$ was 33.4 $\mu\text{mg./min./g.}$ wet wt. with K_m 100 $\mu\text{mg./ml.}$

4. A constant distribution ratio of [$4\text{-}^{14}\text{C}$]cortisol between the particulate and the soluble fractions of liver homogenates (about 6:1) was attained within 5 min. and the results could be expressed as an adsorption isotherm. These experiments, taken together with the characteristics of uptake of cortisol by slices at 4°, suggest that cortisol enters the cell by diffusion. A favourable diffusion gradient at 4° appears to be maintained by the intracellular adsorption of free cortisol.

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