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THE DISTRIBUTION AND DISPOSAL OF CORTISOL IN HUMANS*

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The theory of the distribution and disposal of cortisol proposed in this report is not unique in its general features and resembles that outlined earlier by Samuels, Brown, Eik-Nes, Tyler, and Dominguez (1957)[10] and by Peterson (1959)[8]. However, in contrast to the earlier verbal formulations our theory is developed as a set of differential equations. The quantitative adequacy of the theory will be examined by comparing data obtained by solution of the equations with data from experimental studies in human volunteers. The theory will also be used to analyze two observations; namely, the rapid disposal rate that occurs after administration of a tracer compared to a load dose of cortisol and the apparent decrease in cortisol disposal rate following estrogen administration.

Methods

Normal subjects, and patients with adrenal insufficiency treated with dexamethasone and desoxycorticosterone were studied. The studies on the effects of estrogens were performed on females who had had hysterectomies but were otherwise normal. All subjects were given 1 mg of dexamethasone by mouth at 11 : 00 P.M. the night before the studies and 0.5 mg every six hours during the course of the studies to suppress adrenal glucocorticoid secretion, as described by Nugent, MacDiarmid, Nelson and Tyler (1963)[7]. The methods for constant intravenous infusion and plasma 17-OHCS determination have been described previously by Eik-Nes (1957)[3] and by Nugent, *et al.* (1963)[7]. To establish the composition of the substances estimated by the plasma 17-OHCS method, 2 male volunteers were given six hour infusions containing 5 μc of 4-C^{14}-cortisol together with unlabeled cortisol at a rate of 110 μg per kilogram of body weight each hour (K-hr). Blood specimens were drawn at the end of the infusion and again 45 minutes later. Extraction and purification of the plasma were performed as in the Eik-Nes method. The extracts were chromatographed on paper in the Bush B-5 system. The only Porter-Silber chromogens identified were cortisol and cortisone; these accounted for 77 and 20 per cent of the radioactivity, respectively. It is recognized that under the conditions of these studies the plasma 17-OHCS method measured mainly but not exclusively the concentration of cortisol.

Assumptions made in constructing the model

The model is outlined in Figure 1A. Intravenously injected cortisol is distributed initially throughout the plasma, V_1. Wennesland, Brown, Hopper, Hodges, Guttentag, Scott, Tucker and Bradley (1959)[11] estimate that the plasma volume is 0.035 liter per kilogram of body weight. The total concentration of plasma cortisol is composed of unbound, C_1, albumin bound, C_1A, and transcortin bound cortisol, C_1T. Q indicates

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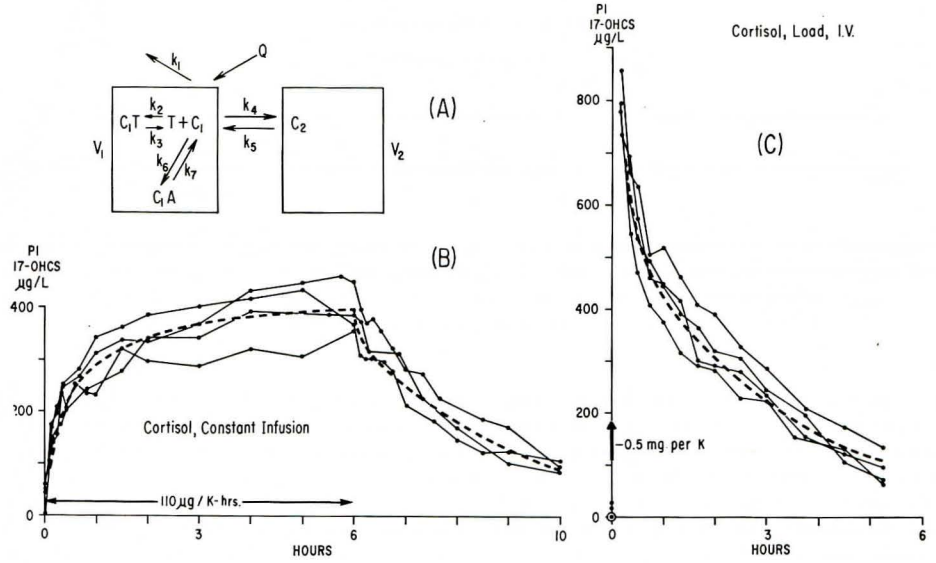

Fig. 1A. A model for cortisol distribution and disposal. The meaning of the abbreviations is given in the text.

Fig. 1B. Plasma 17-OHCS concentrations during and after 6 hour intravenous infusions of cortisol at a rate of 110 μg per K-hr. The experimental data are shown as dots connected by solid lines. The calculated data are shown as dashed line.

Fig. 1C. Plasma 17-OHCS concentrations after sudden intravenous injection of 0.5 mg of cortisol per K. Data plotted as in B.

the rate of infusion of cortisol by a constant infusion pump. The small amount of cortisol of endogenous origin in the plasma of these dexamethasone treated subjects is ignored. The reaction of unbound cortisol with albumin to form C_1A is shown as a unimolecular reaction; this is justifiable since, as shown by Bush (1957)[1], the concentration of albumin is so large that changes in its concentration during the reaction can be ignored. Each of the following values, accepted in this report, is in the range established by the indicated investigator: equilibrium value of C_1A/C_1 equals 2, Daughaday and Mariz (1961)[2]; normal total transcortin binding equals 250 μg per L, Mills (1962)[6]; association constant of C_1T, that is k_2/k_3, equals 0.059, Mills (1962)[6]. Cortisol is also expected to exist outside the plasma volume. It is postulated that the many extravascular compartments (interstitial fluid, liver, muscle, etc.) can be represented as though they were a single compartment, V_2. Extravascular cortisol, bound and unbound, is represented as though it existed unbound at a concentration C_2. It will be assumed that delays due to mixing in V_1 and V_2 are short and can, therefore, be ignored. Unbound cortisol in plasma may be disposed of by many different processes; however, these processes will be described as though they were a single process, with a rate of cortisol removal proportional to C_1 (rate constant k_1). The equations for the theory can now be written.

(1) $V_1dC_1/dt = Q - k_1C_1 - V_1d(C_1T)/dt - V_1d(C_1A)/dt - V_2dC_2/dt$
(2) $V_1d(C_1T)/dt = k_2(C_1)(T) - k_3(C_1T)$
(3) $V_1d(C_1A)/dt = k_6C_1 - k_7(C_1A)$
(4) $V_2dC_2/dt = k_4C_1 - k_5C_2$

Concentrations are in μg per L expressed in terms of equimolar concentrations of cortisol, time is in hours and Q is in μg per K-hr. The dimensions of the remaining terms are shown in Table I.

TABLE I

Values of coefficients of equations 1 to 4

Coefficient	Value	Coefficient	Value
V_1	0.035 L/K	k_3	0.0872 L/K-hr
V_2	2.0 L/K +	k_4	3.75 L/K-hr
$T+C_1T$	250 μg/L	k_5	3.75 L/K-hr
k_1	1.66 L/K-hr	k_6	0.5 L/K-hr
k_2	0.00516 L^2/μg-K-hr	k_7	0.25 L/K-hr

+ The volume selected for V_2 is obviously unrealistically large. Its magnitude implies that much extravascular cortisol is bound so that at certain sites its concentration must be high. The rates of diffusion between V_1 and V_2, regulated by k_4 and k_5, are the rate limiting processes controlling C_2.

Estimation of values of coefficients of equations

The assumptions made above have imposed values on certain coefficients or on their ratios in equations 1 to 4. Within the limits of these restrictions the values of the remaining coefficients were determined after solving the equations by means of an electronic analogue computer. The values of the coefficients were selected so as to obtain the best agreement between the theoretical predictions and the actual data in two different experimental arrangements. One set of data was obtained during and after the infusion of cortisol into 4 subjects at a rate of 110 μg per K-hr for 6 hours (Figure 1B). A second set of data was obtained after infusing cortisol, 0.5 mg per K, intravenously over a two minute period at the beginning of the study (Figure 1C). Using the values of the coefficients shown in Table I it was possible to obtain solutions for the equations that were similar to both sets of experimental data (Figure 1B and C). The values of the pairs of rate constants associated with albumin binding, k_6 and k_7, and with transcortin binding, k_2 and k_3, could be considerably higher without impairing agreement of the calculated and experimental data.

In Figure 1B the most rapid rise in the concentration of plasma 17-OHCS occurs immediately after the onset of the infusion. This rise is due mainly to binding of cortisol with the large amount of free transcortin that is present early in the infusion. After the load-dose of cortisol (Figure 1C) the initial rapid fall in concentration of plasma 17-OHCS is the result of loss of unbound cortisol from the plasma by destruction and by escape into the large extravascular space. The slow fall in concentration of plasma 17-OHCS from 1 to 5 hours after the load-dose is attributable in great part to back diffusion of cortisol from the extravascular space.

If the present theory, stated by equations 1 to 4 and Table I is to have merit, we might reasonably expect that its predictions should conform with results obtained in experimental arrangements not utilized in constructing the theory. Two such experiments will now be discussed.

Rapid disposal rate after tracer doses

After injection of a tracer amount of 4-C^{14}-cortisol Peterson (1955) reported that the disposal rate of the dichloromethane-extractable radioactive material was appreciably greater than the disposal rate of 17-OHCS determined after administration of a load-dose of cortisol. Can this discrepancy be explained within the confines of the present theory or must additional hypotheses be made, perhaps attributing this discrepancy to a temporary overloading of cortisol disposal (k_1) by the load-dose? On the left in Figure 2A are shown the calculated concentrations of plasma cortisol using equations 1 to 4 with the values for the coefficients shown in Table I for a patient given a load-dose of 0.5 mg of cortisol per K. On the right in Figure 2A are the plasma concentrations of radioactive cortisol in the same patient given 0.175 μg of 4-C^{14}-cortisol per K calculated on the basis of the identical values for the coefficients in equations 1 to 4. Fol-

lowing the load-dose there is a rapid fall in the concentration of plasma cortisol so that only a small percentage of the total administered dose remains in the plasma 1 hour after injection. This is attributable to saturation of transcortin binding capacity soon after injection of a load-dose. Therefore, large amounts of unbound cortisol are available for destruction or for escape into the extravascular space from which it diffuses back and sustains the slow late fall in plasma cortisol concentration. On the other hand, after the tracer dose of cortisol a much larger proportion can combine with

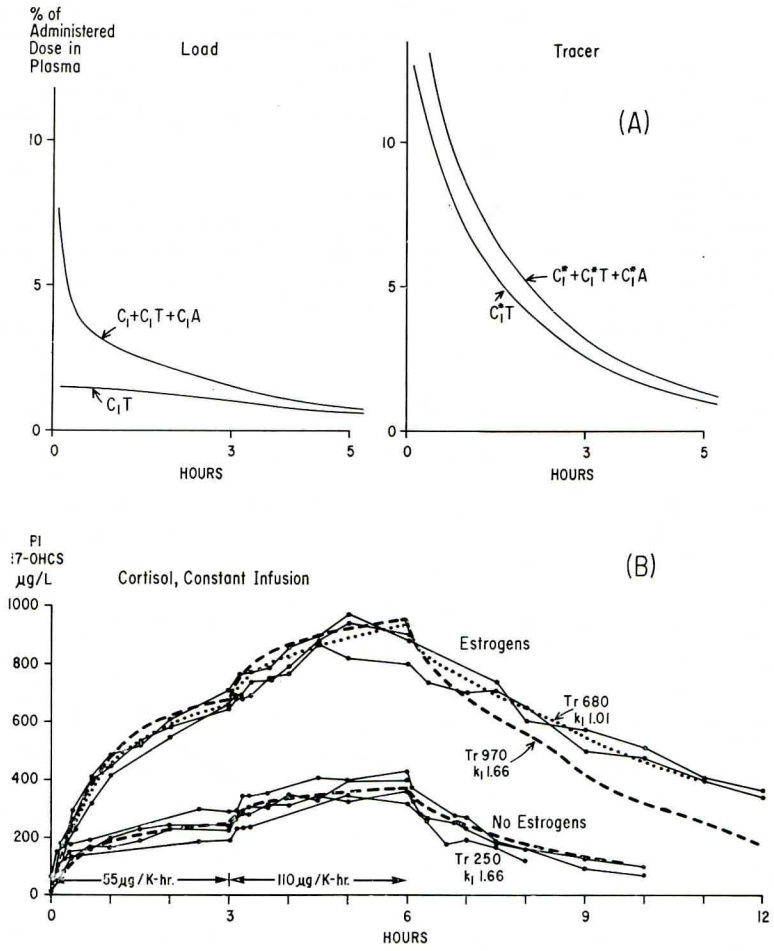

Fig. 2A. Left, calculated concentrations of cortisol ($C_1 + C_1A + C_1T$) and of C_1T in plasma after sudden injection of 0.5 mg of cortisol per K. Right, calculated concentrations of labeled cortisol and of transcortin-bound, labeled cortisol in plasma after sudden injection of 0.175 μg of 4-C^{14} labeled cortisol per K in a patient with an unlabeled cortisol concentration in his plasma of 100 μg per l. Asterisks indicate radioactivity.

Fig. 2B. Plasma 17-OHCS concentrations during intravenous infusions of cortisol at constant rates in 4 subjects studied before and 3 subjects studied after estrogen administration. The cortisol infusion rates were 55 μg per K-hr for the first three hours and 110 μg per K-hr for the subsequent three hours. Data plotted as in Figure 1B. All values used for coefficients in equations 1 to 4 in calculating the theoretical curves were as given in Table I except for patients treated with estrogens. In these patients the value for total amount of transcortin (Tr) was 980 for the dashed line while for the dotted line changes were made in the value of Tr (680) and of the cortisol disposal rate constant, k_1 (1.01).

transcortin rather than being immediately destroyed or escaping into V_2. Consistent with this interpretation is the finding that if the extravascular space, V_2, is removed from the model, one hour after injection of either tracer or load-doses of cortisol there are similar and rapid disposal rates. It is to be emphasized that the curves pictured in Figure 2A are theoretical and not experimental data. The fact that the calculated curves in this and the other figures in this report agree in their general pattern with experimental data does not prove that the theory is correct but only that it is compatible with the experimental data.

Effects of estrogens

A slow apparent rate of cortisol disposal in subjects who are pregnant or ingesting estrogens has been described by Migeon, Bertrand and Wall (1957)[4] and by Peterson, Nokes, Chen and Black (1960)[9]. Is this attributable only to an increase in transcortin binding or is there really a decrease in the disposal rate? In the lower part of Figure 2B, labeled 'No estrogens', are shown the experimental and theoretical data on four subjects infused with cortisol at 2 different rates successively. Under these conditions there is good agreement between the experimental data and those calculated by using the values of Table I. Note that there is a greater increase in plasma 17-OHCS concentration during infusion at the rate of 55 μg per K-hr than when the rate is doubled. This nonlinearity in response is attributable to an increased proportion of the total cortisol being bound to transcortin when the total cortisol concentration is low.

Three of the subjects were then given ethinyl estradiol, 0.5 mg per day, for one month and restudied (upper curves, labeled 'Estrogens', in Figure 2B). When it is assumed in the calculations that the only effect of estrogen is to increase transcortin (see curve marked Tr 970, k_1 1.66) the theoretical rate of disposal after cessation of the cortisol infusion was too fast. However, by assuming that estrogens decrease the disposal rate constant of unbound cortisol and increase transcortin concentration good agreement was obtained between the calculated and experimental data (see curve marked Tr 680, k_1 1.01).

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