Factors Influencing the Metabolism and Distribution of Corticosterone-1,2-^{3}H in the Rat

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SUMMARY

The metabolism and distribution of intravenously administered tracer doses of corticosterone-1,2-^{3}H are not affected by elevated plasma levels of endogenous corticosterone due to cold stress. Pretreatment with methylcholanthrene increased the plasma half-life of the labeled steroid, reflecting a decreased rate of metabolic destruction, but had no effect on the apparent volume of distribution. Pretreatment with phenylbutazone caused an increased volume of distribution with no change in the plasma half-life.

The adrenal venous blood of rats contains only two glucocorticoids, corticosterone and hydrocortisone, in a ratio of approximately 8:1 (1); hence, the plasma level of corticosterone may be considered as an accurate index of adrenocortical function in rats. A number of recent studies of pituitary-adrenocortical activity (2–6) base their interpretations on changing plasma corticosterone levels, a usage which depends upon balanced rates of corticosterone secretion and metabolism. In resting animals, corticosterone secretion is continuous; the plasma content turns over rapidly as shown by a half-life of less than 20 min (5–7). In such a system increased corticosterone secretion by the adrenal cortex would raise the plasma level; the increased plasma level would then be an index of increased adrenocortical activity. However, an increased plasma level could also occur without a change in secretion rate if the rate of enzymic destruction were severely curtailed. Administration of tracer doses of corticosterone-1,2-^{3}H to rats makes it possible to "label" the plasma pool of the steroid. Since the disposition of the labeled corticosterone reflects the disposition of endogenous steroid, it is possible to study factors that influence the endogenous steroid by following changes in the radioactivity.

Corticosterone-1,2-^{3}H (New England Nuclear Corp., specific activity > 700 mC/mmole) was purchased as a solution in acetone–benzene, the solvent was removed by evaporation in a stream of nitrogen at room temperature, and the corticosterone

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residue was dissolved in a small amount of absolute ethanol and diluted with water to a final concentration of 5% ethanol. Adult male Sprague-Dawley rats (about 200 g) were used in all experiments. Each animal received 10 μC of ^{3}H-corticosterone, a dose of <5 μg/rat, in a volume of 0.4–0.6 ml by injection into the tail vein. Rats were stunned, then decapitated, and blood was collected into beakers containing heparin. Blood was transferred to tubes and centrifuged; aliquots of plasma were then frozen and stored at —10° for corticosterone assay.

Endogenous corticosterone and ^{3}H-corticosterone were extracted by a modification of the procedure described by Guillemin *et al.* (8). Two milliliters of plasma was shaken with 5 ml of 2,2,4-trimethylpentane. After centrifugation, the organic phase was removed by aspiration and discarded. The aqueous phase was then diluted with 2 ml of distilled water and the corticosterone was extracted into 15 ml of $CHCl_3$. After centrifugation, the aqueous phase was removed and the $CHCl_3$ phase was washed with 3 ml of 0.1 N NaOH. One aliquot (8 ml) of the $CHCl_3$ phase was treated with 30 N sulfuric acid for fluorimetric assay of corticosterone (8). A second aliquot (3–5 ml) of the chloroform was transferred to glass vials for liquid scintillation counting and evaporated to dryness in a stream of nitrogen at 30–35°. After addition of 15 ml of the phosphor mixture described by Beaven and Maickel (9), the vials were allowed to stand at room temperature for 30–60 min before being counted in a Packard Tricarb scintillation spectrometer. A recount after addition of an internal standard of ^{3}H-toluene permitted all samples to be corrected for efficiency; results are reported as disintegrations per minute (dpm).

The specificity of this assay for ^{3}H-corticosterone was demonstrated, in randomly selected samples of all test groups, by paper chromatography of the chloroform extract; the solvent system described by Maickel *et al.* (5) was used. This system separates corticosterone from its major metabolites. On scanning of the developed strips, >96% of the radioactivity was localized in a single spot corresponding in R_F to authentic ^{3}H-corticosterone. Total recovery of radioactivity was >98%.

After the administration of tracer amounts of corticosterone-1,2-^{3}H to control rats, the plasma levels of the labeled steroid declined exponentially as shown in Fig. 1A. The plasma half-life ($t_{1/2}$) estimated from the slope of the line was 11.3 min; extrapolation of the line to $t = 0$ gave a value of 1.08×10^5 dpm/ml, indicating an apparent volume of distribution (V_d) of 204 ml (Table 1).

TABLE 1

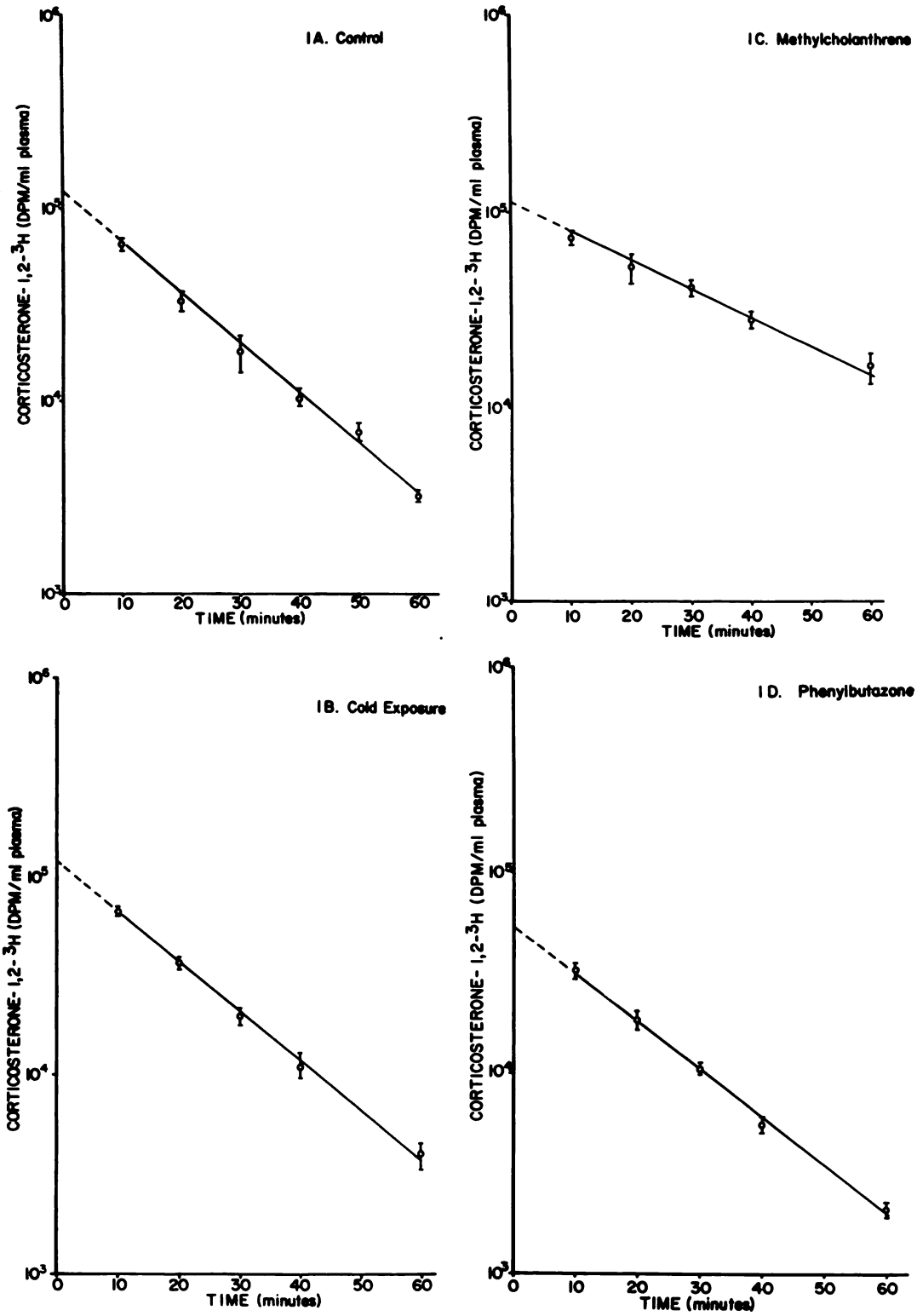
Effect of various treatments on plasma disappearance curve of corticosterone-1,2-^{3}H in rats

Treatment	Endogenous plasma corticosterone (μg/ml ± S.D.)	Corticosterone-1,2-^{3}H $t_{1/2}$ (min)	Corticosterone-1,2-^{3}H V_d (ml)
Control	0.15 ± 0.02	11.3	204
Cold exposure	0.47 ± 0.06	12.0	193
20-Methylcholanthrene	0.26 ± 0.04	22.2	212
Phenylbutazone	0.23 ± 0.04	12.7	408

On exposure of rats to a low environmental temperature (4°), the plasma level of corticosterone rose from the resting value of 0.15 μg/ml to a maximum level of 0.45–0.50 μg/ml within 90 min (5) and remained elevated for many hours. When labeled corticosterone was administered to rats that had been exposed to cold for 4 hr, the $t_{1/2}$

FIG. 1. *Plasma level of radioactivity at various times after administration of corticosterone-1,2-^{3}H*

Each point is the mean value of 8–12 rats. Vertical bars indicate the range of values obtained. (1A) Control rats. (1B) Rats exposed to an environmental temperature of 4° for 4 hr. (1C) Rats pretreated with 20-methylcholanthrene (50 mg/kg s.c., in oil) 24 hr prior to corticosterone-1,2-^{3}H. (1D) Rats pretreated with 5 doses of phenylbutazone (150 mg/kg, s.c., in oil) at 12-hr intervals prior to corticosterone-1,2-^{3}H.


1A. Control
1C. Methylcholanthrene
1B. Cold Exposure
1D. Phenylbutazone
CORTICOSTERONE-1,2-^{3}H (DPM/ml plasma)
TIME (minutes)
0 10 20 30 40 50 60
10^6
10^5
10^4
10^3

of the labeled steroid in plasma was virtually identical with that of normal animals (12 min) (Fig. 1B). Extrapolation of the line to $t = 0$ gave a value for V_d of 193 ml (Table 1). Thus, the increased plasma level of corticosterone cannot be attributed to changes in the plasma half-life or the apparent volume of distribution.

A previous report from this laboratory suggested that the carcinogenic hydrocarbon 20-methylcholanthrene causes a prolonged stimulation of the pituitary-adrenocortical system since plasma levels of corticosterone remained elevated for 48 hr after administration of the drug (10). However, no significant adrenal hypertrophy was observed. When corticosterone-1,2-^{3}H was administered to rats pretreated with 20-methylcholanthrene, the plasma level of labeled steroid fell as described by the curve in Fig. 1C. The $t_{1/2}$ estimated from the slope of this line was 22.2 min, almost double the value for untreated animals. Extrapolation of the line to zero time gave a value of $V_d = 212$ ml, similar to that observed in normal or cold-exposed rats (Table 1). These results suggest that the hydrocarbon treatment had inhibited enzymes responsible for the metabolism of corticosterone.

The data in Fig. 1D show the decline of corticosterone-1,2-^{3}H in rats pretreated with phenylbutazone. Calculation of the plasma half-life from the slope of the line gave a value for $t_{1/2} = 12.7$ min. Extrapolation of the line to $t = 0$ gave a value of $V_d = 408$ ml (Table 1). Thus, phenylbutazone had no effect on the metabolism, but markedly changed the physiological disposition of the steroid.

Glucocorticoids are present in the bloodstream in extremely low concentrations, highly bound to plasma proteins. Since they are continually produced and released by the adrenal cortex, they must be rapidly inactivated to prevent prolonged action on the target organs. At the same time, some small concentrations of the steroids must always be available to act on the target organs and maintain normal function. For adrenocortical steroids, these conditions are met by liver enzymes which rapidly degrade the steroid molecule and by plasma proteins which have a high affinity for the steroids, rapidly transporting them to various body organs in a form which is inactive and protected from metabolic degradation (11). In the rat, the main glucocorticoid secreted by the adrenal cortex is corticosterone. Administration of corticosterone-1,2-^{3}H of high specific activity to rats labels the pool of endogenous corticosterone and permits a study of the physiological fate of the endogenous steroid. For example, in normal rats, the plasma half-life of corticosterone-1,2-^{3}H was found to be 11.3 min, similar to values obtained using larger doses of exogenous, nonlabeled corticosterone (7).

When intact rats are exposed to low environmental temperature (4°), the plasma level of corticosterone increases 3- to 4-fold. No changes are observed in the plasma half-life or the apparent volume of distribution, implying that the increased plasma level of corticosterone is due solely to an increased rate of secretion. After treatment with 20-methylcholanthrene, the endogenous level of steroid is almost doubled, a finding which had previously led to the suggestion that the hydrocarbon caused a prolonged stimulation of the pituitary-adrenocortical system (10). The present studies show that this results from a decreased metabolism of the steroid as the plasma half-life is almost doubled. This finding is in agreement with a recent publication by Conney and Schneidman (12) showing that liver preparations from rats treated with 20-methylcholanthrene have a decreased ability to metabolize steroids.

Animals treated with phenylbutazone have a somewhat elevated (approximately 50%) plasma level of endogenous steroid and a practically normal (12.7 min) plasma half-life, suggesting adrenocortical hyperactivity of a smaller magnitude than that evoked by cold-exposure. However, the plasma level of radioactive steroid at various times after administration of corticosterone-1,2-^{3}H is much lower than expected (Fig. 1D). As a result, the apparent volume of distribution is larger, reflecting an altered distribution of the labeled steroid between blood and tissue. The implications

of this altered distribution must be considered in terms of the normal steady state existence of the plasma corticosterone. In control animals, the plasma concentration of the steroid remains constant, due to an equilibrium between rate of secretion and rate of metabolism. However, when a compound such as phenylbutazone increases the apparent volume of distribution without changing the rate of metabolism, it is obvious that more corticosterone must be produced by the adrenals and the total body content of the steroid must be increased. The action of phenylbutazone may then have two distinct facets: *first,* an increased level of corticosterone in tissues, and *secondly,* an increased secretion of the steroid in the adrenals. The latter action has been observed by Bernauer and Schmitt (13) as an increase in the corticosterone content of adrenal venous blood after administration of phenylbutazone. Studies currently in progress indicate that phenylbutazone interferes with the binding of corticosterone to plasma proteins, resulting in a displacement of steroid molecules and increasing the fraction of corticosterone present in the free (nonbound) form (14).

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The Binding of Xenon to Sperm Whale Deoxymyoglobin

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SUMMARY

Xenon binds to deoxymyoglobin at one specific site within the protein molecule. A major portion of the binding energy is provided by London interactions.

The existence of anesthetic properties among members of the "rare" gases (1) shows that specific structural groupings are not necessary for anesthetic action. Anesthetic ability seems to depend mainly upon the polarizability of the agent molecules.

Solubility studies (2) have shown that anesthetic agents such as cyclopropane, nitrous oxide, and xenon bind to a number

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