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## Peripheral Blood Concentrations of Steroids in Man After Oral Administration of 17-Hydroxycorticosterone.* (20562)

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Recent developments in chromatographic and micro-analytical technics for the resolution and quantitation of steroids make possible the analysis of corticosteroids in peripheral blood of man. A method for analyzing 17-hydrocorticosteroids in peripheral blood has been developed by Nelson and Samuels(1). They have employed Florosil for resolution and a modified phenylhydrazine reaction(2) for quantitation of the steroids. The method of Sweat(3) utilizes silica gel for resolution and sulfuric acid fluorescence for quantitation of corticosterone-like steroids and 17-hydroxycorticosterone. In the present study the technic of Sweat has been applied to the determination of the concentrations of steroids in peripheral blood of man following the oral administration of a single dose of 17-hydroxycorticosterone.

*Methods.* Eight normal adult males were subjects for this study. Immediately following withdrawal of a control blood sample, 6 of the subjects received 50 mg of 17-hydroxycorticosterone (Hydrocortisone, Merck) orally; blood samples were collected at various time intervals thereafter for steroid analysis. In control subjects, blood samples were collected and analyzed, but no hormone was given. Twenty ml of blood were drawn from an antecubital vein, mixed with ⅓ ml of heparin and centrifuged. Ten ml of separated plasma were analyzed for steroid content. The method employed for analysis of corticosterone-like steroids and 17-hydroxycorticosterone in plasma was that developed by Sweat. In brief, this method consists of a) extraction of the plasma sample with 10 volumes of chloroform, b) fractionation of the dried chloroform extract between 70% ethanol and petroleum ether and c) resolution on a silica-gel column by elution with mixtures of ethanol and chloroform in various ratios. The resolved steroids were quantitated by the degree of fluorescence developed in concentrated sulfuric acid. Recovery studies have shown that approximately 95% of 17-hydroxycorticosterone and 80% of corticosterone added to plasma are resolved and recovered by this technic.

*Results.* The individual values obtained for the concentration of 17-hydroxycorticosterone are plotted in Fig. 1. Curve A is a line of best fit based on results obtained in 5 individuals who received 50 mg of 17-hydroxycorticosterone orally. A significant increase in plasma concentration of the hormone occurred 15 minutes following its administration. The peak concentration was attained between 45 and 60 minutes; normal values were reached between 4 and 6 hours. Curve

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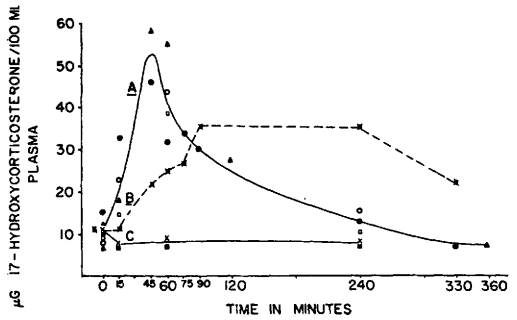


FIG. 1. Plasma concentrations of 17-hydroxycorticosterone in peripheral blood of humans. Curves A and B represent subjects who received 50 mg of 17-hydroxycorticosterone orally. Curve C represents control subjects who received no hormone.

B represents the concentrations of 17-hydroxycorticosterone in a subject who received 50 mg of 17-hydroxycorticosterone orally and whose pattern of response appeared to be quite distinct from that of the other 5 subjects. Results obtained from the two control subjects are represented by curve C. During the 4 hours of collection there was no significant change in plasma concentration of 17-hydroxycorticosterone in these 2 individuals.

Analyses for the corticosterone-like steroids present in human plasma were run simultaneously with the 17-hydroxycorticosterone determinations. In the 6 test subjects the control values for the corticosterone-like steroids were between 2.5 and 5.9 μg per 100 ml of plasma. Following the oral administration of 17-hydroxycorticosterone to these individuals, no significant increase in the plasma concentration of the corticosterone-like steroids occurred. No significant change occurred in the concentration of this fraction in the control subjects during a 4-hour collection period.

*Discussion.* The method of Sweat for blood steroid analysis effectively resolves corticosterone-like steroids and 17-hydroxycorticosterone on a silica-gel column. A number of evidences have been accumulated(4) which strongly suggest that the more polar fraction which fluoresces is 17-hydroxycorticosterone. Other evidences suggest that the material which chromatographs in the corticosterone region and which fluoresces consists of corticosterone and possibly other steroids. Nelson *et al.*(5) have reported blood levels of 17-hydroxycorticosteroids in humans following the oral administration of 50 mg of 17-hydroxycorticosterone acetate. In general, the time pattern of increase and decrease of blood steroid concentrations obtained by these authors appears to be in essential agreement with the data presented in this paper.

In 5 of 6 individuals given 17-hydroxycorticosterone in the present study, there was a rapid increase in plasma concentration of this steroid with maximal levels being attained 45 to 60 minutes following its oral administration. No significant elevation in plasma steroid concentration could be detected after 4 hours. When 17-hydroxycorticosterone is given orally frequent administration is necessary for maintenance of elevated plasma concentrations. In a single individual given oral 17-hydroxycorticosterone (curve B, Fig. 1), a slower increase in plasma concentration of this steroid was observed with the maximal level occurring between 1.5 and 4 hours. These results can best be explained by delayed absorption of the steroid. If the major absorption pathway is via the hepatic portal venous system, results obtained in this study would imply that an appreciable fraction of orally administered 17-hydroxycorticosterone passes through the liver without structural alteration. The possibility exists that 17-hydroxycorticosterone passes from the gastro-intestinal tract into the blood stream by way of the lymphatic system. However, Dull(6), in preliminary studies, has been unable to detect cortisone in thoracic duct lymph subsequent to the introduction of the steroid into the intestinal tract of the dog.

*Summary.* A method which measures blood concentrations of corticosterone-like steroids and 17-hydroxycorticosterone has been employed to determine the plasma concentrations of these steroids in humans following a single oral dose of 17-hydroxycorticosterone. A rapid increase in plasma concentration of this steroid occurred in 5 of 6 subjects; maximal levels were attained between 45 and 60 minutes after administration. The plasma concentration of this steroid decreased rapidly thereafter, reaching normal values between 4 and 6 hours. No significant elevation in plasma concentration of the corticosterone-like steroids could be detected following the

oral administration of 17-hydroxycorticosterone.

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## Effect of Thyroidectomy on Adrenal Weight in Adult Male Rats. (20563)

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There has been some discrepancy in the literature concerning the effect of thyroid deficiency (affected by thyroidectomy or the administration of goitrogens) on adrenal weight. Leblond and Hoff(1) noted a decrease in the size of the adrenals in rats receiving goitrogenic sulfonamide drugs or thiouracil. This was confirmed by Baumann and Marine(2) when they noted involution of the adrenals to half their former size in rats fed thiouracil for 4 months. Zarrow and Money(3) have shown that after 6 weeks or more of thyroid deficiency there is a relative as well as an absolute loss of adrenal weight. Freedman and Gordon(4) reported that thiouracil treatment and thyroidectomy in rats results in adrenal atrophy. On the other hand, Winter and Emery(5) reported that, in rats, thyroidectomy *per se* does not appreciably influence the size of the adrenals, nor does it affect the degree of compensatory adrenal hypertrophy as compared with intact animals. Morris(6) reported that after treatment with thiouracil or thyroidectomy the cockerel shows a marked adrenal hypertrophy, particularly when adrenal weights are calculated on a relative weight basis. The adrenals of young, thyroidectomized rats were found to be similar in weight to those of intact animals when calculated on a relative basis(7). The administration of the goitrogen p-aminosalicylic acid to rats was without effect on adrenal weight(8). The use of Amphenone "B", which inhibits the incorporation of radioiodine into thyroid protein as effectively as propylthiouracil, produced an adrenal enlargement in rats(9).

Since thyroid deficiency in the young growing animal results in the retardation of body growth, the adrenal atrophy of hypothyroidism cited by some investigators may be a reflection of the reduced body weight. This study was designed with that problem in mind. To rule out the effect of thyroid deficiency on growth, adult, fully grown ("plateaued") rats were utilized.

*Methods.* Male rats of the Holtzman strain were used throughout this study. Animals were made thyroid deficient by total thyroid-parathyroidectomy at an age of 5½ months, and together with controls, permitted to take Purina Laboratory Chow pellets and tap water *ad libitum* for an additional 2 months. At autopsy, organs were weighed on a torsion balance and the pituitary and adrenal glands saved for histological study.

*Results.* Although all of the animals had reached a "plateau" in their growth curves, there is still a small, but significant, difference in the body weights of thyroidectomized rats compared with their controls. The thyroidectomized animals weighed about 34 g less than the unoperated ones (Table I). Even with this difference in body weight, the absolute weight of the right adrenals of the thyroidectomized rats did not differ significantly from that of the intact animals. Neither left nor right adrenal glands showed any difference in relative weights between the 2 groups (Table I). There is, however, a significant