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Suppression of Biosynthesis of Adrenal Cortical Steroids in Man By Amphenone. (22964)

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Amphenone (1,2-bis (*p*-aminophenyl) 2-methyl propanone-1-dihydrochloride), one of a series of substituted desoxybenzoins, was synthesized by Allen and Corwin(1), and observed by Hertz *et al.*(2) to produce a marked enlargement of both adrenals of the rat, associated with an increased deposition of lipid material. In the hypophysectomized animal, amphenone does not produce adrenal enlargement. This suggests that amphenone may act directly on the adrenal to interfere with steroidogenesis, and that the induced adrenal enlargement is secondary to ACTH stimulation. In the hypophysectomized dog(3), amphenone depressed the secretion of 17,21-dihydroxy-20-ketosteroids as measured in adrenal vein blood. This effect was also noted during ACTH stimulation of the adrenal. Hertz *et al.*(4) observed an irregular decrease in urinary and plasma 17,21-dihydroxy-20-ketosteroids, and little or no decrease in excretion of urinary 17-ketosteroids in patients

with breast carcinoma and adrenal hyperplasia. Similar observations were also noted by Thorn *et al.*(5) and Hertz *et al.*(6) in a patient with adrenal carcinoma. Gallagher *et al.*(7) reported decreased urinary excretion of dehydroisoandrosterone, androsterone, etiocholanelone, and the 11-oxygenated analogs of both androsterone and etiocholanelone in a patient with metastatic adrenal carcinoma treated with amphenone.

Because of the failure to obtain lowered urinary 17-ketosteroid values after oral amphenone therapy (except Gallagher *et al.*(7)), and the paradoxical findings in some cases of lowered urinary corticoid excretion without significantly lowered plasma hydrocortisone level, there has been some question as to the possibility that amphenone may have interfered, either with steroid determinations or with rate of hepatic or renal clearance of the steroids. It therefore seemed of interest to further evaluate the effects of amphenone on adrenal function, utilizing more specific methods now available. These methods have also made it possible to obtain data of a quantitative nature relating to the effect of amphenone on adrenal steroid synthesis.

Methods. Plasma hydrocortisone and urinary corticosteroids were determined by a modification(8,9) of the Silber-Porter procedure(10). Urinary 17-ketosteroids were determined by a modified Zimmermann procedure(11) that does not require a color correction. Plasma corticosterone was determined by isotope dilution method(12). The turnover rate of hydrocortisone was determined by previously described procedure(13), and the turnover rate of corticosterone by a similar method utilizing the same general principles.

Results. Effect of amphenone on plasma levels of hydrocortisone and corticosterone. Oral or intravenously administered amphenone produces a decrease in concentration of both plasma hydrocortisone and corticosterone (Table I). This effect was more pronounced in patients with adrenal carcinoma unresponsive to ACTH but was also observed in normal subjects. In the normal subject, amphenone lowered the plasma steroid levels,

TABLE I. Plasma Levels of Hydrocortisone (F) and Corticosterone (B) before and after Amphenone Therapy.

		μg %		
		Phenyl-hydrazine	Isotope dilution	
		F	F	B
	Adrenal carcinoma:			
N.G.	Control	70	69	1.7
	Amphenone p.o. 6 g q d × 25	39	38	.2
V.G.	Control	156	—	2.0
	Amphenone i.v. 1 g q hr × 8	46	38	.5
	Post-amphenone	260	240	5.6
G.T.	Control	67	60	1.7
	Amphenone i.v. 1 g q hr × 8	17	15	.5
	Normal:			
H.G.	Control	19	20	1.1
	Amphenone p.o. 5 g q d × 5	9	9	.4
O.W.	Control (4 units ACTH/hr i.v.)	69	63	7.6
	Amphenone i.v. 1.5 g q hr × 4	41	40	2.2

even though intravenous ACTH was being administered at 4 units/hour. It is to be noted that the plasma hydrocortisone concentration, as determined colorimetrically with the phenylhydrazine reagent without prior chromatography agreed well with the more specific isotope dilution assay.

Effect of amphenone on urinary steroid metabolites of hydrocortisone. Fig. 1 shows the effect of amphenone on urinary steroids in a patient with adrenal carcinoma. One week after oral administration of 6 g/day of amphenone, urinary corticoids fell to within the high normal range. This was associated with a fall in plasma levels of hydrocortisone, but to concentrations that were still 2 to 3 times normal. Urinary steroids did not show a further decline after 12 days of treatment. There was no significant fall in total urinary 17-ketosteroids; however, paper chromatographic studies showed that there was a marked decline in level of the 11-oxygenated 17-ketosteroids. These steroids presumably arise as metabolites of hydrocortisone.

Effect of amphenone on rate of metabolism of steroids. The rate of disappearance of

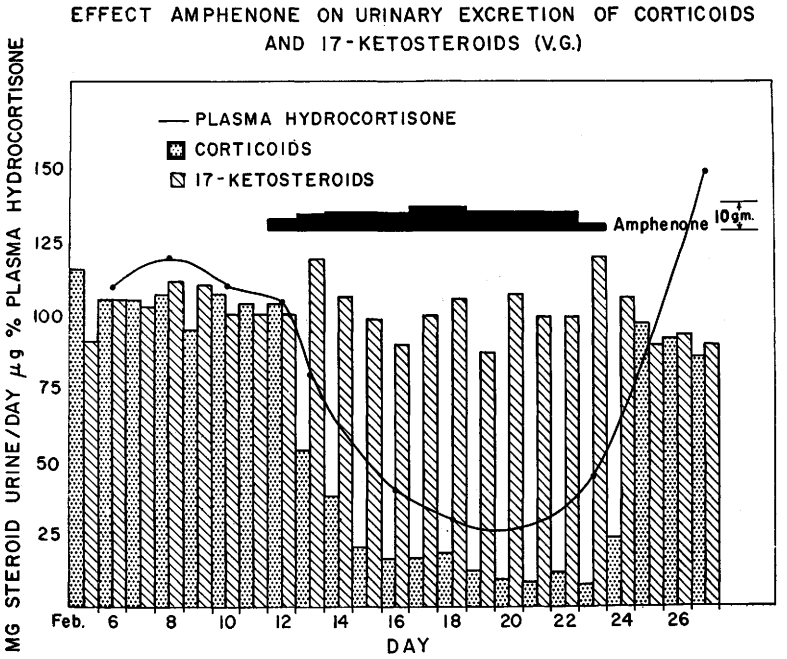

FIG. 1. Effect of orally administered amphenone on concentrations of urinary corticoids and 17-ketosteroids, and plasma hydrocortisone.

intravenously infused hydrocortisone or corticosterone was not altered after administration of amphenone to normal subject (Table II). Also, the rate of disappearance of tetrahydrohydrocortisone, which presumably is a measure of ability of liver to conjugate steroids, was not delayed.

Effect of amphenone on rate of synthesis of hydrocortisone and corticosterone. In patient G.T., with metastatic adrenal carcinoma, the miscible pool and rate of synthesis of hydrocortisone was increased approximately 8-fold over the normal(13) (Table III). The corticosterone pool and turnover rate was increased approximately 2.5 times normal (unpublished observations). Following amphenone therapy, the pools and turnover rates of both hydrocortisone and corticosterone decreased to within normal range. In the normal subject under the influence of maximal exogenous ACTH stimulation, intravenously administered amphenone resulted in a decrease in both pool size and rate of synthesis of hydrocortisone; however, the depression was not as great as in the patient with adrenal carcinoma.

Evaluation of effect of amphenone and its metabolites on the method of assay. The fol-

TABLE II. Biological Half-Times of Steroids before and after Amphenone Therapy.

		$t_{1/2}$ minutes		
		Hydrocortisone	Corticosterone	Tetrahydrohydrocortisone
V.S.	Control	120		35
	Amphenone i.v. 1 g q hr × 4	128		48
H.G.	Control	110		43
	Amphenone p.o. 5 g q d × 6	110		33
O.W.	Control	—		26
	Amphenone i.v. 2.5 g q hr × 3	—		27
T.R.	Control		90	
	Amphenone p.o. 4 g q d × 4		78	

TABLE III. Turnover Rate Hydrocortisone and Corticosterone before and after Amphenone Therapy.

			Miscible pool, mg	K*	mg/hr
O.W. Normal	Hydrocortisone	Control (4 units ACTH/hr i.v.)	15.3	.53	8.1
		Amphenone, i.v., 1.5 g q hr × 6	9.2	.44	4.1
G.T. Adrenal carcinoma	Hydrocortisone	Control	12.2	.72	8.8
		Amphenone, i.v., 1 g q hr × 8	2.0	.51	1.0
	Corticosterone	Control	.52	.55	.29
		Amphenone	.22	.59	.13

* Fraction of the pool replaced per hr.

lowing procedures were used to evaluate the possible interference of amphenone with steroid determinations: Addition of hydrocortisone to plasma from patient who had received amphenone (plasma level of amphenone 20 μg %) and in whom the plasma hydrocortisone was zero[1] resulted in quantitative recovery of the added hydrocortisone. Also, addition of tetrahydrocortisone and androsterone to urine of patient maintained on 6 g of amphenone/day showed no interference with recovery of the steroids as determined by phenylhydrazine or Zimmermann procedures.

Interference of amphenone and its metabolites with color reaction for steroids. Two g amphenone were given orally to a subject in whom the plasma hydrocortisone concentration was zero. Plasma samples were taken, 1, 2, and 4 hours after administration of the amphenone and assayed for hydrocortisone by the phenylhydrazine procedure. No hydrocortisone-like material was measured. Also, the close agreement of plasma hydrocortisone levels as determined by the phenylhydrazine procedure and isotope dilution method (Table I) indicated that amphenone or its metabolites did not contribute any significant amount of color with the phenylhydrazine reagent. Six g of amphenone/day were given to patient who had urinary corticoid level of <0.5 mg/day and urinary 17-ketosteroid level of 2 mg/day. The amphenone medication did not cause an increase in chromogenic material as measured with either phenylhydrazine or Zimmermann reagents.

Discussion. The lowered plasma steroid levels, failure to demonstrate any impairment in ability of liver to metabolize the steroids, and the decreased urinary corticosteroid levels obtained following administration of amphenone all indicated that amphenone affected rate of synthesis of hydrocortisone and corticosterone by the adrenal cortex. The results of the isotope dilution method of assay, because of its specificity, would tend to rule out any possibility of an artifactual lowering or elevation of these levels through interference of amphenone with the assay procedure. The isotope dilution assay has served to confirm the general reliability of the modified Silber-Porter phenylhydrazine colorimetric assay for determination of hydrocortisone in plasma of patients receiving amphenone. It is also of interest to note that in the baseline control plasmas of patients with adrenal carcinoma, the colorimetric method gives essentially the same plasma hydrocortisone concentrations as the isotope dilution method. This would indicate that essentially all free 17,21-dihydroxy-20-ketosteroid in the plasma was hydrocortisone. In general, the plasma hydrocortisone and corticosterone levels decreased to the same degree in all subjects given amphenone.

The results of the studies on phenylhydrazine colorimetric method for plasma and urine and the Zimmermann color method on urine containing amphenone and its metabolites afford no evidence to indicate that amphenone

[1] Plasma hydrocortisone levels of zero and urine corticosteroid levels of <1.0 mg/day were produced by oral administration of 2 mg of $\Delta^{1,9\alpha}$-fluorohydrocortisone/day in divided doses for one or more days. Urinary 17-ketosteroid values of <3 mg/day were produced after 3 or more days of adrenal suppression with $\Delta^{1,9\alpha}$-fluorohydrocortisone.

therapy interfered with the assay in either a positive or negative manner. Thus, decreased levels of corticoids would not appear to be of a spurious nature, and the failure to find a marked decrease in urinary ketosteroids in these patients would appear to be a valid observation.

After amphenone therapy, the miscible pool and rate of synthesis of hydrocortisone and corticosterone were decreased. In the normal subject receiving ACTH, the depressant effect of amphenone was not as marked as in the patient with adrenal carcinoma, whose adrenal tumor was relatively unresponsive to ACTH. Also, decreased rate of synthesis of hydrocortisone observed in the normal subject given ACTH, indicates that amphenone may exert its suppressive effect directly on the adrenal, rather than through suppression of the pituitary.

It thus seems apparent that amphenone affects synthesis of corticosteroids by the adrenal rather than affecting their rate of metabolism. The clinical observations in patients with adrenal carcinoma given amphenone (*viz.* fall in the elevated blood pressure and hyperglycemia, and a decrease in glycosuria and insulin requirement(4,5)) substantiate the laboratory data on adrenal cortical steroid synthesis.

Summary. Administration of amphenone to patients with adrenal carcinoma and to normal subjects caused a fall in concentration of plasma hydrocortisone and corticosterone, and urinary 17,21-dihydroxy-20-ketosteroids. No striking decrease in urinary 17-ketosteroids was noted after amphenone therapy. The rate of metabolism of hydrocortisone, corticosterone, and tetrahydrohydrocortisone was not altered by amphenone therapy. Studies on the miscible pool and turnover rate of hydrocortisone and corticosterone demonstrated that amphenone exerted its effect through inhibition of adrenal cortical synthesis, and this was more marked with adrenal tissue relatively unresponsive to ACTH.

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