Effects of Amphenone in Patients with Disturbed Carbohydrate Metabolism

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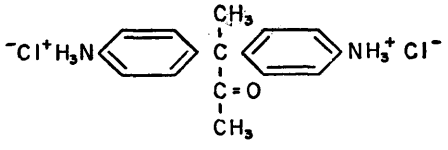
In 1952, Hertz and co-workers selected amphenone (3, 3-di(p-aminophenyl) butanone-2 dihydrochloride,* figure 1) as a compound of potential biological interest because of its structural relation to synthetic estrogens.[1]



3,3-di(p-aminophenyl) butanone-2 dihydrochloride
FIGURE 1

The compound exhibited a partly progestational, partly folliculoid activity in experimental animals and, in addition, surprisingly produced enlargement of both the adrenal and the thyroid glands. This adrenal enlargement was due to hypertrophy of the cortex and, when compared with tissue from normal animals, the adrenal cortical cells from amphenone-treated rats were greatly enlarged and appeared loaded with lipid material, particularly in the region of the zonae fasciculata and reticularis. However, in contrast to the hyperplastic appearance of their adrenals, amphenone-treated animals exhibited symptoms indicative of hypoadrenocorticism, thus suggesting that amphenone administration might have resulted in a hyperplastic but hypoactive adrenal cortex. Conclusive evidence of an inhibitory effect of amphenone on corticoid secretion was then obtained in the hypophysectomized dog since the administration of the compound suppressed the appearance of Porter-Silber chromogens in the adrenal vein, despite maximal adrenocorticotrophic hormone stimulation.[2, 3]

The clinical trial of amphenone has so far been limited to (a) the short term (two to four days) use of the compound as a potential means of assessing the role of adrenal cortical secretions in certain disease states and (b) the more prolonged (up to one month) administration of amphenone in an attempt to produce therapeutic suppression of adrenal cortical function in the presence of hyperadrenocorticism or of metastatic mammary carcinoma. The compound has been administered to a total of approximately sixty patients at dose levels of 3 to 10 gm. per day, and the results obtained may be summarized as follows. In patients with adrenal cortical carcinoma, amphenone administration results in a profound suppression of the biosynthesis or release of all three major types of biologically active adrenal cortical steroids: 17-hydroxycorticoids with glucocorticoid activity, aldosterone and 17-ketosteroids.[4, 5, 6, 7, 8] In patients with normal adrenal function, a decreased urinary excretion of aldosterone with concomitant sodium diuresis can be readily shown;[7, 9] a decreased secretion of 17-hydroxycorticoids is clearly apparent only in the presence of exogenous adrenocorticotrophic hormone stimulation;[6] a clear-cut inhibition of 17-ketosteroid secretion has yet to be demonstrated.

In addition to action on the adrenal cortex, amphenone influences the human thyroid in a manner similar to thiouracil[4, 13] and exhibits undesirable side effects, particularly when administered for prolonged periods.[4] When administered for two to four days these side effects have been limited to anorexia, nausea and sedation. More prolonged administration was further complicated by skin rashes, methemoglobinemia and suggestive evidence of hepatotoxicity.[4]

* Amphenone was previously thought to have the following structure: (1, 2-bis(p-aminophenyl) -2-methylpropanone -1-dihydrochloride). When a supply of the compound was made available to us for the continuation of the present studies by the Upjohn Company, Kalamazoo, Michigan, the probability of the structure represented in figure 1 was indicated. This structure has now been established (Korman, J., and Olson, E. C.: *Journal of Organic Chemistry*, in press; Bencze, W., and Allen, M. J.: *Journal of Organic Chemistry*, 22:352, 1957.

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It is the present opinion of the authors that amphenone itself is not a suitable drug for the therapeutic inhibition of glucocorticoid secretion in patients with normal adrenal function. However, evident clinical interest is attached to the possibility of obtaining a reversible inhibition of adrenal cortical secretion in, for instance, patients with diabetes mellitus. Since amphenone derivatives are being synthesized and may prove to be more suitable, it would seem appropriate to record the observations made to date on the effects of amphenone on some manifestations of glucocorticoid activity and particularly in patients with disturbed carbohydrate metabolism. Most observations were incidental to studies in which amphenone was administered for other purposes. Some of these studies have been reported elsewhere.[5, 6]

METHODS

These studies were performed in the metabolic ward of the Peter Bent Brigham Hospital. Amphenone was administered by mouth and generally given in divided doses at intervals of two to four hours throughout the twenty-four hour period. Urinary 17-hydroxycorticoids were determined by the method of Reddy[11] and urinary 17-ketosteroids by a modification of the method of Drekter and co-workers.[12] Plasma 17-hydroxycorticoids were measured by the method of Nelson and Samuels.[13] Urinary glucose levels were measured enzymatically by the method of Froesch and Renold.[5] Since the urinary metabolites of amphenone are colored, are reducing substances and are at least in part lipid-soluble, special precautions must sometimes be taken for chemical determinations during and immediately following periods of amphenone administration. Treatment of the urine with combined cation and anion exchange resins (IR 120 and IR 45 at neutral *p*H) removed the greater portion of these metabolites. This latter procedure was used prior to urinary glucose determinations to avoid excessively high enzyme blanks.

OBSERVATIONS

Effect of amphenone in two patients with adrenal cortical carcinoma. The effect of amphenone on glucose levels in blood and urine has been followed in two patients with metastatic adrenal cortical carcinoma. S. D. (P.B.B.H. 5H689) a forty-one-year-old housewife with florid Cushing's syndrome including diabetes mellitus, hypertension, muscular wasting, striae, osteoporosis, rounding of the face, amenorrhea and loss of scalp hair, was given amphenone on two occasions. The detailed case history has been previously reported.[5] Amphenone administration resulted in a profound decrease in blood and urine 17-hydroxycorticoids and in a remarkable change of the diabetic state as illustrated in figure 2. Amphenone was administered by mouth, an initial dose of 1 gm. being followed by 500 mg. every three hours throughout the first day, and by 500 mg.

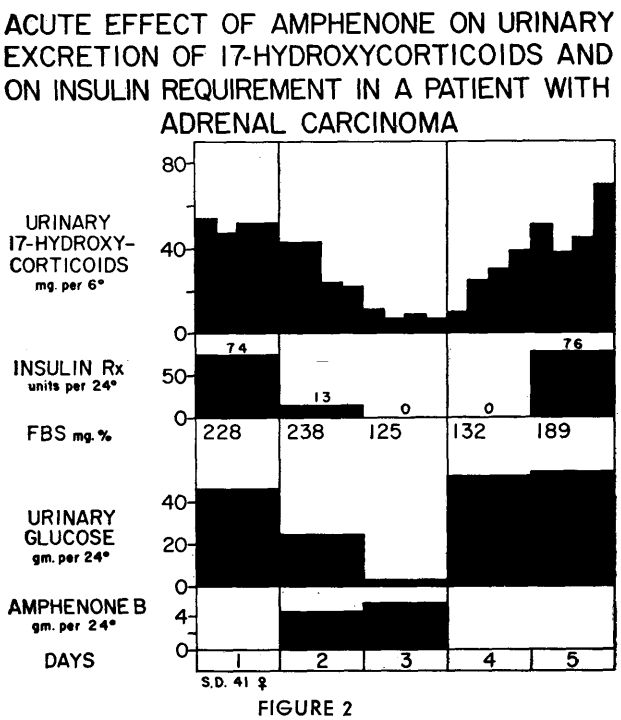

FIGURE 2

every two hours the second day. Marked effects on the urinary excretion of 17-hydroxycorticoids as well as on the measured parameters of carbohydrate metabolism and on the insulin requirements were apparent. Urinary glucose excretion decreased from approximately 40 gm. daily to somewhat over 20 gm. on the first and 2.5 gm. on the second day of amphenone administration, and the fasting blood glucose levels showed a concurrent decrease from values above 200 mg. per cent to 125 mg. per cent. Simultaneously the administered insulin decreased from 74 units on the day before treatment to 13 units on the first and to nothing on the second day of amphenone therapy. After discontinuation of the drug, the pretreatment levels of urinary 17-hydroxycorticoids and urinary glucose were reached within twenty-four hours.

The second patient, S.L. (P.B.B.H. 5J545), a seven-year-old girl, with masculinization of six months' duration, was followed in collaboration with Dr. Sidney Farber and Dr. Edna Sobel from the Cancer Research Institute of the Children's Medical Center in Boston. Exploratory surgery revealed an adrenal cortical carcinoma, and subsequently there appeared pulmonary metastases. After demonstration of some responsiveness of the tumor to adrenocorticotrophic hormone, hypophysectomy was carried out. She subsequently received increasing amounts of amphenone for sixteen days (figure 3). Before amphenone administration she excreted between 70 and 116 mg. of urinary 17-hydroxycorticoids per day. This decreased to 27 mg. per day by the thirteenth day of amphenone therapy, a definite rebound occurring after discontinuation of the drug. Glucose excretion varied from 400 to 600 mg. per twenty-four hours before amphenone therapy (clearly elevated values for enzymatically determined glucose[14]) and continued at that level until the amphenone dose reached 6 gm. daily. From then on, urinary glucose excretion was less than 100 mg. per twenty-four hours until one day after discontinuation of therapy when it returned

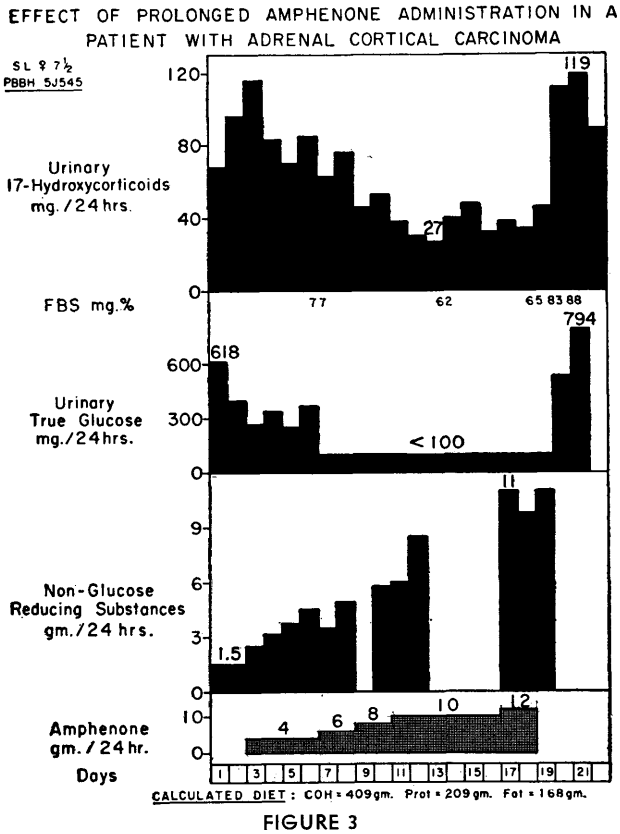

FIGURE 3

to 500 and 800 mg. in twenty-four hours. It should be noted that the patient excreted large amounts of nonglucose reducing substances during the period of amphenone therapy thus examplifying the need for specific glucose determinations during amphenone administration.

Effect of amphenone on the adrenal response to adrenocorticotrophic hormone. K. H. (P.B.B.H. 1J613) a twenty-year-old male with mild Cushing's syndrome due to bilateral adrenal cortical hyperplasia received standardized intravenous infusions of adrenocorticotrophic hormone both before and during amphenone administration. As shown in figure 4, the base line excretion of 17-hydroxycorticoids was 9 mg. per twenty-four hours. Following adrenocorticotrophic hormone administration, the urinary excretion of 17-hydroxycorticoids increased to 47 and 54 mg. per twenty-four hours, a "hyperactive" response compatible with adrenal cortical hyperplasia.[15] Urinary glucose excretion was moderately and significantly elevated at rest and adrenocorticotrophic hormone increased to 9 and 13 gm. per twenty-four hours. When these measurements were repeated before and during adrenocorticotrophic hormone stimulation while the patient was also receiving 6 gm. of amphenone daily, no decrease in the base line excretion of either glucose or 17-hydroxycorticoid was noted. During maximal adrenocorticotrophic hormone stimulation, however, both the urinary excretion of 17-hydroxycorticoids and the urinary glucose excretion increased only to approximately half the levels reached on adrenocorticotrophic hormone alone. Since the dosage of adrenocorticotrophic hormone infused (25 units over eight hours) has been shown to produce maximal stimula-

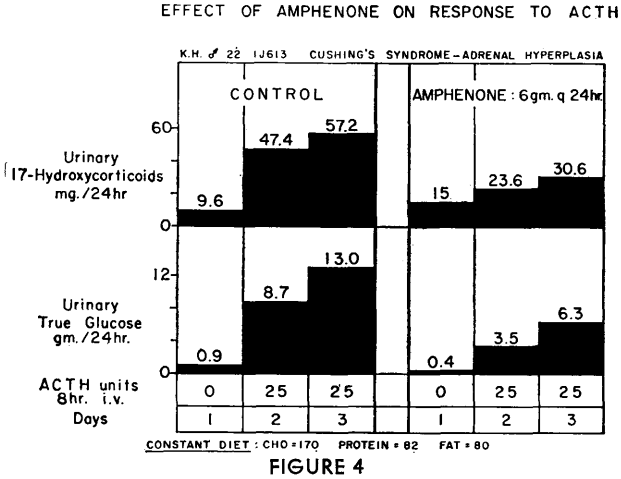

FIGURE 4

tion[16, 17] one may conclude that, in this patient and at this dose level, amphenone administration resulted in an approximate 50 per cent inhibition of glucocorticoid secretion and in an approximate 50 per cent inhibition of its biological effect.

Similar observations were made in subject R. A. (P.B.B.H. 7B948) a forty-year-old male volunteer with normal adrenal function undergoing prolonged stimulation with Armour ACTH gel, 20 units being administered daily by intramuscular injection (figure 5). During the period of adrenocorticotrophic hormone administration a marked gradual increase in the urinary excretion of 17-hydroxycorticoids occurred as well as a definite increase in urinary glucose excretion. The urinary 17-hydroxycorticoid excretion leveled off at 40 to 50 mg. per twenty-four hours and urinary glucose excretion at about 600 mg. per twenty-four hours. When 6 gm. of amphenone was administered in divided doses for one twenty-four-hour period, the urinary excretion of 17-hydroxycorticoids dropped to 20 mg. per twenty-four hours while the glucose excretion reached a minimum of 145 mg. on the following day. After discontinuation of amphenone a "rebound" excretion of 17-hydroxycorticoids occurred with a maximum of 67 mg. per twenty-four hours; this was paralleled by an increase of glucose excretion to 3.2 gm. in twenty-four hours. Again the correlation between adrenal cortical activity and urinary glucose excretion is striking.

Effect of amphenone on the glucosuria of patients with dia-

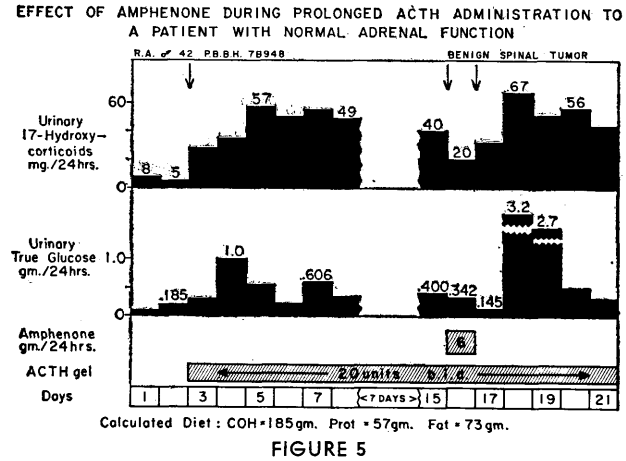

FIGURE 5

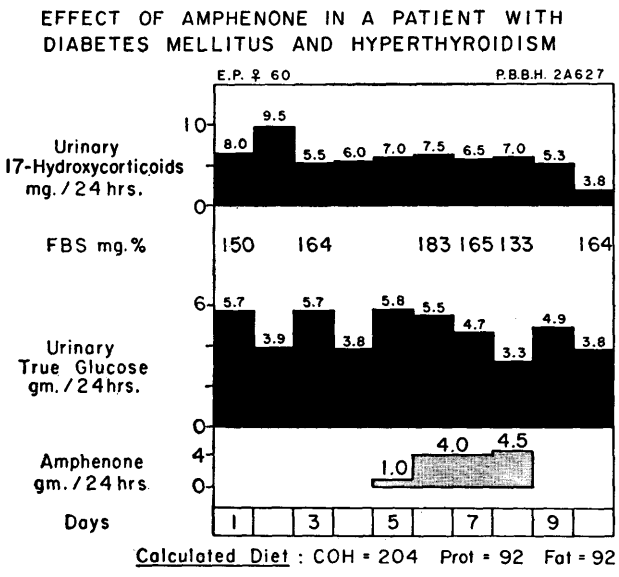

FIGURE 6

betes mellitus. Figure 6 illustrates the effect of amphenone on adrenal cortical secretion and urinary glucose excretion in a sixty-year-old patient with diabetes mellitus and thyrotoxicosis (E. P.; P.B.B.H. 2A627), both of mild degree. Control glucose excretion varied from 3 to 6 gm. daily and the fasting blood glucose varied around 155 mg. per cent. The administration of up to 4.5 gm. of amphenone daily produced little change in the 17-hydroxycorticoid excretion, in the urinary excretion of glucose, or in the blood glucose levels. Also there was no "rebound" after discontinuation of the drug. Similar observations were made in two further patients with diabetes mellitus and in one patient with diabetes mellitus who had previously undergone adrenalectomy and was maintained on a constant dose of cortisone. In no instance did a significant decrease in urinary 17-hydroxycorticoid excretion or in urinary glucose excretion occur. It should be stressed that the total daily amphenone dosage did not exceed 6.0 gm. in any of these patients.

DISCUSSION

Amphenone interferes with the secretion and probably with the biosynthesis[18] of adrenal cortical steroids. It would appear at present that, in man, amphenone administration results in an effective decrease in glucocorticoid secretion whenever endogenous regulation of their secretion is impaired, as in the case for malignant adrenal cortical tissue or during exogenous adrenocorticotrophic hormone administration. The effective, and at times, dramatic suppression of the secretion of 17-hydroxycorticoids under these circumstances is accompanied by equally impressive changes in their biological effects, such as normalization of previously present hyperglycemia and glucosuria, and decreased insulin requirement. The concurrence of decreased levels of 17-hydroxycorticoids in blood and urine with appropriate changes in biological indices of their effectiveness greatly strengthens our confidence in the validity of hormonal measurements.

In the presence of a normal pituitary-adrenal system, however, it is difficult to demonstrate an effective suppression, or indeed any suppression of glucocorticoid secretion as a result of amphenone administration. Again this statement is based both on hormonal measurements and on evaluation of their biological effect. These observations can be reconciled if one considers that, in the presence of a normally functioning pituitary-adrenal system, the adrenal cortical reserve with regard to 17-hydroxycorticoid secretion exceeds basal needs by a factor of the order of magnitude of five to ten.[16, 17] Even a slight initial decrease in circulating 17-hydroxycorticoids, as a result of amphenone administration, would be expected to produce a compensatory increase in adrenocorticotrophic hormone secretion[19] which in turn would lead to a compensatory increase in the secretion of 17-hydroxycorticoids by the adrenal cortex, and in a return to normal of circulating 17-hydroxycorticoid levels. Since, with the doses of amphenone which have been employed, the inhibition of glucocorticoid biosynthesis appears to be of the order of magnitude of 50 per cent (figure 4) a modestly increased adrenocorticotrophic hormone secretion would suffice to restore basal secretion. The finding of adrenal hypertrophy following amphenone administration,[1, 4] and the dependence of this hypertrophy on the presence of the pituitary,[1] support the concept of increased adrenocorticotrophic hormone release during amphenone action.

It is of interest that a suppressing effect of amphenone on the excretion, and presumably on the biosynthesis and secretion of aldosterone, can be readily demonstrated even in the presence of a normal pituitary-adrenal system.[7, 8] This observation may be related to the lesser responsiveness of aldosterone secretion to changes in circulating levels of adrenocorticotropic hormone. The compensatory increase in its secretion postulated above could easily result in normalization of 17-hydroxycorticoid but not of aldosterone levels.

Finally, it should be stressed that as yet the number of patients studied is small and that the side effects of amphenone have been sufficiently bothersome to prevent the use of larger and perhaps adequate doses. Further, more complete evaluation of the use of amphenone as a means of suppressing glucocorticoid production by the adrenal cortex and, therefore, as a means of producing a reversible medical adrenalectomy will have to await the availability of more suitable and less toxic derivatives. Further study of this and related compounds appears clearly indicated.

SUMMARY

The effect of amphenone on glucocorticoid secretion in man, as measured by chemical indices, has been compared with the effect of the compound upon biological indices of glucocorticoid activity. In patients with adrenal cortical carcinoma and in patients undergoing adrenal cortical stimulation with exogenous adrenocorticotrophic hormone, amphenone administration resulted in a striking and immediate suppression of adrenal cortical secretion as measured by both chemical and biological indices. In patients with normal pituitary and adrenal function, however, an effective suppression of glucocorticoid secretion was not achieved. It would appear that the functional reserve of the normal pituitary-adrenal system, with respect to glucocorticoid secretion, is sufficient to overcome the suppression resulting from the doses of amphenone used in this study. Limitation of the dosage was imposed by the occurrence of nausea and sedation. More complete evaluation of amphenone as a means of suppressing glucocorticoid production by the adrenal cortex will have to await the availability of less toxic derivatives.

SUMMARIO IN INTERLINGUA

Effectos de Amphenona in Patientes con Dysfunction del Metabolismo de Hydrato de Carbon

Le effecto de amphenona super le secretion de glucocorticoide in humanos — mesurate per indices chimic — esseva comparate con le effecto del mesme composito super le indices biologic del activitate de glucocorticoide. In patientes con carcinoma adrenocortical e in patientes subjicite a stimulation adrenocortical con corticotropina exogene, le administration de amphenona resultava in un frappante suppression immediate del secretion adrenocortical, mesurate tanto per indices chimic como etiam per indices biologic. In patientes con functionamento normal del glandulas pituitari e adrenal, del altere latere, un efficace suppression del secretion de glucocorticoide non esseva effectuate. Il pare que le reserva functional del normal systema pituitario-adrenal con respecto al secretion de glucocorticoide suffice pro invalidar le suppression que resulta ab le doses de amphenona usate in iste studio. Un limitation del dosage esseva imponite per le occurrentia de nausea e sedation. Un plus complete evalutation de amphenona como medio pro supprimer le production de glucocorticoide per un normal cortice adrenal debe attender usque minus toxic derivatos de illo deveni disponibile.

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