Plasma 17-Hydroxycorticosteroid Concentrations in Fasted Dogs Following Oral Administration of Hydrocortisone Esters. (23804)

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Previous studies by Collins(1) and others (2,3,4) have indicated that (a) the rates of gastrointestinal absorption of hydrocortisone (F) and certain F analogues are not appreciably different and (b) the higher and more prolonged elevations of plasma 17-hydroxycorticosteroid (17-OHCS) concentrations after F analogues result from a decreased rate of metabolism.

In the rat, oral administration of certain F esters results in an enhanced liver glycogen deposition activity after 7 or 24 hours(5), suggesting that esterification of F delays intestinal absorption and/or degradation of the hormone. This possibility has been evaluated experimentally in the dog by an examination of plasma 17-OHCS concentrations as a function of time following oral administration of several F derivatives.

Methods. Laboratory-trained, male, mongrel dogs weighing 15-20 kg were used in these studies. The animals were fasted for 16-18 hours prior to steroid administration and food but not water was withheld during the blood sampling period. Each of the dogs was given various F esters at a dose equivalent to 20 or 40 mg of F. Following administration of the drug, blood samples were collected from the external jugular vein without anesthesia at hourly intervals for 5 hours. Plasma 17-OHCS concentrations were determined by a modification(1,6) of the Nelson-Samuels procedure(7,8). Each animal was tested at 10-day intervals. The following esters were studied: hydrocortisone cyclopentylpropionate (FCP), hydrocortisone acetate (FAc), hydrocortisone α-ethylbutyrate (FEB), and hydrocortisone α-ethylisovalerate (FIV).

Results. In the present studies only free 17-OHCS in the plasma were measured. Previous work has shown that neutral F esters of the type discussed are separated from free 17-OHCS during Florisil chromatography (6).

To test for the presence of intact ester in the plasma, chloroform extracts of 30-40 ml of dog plasma, collected 1 hour after a 60 mg dose, were examined by paper chromatography in the benzene-formamide system(9). No ester was detectable in the samples. Similar observations in the human have been reported (6). With the methods employed, intestinal hydrolysis of the esters prior to absorption cannot be differentiated from rapid hydrolysis by blood and liver esterases following the absorption of the intact esters.

Mean plasma 17-OHCS concentrations as a function of time following oral administration of F and the F esters studied are shown in Fig. 1. At a 20 mg dose, the esters produced maximum plasma 17-OHCS concentrations which were 45-73% of the value observed after F. In no case at this dose did the rate of removal of 17-OHCS from the plasma appear to be slower after ester than after free alcohol administration. At the 40 mg dose, the esters produced maximum plasma 17-OHCS concentrations which were 20-49% of that observed after 40 mg of F and were equal to (FCP) or less than the maximum elevation following 20 mg of F. However, at the higher dose, the disappearance of plasma 17-OHCS following ester administration was considerably slower (except for FAc) than after F. These results indicate a slower rate of absorption of steroid after administration of the F esters studied than after F itself.

Maximum plasma 17-OHCS concentrations as a function of the dose are shown in Fig. 2. Over the range studied a linear response was observed for F. However, doubling the dose gave only about a 50% increase in maximum plasma 17-OHCS with FCP and an even smaller increase with the other esters. It appears that maximum absorption rate is reached at a much lower dose of ester than of F.

Discussion. Plasma 17-OHCS curves such as those presented (Fig. 1) represent the resultant of factors tending to increase plasma

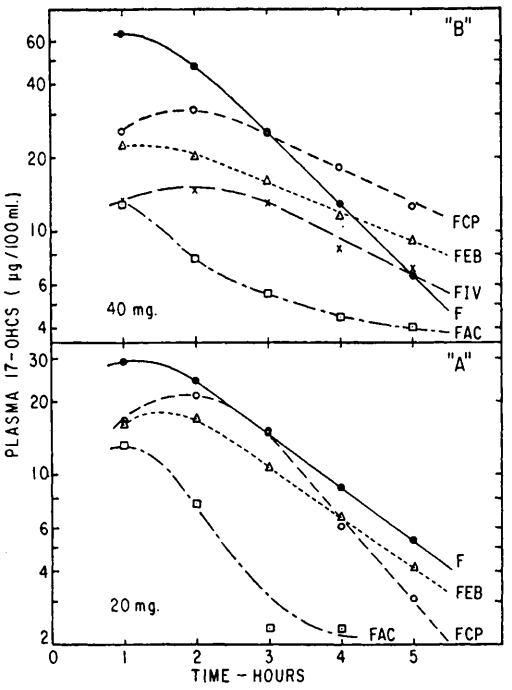

FIG. 1. Plasma 17-OHCS conc. *vs* time following oral administration of F and F esters.

levels, that is solution and absorption of the drug, and factors decreasing the levels, that is metabolism and excretion. Failure to detect intact esters in the plasma indicates that the free alcohol is the species metabolized. Therefore, any differences in the rate of disappearance of plasma 17-OHCS following administration of the F esters reflects a difference in absorption.

At the 20 mg dose, the lower maximum plasma 17-OHCS concentrations after F esters compared to F indicates a slower rate of absorption. At this dose of ester there is no alteration in the disappearance rate from the plasma from that observed with F (Fig. 1A). At the 40 mg dose, the slower rate of absorption is even more pronounced with the esters. In addition, the apparent disappearance rate of plasma 17-OHCS is reduced, compared to the rate after F, indicating continuing absorption from the gastrointestinal tract for a longer period of time (Fig. 1B). It appears that a maximum absorption rate is reached at a much lower dose with the esters than with F itself (Fig. 2). Increasing the dose does not greatly increase the maximum plasma 17-OHCS levels attainable but does maintain elevated 17-OHCS concentrations for a longer period. The rate of absorption of the F esters may be controlled by one or more of the following: (a) rate of solution of the esters, (b) solubility of the esters, (c) rate of enzymatic and/or non-enzymatic hydrolysis of the esters prior to absorption. The present experiments do not permit a choice among these possibilities. It may only be concluded that esterification modifies the intestinal absorption of orally administered steroid.

The efficient absorption of FCP is in agreement with the demonstrated clinical efficacy of this drug in the human(6). The poor absorption observed for FAc is as expected in view of the low order of activity of orally administered FAc in the dog(6).

Summary. Plasma 17-OHCS concentrations in the dog have been compared following oral administration of hydrocortisone, hydrocortisone cyclopentylpropionate, hydrocortisone acetate, hydrocortisone α-ethylbutyrate, and hydrocortisone α-ethylisovalerate. Prompt elevation of plasma 17-OHCS occurred within an hour after administration of any of these steroids. The magnitude of the response varied with the ester and dose but was always less than observed after an equivalent dose of hydrocortisone. At higher doses, the esters displayed a more prolonged elevation of plas-

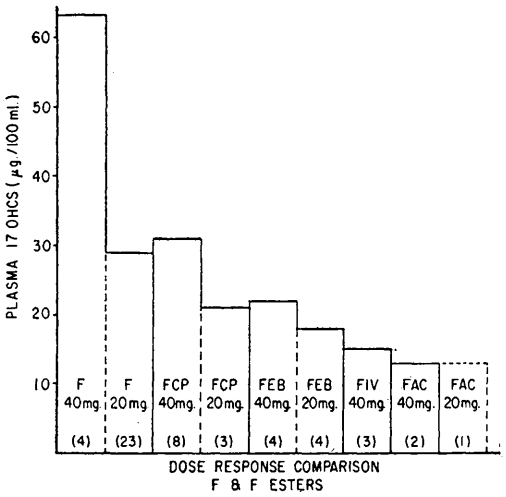

FIG. 2. Maximum plasma 17-OHCS conc. after oral administration of F and F esters. Results are avg of No. of animals indicated in parentheses.

ma 17-OHCS than found after hydrocortisone. In this respect, hydrocortisone cyclopentylpropionate was superior to other preparations studied. It is concluded that esterification alters the rate of steroid absorption.

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Teratogenic Changes in Early Chick Embryos Following Administration of Antitumor Agent. (Azaserine)* (23805)

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It has been previously reported that certain viruses, when inoculated in high concentration over the blastoderms of 48 hour chick embryos, produce specific teratogenic changes which are apparent on gross observation within 24 hours after inoculation(1-6). It has also been observed that antitumor agents often produce significant teratogenic changes in various embryonic organ systems. In recognition of the possible value of teratogenic studies in screening for antitumor agents, direct exposure of the frog embryo beginning at the 4-celled stage(7), and yolk sac inoculations of the chick embryo chiefly on fourth day(8), have been employed to determine teratogenic activity of various chemotherapeutic agents. The 2 methods, using different embryonic systems, however, have not always yielded comparable results for the same compound tested. It appeared that the 48-hour chick embryo, which consists of tissues in widely varying stages of proliferation with accompanying differentiation, might provide a sensitive testing system. If the changes should occur as rapidly as those following virus inoculations, this method would be particularly valuable in conserving time as well as laboratory space. Inoculations may be made directly over the cells of the developing blastoderm at this stage, thus avoiding possible interference by unknown variables such as absorption from the yolk sac, faulty access to susceptible organs via the circulatory system, or alteration of the compound *en route*. The substituted serine, O-diazoacetyl-L-serine (azaserine), having known antitumor activity (9), was selected for preliminary testing. This compound has previously been shown to produce developmental defects in the chick embryo following yolk sac inoculation at 4 days incubation(10), but produced less satisfactory results when inoculated into the yolk sac of unincubated eggs and eggs of 2 days incubation.

Materials and methods. The azaserine was obtained in pure form from National Institute of Health. As a control for specificity of action of the azaserine, experiments were also done using pure L-serine, obtained from Nutritional Biochemicals, Inc. For inoculations, the compounds were dissolved in saline buffered at pH 8.0. All materials used for inoculations were sterilized in routine manner except the compounds to be tested. The compounds were not sterilized because of the possibility of alteration by the process. Addition of antibiotics was omitted to avoid another variable. Broth cultures were made of all

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