Although the fasting state may facilitate absorption of vit. B_{12}(6) from the gastrointestinal tract, absorption occurred independent of the fasting state in our patients.

The action of oral preparations containing both intrinsic factor and vit. B_{12} cannot *a priori* be attributed to their intrinsic factor content, since as has been shown above, remission may follow the use of the vit. B_{12} alone.

Studies on vit. B_{12} metabolism in this group of patients are still in progress. Preliminary results in some of these patients suggest that remission occurs without a rise of the serum vit. B_{12} level above relapse levels (less than 20 $\mu\mu$g/ml).

Summary. 1. Hematologic and clinical remissions were induced 11 times in 8 patients with pernicious anemia in relapse, and in 1 patient with non-pernicious megaloblastic anemia, by small oral doses of purified vit. B_{12} without added intrinsic factor. The effective dosage range was 5 to 16.8 μg per day. 2. Unequivocal failure occurred in 1 case of relapsed pernicious anemia. 3. These results further confirm the suggestion that in pernicious anemia there is a quantitative rather than a qualitative change in gastric secretion, and that variations in the amounts of intrinsic factor remaining in pernicious anemia patients may be responsible for varying responses to oral vit. B_{12}. 4. A trial period of vit. B_{12} alone should precede any study with a vit. B_{12} plus intrinsic factor preparation.

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Δ^1-Hydrocortisone: Plasma 17-Hydroxycorticosteroid Concentrations Following Oral and I. V. Administration.* (22307)

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During the past year the Δ^1-steroids have been employed clinically to a considerable extent. These compounds are synthetic derivatives of hydrocortisone and cortisone, differing from the parent steroids by the insertion of a double bond in the 1-2 position of the steroid nucleus. Δ^1-hydrocortisone ($\Delta^{1,4}$-pregnadiene-11β, 17α, 21-triol-3,20-dione) has been produced under various commercial names: metacortandralone, prednisolone, delta-cortef, hydeltra, meticortelone, and sterane.

Clinically, the Δ^1-steroids appear to be 3 to 5 times as effective as the parent compounds(1-3). A similar ratio has been found for glucocorticoid activity (liver glycogen deposition, eosinophil response)(3,4). However, results of *in vitro* studies of anti-inflammatory potency have not been reported. It is possible that the apparent advantages of the Δ-steroids over the parent compounds are attributable to differences in absorption or

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metabolism which permit more efficient utilization of the former steroids. This possibility has been investigated in the present study by measuring in human subjects the influence of oral or I. V. Δ^1-hydrocortisone administration on plasma 17-hydroxycorticosteroid (17-OHCS) concentrations.

Materials and methods. Normal young adults were the subjects used in all the following studies. In the study of oral administration each of 9 subjects was given a dose of 45 mg Δ^1-hydrocortisone§ orally. Plasma samples were obtained before and at 1, 2, 4, 6, and 8 hours after administration of the test dose and 17-OHCS concentrations determined by the method of Nelson and Samuels(5,6), using hydrocortisone as the reference standard. In another group of 6 subjects each was given a dose of 40 mg free hydrocortisone orally and plasma samples were obtained for 17-OHCS determinations at 0, 2, 4 and 6 hours. In a third group of 11 subjects each was given 100 mg free hydrocortisone orally, and plasma samples were obtained at 0, 1, 2, 4 and 8 hours for 17-OHCS determinations. In the study of I. V. administration each of 8 subjects was given a dose of 0.5 mg/kg Δ^1-hydrocortisone intravenously as a 125 ml infusion over 30 minutes. Plasma samples for 17-OHCS concentration were obtained 1, 2, 3, and 4 hours after administration. From these data a regression line of best fit was determined by the method of least squares and the 17-OHCS half-life value calculated from the slope of the line.

Results. To determine whether the Δ^1-steroid was measured quantitatively by the Nelson and Samuels technic a comparison of the color development with the phenylhydrazine-sulfuric acid reagent was made using standard hydrocortisone and Δ^1-hydrocortisone. The resulting absorption curves for the two steroids were similar over a wave length range of 340-480 mμ, differing in that the

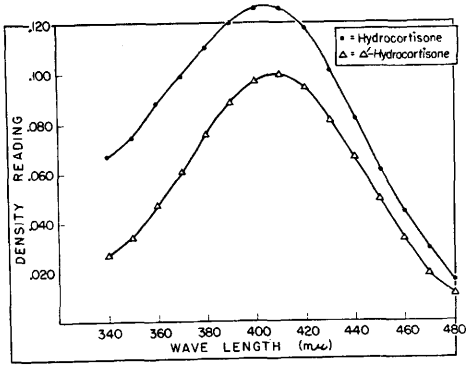

FIG. 1. Δ^1-hydrocortisone vs hydrocortisone: Absorption curves using phenylhydrazine-sulfuric acid reagent.

curve for hydrocortisone had a greater density throughout (Fig. 1). After a correction factor for background influence was applied the density values at 410 mμ were essentially superimposed. The density values at 410 mμ for different amounts of steroid (Fig. 2) were shown to be the same for Δ^1-hydrocortisone as for hydrocortisone. Also, recovery following chromatography was almost the same, averaging 70% for Δ^1-hydrocortisone and 76% for hydrocortisone.

In Fig. 3 is shown a comparison of mean plasma 17-OHCS values at various time intervals following the oral administration of Δ^1-hydrocortisone and of free hydrocortisone. Following 45 mg Δ^1-hydrocortisone the peak 17-OHCS concentrations were greater than those following 40 mg free hydrocortisone, but less than those following 100 mg free hydrocortisone. In those subjects given 40

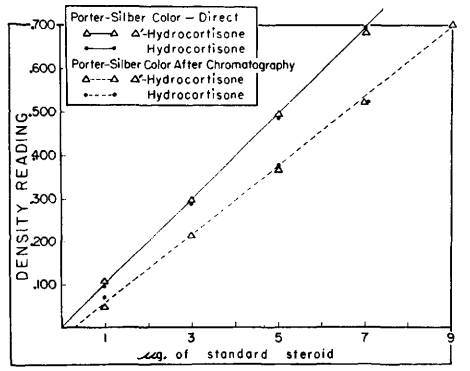

FIG. 2. Δ^1-hydrocortisone vs hydrocortisone: phenylhydrazine-sulfuric acid color development (410 mμ) directly and after column chromatography.

§ C. T. Delta-Cortef® supplied through courtesy of Dr. C. J. O'Donovan, Upjohn Co., Kalamazoo, Mich.

‖ C. T. Cortef® supplied through courtesy of Dr. C. J. O'Donovan, Upjohn Co., Kalamazoo, Mich.

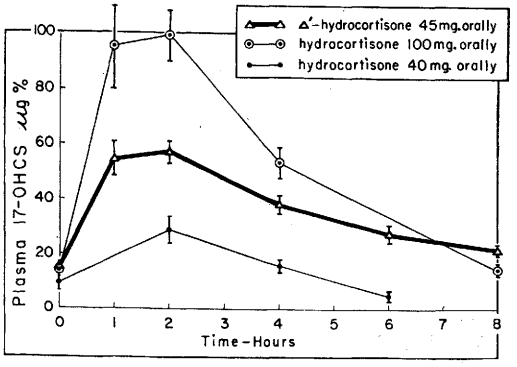

FIG. 3. Δ¹-hydrocortisone vs hydrocortisone: Plasma 17-OHCS concentrations following oral administration.

mg free hydrocortisone orally the plasma 17-OHCS concentrations were at or below control levels by 6 hours. A similar decline is seen in the curve representing the plasma 17-OHCS concentrations following the administration of 100 mg hydrocortisone orally, a return to normal values occurring by 8 hours. In contrast, the Δ¹-hydrocortisone curve falls more slowly; at 8 hours the mean 17-OHCS concentration is still well above the control value—and is significantly higher than the corresponding value following 100 mg hydrocortisone ($p < .01$).

The influence of intravenous administration of Δ¹-hydrocortisone on plasma 17-OHCS concentrations is indicated by the half-life data in Table I. The mean half-life obtained in 8 subjects was 192 minutes. In contrast to this, data reported from this laboratory(7) and elsewhere(8,9) for the mean half-life of hydrocortisone indicate that this is significantly less, ranging from 90 to 115 minutes. Thus, intravenous administration of Δ¹-hydrocortisone produces a more prolonged elevation of plasma 17-OHCS than does the intravenous administration of hydrocortisone.

Discussion. It has been shown that orally administered Δ¹-hydrocortisone, in doses roughly equivalent to those of free hydrocortisone, caused plasma 17-OHCS elevations both of greater magnitude and longer duration than did the latter steroid. When the dose of hydrocortisone was increased 2½ times the magnitude of 17-OHCS elevations became greater than that following Δ¹-hydrocortisone. However, even following the administration of this larger dose, these elevations disappeared more rapidly than those following the Δ¹-steroid. In addition, the 17-OHCS half-life in subjects receiving Δ¹-hydrocortisone intravenously was more prolonged than in those receiving hydrocortisone. These observations indicate that no appreciable differences in absorption exist but that the metabolism of Δ¹-hydrocortisone proceeds at a rate slower than that of hydrocortisone.

Results of *in vitro* experiments(10,11) are in accord with this interpretation. The biologically active adrenal corticosteroids contain the Δ⁴-3-ketone structure in ring A, and in their metabolism this ring is reduced at carbons 4, 5, and then 3. The Δ¹-steroid must first undergo saturation of the 1-2 double bond to form the Δ⁴ compound. Only following this is the Δ⁴-3-ketone reduced to form the tetrahydro steroid and subsequently conjugated.

These data from both *in vivo* and *in vitro* experiments lend support to the possibility that differences in the rate of metabolism may account for the equivalent clinical effectiveness of smaller doses of the delta forms than of hydrocortisone or cortisone. To date, Δ¹-hydrocortisone represents the only steroid

TABLE I.

Plasma 17-OHCS Half-Life Values following Intravenous Δ¹-Hydrocortisone.

Normal patients	Half-life (min.)
D.C.	217
F.B.	189
T.H.	141
E.H.	205
J.K.	185
J.N.	232
L.O.	171
A.D.	197
Mean	192 ± 10

Plasma 17-OHCS Half-Life following Intravenous Hydrocortisone.

Author reference	No. normals	Mean half-life (min.)
Done(7)	9	90 ± 7.4
Brown(8)	11	111 ± 5.3*
Peterson(9)	18	115

* Calculated from author's individual 17-OHCS data.

which has been reported to have a half-life longer than that of hydrocortisone. Others studied include tetrahydro-hydrocortisone, 20-hydroxy-hydrocortisone, cortisone, and corticosterone(8,9); each of these has a half-life shorter than that of hydrocortisone. Of the steroids mentioned only cortisone, hydrocortisone, and Δ^1-hydrocortisone have a Δ^4-17, 21-dihydroxy-3,20-keto configuration and are potent glucocorticoids. Among these 3, the glucocorticoid potency and the half-life are related in such a way as to suggest that glucocorticoid potency is inversely proportional to the rate of metabolism.

Summary. The influence of oral or intravenous administration of Δ^1-hydrocortisone on plasma 17-OHCS concentrations was studied. With comparable oral doses Δ^1-hydrocortisone produced 17-OHCS elevations of greater magnitude and longer duration than did hydrocortisone. Also, the half-life of the Δ^1-steroid was longer than that of hydrocortisone. It was concluded that a slower metabolism of the Δ^1 form occurs and postulated that this might contribute to the greater clinical effectiveness of this steroid.

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A Non-hypophyseal Sex Difference in Estrous Behaviour of Mice Bearing Pituitary Grafts.* (22308)

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Differences in size of the pituitary gland related to sex have been described in some species, the female gland being larger and heavier than that of the male. Similarly, the gonadotrophic potency of injected hypophyseal extracts from males is greater than those prepared from female pituitaries(1,2). Castrated male guinea pigs with grafted ovaries showed a greater stimulation of the mammary glands and other sexual structures, than intact females(6). The same was true in castrated male mice bearing ovarian and vaginal grafts. There was a continuous non-cyclic stimulation of the vaginal tissue(5). However, Harris and Jacobson(3,4) have demonstrated that hypophysectomized female rats bearing male hypophyseal grafts from newborn donors, had normal estrous cycles, became pregnant and delivered their young at term. This would indicate that the characteristic cyclic gonadal function of the female, as compared to the non-cyclic of the male, would not be related to the sex of the pituitary, but rather to a sex difference in some other structure. Since functional pituitary grafts can be placed in hypophysectomized mice by means of a rather simple one-stage operation, we have

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