non-specific material to be brought down in the first of a series of antigen-antibody precipitations.

It is possible to estimate the half life of the different serum constituents from curves similar to those of Fig. 1 and 2 if it is assumed that the radioactivity is not part of a molecule that is reutilized to make more of the same material. However, as large samples of blood were taken at each bleeding the data are not too significant in this regard. It is interesting to note that the red cells are still incorporating activity when the activity of the other serum constituents is decreasing rapidly.

These results confirm those of Schoenheimer and Heidelberger. The normal constituents of a rabbit's serum incorporate radioactivity given in the form of an amino acid at a very rapid rate. Passively transferred antibodies either do not incorporate this radioactivity to any marked extent or else do so only upon the loss of their specific antibody characteristics.

Summary. C^{14}-labeled leucine has been administered to immune rabbits. It has been found that while the normal serum components incorporate the C^{14} activity passively transferred antibody does not.

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Studies of 11-Alpha Hydrocortisone* in the Chick Embryo and on Skin Lesions in Man.† (20576)

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Cortisone acetate has a marked, characteristic effect in modifying and inhibiting growth of the developing chick embryo(1). Because of the specificity of this effect, the chick embryo has been used to examine related steroids for "cortisone activity"(2,3), and affords a useful and simple method for testing compounds for adrenocortical activity. Compound F (17-hydroxycorticosterone) acetate was found to be 50 to 100 times more active than cortisone acetate in the chick embryo and the newly-hatched chick(4); this marked difference was not noted in comparative studies on mammals.

The growth-inhibiting dose of hydrocorti-

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TABLE I. Effects of Hydrocortisone Acetate and 11-alpha (Hydroxy Epimer) of Hydrocortisone ("Epimer") when Injected onto Chorioallantoic Membrane of the 8-Day-Old Chick Embryo.

Dose/egg, mg	No. eggs	No. surviving, sacrificed at 18 days	Avg wt, g	Avg steroid effect "Stage"
Controls	20	17	17.4 ± 2	0
"Epimer"				
.5-1	6	5	17.8 ± .8	0
2	11	11	18.7 ± 1.7	0
5	27	13	15.4 ± 2.8	0
Hydrocortisone acetate				
.005-.01	13	11	16.7 ± 3.24	0
.02	28	21	10.0 ± 2.4	1.5 ± 1
Combination Epimer, 5 / Hydrocortisone acetate, .02	20	10	9.0 ± 2.2	2 ± 1

sone acetate is in the range of 0.01 to 0.02 mg/egg; free hydrocortisone is about 1/5 as active (0.1 mg/egg). The high activity of hydrocortisone in the chick embryo made this organism particularly useful for studying the effects of analogues of hydrocortisone. This report describes the inactivity of the 11-alpha hydroxy epimer of hydrocortisone in the chick embryo; this was also confirmed by another procedure, whereby the 11-alpha epimer, hydrocortisone and cortisone were injected into human skin lesions in which the latter steroids previously had been found to induce regressions(5).

Materials and methods. White Leghorn embryos were used and incubated at 38°C at a relative humidity of 85%. The test steroid was suspended in saline and inoculated on the chorioallantoic membrane (CAM) of the 8-day embryo. In some cases, where it was necessary to test large amounts of steroid, the substance was applied directly as a powder onto the CAM. The CAM was examined when the embryo died or was sacrificed and, in most cases, it was grossly clear, indicating the steroid had been absorbed. The eggs were candled daily and the dead embryos examined grossly. Embryos surviving to 18 days were weighed, and the degree of steroid effect graded according to the method previously described(1). This grading system extends from Stage I (slight effect), Stage II (definite effect), to Stages III and IV (the maximum degree of adrenocortical steroid effect). Several experiments were done with each compound. The results obtained were pooled and analyzed together. While the trend of the experiments was similar, the fact that several experiments were pooled explains in part the wide standard deviations in the data.

For the clinical study, two patients were selected with multiple skin lesions, measuring 5 to 15 mm. One was a 27-year-old colored female with disseminated Boeck's sarcoid of 5 years duration; the other a 72-year-old white male with generalized Hodgkin's disease. The nature of the lesion was established by microscopic examination of a skin biopsy. Selected skin lesions were infiltrated with 0.1 cc of a suspension of the following: a) 5 mg (50 mg/cc sol.) and 2.5 mg (25 mg/cc) of hydrocortisone acetate. b) 5 mg (50 mg/cc) and 2.5 mg (25 mg/cc) of cortisone acetate. c) 5 mg (50 mg/cc) and 2.5 mg (25 mg/cc) of the 11-alpha epimer of hydrocortisone. d) 0.1 cc of the cortisone and hydrocortisone suspending agent, and 0.1 cc of a 1% solution of carboxymethylcellulose used to suspend the 11-alpha hydroxy epimer of hydrocortisone.

Results. 1. Lack of adrenocortical activity of 11-alpha hydroxy epimer of hydrocortisone in the chick embryo. The "epimer" was applied to the CAM in a solution, in most cases, but as a powder at the high doses. Table I shows that doses of "epimer" ranging from 0.5 to 5 mg per egg were without demonstrable growth-inhibiting or adrenocortical activity in the chick embryo. At 5 mg there was a

slight suggestion of growth-inhibition. Embryos did not tolerate larger doses of the "epimer" in most cases. Three embryos, however, that survived 10 mg doses of the "epimer" showed slight growth-inhibition and questionable evidence of an adreno-steroid effect. It is concluded that the 11-alpha hydroxy epimer of hydrocortisone at tolerated doses is without appreciable adrenocortical-like activity in the chick embryo.

2. *Lack of effect of the "epimer" on skin lesions in man.* The skin lesions infiltrated with steroids gave the following results: a) hydrocortisone acetate caused complete or nearly complete regression within 7 days. b) cortisone acetate produced a significant reduction of 50 to 70% in size within 7 to 10 days, but the response was somewhat less than with hydrocortisone. c) the "epimer" and the suspending agents produced no measurable change. The untreated lesions were unchanged.

3. *Failure of 11-alpha hydroxy epimer of hydrocortisone to modify the action of hydrocortisone acetate in the chick embryo.* In Table I it is observed that 0.02 mg Compound F acetate produced a consistent modification in the growth of the chick embryo. In a group of embryos, hydrocortisone acetate, 0.02 mg per egg, was given together with 5 mg of the "epimer". When the surviving embryos were sacrificed at 18 days there was no evidence of any antagonistic effect of the "epimer" in interfering with the activity of hydrocortisone acetate. If anything, the embryos receiving the combined treatment were slightly more affected, suggesting either that the 2 agents may have had an additive effect on the chick embryo, or that the "epimer" contained a small percentage of hydrocortisone as an impurity. This difference, however, was not statistically significant, and it was concluded that the 11-alpha hydroxy epimer does not significantly antagonize or synergize the effect of hydrocortisone acetate in the chick embryo.

Discussion. Our experience with 11-α-hydroxycorticosterone indicates that it was without appreciable adrenocortical activity in the chick embryo, even when given at 200 to 300 times the effective dose of hydrocortisone acetate and 40 to 50 times that of free hydrocortisone. This loss of biological activity is presumably due to the fact that the hydroxy group in the 11 position has been changed from the natural beta orientation to the unnatural alpha configuration. It is of interest that this change in the orientation of the 11-hydroxyl group appears to inactivate the molecule almost completely. On the other hand, 11-desoxy hydrocortisone acetate (Compound S), that is, hydrocortisone without an 11-hydroxy group, has a definite inhibitory effect on chick embryo development; the minimum effective dose necessary to produce stunting of the embryo is 2.7 mg/egg as compared to 0.01 to 0.02 for hydrocortisone acetate(3). It is thus evident that the 11-alpha hydroxy group not only inactivates hydrocortisone but is actually a hindrance to biological activity, since the 11-alpha hydroxy epimer is even less effective than the analogously constituted 11-desoxy steroid (Compound S).

Summary and conclusions. 1. The 11-alpha hydroxy epimer of hydrocortisone does not inhibit the growth of the chick embryo when given at doses up to 5 mg/egg; this is 200 to 300 times the dose level at which hydrocortisone acetate (0.01 to 0.02 mg/egg) and 50 times the level at which free hydrocortisone (0.1 mg/egg) interfere with embryonic development. The unnatural orientation of the 11-alpha hydroxy group thus inactivates the molecule as far as inhibiting embryonic growth is concerned. This unnatural configuration actually inhibits biological activity, since an analogous compound, 11-desoxy corticosterone acetate (Compound S) inhibits embryonic growth at a dose level of 2.7 mg/egg. The epimer does not diminish or potentiate the action of hydrocortisone acetate in the chick embryo.‖ 2. The 11-alpha hydroxy epimer of hydrocortisone in

‖ After this work was submitted for publication, a report by Segaloff, A., and Horwitt, B. N., *Science,* 1953, v118, 220 appeared, describing the limited activity of "epi-F" in causing liver glycogen deposition in adrenalectomized mice, and its inability to interfere with the glycogen deposition produced by hydrocortisone. These results are in agreement with our own.

contrast to hydrocortisone acetate and cortisone acetate is also inactive by intra-tumoral injection in producing involution of the skin lesions in patients with Boeck's sarcoid and Hodgkin's disease.

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Excretion of Ether-Soluble Acids by Rats on Necrogenic Diet with and Without Supplements of Antibiotics.* (20577)

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Antibiotics such as aureomycin and penicillin when added to a necrogenic diet prolong the life of rats by delaying the onset of liver necrosis(1). Regardless of whether the antibiotics act by systemic action on the animal or by interaction with the intestinal flora, indirect effects should appear in the metabolism of the host. For this reason a comparison of the excretion products of rats on a necrogenic diet with those of animals receiving supplements of aureomycin or penicillin is of interest. The present study deals with the effect of the addition of these antibiotics to the basal necrogenic diet on the ether-soluble acid excretion of the rats.

Animals and methods. Male weanling rats (Sprague-Dawley or Carworth Farms) initially weighing approximately 45 g were kept in individual cages and were fed the necrogenic basal diet† with a daily supplement of vitamins(1). Aureomycin-HCl (Lederle) 25 mg, penicillin (N N'-Dibenzylethylene diamine-Wyeth) 5 mg, or vit. E 1 mg, were added to the daily rations of the treated animals. Control animals were offered 8.0 g of diet daily and the treated animals were pair-fed. For the *collection of urine* rats were placed in metabolic cages and kept on regular rations with water *ad lib.* Urine was collected under toluene. Funnels were washed down with hot water every day and the urine filtered and frozen. Analyses were run in duplicate on aliquots of combined 2- or 3-day samples of non-hydrolysed urine. *Nitrogen* was run by a semi-micro Kjeldahl method(2). Creatinine was run by the Jaffe reaction(3). Ammonia and urea after hydrolysis were determined by aeration and titration(4). Para aminobenzoic acid was determined by the usual diazotization method(5). Ether-soluble acids in urine were extracted by the method of Kanzaki(6) as modified by Bray *et al.*(7). Urine samples (10 ml) acidified with sulfuric acid (1 cc, 1 N) were continuously extracted with ether for 3 hours, the ether evaporated, the residue taken up in water and titrated with 0.1 N NaOH solution. Duplicate extracts of the same urine gave closely similar titration values. Hippuric acid (20 mg) added to a sample of urine was recovered 95-100% under the conditions of the experiments. Hippuric acid was determined after destruction of urea of the ether-soluble extract(8). Chromatograms of ether-soluble acids were run by descending technic with a butanol-acetic acid-water mixture (4:1:1) as solvent(9). Spots were developed with an alcoholic solution of brom cresol green.

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† Basal diet consists of: Corn starch 79; Baker's yeast 18 (British "D.C.L. Vit. B_1 yeast" from Distillers Corp., Ltd.); Salt Mix No. 2 USP 3; Peanut oil 0.5.