usually non-lethal syndrome is not known and is under investigation.

The guinea pigs which were sensitized with Ea in saline only and which had small amounts of circulating antibody died within a relatively short period of time. At necropsy, there was both emphysema of the lungs and stasis in the gastrointestinal tract.

*Summary.* 1. A massive hemorrhagic reaction was observed at site of subcutaneous injection of large amounts of egg albumin in guinea pigs sensitized with egg albumin incorporated in water-in-oil emulsions containing killed *M. tuberculosis* or killed *M. butyricum.* No hemorrhages were seen in guinea pigs sensitized with paraffin oil adjuvants without mycobacteria, although these animals had circulating antibodies in amounts comparable to those in guinea pigs sensitized using mycobacteria. 2. Guinea pigs sensitized by intracutaneous injection of egg albumin in paraffin oil adjuvants with or without mycobacteria underwent a protracted but usually non-lethal anaphylactic shock after a subcutaneous eliciting injection of egg albumin. On the contrary, in guinea pigs sensitized by intracutaneous injection of egg albumin in saline alone, death occurred in 39 to 66 minutes and at necropsy there were manifestations of a combination of symptoms of acute (pulmonary emphysema) and protracted shock. In accordance with previous work, prolongation of the anaphylactic syndrome was associated with the presence of antibody in the circulation. 3. Protracted anaphylaxis did not cause grossly apparent hemorrhages at sites of tuberculin reactions to PPD. Intravenous injection of *S. typhosa* or *E. coli* endotoxin 24 or 48 hours after the intracutaneous injection of PPD caused hemorrhages at the sites of tuberculin reactions.

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## Biological Activity of 9α-Fluoro-11β-Hydroxy-Δ⁴-Androstene-3,17-Dione.*† (24342)

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Considerable interest in 9α-halogenated $C_{21}$-steroids has resulted from the findings of Fried and co-workers(1) that substitution of the 9α-hydrogen by fluorine results in compounds having intensified adrenocortical activity. Thus the 9α-fluoro derivative of cortisol has a glycogenic activity approximately 5 times greater than cortisol in the adrenalectomized rat. The 9α-fluoro derivative of corticosterone has a sodium retaining activity 15 to 30 times that of desoxycorticosterone, and actually of the same order as aldosterone. The synthesis of 9α-fluoro-11β-hydroxy-Δ⁴-andro-

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TABLE I. Sodium Excreting Activity of FHA in Adrenalectomized Rats.

| Steroid | Total dose, μg | No. of rat pairs | Na excretion, mg/100 g/hr | Significance of diff of mean compared with that of solvent controls P |
|---|---|---|---|---|
| 0 | 0 | 14 | 1.98 ± .12* | |
| Desoxycorticosterone | 6 | 16 | 1.33 ± .08 | <0.01 |
| | 25 | 16 | .79 ± .08 | " |
| FHA | 1 | 4 | 2.52 ± .21 | |
| | 6 | 16 | 1.54 ± .09 | " |
| | 25 | 11 | 1.29 ± .15 | " |

* Mean ± stand. error of mean.

stene-3,17-dione(2) (FHA)[2] has made possible a detailed biological study with findings that this steroid retains sodium in the adrenalectomized rat, and that introduction of 9α-fluoro group increases uterotrophic activity while androgenicity of the compound is decreased.

*Experimental. Sodium excreting activity.* The sodium excreting activity was determined by a modification of the method of Marcus *et al.*(3). Bilaterally adrenalectomized male rats weighing between 135-150 g received a sodium chloride load of 45 mg administered intraperitoneally together with the test material administered subcutaneously. Urine was quantitatively collected over a 6-hour period and sodium determined by flame photometer (Perkin-Elmer Model 52-C Instrument), using lithium as an internal standard. In any one experiment, both solvent controls (olive oil) and desoxycorticosterone treated animals were employed. These groups were studied in parallel with FHA.

Table I summarizes results of the electrolyte studies. In a solvent group of 14 pairs of rats, by this method, sodium excretion was 1.98 ± 0.12 expressed as mg/100 g body weight/hour. Administration of FHA at the 1 μg level gave no significant changes in sodium excretion. However, administration of 6 and 25 μg doses of FHA significantly decreased sodium excretion. These effects were not as intense as those found for desoxycorticosterone studied simultaneously.

*Thymolytic and anti-inflammatory.* Neither thymolytic nor anti-inflammatory activity could be demonstrated for FHA. These activities were studied in 25-day-old bilaterally adrenalectomized rats. Two 5 mg cotton pellets were implanted subcutaneously in each rat at operation. The animals were injected once daily for 2 consecutive days with steroids suspended in Tween so that the daily dose was contained in 0.1 ml of diluent. Autopsies were performed one day after last injection, at which time body weight and thymus weights were determined. The cotton balls with adhering tissue were removed from the subcutaneous tissue, dried at 100°C for 24 hours and weighed.

A total dose of 0.5 mg of cortisol exhibited significant thymolytic and anti-inflammatory activity while 1 mg of FHA was inactive in both tests.

*Uterotrophic activity.* The estrogenic activity of FHA was studied by its ability to increase uterine weight in the immature mouse using the method of Rubin *et al.*(4). The steroid was administered daily in a corn oil solution for a period of 3 days, the daily dose of both estrone used as a standard and FHA were contained in 0.1 ml of solution. At autopsy, 24 hours after last injection, the uterus was dissected and weighed.

The results are given in Table II. The solvent control group of Exp. A had a mean uterine ratio (uterine wt in mg to body wt in g) of 0.93 ± 0.10. Administration of estrone at levels of 0.05 and 0.1 μg total dose resulted in uterine ratios of 1.91 ± 0.13 and 4.02 ± 0.4, respectively. FHA gave a significant response at the 50 μg level as judged by a uterine ratio of 1.85 ± 0.16. At the 5 μg level no activity could be detected.

Exp. B also shows that FHA exhibits distinct estrogenic activity. Whereas 11β-hydroxy-Δ⁴-androstene-3,17-dione at 100 μg caused no significant increase in the uterine

TABLE II. Uterotrophic Activity of FHA (Immature Mouse—Injection).

| Exp. | Steroid | Total dose, μg | No. of mice | Uterine ratio |
|---|---|---|---|---|
| A | 0 | .0 | 9 | .93 ±.10* |
| | Estrone | .05 | 8 | 1.91 ±.13 |
| | | .10 | 8 | 4.02 ±.40 |
| | FHA | 5. | 8 | .97 ±.10 |
| | | 50. | 9 | 1.85 ±.16 |
| B | 0 | .0 | 11 | .92 ±.05 |
| | Estrone | .05 | 10 | 1.88 ±.09 |
| | | .10 | " | 2.89 ±.13 |
| | | .20 | " | 4.89 ±.30 |
| | 11β-Hydroxy-Δ⁴-androstene-3,17-dione | 100. | " | 1.09 ±.06 |
| | FHA | 100. | " | 1.71 ±.07 |

* Mean ± stand. error.

ratio (1.09 ± 0.06), FHA at the same dosage level caused a significant increase in uterine stimulation. This group of mice had a uterine ratio of 1.71 ± 0.07 as compared to 0.92 ± 0.05 for the solvent controls.

*Androgenic activity.* Androgenic activity was estimated after daily local application of corn oil solutions steroid to the chick's comb for 7 days(5). The daily volume of solvent was 0.05 ml. Twenty-four hours after last application the animals were sacrificed and the combs removed and weighed on a torsion balance. Body weights were determined and the response expressed as the ratio of comb weight in mg to body weight in g. Table III compares androgenic activity of FHA to that of Δ⁴-androstene-3,17-dione and 11β-hydroxy-Δ⁴-androstene-3,17-dione. 11β-Hydroxy-Δ⁴-androstene-3,17-dione was significantly less active than Δ⁴-androstene-3,17-dione, and FHA (which has the 9α-fluoro substitution as well as the 11β-hydroxy grouping) was inactive.

TABLE III. Androgenic Assay of FHA (Chick Comb—Inunction).

| Steroid | Total dose, μg | No. of chicks | Comb ratio |
|---|---|---|---|
| 0 | 0 | 27 | .36 ± .01* |
| 11β-Hydroxy-Δ⁴-androstene-3,17-dione | 80 | 18 | .38 ± .02 |
| | 160 | 15 | .50 ± .03 |
| Δ⁴-Androstene-3,17-dione | 40 | 19 | .48 ± .03 |
| | 80 | 18 | .64 ± .05 |
| | 160 | 19 | .73 ± .04 |
| FHA | 160 | 18 | .35 ± .02 |
| | 320 | 20 | .37 ± .02 |

* Mean ± stand. error.

*Summary and conclusions.* 1) FHA (9α-fluoro-11β-hydroxy-Δ⁴-androstene-3,17-dione) was an effective sodium retaining substance but was inactive in thymolytic and anti-inflammatory tests. This is the first instance of a highly active sodium retaining substance in the $C_{19}$ series. 2) Introduction of fluorine at the 9α-position in 11β-hydroxy-Δ⁴-androstene-3,17-dione resulted in a compound essentially inactive as an androgen while the uterotrophic activity was intensified as compared to the non-fluorinated steroid.

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