bits. An *in vitro* secondary response, as measured by the uptake of tritiated thymidine, was elicited in spleen or lymph node cultures when incubated with the immunizing antigen. The uptake of tritiated thymidine represented an increased rate of DNA synthesis and was associated with division of the responding cells. A similar response was not seen in cultures of thymus cells, peripheral leukocytes or alveolar macrophages. The rate of DNA synthesis in cultures of bone marrow cells was depressed when they were incubated with either the immunizing antigen or phytohemagglutinin.

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## Effect of Treatment with Gonadal Steroids on Conversion of $^{14}$C-Estradiol to Estriol by Rat Liver.* (29675)

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Studies on urinary estrogens have indicated that the proportion of estriol in the total estrogen excretion differs between men and women(1) and between individuals of different race or environment(2). Attempts have been made to relate these differences to disease states, in particular, coronary disease(3,4).

Hagopian and Levy(5) have shown that estriol is formed from estrone by rat liver tissue *in vitro,* and the object of the present study was to compare the extent of the conversion of estrone to estriol by liver tissue from male, female, and castrated rats and to determine the effect on this conversion of treatment of the animals with sex hormones.

*Materials and methods.* Sprague-Dawley rats were housed in separate cages and fed Purina rat diet *ad libitum.* Castration was performed on the 14th day of age. Hormones in oil solution were injected into the rump twice weekly for 13 weeks beginning at the 22nd day of age. The total dosages per animal were: Testosterone propionate 65 mg; Estradiol-17$\beta$ 6.5 mg; Progesterone 130 mg. The animals were killed by decapitation 108 days after the start of the hormone treatment. The livers were immediately excised and used for incubation.

In a typical experiment, liver slices weighing 2 to 5 g were incubated with $^{3}$H-estrone or $^{14}$C-estradiol-17$\beta$ in Krebs-Ringer phosphate buffer at pH 7.4 in a Dubnoff shaker at 37°C under 95% $O_2$ and 5% $CO_2$ for 4 hours. Incubation was terminated by addition of acetone, and the incubate was placed in the refrigerator overnight. The filtered water-acetone extract was concentrated *in vacuo* to an aqueous residue, which was acidified with 10% HCl to pH 1 and extracted 6 times with 50 ml of chloroform. The chloroform extracted was evaporated to dryness and partitioned between water-methanol 3:7 and hexane. The hexane was discarded, and the aqueous methanol was evaporated to dryness. The residue was partitioned between chloroform and water. The chloroform extract was evaporated to dryness and dissolved in absolute methanol for chromatography.

In each extract the estrone, estradiol and estriol were separated on one of the following paper chromatographic systems:

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TABLE I. Effects of Castration and of Treatment with Gonadal Hormones on Interconversion of Estrogens by Rat Liver *in vitro*. Figures in parentheses are number of animals for experiment.

| | Sex | % Conversion of $^{3}$H-estrone to: Estradiol | Estriol | % Conversion of $^{14}$C-estradiol to: Estrone | Estriol |
|---|---|---|---|---|---|
| Normal | ♂ | 21.5(3) ± 2.3* | 39.8(3) ± 4.2 | 34.1(1) | 40.6(1) |
| | ♀ | 25.4(3) ± 2.5 | 34.5(3) ± 5.3 | 31.7(2) ± 1.4 | 43.4(2) ± 1.2 |
| Normal + testosterone | ♂ | 29.6(2) ± 4.1 | 33.4(2) ± .0 | 27.4(2) ± 2.9 | 38.0(2) ± 2.1 |
| | ♀ | 25.4(4) ± 1.6 | 30.5(4) ± 1.3 | 32.4(2) ± 3.6 | 34.7(2) ± 2.4 |
| Normal + progesterone | ♂ | 26.0(1) | 39.7(1) | 22.7(1) | 47.8(1) |
| | ♀ | 33.0(1) | 25.4(1) | 27.5(2) ± 2.5 | 52.2(2) ± .7 |
| Normal + estrone | ♂ | 28.2(2) ± 5.3 | 21.5(2) ± 6.4 | 35.0(3) ± 3.3 | 35.4(3) ± 2.6 |
| | ♀ | 35.7(2) ±10.3 | 19.4(2) ± 2.6 | 28.9(3) ± 3.0 | 31.5(3) ± 4.5 |
| Castrated | ♂ | 27.1(5) ± 2.4 | 33.9(5) ± .9 | 34.0(3) ± .4 | 35.0(3) ± 1.0 |
| | ♀ | 26.9(5) ± 2.4 | 37.2(5) ± 3.0 | 28.1(3) ± 6.8 | 34.7(3) ± 1.4 |
| Castrated + testosterone | ♂ | 24.4(3) ± 3.0 | 35.7(3) ± 3.4 | | |
| | ♀ | 27.8(3) ± 3.5 | 29.6(3) ± 3.8 | 36.8(1) | 40.4(1) |
| Castrated + progesterone | ♂ | 26.2(1) | 38.0(1) | | |
| | ♀ | 28.6(2) ± 3.7 | 25.2(2) ± 4.8 | 30.6(1) | 42.6(1) |
| Castrated + estrone | ♂ | 26.9(3) ± 4.1 | 26.7(3) ± 5.1 | 34.2(2) ± 6.4 | 39.0(2) ± 4.9 |
| | ♀ | 29.8(3) ± 3.3 | 22.3(3) ± 3.4 | 28.5(2) | 44.8(1) |

* Standard deviation.

A. Hexane-benzene-methanol-water 2:2:3:1 (v/v).

B. Chloroform formamide. Impregnation of the papers was carried out as described by Layne and Marrian(6).

The extracts obtained from the incubations were chromatographed in parallel with reference amounts of estrone, estradiol, and estriol. The chromatograms were sprayed with Folin-Ciocalteu reagent(7). The areas corresponding to the estrogen spots were cut out, cut into small pieces, and placed in scintillation vials. Methanol (0.2 ml) was added to each vial, then 10 ml of scintillation solution(8). The solutions were assayed for radioactivity in a Packard liquid scintillation spectrometer with appropriate correction for quenching. The recovery of radioactive estrogens carried through the entire procedure of extraction, chromatography and counting was of the order of 80%.

Control experiments with estrone and buffer and with liver and buffer only were run through the complete procedure.

*Results.* Pooled estriol areas from chromatograms of several incubations of 200 $\mu$g each of $^{14}$C-estradiol were crystallized and found to have an infrared spectrum identical to that of authentic estriol. Table I presents a summary of the results, showing the percentage of $^{3}$H-estrone converted to $^{3}$H-estriol, and the percentage of $^{14}$C-estradiol-17$\beta$ converted to $^{14}$C-estrone and $^{14}$C-estriol. In addition to t tests between the means of the various groups, pooled variances were calculated between male and female animals, between hormone treated and control animals, and between castrate and intact animals. No significant alteration was observed as a result either of castration or of treatment with gonadal hormones on the conversion of either estrone or estradiol to estriol. Animals treated with progesterone converted more estradiol to estriol than did either control animals or those treated with androgen or estrogen, but this effect was not significant. The statistical evidence indicates that (a) male livers convert more estrone to estriol than do female livers; (b) livers of estrogen-treated animals convert less estrone to estriol than do livers of control animals and of those treated with either testosterone or progesterone.

*Discussion.* These results indicate that the rat liver *in vitro* can effect a major transformation of both estrone and estradiol to

estriol. The conversions found in these experiments amount to 30 to 40% of the added tracer dose.

The larger production of estriol by male livers is of interest, but the results on the animals treated with steroids give no indication as to whether this effect is under hormonal control. It is possible that the smaller conversion of estrone by the livers of the estrogen-treated rats (Table I) and the higher conversion of estradiol to estriol in the progesterone-treated animals (Table I) may represent real effects, even though significance was not attained in the present experiments.

*Summary.* The object of the present study was to prove that rat-liver tissue will convert either estrone or estradiol to estriol *in vitro* and to study and compare the extent of this conversion by liver tissue from male, female, and castrated rats and the effect on this conversion of treatment of the animals with sex hormones. While there is no doubt as to the *in vitro* conversion of estradiol and estrone to estriol, which amounts to about 30 to 40%, there are no statistically significant effects of gonadectomy or treatment with sex hormones on rate of conversion. A significantly larger conversion of estrone to estriol was observed in livers from male rats than in those from females.

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## Pancreatic Enzyme Levels in Chicks Fed Unheated Soybean Meal.* (29676)

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The presence of a heat-labile component in soybean meal which causes pancreatic hypertrophy and growth depression has been well established(6,8,9). Histological examinations show pancreatic hypertrophy to be associated with an accumulation of zymogen in the acinar cells when unheated soybean meal is fed to chicks(21). Further, pilocarpine injections did not cause release of zymogen from the pancreas of those chicks, suggesting an impairment in the secretory ability of the pancreas.

Two groups have prepared a water insoluble residue which contains much less trypsin inhibitor, but which will cause growth depression and pancreatic hypertrophy in rats (17) and chicks(20.) It has been shown that dietary trypsin inhibitor increases intestinal levels of enzymes in rats(8,13,14), but similar results have not been reported for chicks. There are two reports that chick growth is not impaired by feeding concentrates of soybean meal containing trypsin inhibitor(4,20), while another report claims that crystalline trypsin inhibitor causes growth impairment and pancreatic hypertrophy(15). Thus, the effects of trypsin inhibitor have not been fully elucidated. Furthermore, pancreatic hypertrophy and growth depression may be caused by some material in soybean meal which has not been characterized.

The purpose of these studies was to establish the relationship between dietary unheated soybean meal and the level of 2 enzymes,

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