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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trypsin and amylase, in the pancreas. A substrate specifically hydrolyzed by trypsin, p-toluene-sulfonyl-*L*-arginine methyl ester (TAME), was selected to permit the study of one enzyme while excluding other proteolytic enzymes.

Experimental. Day-old chicks were divided into 2 groups and fed either heated or unheated soybean oil meal as 50% of the diet, according to diets and conditions previously described(19,21). After 4 weeks on a diet, all chicks were fasted for 16 hours, then half of each dietary group was fed the alternate diet while the other half was continued on the same diet. Two chicks from each group were sacrificed after 0, 2 and 6 hrs on feed. Pilocarpine (0.016 mg in saline per chick) was injected intraperitoneally into 2 chicks from each diet at zero time, these chicks being sacrificed 30 minutes later without feed. The pancreatic tissue was immediately frozen and stored at —15°C. The frozen tissue was minced, homogenized in 10 volumes of distilled water and centrifuged at 5,000 $\times$ *g* for 20 minutes (0°C). Aliquots of the supernatant which were within the region of linear response to enzyme concentration were used for enzyme assays.

Two other groups of day-old chicks were fed either raw or heated water insoluble residue, prepared as previously described(17,20). The residue was 20% of the diet, added at the expense of glucose in the basal diet(19). After the 2 week feeding period, the chicks (15 per group) were fasted 16 hours then fed the same diet until sacrificed. The supernatants from pancreatic homogenates were lyophilized and stored at —15°C.

Three additional groups, 10 chicks per group, were fed either 50% heated meal, 50% raw meal, or 50% heated meal plus 20% raw residue replacing equivalent glucose(19). The body and pancreas weights were measured after 2 weeks on diet from day of age.

Amylase activity was measured by the 3,5-dinitrosalicylate method(3). The reaction time was 3 minutes at 30°C for 5 microliter aliquots (about 0.1 mg protein). The unit of amylase activity was defined as the micromoles of maltose formed per minute, although alpha-amylase converts starch to dextrins.

Trypsin was assayed by following the hydrolysis of TAME spectrophotometrically at 247 mμ (Beckman DU) at 30°C(11). Twenty-five microliter aliquots (about 0.5 mg protein) were adequate for assay. Trypsinogen was converted to trypsin by treating with enterokinase (2.0 mg per 50 mg tissue in 0.5 ml) for 2 hours at 30°C. The trypsin unit was defined as the amount of enzyme causing an absorbency increase of 0.001 per minute.

Trypsin inhibitor was determined in aliquots from material pulverized (mortar and pestle) and suspended in pH 8.1, 0.04 M Tris buffer which was also 0.01 M $CaCl_2$. The trypsin inhibitor unit was defined as the loss in trypsin activity in terms of the trypsin unit.

Protein in the supernatant was determined by measuring absorbency at 280 and 260 mμ to correct for nucleic acids(12). Protein in the lyophilized samples was determined by the micro-Kjeldahl method. Specific activity is defined as enzyme units per mg protein. Each point in both figures represents an average of 4 or 5 determinations. The observed differences in specific activity for each enzyme in the 2 experiments probably reflects the age of the chicks.

Results and discussion. Feeding raw residue did not depress growth in these experiments, yet the pancreatic weight was about the same as when raw soybean meal was fed (Table I). If trypsin inhibitor is solely responsible for pancreatic hypertrophy, then considerably less inhibitor than the 0.3% previously reported(15) can cause hypertrophy since the raw residue diets contain 0.024% inhibitor, calculated on the basis of trypsin inhibited.

The variation of pancreatic enzyme activity with time is shown in Fig. 1. The histological studies(21), which had demonstrated an abnormal pancreatic response to pilocarpine by chicks fed raw soybean meal, are confirmed by the enzyme studies. Actually, the specific activity of both enzymes increased 30 minutes after injecting pilocarpine

TABLE I. Effect of Feeding Soybean Meal Preparations to Chicks.

Exp No.	Material	Inhibitor: Units/mg material	Inhibitor: Units/mg diet	Body wt (g)	Pancreas (g)	Pancreas/body $\times 10^4$
2	Heated residue	—	—	138	.65	48
	Raw residue	133	27	124	.75	61
3	Heated meal	—	—	131	.59	46
	Raw meal	1180	590	106	.87	87
	Raw residue	180	36	128	.90	70

into chicks on raw diets, whereas the histological study indicated only that zymogen was not released by the pancreas. The increase in specific activity of both enzymes suggests that synthesis of zymogen still occurs.

Chicks with a hypertrophic pancreas have a higher trypsin, but lower amylase, specific activity than chicks with a normal pancreas after the fasting period. Chicks fed raw meal both before and after the fasting period maintained a fairly constant level of both enzymes, which contrasts to the variations in activities observed for chicks fed heated meal. These variations seem to reflect the pre-fasting diet, especially for chicks changed from raw to heated meal diets, since the pancreatic levels of both enzymes approach the levels for chicks continually fed heated meal. The change from heated to raw meal is reflected by lower level of both enzymes, which might be interpreted as an increased release of enzymes from the pancreas. An equally valid interpretation involves an altered rate of synthesis of pancreatic zymogen. Neither interpretation can be dogmatically asserted without further experimentation. The effect of feeding raw meal seems to be cumulative, but the tendency towards reversal is observed between 2 and 6 hours after ending the fast when feeding the alternate diet. This observation correlates with an earlier observation wherein reversals of pancreatic hypertrophy were noted within 72 hrs after changing diets(21).

Feeding chicks an insoluble fraction from soybean meal which contains less trypsin inhibitor than raw meal and adds about one-fifteenth the amount of inhibitor to the diet caused a different pancreatic response, as shown in Fig. 2. There was little effect on

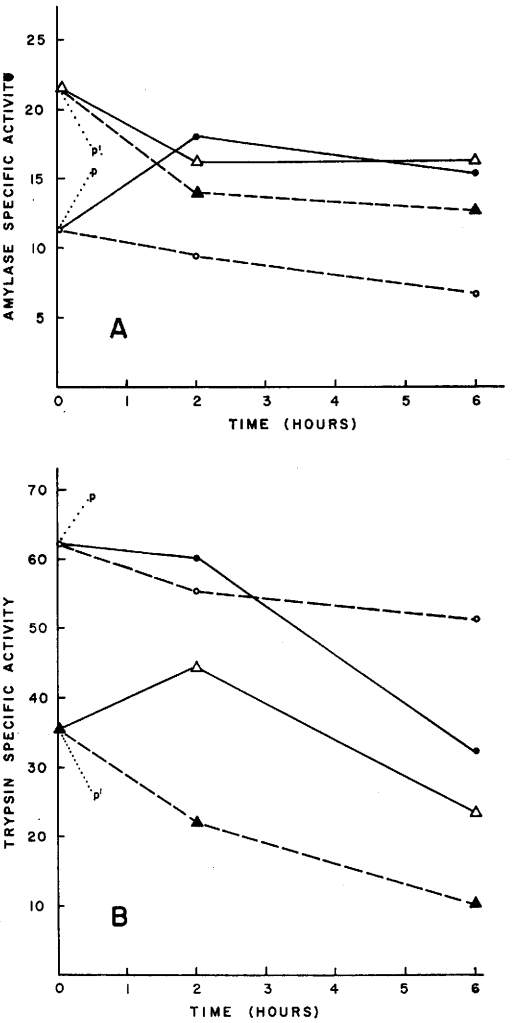

FIG. 1. Specific activity of amylase (A) and trypsin (B) in pancreatic homogenates. Chicks fed either raw or heated soybean meal for 4 weeks prior to 16 hr starvation period. Points p and p^1 indicate activity 30 min after pilocarpine injection. Solid lines indicate heated meal and dashed lines raw meal fed after starvation. ○, Continued on raw; ●, changed from raw to heated; △, continued on heated; ▲, changed from heated to raw.

amylase levels. The effect on trypsin levels was much the same as feeding raw meal, although there was a decrease in activity between the second and fourth hours which was not observed in the previous experiment.

The observation that zymogen levels are higher in the hypertrophic pancreas complements 2 earlier reports from this laboratory. One report showed an accumulation of zymogen in enlarged acinar cells which, in contrast to the controls, did not diminish upon stimulation by pilocarpine or by feeding(21). The second showed hypertrophy to be produced when a fraction containing little trypsin inhibitor was fed(20). These 3 reports suggest that pancreatic hypertrophy, accompanied by an accumulation of zymogen, may be caused by some component(s) of raw soybean meal other than the several trypsin inhibitors(16).

The change in enzyme levels of the hypertrophic pancreas might simply reflect changes in the ratio of dietary components, as has been shown for rats(7,10), especially since the residual fraction was fed at the expense of glucose. However, the difference in trypsin levels between chicks fed heated or nonheated residue suggests that the pancreas is responding to something other than changes in dietary composition.

A recent report of histological and proteolytic enzyme studies with 6-week old cockerels(2) might be considered contradictory to histological studies on younger chicks in our laboratories(21), although our earlier work was not cited. Discrepancies probably reflect age differences because there is less response to the dietary change as the bird matures (5,18).

Summary. An unheated, water insoluble fraction of soybean meal, while adding only .024% trypsin inhibitor to the diet, causes pancreatic hypertrophy in the chick. The hypertrophic pancreas has a higher trypsin specific activity than controls. Amylase specific activity was lower after feeding raw soybean meal but there was no difference after feeding the water insoluble fraction. These observations suggest that a heat-labile component other than trypsin inhibitor may be responsible for pancreatic hypertrophy and associated changes in enzyme levels in animals fed raw soybean meal.

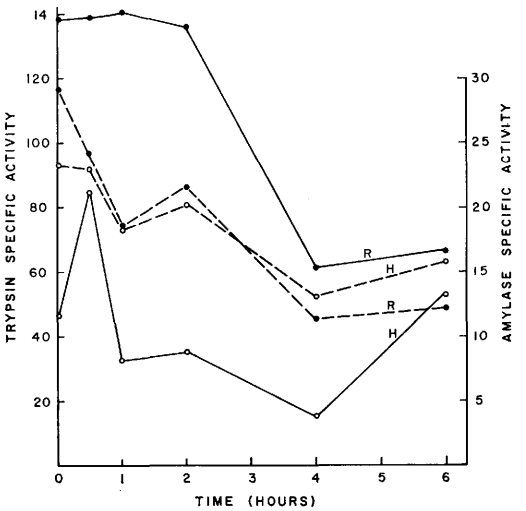

FIG. 2. Specific activity of trypsin and amylase in lyophilized pancreas from chicks fed either raw or heated water insoluble residue for 2 weeks prior to starvation. Dashed lines indicate amylase; solid lines, trypsin. R and H indicates raw or heated diets.

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Conversion of 4-Androstene-3β,17β-diol-4-C^{14} by Human Adrenals *in vitro*.* (29677)

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Recent interest in the conversion of the Δ^4-3-hydroxyl to the Δ^4-3-ketone(1,2) has been generated by the isolation of Δ^4-3-hydroxyl compounds(3) or their artifacts from natural sources(4) and by demonstration of the conversion of the Δ^4-3-ketone to the Δ^4-3-hydroxyl under *in vitro*(5) and *in vivo*(3) conditions.

The conversion of Δ^4-androstene-3β,17β-diol and its 3α-isomer to testosterone had previously been shown to occur in both rat and chicken liver homogenates(1,2). The conversion of these isomers to testosterone and 4-androstene-3,17-dione occurred in both the supernatant fraction and thrice washed particulates from the 5000 $\times$ *g* fraction of rat and chicken liver acetone powders. The latter residue fraction, however contained only 30% of the activity of the supernatant. A stimulation by the pyridine nucleotides was also demonstrated.

In addition the conversion of the Δ^4-hydroxyl group to the Δ^4-3-ketone has been shown to occur in the testis tissue of the mouse(6). The present report demonstrates that human adrenal tissue also has the capacity to convert 4-androstene-3β,17β-diol to testosterone and 4-androstene-3,17-dione.

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Methods. A total of 11 g of hyperplastic human female adrenals were homogenized with 80 ml of Krebs-Ringer bicarbonate buffer, pH 7.3, in a Potter-Elvehjem homogenizer. In a series of incubations, 2.5 ml of homogenate were placed in 50 ml Erlenmeyer flasks along with 5 μM ATP, 6 μM NADP, and 1 mg (73,000 cpm) of 4-androstene-3β,17β-diol-4-C^{14} (prepared by Dr. M. Gut, Worcester Foundation for Experimental Biology). The final volume was 2.8 ml. The flasks were incubated at 37° in a Dubnoff Metabolic Shaking Incubator (Precision Scientific Co.) under an atmosphere of 95% O_2 and 5% CO_2. The incubator was set at 100 oscillations per minute. Incubations were stopped with 10 volumes of acetone at zero time, 15, 30, 60, and 180 minutes.

The acetone extracts were filtered on a sintered glass filter funnel and the tissue residue was freed of residual steroids with successive washings of acetone, ethyl acetate and methanol. After removal of these solvents under vacuum the lipids were removed by partition between petroleum ether and 70% aqueous methanol. The methanol was removed and the steroids were extracted from the aqueous phase with ethyl acetate. Aliquots of the extracts were then analyzed by thin-layer and gas chromatography.

Chromatoplates were prepared for thin-layer chromatography by applying a slurry of silica gel GF (Merck 7730) in water (1:2)