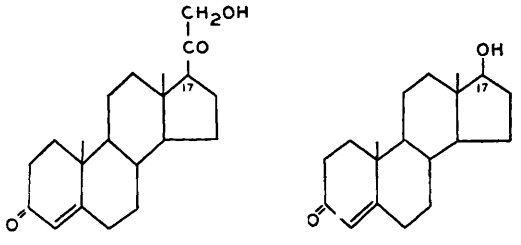

DESOXYCORTICOSTERONE TESTOSTERONE

FIG. 2. Comparison of structures of desoxycorticosterone and testosterone.

the former has a side chain at C17, while the latter does not. It may be that the presence of this side chain is essential for the alteration of responsiveness to insulin.

Summary. The administration of testosterone propionate in doses of 10, 25, or 50 mg daily for a period of 10 days to 13 patients with diabetes mellitus failed to alter significantly the responsiveness to insulin in all but one. It is suggested that the presence of the side-chain attached to carbon atom 17 in the steroid molecule may be essential for this activity.

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Nephrotoxic Action of dl-Ethionine.* (18879)

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Ethionine, a presumed antagonist of methionine, induces fatty changes in the liver(1), influences protein metabolism(2), and causes destructive changes in the pancreas(3,4). In the course of experiments in which enhancement of the pancreas-damaging effect of ethionine was found by the use of a protein depletion diet(5), renal changes were also observed. These are the subject of the present communication.

Experiments and results. Young male and female albino rats of the Wistar strain weighing from 125 to 250 g were used. One group of male and female rats was fed a complete Rockland rat diet. A second group was put for a period of 7 to 14 days on a protein depletion diet consisting of 77.6 parts corn starch, 15 parts hydrogenated cotton seed oil (Crisco), 4 parts salt mixture U.S.P. XIV, 4 parts ruffex, and 0.4 part Brewers yeast powder (Nutritional Biochemical Corp., Cleveland, Ohio). The yeast, according to the manufacturer, contains 50.3% protein. The methionine content based on 16% N averages around 1.8%. The protein depletion diet used contained therefore approximately 0.2% protein and 0.0036% methionine respectively. The animals received in addition one drop of cod liver oil twice weekly. All rats were given 1 mg dl-ethionine (α-amino-γ-ethylthyol butyric acid) (Nutritional Biochemical Corp.) per g body weight, divided in 3 doses, intraperitoneally. Animals on a complete diet were fasted for 16 hours prior to the administration of the drug. The possible protective effects of dl-methionine, glycine, and dl-alanine were tested by the administration of equimolecular amounts of these amino acids which were given intraperitoneally in 3 divided doses half an hour prior to the injections of ethionine to rats on the protein depletion diet. These rats were sacrificed 24 hours later.

Renal damage was seen in almost all of the

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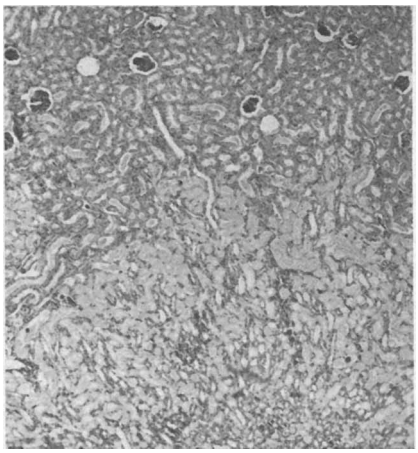

FIG. 1. Sections of the kidney of a male rat on the protein depletion diet for 12 days and sacrificed 24 hr after ethionine administration. Necrosis of the distal portions of the proximal convoluted tubules in the inner cortical zone is widespread. Hematoxylin-eosin stain × 40.

animals on the protein depletion diet. Distinct changes in tubules located in the inner portion of the renal cortex were noticed as early as 6 hours following the administration of ethionine. There was marked condensation of the basal portion of the cytoplasm which stained intensely eosinophilic with the hematoxylin and eosin stain. The luminal portion of the cytoplasm was often fringed, hazy in outline and took the stain poorly. The nuclei were shrunken and pyknotic. After 13 hours some of the tubular cells had become frankly necrotic. In rats sacrificed 24 to 48 hours following the administration of ethionine destructive changes in the epithelium of tubules located in the inner zone of the renal cortex had become widespread in some of the kidneys (Fig. 1). The epithelium in damaged tubules was completely necrotic and the nuclei had disappeared entirely. Occasionally, however, pyknotic nuclei were still present in some of the damaged tubules. These changes resembled closely those seen following the administration of dl-serine(6). In animals that were allowed to live for 3 days evidence of early repair was found. Tubular epithelium started to regenerate and mitotic figures became numerous. Within the next day the necrotic tubular cells were partially replaced by flat cells which had a more basophilic staining cytoplasm. Due to the flatness of these cells the tubular lumen appeared dilated. In rats that lived for 7 days almost all of the destroyed tubular epithelium had been replaced but remnants of necrotic cells were still occasionally visible. Calcification was not seen. Casts were not noticed and the glomeruli remained intact. In an attempt to localize the sites of the renal damage, single nephrons were isolated and appropriately stained. The isolation of single renal tubules was performed by Dr. Jean Oliver. Necrosis was limited to the terminal portion of the proximal convoluted tubules. No other part of the nephron was involved. The extent of the renal involvement varied considerably. Its degree was expressed as 0, ±, 1+, 2+, and 3+. In kidneys considered to show ± damage only very few necrotic tubules could be observed on appropriate sections.

As can be seen from Table I, there is considerable difference between male and female rats and between animals on a complete or a protein depletion diet in the susceptibility of the renal cortex to the damaging action of ethionine. Male animals on both diets, showed more extensive involvement than female ones. On the complete diet, however, renal involvement was only light in the male and absent in the majority of the female rats. There was a marked increase in renal toxicity in rats on the protein depletion diet. A similar enhancement of the pancreas-damaging action in animals on a protein depletion diet has been described previously(5). Almost complete protection was observed in both male and female animals when methionine was given, while glycine and alanine were without significant influence.

Comment. Renal lesions were found on a diet low in protein with either carrots or turnips as the primary constituents(7,8). Since, however, rats on a diet containing 0.5%(8) or 1%(9) casein as protein source did not pre-

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TABLE I. Effect of Sex, Diet and Several Amino Acids on Renal Toxicity of DL-Ethionine.

Sex	Diet	Administered: Ethionine	Administered: Additional	Total No. rats	No. of rats showing lesions: 0	±	1+	2+	3+
Male	Complete	+	—	10	2	3	1	3	1
Female	"	+	—	12	8	2	0	2	0
Male	Protein depletion	—	—	7	7	0	0	0	0
"	" "	+	—	20	0	0	1	13	6
Female	" "	+	—	17	1	2	5	9	0
Male	" "	+	DL-methionine	8	6	2	0	0	0
"	" "	+	Glycine	6	0	0	1	3	2
"	" "	+	DL-alanine	6	0	0	1	3	1
Female	" "	+	DL-methionine	10	9	1	0	0	0
"	" "	+	Glycine	6	2	1	1	2	0
"	" "	+	DL-alanine	7	0	0	2	4	1

sent comparable renal changes, it was concluded that it was not the lack of protein but the persistent alkaline urine containing large amounts of calcium and phosphate which was responsible for the renal lesions(9). The depletion diet used in these experiments containing only 0.2% protein likewise did not induce renal damage. Protein depletion led, however, to marked increase in the susceptibility of the kidney to the nephrotoxic action of ethionine. In view of the fact that female rats on a complete diet very often failed to show any renal lesions the negative findings of Simpson, Farber, and Tarver(2) are thus easily explained.

Ethionine has been shown to inhibit to a marked degree the incorporation of radioactively labeled methionine and glycine into the proteins of the liver and kidneys of intact rats(2). These inhibitions as well as the fatty infiltration of the liver caused by ethionine are prevented by methionine but not by other amino acids including glycine and alanine(1). Methionine prevents also the pancreatic damage(3). The nephrotoxic action of ethionine is influenced in a similar manner by methionine but not by glycine or alanine. It is noteworthy that tissue repair in the kidney should set in 48 to 72 hours after the administration of ethionine, in spite of the fact that the animals remained on a diet that contained only traces of methionine.

Under different experimental conditions ethionine was found to exert an opposite effect. Mixed with the food it prevented the accumulation of liver fat and the hemorrhagic kidney degeneration on a low methionine, low choline diet(10). This is not necessarily contradictory to the present findings since much larger amounts of the drug reach the blood stream and kidneys when given by the parenteral route than when mixed with the diet. The beneficial effect of ethionine on the renal damage caused by choline deficiency probably involves a different biochemical mechanism.†

The greater susceptibility of male rats to the nephrotoxic action of ethionine is in agreement with previously established findings. dl-Serine(6,11) carbon tetrachloride(12), and chloroform(13) are more toxic to male than to female animals. The hemorrhagic necrosis in acute choline deficiency is likewise more pronounced in young male than female rats (14).

Summary. Ethionine induces necrosis of the distal portion of the proximal convolutions in the rat kidney. On a normal diet renal involvement is seen more frequently and to a greater extent in male animals. A pro-

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† No renal damage was found by Stekol and Weiss (*J. Biol. Chem.*, 1949, v179, 1048) in male rats given at the age of 24 to 35 days a complete diet containing 25% casein and 0.14 to .55% ethionine for a period of 27 days. Feeding of ethionine together with choline or methionine over a period of 3 months resulted in liver and kidney damage.

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tein depletion diet increases very markedly the susceptibility of the kidney to the nephrotoxic action of ethionine in male and female rats. Methionine inhibits the renal toxicity of ethionine while glycine and alanine are without effect.

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Differences of Cortical/Medullary Ratio in the Adrenals of Normal Rats, Normal Rats treated with ACTH, and Renal Hypertensive Rats.* (18880)

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The existence of a direct relationship between heart weight and adrenal weight in male rats made hypertensive by the removal of one kidney and constriction of the other has been reported(1,2). It was shown that the relative degree of enlargement of the medulla exceeded that of the cortex, although by far the greater part of the weight increase of the whole gland was due to increase in size of the cortex(2).

The assumption that enlargement of the adrenals under conditions of stress is due to the effect of hypophyseal corticotrophin has been generally accepted(3). If increase in the size of the adrenals in severe or prolonged stress is due solely to the effect of hypophyseal corticotrophin, and if enlargement of the adrenals in renal hypertensive rats is an instance of the reaction to stress, then it would be expected that the administration of adequate doses of ACTH would bring about changes in the adrenals of normal rats similar to those found in renal hypertensive rats. The aim of the present report, in part, is to show that such is not the case. ACTH was given to normal rats and found to induce cortical but not medullary growth, within the limits of accuracy of the method. If any change in medullary size occurred it was very small. In contrast, the adrenal medulla of the renal hypertensive rat has been shown to increase to as much as 4 times the normal size. This is due to both cell hypertrophy and cell hyperplasia(2).

Experimental procedure. Four male rats of the Stanford colony were given 0.5 mg of ACTH (Armour) intramuscularly 4 times daily to a total dose of 25 mg each. They were then killed by exsanguination from the aorta under ether anesthesia and their adrenals weighed to the nearest milligram on a torsion balance. Seven normal control rats were killed similarly, as were 6 renal hypertensive males. Cortical/medullary volume ratios were determined by dividing each adrenal into 3 discs, embedding these in paraffin after fixation in Bouin's fluid and dehydration in dioxane, and filling several slides with sections. The slides were projected onto paper at 37 diameters magnification, drawn in outline, and the areas of cortex and medulla measured with a planimeter. A correction was made for vascular space in the medulla only. The individual cortical/medullary ratios given are based on a minimum of 100 sections through the adrenals and their statistical accuracy has been tested and verified by plotting the course of cumulative ratios(2).

Results. The data are given in Table I. The 32% increase of the ACTH treated rats over the normal rats in adrenal weight expressed as percent of a predicted value based on body weight, is statistically significant ($P < 0.02 > 0.01$). No significant change in medullary weight seems to have been produced

* This investigation was supported by USPHS Grant No. H-811.

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