was tried to see if it would increase the effect of the estrin. Each rat was injected 2 or 3 times with Proluton Schering, in amounts varying from 0.01 to 0.10 I.U., either in a single injection or in 4 injections given in one afternoon. In no case was there either increase or decrease in the estrin-maintained behavior level. The highest dose used is one-half the effective dose for the guinea pig,[1] an animal about 3 times the size of the rat.

Obviously the experiments with LH will have to be repeated with variations to determine whether it produces a truly specific effect on sex behavior. The failure to obtain any effect from progestin in the rat is, perhaps, open to the criticism that this species may be relatively insensitive to this hormone as regards behavior. Nevertheless, the observations reported bring the endocrinology of heat behavior in the rat, so far as it is known, into line with what it is reasonable to expect from the physiology and time relations of the ovarian and behavioral events occurring at estrus.

9002 C

An Attempt to Induce Nephrotoxins and Experimental Glomerulonephritis by Injections of Homologous Renal Tissue.

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The rôle of allergy and nephrotoxins in the production of glomerulonephritis has been studied extensively by many investigators. The results of these studies may be found in excellent reviews of the literature by Pearce,[1] Leiter,[2] Masugi,[3] and Fishberg.[4] Recently Masugi has shown that if the blood serum of ducks that have received repeated injections of rabbit kidney be injected intravenously into rabbits, a glomerulonephritis results. Schwentker and Rivers[5] have demonstrated, further, that by autolyzing rabbit brain it is possible to render it antigenic to rabbits so that its repeated in-

[1] Pearce, R. M., *Arch. Int. Med.*, 1910, **5**, 113.

[2] Leiter, L., *Arch. Int. Med.*, 1924, **33**, 611.

[3] Masugi, M., *Ziegler's Beitr.*, 1934, **92**, 429.

[4] Fishberg, A. M., *Hypertension and Nephritis*, Philadelphia, Lea and Febiger, Ed. 3, 1934.

[5] Schwentker, F. F., and Rivers, T. M., *J. Exp. Med.*, 1934, **60**, 559.

jection leads to the production of complement fixing antibodies. Consideration of these findings led us to believe that by autolyzing rabbit kidney, it might be made antigenic to rabbits, and that repeated injections might lead to the production of an experimental glomerulonephritis.

Sixteen male rabbits were injected intraperitoneally with emulsions of autolyzed rabbit kidney. Eight of these rabbits were injected with emulsions made from the kidneys of sacrificed rabbits, whereas the remaining 8 were injected with emulsions made from one of their own kidneys that had been removed by unilateral nephrectomy. Thus it was attempted to study the production of "isonephrotoxins" by the injection of modified renal tissue from the same species of animal, and the production of "auto-nephrotoxins" by the injection of modified renal tissue from the same animal.

Strict aseptic precautions were observed in preparing and handling the 16 emulsions used for the injections. Each emulsion represented the tissue of an entire kidney obtained by operation. No attempt was made to wash the blood from these organs for fear that the additional handling might promote bacterial contamination. Following removal, the kidney was held in boiling water for about 3 seconds and was then placed in a sterile stoppered bottle, where autolysis *in toto* was allowed to proceed. Smears stained by the Gram method were made from the interior of these organs at various stages in their autolysis and were examined microscopically for evidence of contamination. Preparations showing more than slight contamination were discarded and new ones substituted. When the desired degree of autolysis had been reached, each kidney was cut up finely with scissors, triturated in a mortar and emulsified by mixing with 35 cc. of sterile normal saline solution.

It was thought that the degree of autolysis of the renal tissue might influence the antigenic properties of the emulsions, and for this reason emulsions were prepared from kidneys autolyzed to different degrees. Those from the injection of Rabbits Nos. 1 to 4 were made from kidneys completely autolyzed by immersion in a water bath for 6 days; of Rabbits Nos. 5 to 8 from kidneys incompletely autolyzed at room temperature for 5 days; of Rabbits Nos. 9 to 12, from kidneys allowed to stand for 14 days; and those for the injection of Rabbits Nos. 13 to 16, from kidneys kept at room temperature for 21 days.

The appropriate emulsion was injected intraperitoneally into each rabbit in doses of 6 to 7 cc., on 4 different occasions, 5 days apart.

The emulsions employed for injecting Rabbits Nos. 7, 10, and 16 were made from kidneys showing slight bacterial or fungus contamination, but the number of organisms seen on repeated smear examinations were so few that rejection was not considered necessary.

The blood sera of the injected rabbits were tested repeatedly by means of the complement fixation reaction to detect the presence of antibodies to the emulsions of autolyzed renal tissue by a modification of a standardized method for performing the Wassermann reaction described by Hinton.[6] The antigen employed in each test was a freshly prepared 1:20 or 1:40 dilution (without filtering or centrifugalization) of the emulsion used for the injections. The blood sera from the normal control and the injected rabbits was obtained by bleeding from an ear vein and was inactivated before use by heating in a water bath at 57°C. for 30 minutes.

The complement was titrated prior to the actual complement-fixation test on each test day, to determine the smallest quantity of complement which caused hemolysis of 0.5 cc. of sensitized sheep erythrocytes (a mixture of equal parts of standardized sheep red blood cells and dilute anti-sheep cell amboceptor). Twice this amount of complement (or 2 units) was used in the subsequent procedures.

The antigens were titrated prior to every complement-fixation test to determine the largest quantity of each antigen which, in the presence of 2 units of complement and 0.5 cc. of sensitized sheep cells caused no inhibition of hemolysis. This and fractional amounts of each antigen were used in the tests themselves. Controls were employed to check against the possibility that inhibition of hemolysis in the test system was due to some factor other than the complement-fixing ability of the treated rabbit serum used. Normal saline was used in all tubes to dilute to constant volume, and 0.5 cc. of sensitized sheep cells was added to each tube after incubation.

Simple urinalysis and determination of the phenolsulphonephthalein excretion were carried out occasionally on each treated rabbit, both before and after the injection of the emulsions, in order to detect any signs of renal damage resulting from the injections.

Results—Pathological Studies. Of the 16 rabbits, 4 died spontaneously at intervals varying from 2 to 66 days after their last injection of emulsion. Each of the 4 displayed symptoms of weakness, loss of weight, diarrhea and collapse. Though only one of these rabbits (No. 16) was injected with a visibly contaminated

[6] Hinton, W. A., *Am. J. Syphilis*, 1920, **4**, 598.

emulsion (fungus), all 4 showed a variable degree of localized or diffuse inflammation of the peritoneum. The remaining 12 showed no evidence of disease during the course of the experiments, and were in good condition when killed. Except for the 4 instances of peritonitis and hypertrophy of a remaining kidney, the autopsies revealed nothing remarkable. Previous operative wounds in the nephrectomized animals were entirely healed. In all animals the organs other than the kidneys were normal in the gross. None of the kidneys from the 16 animals showed any evidence of diffuse nephritis or other abnormalities on gross inspection. Microscopic examination of the kidney sections revealed, in almost every case, a variable degree of interstitial nephritis, characterized by local infiltrations of round cells and focal areas of dilated tubules lined with flattened epithelium. In no instance were there diffuse proliferative or exudative changes in the glomeruli, or any evidence of vascular damage.

Complement-fixation Tests. All sera obtained prior to the injection of the emulsions showed negative reactions. Among the sera obtained 10 days or more after the last injection of the emulsions, those from 4 animals (Rabbits Nos. 1, 4, 5, and 8) showed definite fixation of complement in low titer, on one or more occasions. Such positive results, however, were obtained in only one out of 5 tests for Rabbit No. 1, one out of 5 tests for Rabbit No. 4, one out of 7 tests for Rabbit No. 5, and 2 out of 4 tests for Rabbit No. 8. In every instance later tests on the same animals gave negative results. Altogether out of a total of 50 complement-fixation tests made on the sera of 14 treated rabbits, after the last injection of emulsion, positive fixation of complement was observed for only 4 animals in but 5 instances, and always in low titer.

Renal Function Tests. The urinalysis of the treated rabbits, both before and after the injection of the emulsions, showed transient albuminuria and occasional epithelial cells and casts in sediment. Such abnormalities, however, never were permanent. Later tests showed entirely normal urines from each animal.

The tests for phenolsulphonephthalein excretion made on each rabbit prior to the first injection of the emulsions showed an excretion of 60% or more of the dye in the 2-hour period, except in 2 instances where the excretion was only 24% (Rabbits Nos. 3 and 15). On tests made after the last injection of the emulsions each animal showed excretions of the dye closely similar to its previous result.

Conclusion. An attempt to induce in rabbits complement-fixing

antibodies and an experimental glomerulonephritis by injections of autolyzed homologous kidney was essentially negative in its results.

9003 C

Stability of Vitamin C, and Absence of Ascorbic Acid Oxidase in Citrous Fruits and Milk.

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It has been shown recently that in certain plants such as Hubbard Squash and Summer Squash, a powerful enzyme is present which oxidizes rapidly vitamin C[1, 2] although these plants contain almost none of this vitamin. Similar is the case with cucumbers. Statements have appeared more recently that "plant tissues which contain ascorbic acid apparently also contain an ascorbic acid oxidizing enzyme," and that the partial destruction of vitamin C in cow's milk is also brought about by ascorbic acid oxidase. The present writer could not find ascorbic acid oxidase in mammalian tissue,[3] and Roe and Barnum[4] found in human and rat blood cells and plasma an enzyme which reduces the reversibly oxidized form of ascorbic acid, thus having just the opposite effect from the ascorbic acid oxidase of plant tissues.

The aim of the present work was to find out whether the vitamin C content of juices of citrous fruits, which are excellent sources of the vitamin, is exposed to the destructive action of the ascorbic acid oxidase. In other words, whether these juices contain the oxidase.

Table I shows that the vitamin C content of the juices of oranges,

TABLE I.
Analysis of Vitamin C per cc. of Fruit Juice.

	After 5 hr. at 6° mg.	After 5 hr. at 38° mg.
Orange Juice (type *t*)	.51	.46
Lemon " (" *t*)	.56	.53
Tangerine " (" *a*)	.39	.33
Grapefruit " (" *a*)	.46	.44

1 Tauber, H., and Kleiner, I. S., PROC. SOC. EXP. BIOL. AND MED., 1935, **32**, 577.

2 Tauber, H., Kleiner, I. S., and Mishkind, D., *J. Biol. Chem.*, 1935, **110**, 211.

3 Tauber, H., *Experimental Enzyme Chemistry*, Burgess Pub. Co., Minneapolis, 1936.

4 Roe, J. H., and Barnum, G. L., *J. Nutrition*, 1936, **11**, 359.