showed signs of anaphylactic shock and the incidence of fatalities (78%) did not differ significantly from that observed in the original sensitized control group (Group I).

The duration of the inhibitory effect of a single intramuscular injection of Cortisone was tested in additional groups of sensitized mice. Complete protection from fatal anaphylaxis was observed as early as 6 hours after the administration of 3.0 mg of Cortisone and this persisted for at least 48 hours. After 96 hours, however, the protective effect had largely been dissipated since at this point the challenging dose of antigen produced fatal anaphylactic shock in approximately 40% of the animals.

Summary. The administration of Cortisone to 42 sensitized mice 18 hours before the intravenous injection of a challenging dose of antigen prevented fatal anaphylactic shock in 38 animals. In contrast, 28 of 34 sensitized but unprotected control mice died in anaphylactic shock.

The authors with to thank Mr. Bernard K. Friedman and Mrs. Jacqueline Isola for their technical assistance in this work.

The Cortisone used in this investigation was purchased by funds from The United States Public Health Service.

Received August 18, 1950. P.S.E.B.M., 1950, v75.

Effect of Testosterone Propionate on Total Urinary Nitrogen Excretion of the Rat Following Burns.* (18139)

J. W. BRAASCH, G. E. WAKERLIN, J. H. BELL, S. M. LEVENSON.

From the U. S. Army Medical Nutrition Laboratory, an Installation under the Jurisdiction of the Surgeon General; the Departments of Physiology, and of Surgery, University of Illinois College of Medicine.

Trauma is frequently followed by an increased total urinary nitrogen excretion(1-4). It is the consensus that this increased excretion of nitrogen represents increased tissue breakdown or failure of tissue formation with subsequent signs and symptoms of protein depletion. A substance which would promote tissue synthesis might be of value to prevent or ameliorate the accompaniments of protein depletion in damaged individuals. Testosterone and related compounds are substances which have been shown to be capable of lowering urinary nitrogen excretion(5-7) and causing muscle tissue and organ hypertrophy with concomitant increase in total protein and water content of these tissues(8-10).

In view of these facts it was considered important to determine the effect of testosterone propionate on the urinary nitrogen excretion following burns. It was also hoped that such a study might aid in the elucidation of the mechanism of the metabolic re-

* The opinions expressed in this paper are those of the authors and do not necessarily represent the official views of any governmental agency.

1. Lucido, J., *Ann. Surg.*, 1940, v111, 640.

2. Hirshfeld, J. W., Abbott, W. E., Pilling, M. A., Heller, C. G., Meyer, F., Williams, H. H., Richards, A. J., and Obi, R., *Arch. Surg.*, 1945, v50, 194.

3. Cuthbertson, D. P., *Biochem. J.*, 1930, v24, 1244.

4. Brunschwig, A., Clark, D. E., and Corbin, N., *Ann. Surg.*, 1942, v115, 1091.

5. Kenyon, A. T., Knowlton, K., Sandiford, I., Koch, F. C., and Lotwin, G., *Endocrinology*, 1940, v26, 26.

6. Kochakian, C. D., and Murlin, J. R., *J. Nutrition*, 1935, v10, 437.

7. Kochakian, C. D., and Moe, J., Josiah Macy Conference on Metabolic Aspects of Convalescence, New York, Josiah Macy, Jr. Foundation, 1946, v12, 104.

8. Korenchevsky, V., Hall, K., and Ross, M. A., *Biochem. J.*, 1939, v33, 213.

9. Kochakian, C. D., Humm, J. H., and Bartlett. M. N., *Am. J. Physiol.*, 1948, v155, 242.

10. Kochakian, C. D., and Stettner, C. E., *Am. J. Physiol.*, 1948, v155, 255.

TABLE I. Composition of Liquid Diet.

Sucrose	860 g
Egg albumin (Merck)	160 "
Cellu flour (soy bean)	120 "
Dried brewers' yeast powder	100 "
Salt mixture*	40 "
Cod liver oil	30 "
Mazola oil	30 "
Wheat germ oil	30 "
Water to make	2000 ml

* Salt mixture: Primary sodium phosphate, 14.0 g; calcium chloride, 12.0 g; monobasic potassium phosphate, 5.2 g; cupric sulfate, 4.0 g; ferric citrate, 3.0 g; magnesium sulfate, 1.0 g; manganese sulfate, 0.8 g; zinc chloride, 10 mg; potassium iodide, 10 mg.

sponse to damage. Previous work on this problem has been inconclusive. The clinical investigations(11-15) were of necessity inadequately controlled and in one unsatisfactory study with animals(16) only 2 dogs were used.

Methods. Adult female and young adult male albino rats of Harlan stock were adapted for 6 days to the force-feeding(17) of a liquid diet (Table I) modified from Ingle *et al.*(18). From the end of the adaptation period to the end of the experiment each of the animals was given 11 ml of this diet 2 times daily. By calculation the diet yielded about 0.2 calorie per g of body weight per day and was made up, on a caloric basis, of 18% protein, 63% carbohydrate, and 19% fat. During the experimental period the animals were kept individually in metabolism cages from which the urine was collected every 2 days. Before collection it was preserved with dilute sulfuric or oxalic acid and toluol, and after collection it was kept in a refrigerator or deep freeze until the total nitrogen determinations were carried out by the macro- or micro-Kjeldahl procedure.

Five groups of animals were studied, of which 3 were burned. After a suitable control period, the first group, female animals, was given daily subcutaneously either 2.0 mg of testosterone propionate in 0.2 ml of peanut oil (7 animals) or just 0.2 ml of peanut oil (5 animals). Two days after the onset of this medication they were burned. The second and third groups, male animals, varied only in the time sequence of the start of the medication with respect to the burn. In one, the medication was started 12 hours before the burn (7 and 5 animals) and in the other, 4 days after the burn (7 and 6 animals). The fourth group consisted of females which were just given 2.0 mg of testosterone propionate in 0.2 ml of peanut oil (6 animals), and the fifth group consisted of males which were given similar doses of testosterone propionate in peanut oil (5 animals) or just peanut oil (5 animals). Under intraperitoneal pentobarbital anesthesia in Groups 1 and 2 and ether anesthesia in Group 3, an approximately 30% area burn was produced by dipping the clipped backs of the rats into a constant temperature water bath at 73°C for 30 seconds after the method of McCarthy (19). The total urinary nitrogen excretion was followed after the burn procedure for a length of time sufficient for the effectiveness of the testosterone propionate to become statistically significant and then disappear. A period of 10 to 12 days usually sufficed. In the case of the animals not burned but just given testosterone propionate in peanut oil or peanut oil alone, the urinary nitrogen excretion was followed after the on-

11. Abbott, W. E., Hirshfeld, J. W., Williams, H. H., Pilling, M. A., and Meyer, F. L., *Surgery,* 1946, v20, 284.

12. Howard, J. E., Winternitz, J., Parson, W., Bigham, R. S., and Eisenberg, H., *Bull. Johns Hopkins Hosp.,* 1944, v75, 209.

13. Browne, J. S. L., Josiah Macy Conference on Metabolic Aspects of Convalescence, New York, Josiah Macy Jr. Foundation, 1943, v2, 48.

14. Stevenson, J. A. F., and Browne, J. S. L., Josiah Macy Conference on Metabolic Aspects of Convalescence, New York, Josiah Macy Jr. Foundation, 1943, v5, 50.

15. Schenker, V., and Browne, J. S. L., Josiah Macy Conference on Metabolic Aspects of Convalescence, New York, Josiah Macy Jr. Foundation, 1942, Supp. 1, 44.

16. Meyer, F. L., Hirshfeld, J. W., and Abbott, W. E., *J. Clin. Invest.,* 1947, v26, 796.

17. Reinecke, R. M., Ball, H. A., and Samuels, L. T., PROC. SOC. EXP. BIOL. AND MED., 1939, v41, 44.

18. Ingle, D. J., Sheppard, R., Evans, J. S., and Kuizenga, M. H., *Endocrinology,* 1945, v37, 341.

19. McCarthy, M. D., *J. Lab. Clin. Med.,* 1945, v30, 1027.

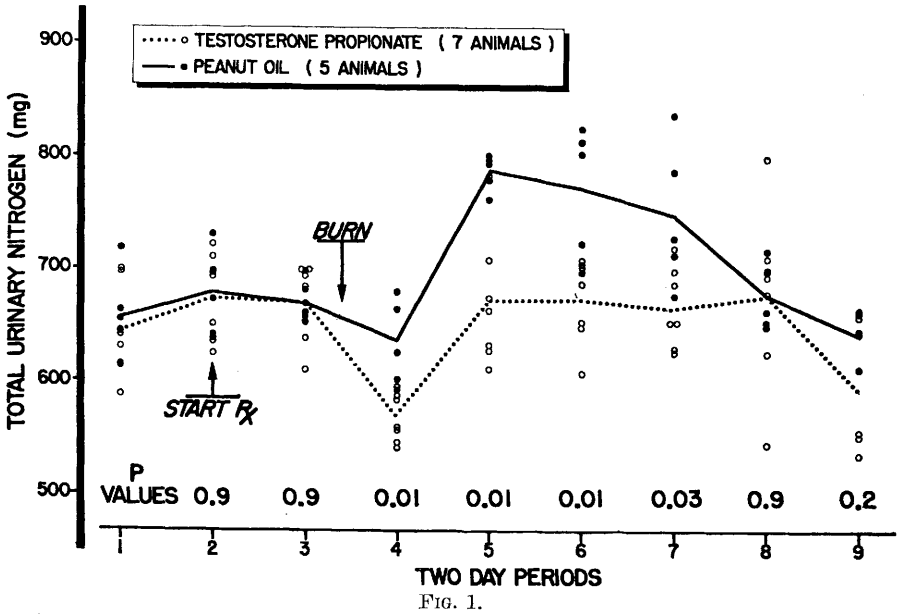

FIG. 1.

Effect of thermal injury and subcutaneous testosterone propionate and peanut oil administration on urinary nitrogen excretion of adult female rats.

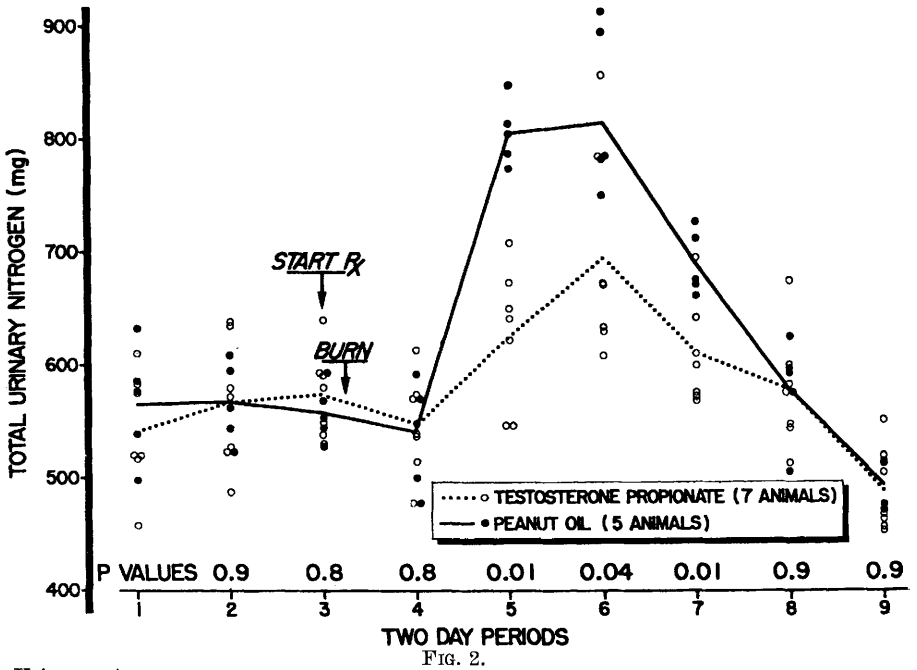

FIG. 2.

Urinary nitrogen excretion following thermal burns of young adult male rats. Effect of subcutaneous testosterone propionate and peanut oil administration started 12 hours before the burn.

set of the medication until it returned to the control level, spontaneously in the case of the males (14 days), or after stopping the medication in the case of the females 18 days after its inception.

Results. The burn procedure used re-

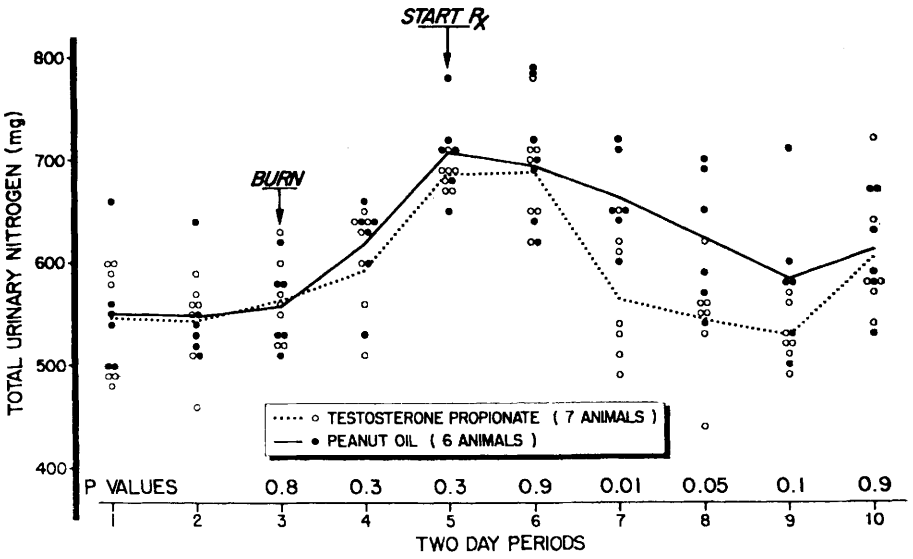

FIG. 3.

Urinary nitrogen excretion following thermal burns of young adult male rats. Effect of subcutaneous testosterone propionate and peanut oil administration started 4 days after the burn.

sulted in a fairly uniform third degree burn of approximately a third of the total body surface area as judged by histologic examination† and an estimate of the total area of skin showing changes characteristic of a burn of this degree. At about the eighth post-burn day the involved skin appeared as a thick, dark crust without gross evidence of infection.

Fig. 1, 2, and 3 depict the data obtained on the urinary nitrogen excretion of burned animals. In the control peanut oil group there was an increase in total urinary nitrogen excretion beginning approximately the second to fourth post-burn day and ending approximately on the tenth to twelfth post-burn day in both sexes. When the nitrogen excretion of those animals which were burned but given testosterone propionate is compared with the foregoing, it can be seen that the rise expected after burning is markedly depressed by the testosterone propionate administration in both sexes beginning two to three days after the onset of the medication.

Fig. 4 and 5 show that the administration of 2.0 mg of testosterone propionate daily to normal male and female animals resulted in a maximal fall in urinary nitrogen excretion of approximately 140 mg of nitrogen per two-day period in the female and approximately 100 mg of nitrogen per two-day period in

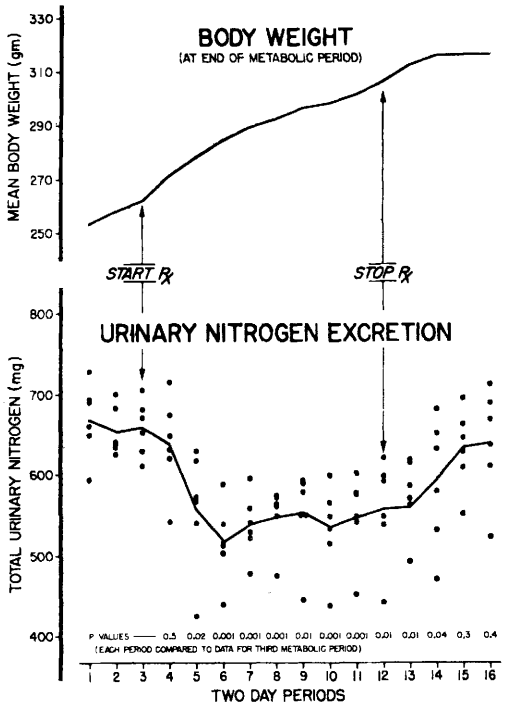

FIG. 4.

Effect of subcutaneous testosterone propionate administration on body weights and urinary nitrogen excretion of 6 adult female rats.

† Kindly performed by Dr. C. L. Pirani.

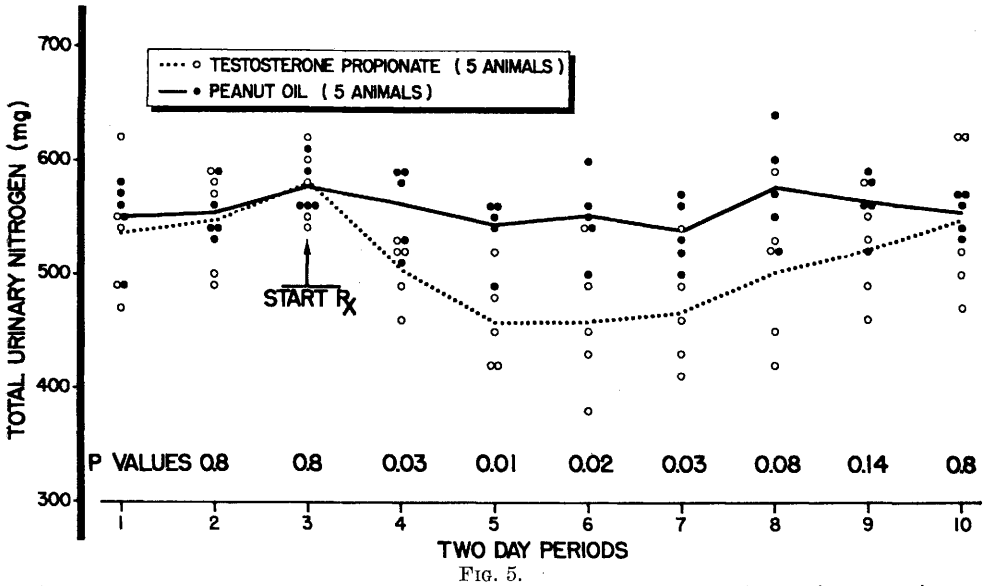

FIG. 5.

Effect of subcutaneous testosterone propionate and peanut oil administration on urinary nitrogen excretion of young adult male rats.

the male animals. The depression began in both sexes two to four days after the onset of the medication. It continued for from 10 to 14 days in the case of the males after which time it apparently spontaneously disappeared. The depression in the urinary nitrogen excretion of the females continued for as long a time as the drug was given (18 days). After this time, the drug was discontinued and the nitrogen excretion rose toward the pre-injection levels.

On comparing the calculated effect of testosterone propionate in the burned animals with its effect in the normal animals, in both sexes it appears to be essentially unchanged by the burn for about 8 days, but thereafter disappears. In the unburned males the effect possibly lasts longer than in the burned, but in the females it certainly lasts longer in the unburned than burned.

The results of a statistical analysis using Fisher's "t" test of significance of the aforementioned changes are shown in Fig. 1-5. According to these calculations the changes described are significant.

Discussion. It has been shown in this study that for a certain time interval the effect of testosterone propionate on the urinary nitrogen excretion of the burned rat is essentially equivalent to its effect in the normal animal. If the albino rat and the human being are sufficiently similar as to their metabolic reaction to thermal trauma and testosterone propionate administration, one might predict that for a certain period post-burn, the administration of testosterone propionate would save the individual approximately 0.03 to 0.1 g of nitrogen per kg per day(5,20), or in the case of a 70 kg subject, from 57 to 200 g of tissue per day(21).

Whether this result would be of benefit is questionable in view of some possible side effects of the drug. These are the enhancement of signs of masculinization following burns(22), a possible adverse effect on healing at the burn site, and an increase in the retention of water and salts following trauma (5,23).

Following thermal injury(22) and other types of damage(15) the 17-ketosteroid ex-

20. Albright, F., Parson, W., and Bloomberg, E., *J. Clin. Endocrinol.*, 1941, v1, 375.

21. Hastings, A. B., and Eichelberger, L., *J. Biol. Chem.*, 1937, v117, 73.

22. Cope, O., Nathanson, I. T., Rourke, G. M., and Wilson, H., *Ann. Surg.*, 1943, v117, 937.

23. Browne, J. S. L., Karady, S., and Selye, H., *J. Physiol.*, 1939, v97, 1.

cretion of female and male patients is at first briefly increased, then markedly decreased. Two explanations of this decrease in 17-ketosteroid excretion are more acceptable than others. They are that either there is a decreased production and secretion of the precursors of these compounds or that these precursors are metabolized differently following damage than in the normal organism. It has been demonstrated that in eunuchoid humans(5) and castrate albino rats(7) with a subnormal production of testosterone-like compounds, the effect of administered testosterone on nitrogen excretion is increased over that found in the normal organism. Therefore, if there was a decreased secretion of the 17-ketosteroid precursors following damage, one might expect the quantitative action of testosterone propionate to be increased in the burned organism over the normal response. It has been shown in this study that the effectiveness of testosterone propionate on the urinary nitrogen excretion is markedly shortened in female and possibly shortened in male animals following burns. There are no data available as to the quantitative changes of the 17-ketosteroid excretion of rats following burns. If the pattern is similar to that described in humans, the findings in this study suggest the second of the two postulates, that the testosterone compounds are metabolized differently following damage. This possibility is further borne out by the findings of Forsham *et al.*(24) and Mason *et al.*(25). These workers found increased excretions of 17-ketosteroids and 11-oxysteroids following the injection of purified ACTH into humans. That the hypophyseal, adrenal cortex mechanism is activated following burns has been demonstrated by Harkins and Long(26) and Venning *et al.*(27). Therefore, that there is an increased production of 17-ketosteroid precursors following burns seems possible.

It can be seen in Fig. 1 and 2 that in the case of the burned females and the first group of burned males the disappearance of effectiveness of testosterone propionate coincides with the return of the nitrogen excretion of the control animals to the pre-burn level. Whether this temporal relationship is causal or coincidental is unknown. It is interesting to note that in the case of the second group of burned males (Fig. 3) in which the medication was begun four days after the burn, the response to testosterone is similar to that in the first group of male animals in which the testosterone was started twelve hours before the burn. We have tried to extend this investigation to include observations on the effectiveness of testosterone propionate when started 10 to 20 days after the burn. These attempts have been seriously handicapped by several factors. These include a high mortality among animals force-fed at this time, an extreme variability in nitrogen excretion, and a tendency for a secondary rise in nitrogen excretion to occur beginning approximately 14 days post-burn.

Summary. The subcutaneous administration of testosterone propionate depressed the rise in urinary nitrogen excretion following burns in male and female rats. The effect of testosterone propionate in reducing the total urinary nitrogen excretion of male and female adult rats is essentially unchanged by a thermal burn for a period of 8 days after the burn or onset of the testosterone medication. For 2 to 4 days after this period the effect of the drug post-burn is no longer evident. In normal female animals the effectiveness of testosterone propionate continues for at least 18 days whereas in normal males it disappears at 12 to 14 days. Possible reasons for this wearing-off of the effectiveness of testosterone propionate after burns are considered. Various problems connected with the possible use of testosterone

24. Forsham, P. H., Thorn, G. W., Prunty, F. T. G., and Hills, A. G., *J. Clin. Endocrinol.*, 1948, v8, 15.

25. Mason, H. L., Power, M. H., Rynearson, E. H., Ciaramelli, L. C., Li, C. H., and Evans, H. M., *J. Clin. Endocrinol.*, 1948, v8, 1.

26. Harkins, H. N., and Long, C. N. H., *Am. J. Physiol.*, 1945, v144, 661.

27. Venning, E. H., Browne, J. S. L., and Schenker, V., Josiah Macy Conference on Metabolic Aspects of Convalescence, New York, Josiah Macy Jr. Foundation, 1945, v11, 133.

propionate in the damaged patient are discussed.

A part of this report is based on work done by Capt. Braasch in the Department of Physiology of the University of Illinois School of Medicine in partial fulfillment of the requirements for the degree of Master of Science in Physiology. The authors wish to thank the Schering Corporation for a generous supply of testosterone propionate.

Received July 21, 1950. P.S.E.B.M., 1950, v75.

Blood Amino Acid Level and Adrenal Cholesterol Content During "Tourniquet Shock" in the Rat. (18140)

JOHN L. GRAY, ALBERT L. BOTKIN, ELIZABETH J. MOULDEN AND H. JENSEN.

From the Medical Department Field Research Laboratory, Fort Knox, Ky.

It has been established that "shock" will produce an elevation of the blood amino acid level and a decrease in adrenal cholesterol content(1,2). The "sparing" effect of administration of isotonic sodium chloride solution in lowering mortality in traumatic shock has been recorded by various investigators (3-5). It was of interest to determine the effects of saline therapy on the blood amino acid level and the adrenal cholesterol content of rats in "tourniquet shock." The findings of this investigation are presented in this paper.

Experimental. White male albino rats of the Sprague-Dawley strain, weighing 220 to 290 g and fed on Purina dog chow checkers, were used. The procedure for shock production in mice developed by Rosenthal(4) and used by Harkins(5) for rats, was employed (6). The animals were fasted for 16 hours before the experiment but were permitted water. With the animals under light ether anesthesia, elastic rubber band (Eberhard Faber No. 30) tourniquets were applied to the limbs and the teeth were clipped to prevent tourniquet removal or self-laceration. The animals were placed in individual cages without access to food or water. Depending on the type of experiment, the animals were sacrificed by decapitation without tourniquet release or tourniquets were removed and the animals replaced in the individual cages to be sacrificed at varying periods of time following release. For the experiments reported here, the occlusion was of both hind-limbs for 4½ hours and of hind- and fore-limb unilaterally for 3 hours or for 5 hours. For treatment of the induced "shock," a 0.9% NaCl solution was given intraperitoneally, 10 ml immediately following tourniquet release, 10 ml 2 hours later and 3½ hours after release the amount necessary to bring the total administered to 10% of the body weight. All experiments were conducted at room temperatures of 20 to 25°C. After decapitation of the animal, the adrenal glands were immediately dissected, weighed and analyzed for cholesterol according to the method of Schoenheimer and Sperry(7). The total cholesterol was determined in each case and the amount of cholesterol is reported as mg/100 mg of fresh wet adrenal tissue. Blood amino acid(8), urea(9), and hematocrit determinations were carried out on blood ob-

1. Davis, H. A., Shock and Allied Forms of Failure of the Circulation. New York, Grune and Stratton, 1949.

2. Wilhelmi, A. E., *Ann. Rev. Physiol.*, 1948, v10, 259.

3. Meyer, J., Lendrum, B., and Katz, L. N., *Am. J. Physiol.*, 1945-46, v145, 151.

4. Rosenthal, S. M., *Pub. Health Rep.*, 1943, v58, 1429.

5. Harkins, H. N., *Am. J. Physiol.*, 1947, v148, 538.

6. Hamolsky, M. W., Gierlach, Z. S., and Jensen, H., *Am. J. Physiol.*, in press.

7. Schoenheimer, R., and Sperry, W. M., *J. Biol. Chem.*, 1934, v106, 745.

8. Frame, E. G., Russell, J. A., and Wilhelmi, A. E., *J. Biol. Chem.*, 1943, v149, 255.