fluence is on carbohydrate metabolism. An approach to this problem is made possible by the development of technics for the quantitative analysis of steroids in adrenal venous blood.

Summary. Adrenal venous blood of the dog has been quantitatively analyzed by paper chromatography for steroid components. Fourteen fractions have been isolated; 3 have been identified as 17-hydroxycorticosterone, corticosterone and 11-desoxy-17-hydroxycorticosterone; 7 of the unidentified fractions exhibit chemical reactions characteristic of adrenocorticosteroids.

ADDENDUM. In a subsequent experiment conducted in collaboraton with Dr. Hans Hirschmann a sample containing Fractions 7 and 8 was acetylated and chromatographed on paper 90½ hours, employing the system propylene glycol-hexane described by Savard. Five distinct zones were found by examining the strip under ultraviolet light. These subfractions (numbered 1-5 in order of their position on the chromatogram, starting at the origin) were eluted with methanol and were examined spectroscopically in this solvent and after reaction with phenylhydrazine-sulfuric acid reagent. Subfractions 1, 2, and 3 absorbed maximally near 240 mμ; subfraction 4, near 235 mμ, while subfraction 5 showed only a shoulder in this region. With phenylhydrazine reagent subfractions 1 and 5 absorbed maximally at 390-400 mμ, whereas subfractions 2, 3, and 4 gave products which did not exhibit absorption peaks in this region. Each subfraction was treated with acetylcholinesterase in glycylglycine buffer at pH 7.5 for 4 hours at 30°C, and assayed for sodium-retaining activity by the method described in an earlier communication(5). All subfractions were inactive except subfraction 4 which possessed high sodium-retaining activity. No claim of homogeneity is made for any of these subfractions, but it would appear that the sodium-retaining activity and the chromogenicity in the phenylhydrazine reagent shown by our previously described Fraction 8 are not due to the same substance.

1. Burton, R. B., Zaffaroni, A., and Keutmann, E. H., *J. Biol. Chem.*, 1951, v188, 763.

2. Reich, H., Nelson, D. H., and Zaffaroni, A., *J. Biol. Chem.*, 1950, v187, 411.

3. Bush, I. E., *Biochem. J.*, 1952, v50, 370.

4. Zaffaroni, A., and Burton, R. B., *Arch. Biochem. and Biophysics*, 1953, v42, 1.

5. Farrell, G. L., and Richards, J. B., PROC. SOC. EXP. BIOL. AND MED., 1953, v83, 628.

6. Simpson, S. A., Tait, J. F., and Bush, I. E., *The Lancet*, 1952, v2, 226.

Received September 3, 1953. P.S.E.B.M., 1953, v84.

Increased Excretion of Dehydroascorbic and Diketogulonic Acids in Urine of Rats after Standardized Temperature Shock. (20550)

MARY MILLS MONIER AND ROSLYN J. WEISS. (Introduced by Joseph H. Roe.)

From the Department of Biochemistry, School of Medicine, The George Washington University, Washington, D.C.

Increased urinary excretion of 2 oxidation products of ascorbic acid (AsA), namely dehydroascorbic acid (DHA) and diketogulonic acid (DKA) has been observed in rats during exposure to an environmental temperature of 0°C(1) and after exposure to high doses of X-ray(2), as compared to control excretions on comparable rats under normal conditions. A third type of stress, namely standardized temperature shock(3), has been tested and the results observed are reported here.

Experimental. The experimental animals were adult albino rats of both sexes, belonging to the same strain as those employed in the previous work(1,2), which were housed in individual metabolism cages as previously described. At the beginning of the control period, each animal was anesthetized with nembutal, and immersed up to the neck in a water-bath maintained at 37°C for 15 seconds.

TABLE I. Urinary Excretion of Ascorbic, Dehydroascorbic and Diketogulonic Acids by Rats before and after Severe Temperature Shock.

Fraction	No. of analyses: Control	No. of analyses: Temperature shocked	Avg 24 hr excretion, μg with S.D.: Control	Avg 24 hr excretion, μg with S.D.: Temperature shocked	"F"
AsA	99	87	441 ± 136	485 ± 178	3.7
DHA	99	87	2 ± 5	43 ± 39	112 *
DKA	99	87	0 ± 0	48 ± 41	79 *

* Probability of variance ratio, "F," less than .01.

The animals were then placed in their individual cages and the excretion of AsA, DHA and DKA was determined each 24-hour period for 7 to 10 days following the mock temperature shock by the method previously employed(4). When the control excretion for each animal had been established, the experimental period began. Each animal was anesthetized with nembutal, and immersed to the neck in a water-bath held at 60°C for 15 seconds; the animals were replaced in their cages and the daily excretion of AsA, DHA and DKA determined for 7 to 10 days following the temperature shock. There were no fatalities during this period.

Results. The results obtained are summarized in Table I. As with cold environment and X-ray irradiation, exposure to this degree of temperature shock caused a marked increase in urinary excretion of DHA and DKA, which is highly significant. There was a slight increase in AsA excretion, which did not attain the level of significance.

Discussion. It appears that this third type of stress, which differs from the 2 previously reported in physiologic mechanism, has the same effect of increasing excretion of oxidized ascorbic acid in the urine. The physiologic mechanism responsible for this remains unknown. Attempts to demonstrate the adrenal glands and the kidneys as the site of formation have been unsuccessful(5).

Summary. 1. Adult albino rats were subjected to temperature shock by immersion in water at 60°C for 15 seconds. 2. Urinary excretion of dehydroascorbic and diketogulonic acids increased greatly over control values established before temperature shock.

1. Monier, M. M., and Weiss, R. J., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 446.

2. ———, *ibid.*, 1952, v81, 598.

3. Hazan, S. J., and Treadwell, C. R., PROC. SOC. EXP. BIOL. AND MED., 1948, v68, 684.

4. Roe, J. H., Mills, M. B., Oesterling, M. J., and Damron, C. M., *J. Biol. Chem.*, 1948, v174, 201.

5. Monier, M. M., Byer, S., and Weiss, R. J., USAF Rep. 21-1208-0003.

Received September 16, 1953. P.S.E.B.M., 1953, v84.

Cortisone on Oxygen Consumption of Granulation Tissue from the Rabbit. (20551)

DANTE G. SCARPELLI, RALPH A. KNOUFF, AND CLIFFORD A. ANGERER.

From the Departments of Anatomy and Physiology, The Ohio State University, Columbus.

It has been shown that cortisone and adrenocorticotrophin (ACTH) produce an inhibitory effect on growth of granulation tissue in rabbits(1) and around turpentine abscesses in rats(2); that ACTH causes a retardation of wound healing in humans as studied by serial biopsies(3). Histological studies of the latter reveal a lack of fibroblasts, capillaries and ground substance; a condition not unlike that found in scorbutic animals(3). This suggests an inhibition of anabolic processes which are of primary importance in tissue repair. The literature reveals no data exist relative to the action of cortisone, ACTH, or adrenocortical extract (ACE) on the metabolism of granulation tissue. This report in-