ly increased the period of survival. 5. Under completely anaerobic conditions the schistosomes survived for 5 days.

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Changes in Carcass Urea Following Evisceration in the Rat. (17618)

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The generally accepted conclusion that liver is the only mammalian tissue that can synthesize urea is based in major part upon the classical studies of Bollman, Mann and Magath(1) who noted that the level of blood urea remained steady following hepatectomy-nephrectomy in the dog, of Bollman and Mann(2) who found no evidence for the conversion of ammonia to urea in the liverless dog, and of Krebs and Henseleit(3) who found that the liver slice was the only tissue which could convert ammonia into urea. Maddock and Svedberg(4) found no evidence for urea synthesis in the liverless monkey. Observations upon the liverless animal have been limited to a few species and to survival periods of a few hours. We have studied changes in the blood and tissues of eviscerate rats that survive for periods of 48 hours and longer. Ingle, Prestrud and Nezamis(5) noted that the blood urea of the rat infused with solutions of glucose and insulin did not fall during the first 24 hours following evisceration although the kidney continued to excrete urea and the animal was in positive water balance, both of which might be expected to lower the urea of the body-water in the absence of urea synthesis. Since the circulation and water metabolism of the eviscerate rat are greatly changed from normal, it would be unsafe to assume that the urea content of its blood is representative of its distribution in the body water or that these observations on the rat provide valid evidence for the extra-hepatic synthesis of urea.

We have reinvestigated the problem by determining the urea content of the carcass and urines of rats at the time of evisceration and of similar rats 24 and 48 hours following evisceration. There was a decrease in the total urea of the carcass and urine following evisceration.

Methods. Male rats of the Sprague-Dawley strain were fed Archer Dog Pellets. At attainment of a weight of 185 to 205 g, the inferior vena cava was ligated between the liver and kidneys in order to cause the development of a collateral circulation. When the animals reached a weight of 250 ± 2 g they were anesthetized with cyclopal and eviscerated by the method of Ingle(6). Asepsis was preserved in both stages of the operation. A solution containing glucose, insulin, and 0.9% sodium chloride was infused into the saphenous vein of the right hind leg by means of a constant injection machine which delivered fluid from each of 6 syringes at the rate of 20 cc in 24 hours. The glucose load was 44 mg per 100 grams of rat per hour and 4 units of regular insulin (Lilly) per rat per 24 hours. Temperature was constant at 26.5°C.

The total urea of the carcass was determined as follows: The tail of the eviscerate rat was cut into small pieces and placed in

1. Bollman, J. L., Mann, F. C., and Magath, T. B., *Am. J. Physiol.*, 1924, v69, 371.

2. Bollman, J. L., and Mann, F. C., *Am. J. Physiol.*, 1930, v92, 92.

3. Krebs, H. A., and Henseleit, K., *Klin. Wochschr.*, 1932, v11, 757.

4. Maddock, S., and Svedberg, A., *Am. J. Physiol.*, 1938, v121, 203.

5. Ingle, D. J., Prestrud, M. C., and Nezamis, J. E., *Am. J. Physiol.*, in press.

6. Ingle, D. J., *Exp. Med. and Surg.*, 1949, v7, 34.

TABLE I.
Total Carcass Urea of Eviscerate Rats. Means and Standard Errors of the Means and Differences Between Means.

Hr following evisceration	No. rats	Total urea, mg	Difference from controls
0	26	203.2 ± 6.3	
24	24	185.6 ± 4.6	17.6 ± 7.8
48	16	180.5 ± 7.7	22.7 ± 10.0
24- and 48-hr groups combined	40	183.3 ± 4.1	19.9 ± 7.5

the body cavity. The carcass was chilled in a deep freeze for one hour. Freezing of the carcass was avoided since it hindered grinding. The carcass was then passed twice through a power grinder which had been cooled by grinding dry ice in it. It was necessary to remove skin from the blade after the first grinding and to cut it into smaller pieces. Finely powdered dry ice was sprinkled over the tissue whenever a delay occurred. The ground tissue was transferred to a Waring blendor containing 150 cc of chilled saline plus the urine excreted by the rat following evisceration. After an initial stirring, 150 cc more saline was added, the blendor covered, and the mixture homogenized for 2 minutes. Two 50 cc aliquots were each added to 400 cc N/12 H_2SO_4 in 2-liter Erlenmyer flasks and allowed to stand one hour with occasional shaking. To each flask was added 50 cc of 10% sodium tungstate, the flask stoppered, the contents shaken and allowed to stand 30 minutes before filtering. The remaining homogenate was measured and the total volume recorded. The Folin-Wu filtrate was analyzed for urea by gasometric measurement of the carbon dioxide formed by the action of urease according to the procedure of Van Slyke(7).

Experiments and results. The total carcass urea was determined on 26 rats immediately following evisceration, on 24 rats that were infused for 24 hours following evisceration, and on 16 rats that were infused for 48 hours following evisceration. All of the urine excreted by each rat following evisceration was added to the carcass at the time of homogenization. The data are summarized in Table I. When the 24 and 48 hour groups are combined and compared with the control animals, the ratio between the difference in means and the standard error of the difference is 2.65. From the statistical standpoint, the chances that there was a true decrease in total urea following evisceration are greater than 99 in 100. The acceptance of statistical analyses of data requires the assumption that all of the uncontrolled variables which affected the results were randomized. We are not aware of any incidental errors or of any extra-renal means whereby urea could have been lost from the carcass, but such may have existed.

The acceptance of this evidence that urea disappears from the carcass of the eviscerate rat to a greater extent than can be accounted for by urinary excretion seems to require the conclusion that urea can be metabolized in the absence of the liver. Bollman and Mann(2) observed that the administration of urea to the hepatectomized dog caused a rise in urinary ammonia, thereby adding support to several lines of indirect evidence that the kidney can convert urea to ammonia. The studies relating to the origin of ammonia have been reviewed by Peters and Van Slyke(8) who concluded that urea is not a precursor of ammonia. Leifer, Roth and Hempelmann(9) have reported that the carbon of urea labeled with C_{14} is excreted in the expired air of the normal rat. This observation does not prove the catabolism of urea since there may have been an exchange of carbon between urea and CO_2 without a decrease in total urea. Rose, Smith, Wormack and Shane(10) have shown that urea can be

7. Van Slyke, D. D., *J. Biol. Chem.*, 1927, v73, 695.

8. Peters, J. P., and Van Slyke, D. D., *Quantitative Clinical Chemistry. Interpretations,* Williams and Wilkins, Baltimore, 1946, v1, 873.

9. Leifer, E., Roth, L. J., and Hempelmann, L. H., *Science,* 1948, v108, 748.

utilized for the synthesis of non-essential amino acids by the intact young rat. The possibility that urea can be utilized in anabolic processes in the eviscerate rat should be considered.

The possibility that urea can be metabolized in the eviscerate rat weakens one point of evidence that urea synthesis occurs only in the liver. The earlier studies(1) on nephrectomized-hepatectomized animals have shown that the blood and tissue urea did not change during the times that these observations were made. The assumption that urea could not be metabolized therefore required the conclusion that no extra-hepatic synthesis of urea could have occurred since the concentration in the body fluids remained unchanged. However, if urea can be metabolized, it is hypothetically possible for extra-hepatic urea synthesis to coexist with an equal or higher rate of urea metabolism without increasing the concentration of urea in the body. As an analogy, it was first assumed that gluconeogenesis occurs only in the liver because the blood glucose falls following hepatectomy. It has now been clearly established that glucose can be formed in the kidney, but that this extra-hepatic gluconeogenesis is masked by a higher rate of glucose utilization(11). Evidence has been reported by Graham, Houchin and Turner(12) that the active mammary gland of the goat can synthesize urea.

Summary. Male rats of the Sprague-Dawley strain were eviscerated at a weight of 250 g and were given intravenous infusions of solutions of glucose, insulin and 0.9% sodium chloride for periods up to 48 hours. Comparisons were made of the total carcass plus urinary urea in 26 control rats taken immediately following evisceration, 24 rats which were killed 24 hours following evisceration and 16 rats which were killed 48 hours following evisceration. The average total carcass urea of the 24-hour rats was lower than that of the controls, and the average value for the 48-hour group was still lower. From the statistical standpoint, it is highly probable that there was some extra-renal loss of urea following evisceration. These results are indirect evidence for the occurrence of urea catabolism. The results are in agreement with observations on other species in showing that there is no rise in the urea concentration of the body in the absence of the liver.

10. Rose, W. C., Smith, L. C., Wormack, M., and Shane, M., *J. Biol. Chem.*, 1949, v181, 307.

11. Reinecke, R. M., *Am. J. Physiol.*, 1943, v140, 276.

12. Graham, W. R., Houchin, O. B., and Turner, C. W., *J. Biol. Chem.*, 1937, v120, 29.

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Increased Tolerance of Mice to Lethal X-Radiation as a Result of Previous Sublethal Exposures.* (17619)

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The pathogenesis of acute radiation illness is poorly understood. The discovery of factors that may influence the survival after exposure to doses of radiation in the lethal range will help considerably in the understanding of the mechanisms that produce this illness. The release of heparin and histamine in irradiated animals has been reported(1,2,3). This suggests an anaphylactoid response. In fact a similarity between hypersensitive states and

* The opinions or assertions contained herein are the private ones of the authors and are not to be construed as official or reflecting the views of the Navy Department or the naval service at large.

1. Allen, J. G., *et al., University of Chicago Metallurgical Laboratory*, Nov., 1946.

2. Cronkite, E. P., *Am. J. Path.*, 1947, v23, 891.

3. Ellinger, F., *Science*, 1946, v104, 502.