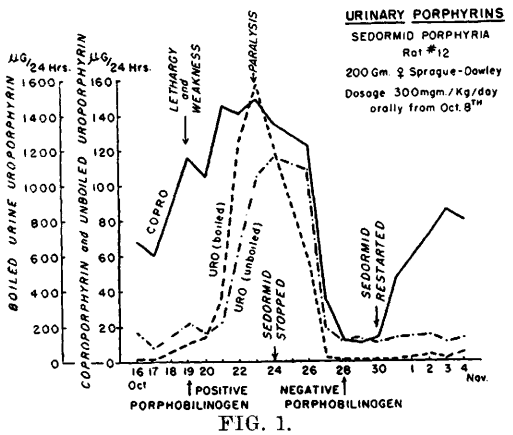

FIG. 1.

phyrin methyl ester suggest that it is probably uroporphyrin type III or the Waldenström uro-type porphyrin.

Subcutaneous injections of Sedormid in dosages that are effective orally caused no disorder of porphyrin excretion (Table I). Propylene glycol given alone by stomach tube likewise produced no change in urinary porphyrins.

The clinical features presented by Sedormid treated animals, the presence of porphobilinogen, increased amounts of predominantly type III coproporphyrin, and enormous amounts of uroporphyrin in the urine very closely approximate the criteria considered diagnostic of porphyria in human adults. The ready availability of extensive biochemical data on rat liver seems to make rats preferable to rabbits for the study of intermediary porphyrin metabolism. Furthermore, the similarity of our findings to those of the University of Minnesota investigators indicates that the effect of Sedormid is not limited to rabbits. The work is being continued.

Summary. Sedormid administered to rats by stomach tube has caused marked increases in urinary uroporphyrin, coproporphyrin, and porphobilinogen, and concurrent clinical changes of paralysis, lethargy and gastrointestinal dysfunction. These findings constitute a syndrome closely resembling human adult porphyria. The disorder thus produced resembles that recently reported in rabbits.

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Rat Plasma Aminopeptidase Activity in Hemorrhagic Shock.* (20420)

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As part of an investigation of the biologic significance of peptidases of blood and tissue, plasma peptidase activity was studied in rats subjected to hemorrhagic shock. The striking alterations which appeared are presented in this report.

Materials and methods. Shock was established in rats by bleeding from the cut tail as described previously(1). Serial samples of nonhemolyzed heparinized plasma were obtained by centrifugation of blood removed from

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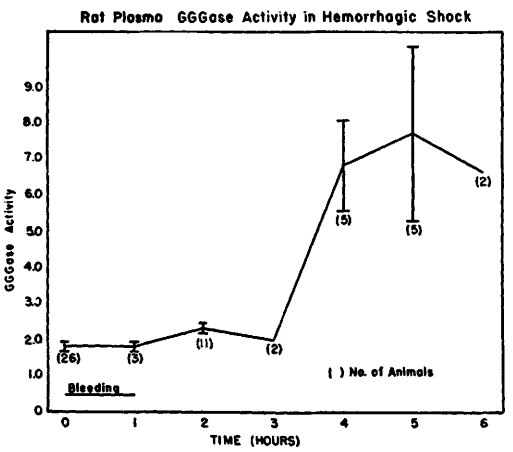

FIG. 1. GGGase activity (see text) at 4 and 5 hr is significantly higher ($p<0.01$) than that measured at zero, one and 2 hr. Vertical lines represent stand. errors.

the cut tail or, terminally, from the abdominal aorta. Plasma aminopeptidase (GGGase) activity was determined by measuring the rate at which a synthetic substrate, glycylglycylglycine (GGG)‡ was hydrolyzed by rat plasma. The enzyme-substrate mixture, consisting of 0.5 ml of 0.1 M GGG, 0.2 ml plasma, 0.5 ml of 0.02 M veronal buffer (pH 7.8) and 0.8 ml of 0.85% sodium chloride, was shaken constantly in a bath maintained at 38°C. Samples were withdrawn for analysis at one and 2 hours after initiation of enzymic hydrolysis. Measurements were obtained with a photometric technic(2). The mean of the one and 2 hour determinations was used to estimate plasma GGGase activity, defined as the number of μM of GGG hydrolyzed per hour per 0.1 ml of plasma per ml of hydrolysis mixture. Calculations were made on the assumption that there is cleavage of only one peptide bond for each molecule of substrate. This assumption is strengthened by the finding that, after hydrolysis had proceeded for 18 hours, the per cent of substrate split never exceeded 100%. Plasma α-amino nitrogen was determined by the method of Frame, Russell, and Wilhelmi(3).

Results. The effect of the production of hemorrhagic shock in rats on plasma aminopeptidase activity is charted in Fig. 1. No change in GGGase activity was discernible during the first 3 hours after the start of the bleeding procedure. After this period, however, a striking but variable rise appeared and persisted through the second 3-hour interval.

That plasma amino nitrogen increases in shock is well established. In 12 animals subjected to shock, GGGase activity and α-amino nitrogen were determined on the same plasma samples in 29 instances. As indicated in Fig. 2, despite considerable individual variation, the 2 sets of values tended to increase proportionately, so that there was a general positive correlation which is probably significant as suggested by the correlation coefficient of 0.45 with $p < 0.05$.

Discussion. The rise in plasma aminopeptidase during hemorrhagic shock becomes meaningful when viewed in the context of previous studies.

Interest in serum peptidases was stimulated by the search for a burn toxin. Zamecnik, Stephenson, and Cope(4) found that in dogs and calves subjected to burns, there is a rise in aminopeptidase activity in the serum and in the lymph draining the burned area. Beloff and Peters(5) made the complementary observation that proteinase concentration of skin decreases in areas where burns were produced by temperatures which did not inactivate this enzyme. More recently, Stern *et al.*

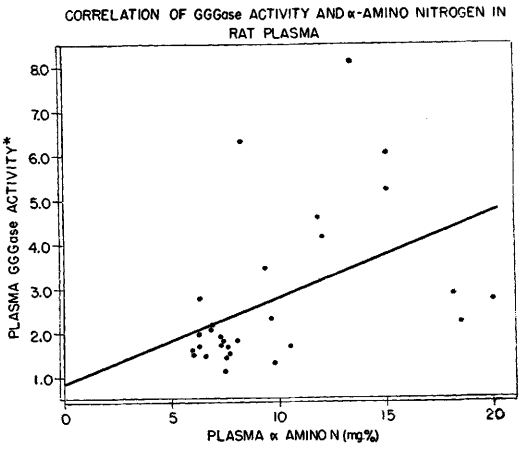

FIG. 2. Positive correlation between GGGase activity and α-amino nitrogen is shown ($r = 0.45$, $p<0.05$) for 29 plasma samples taken from 12 animals. The linear regression curve has the equation $Y = 0.193X + 0.854$.

* See text.

‡Provided through the courtesy of Dr. R. J. Floody, Hoffmann-LaRoche.

(6) found in patients who had suffered fractures, a rise in serum aminopeptidase followed by a fall to normal during convalescence. Several groups of investigators(4,7,8) showed clearly that the mammalian erythrocyte is a rich source of peptidases and that hemolyzed serum is abnormally high in peptidase activity. Smith and associates(9) found significant increases in a variety of serum peptidases following the administration of hemolyzing doses of phenylhydrazine to dogs. In addition, we have noted previously(10) that in certain patients having high plasma peptidase activity, clinical improvement following ACTH or cortisone therapy was paralleled by a fall in plasma peptidase activity to normal values. This observation has been confirmed (11). Thus, it seems clear that tissue destruction may be associated with elevated plasma peptidase activity and that with healing there is a return to normal peptidase levels. This is in accord with the conclusion of Cullen *et al.*(12) that "pathological conditions which are associated with cellular damage result in a high serum peptidase activity, apparently due to a leakage of these enzymes out of the cells."

Among the experimental studies just cited, increased plasma peptidase activity appeared as a consequence of clear-cut cell destruction. In contrast, it is interesting that, in the present investigation, striking elevation in plasma GGGase activity occurred under conditions in which there was no obvious mechanical disruption of cellular elements. Instead, cell damage is attributable to tissue hypoxia. The degree of reversibility of this form of metabolic injury is not well defined but, in a concurrent study(1), it was clearly shown that, when exposed to oxygen, surviving diaphragms of rats subjected to hemorrhagic shock exhibit changes which may be interpreted as evidence of "recovery" from the shocked state.

Summary. When profound shock is produced in rats by severe hemorrhage, a striking rise in plasma aminopeptidase activity occurs. This elevation of plasma GGGase activity is roughly correlated with the increment in plasma amino nitrogen.

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