further clinical trials (1,14). It is the most promising agent available for situations in which a decrease in corticosteroid production is desirable. Its *in vitro* action affords a unique opportunity for qualitative and quantitative study of the effect of ACTH on steroidogenesis, since the tropic hormone is believed to enhance steroidogenesis through its effect on 20-hydroxylation of cholesterol (15–20). Although glutethimide is less than 1% as potent as aminoglutethimide, its inhibitory action must be reckoned with in cases of attempted suicide with large doses of the drug.

Summary. Aminoglutethimide was found to be a competitive inhibitor of the enzymatic conversion of cholesterol to pregnenolone by the adrenals. This effect is due wholly or in part to an inhibition of the 20-hydroxylation of cholesterol.

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Histochemical Investigation of Hepatic Adenosine Triphosphatase and Glucose-6-phosphatase Activity in Hemorrhagic Shock* (32878)

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The profound effects of hemorrhagic shock upon carbohydrate metabolism and upon hepatic glycogenolysis have been repeatedly documented (1,2). The initial response to hemorrhage is hyperglycemia with an accompanying and progressive increase in the blood levels of lactic and pyruvic acids. In termi-

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nal hemorrhagic hypovolemia, hypoglycemia is a consistent finding, hepatic glycogen is depleted, and glucose utilization in peripheral tissues, mainly muscle, is increased (3). There are several recent reviews of the alterations in carbohydrate metabolism (4,5,6). Over 20 years ago, McShan and co-workers (7) suggested that failure of cellular energy production is due to inefficient production of adenosine triphosphate (ATP) or an increased destruction of ATP secondary to the disordered carbohydrate metabolism. The increased blood levels of lactic and pyruvic acids have been considered to reflect a change to anaerobic glycolysis, a less efficient means of energy production. It was the purpose of this investigation to detect possible aberrations of enzyme activity in the liver and to correlate these changes with the altered hepatic structure observed in hemorrhagic shock (8).

Methods. Hemorrhagic shock was produced in 12 male Sprague-Dawley rats by a modification of the Wiggers-Selkurt Method (9). The animals were anesthetized with intraperitoneal pentobarbital, 4 mg/100 gm rat, and additional amounts were given when required by intraperitoneal instillations. The external iliac arteries were cannulated, connected to a mercury manometer and to a 10-ml syringe used as a reservoir. The animals, tubes, and syringes were heparinized. Rectal temperature was monitored continuously and external heat was applied periodically from a light source to maintain the temperature between 34.4 and 37.5°C.

The rats were bled over a 10-min period to a mean arterial blood pressure (MABP) of 50 mm Hg for 30 or 60 min, and then bled to a MABP of 30 mm Hg for 30 or 60 min. After 60, 90, and 120 min from the inception of hypovolemia, the animals were sacrificed by performing a midline abdominal incision and rapid removal of the right hepatic lobe. A section 1–2 mm thick of the hepatic lobe was quickly frozen in liquid nitrogen cooled isopentane and stored in liquid nitrogen for further sectioning. Nonbled anesthetized rats were simultaneously cannulated and sacrificed at similar intervals as controls for the hypovolemic rats. Hepatic tissue from shocked and control nonbled rats was processed synchronously for histochemical study.

Sections of the frozen liver were cut at 8 μ on an International model CTI cryostat. Adenosine triphosphatase (ATPase) activity was determined by a modification of the method described by Ashworth (10): sections were fixed in buffered 10% formalin, pH 7.2, at —4–0°C for 60 min, rinsed in half strength Tris buffer at pH 7.2 at —4–0°C for 5 min, and incubated in Wachstein-Meisel ATPase medium (11) at 37° for 30 min. Sections were placed in dilute amonium sulfide at room temperature for 30 sec. Glucose-6-phosphatase (G-6-Pase) activity was determined by a modification of the method of Chiquoine (12) and Wachstein and Meisel (13): cryostat sections of 8 μ were fixed in buffered hydroxyadipaldehyde (14), pH 7.5 at —4–0°C for 60 min, rinsed in half strength Tris buffer, pH 6.7 at —4–0°C for 5 min, incubated in Wachstein-Meisel glucose-6-phosphatase medium at 37°C for 15 min, and placed in dilute ammonium sulfide as above. In order to check the specificity of the histochemical technique for ATPase and G-6-Pase activity, parallel control sections were incubated using either a medium without substrate or one containing β-glycerophosphate. All sections were studied by light microscopy.

Results. The ATPase activity in normal rat liver was found to be localized mainly in bile canaliculi, bile ducts, and endothelium of blood vessels. The ATPase activity was noted along borders of the hepatic sinusoids, but it was not possible to determine with certainty by light microscopy whether the reaction product indicative of ATPase activity was mainly within Kupffer cells or within the vascular pole of the hepatic parenchymal cells. The ATPase localization was more intense in periportal areas than in the centrilobular zones, giving a well-defined lobular pattern.

Histochemical alterations from normal appearance were not consistent until rats had been subjected to a minimum of 90 min of hypovolemia. The changes became striking in animals after 120 min of hypovolemia. In the bled animals, there was a more intense centrilobular ATPase reaction, with a general increase in the deposition of reaction product throughout the liver. The general increased staining found in hepatic tissue after hypo-

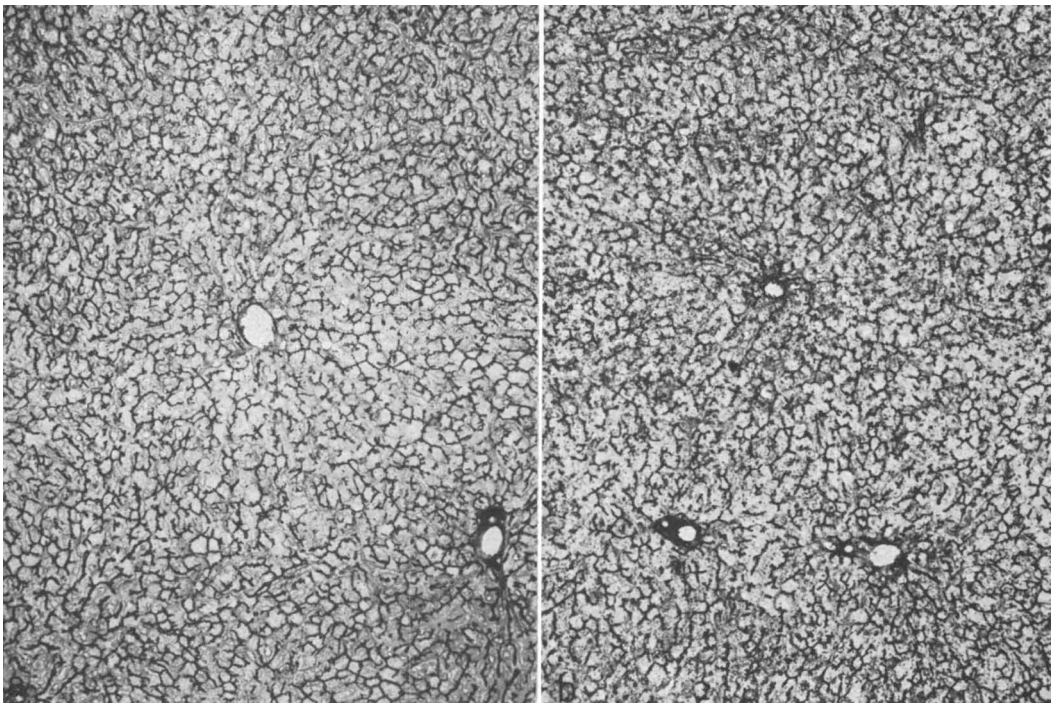

FIG. 1. a. Rat liver, ATPase reaction 80 ×. Note the normal localization of ATPase activity in bile canaliculi with more intense activity in periportal areas. b. Rat liver, hypovolemia 120 min ATPase reaction 80 ×. Note increased ATPase activity especially in centrilobular zone.

volemia was characterized by thickening and granularity of the normally delicate canalicular ATPase reaction and by accumulations of intracellular reaction products especially in the centrilobular zones (Fig. 1a and b).

Glucose-6-phosphatase activity in the control rats was found to be intracytoplasmic and more pronounced in the periportal areas. After hemorrhagic shock of 90-min duration, there was overall more intense staining of the tissue. The normal lobular pattern was preserved, but the reaction product was more dense in both centrilobular and periportal areas (Fig. 2a and b).

Discussion. A precise physiologic explanation of these observations in terms of disordered carbohydrate metabolism is limited by imprecision of histochemical methods. The apparent increase in enzyme activity might be related to several factors which alter histochemical reactivity. Hepatic centrilobular necrosis after prolonged hemorrhage may cause an increase in phosphatase activity as observed by Wieleng *et al.* (15) in areas of infarcted trophoblast. Altered membrane or tissue permeability produced by prolonged ischemia could permit more reactants to penetrate with a consequent greater apparent histochemical activity. The Wachstein-Meisel medium for demonstration of membrane localized ATPase has several important limitations which have been alluded to recently in the literature. At a concentration of lead nitrate of 3.6 m*M*, nonenzymatic hydrolysis of ATP has been shown to occur (16). This process is affected by the concentration of other divalent cations; if these are altered at the cell membrane due to hemorrhage, an apparent increase in ATPase activity might result. It has been established that ATPase activity using the Wachstein-Meisel medium is not Na^+–K^+ sensitive (17). The precise role of this ATPase activity in the physiologic transport of water and ions is not as yet clear (18). It is possible that changes in intracellular and extracellular ionic concentrations and cell membrane damage as a result of hemorrhage might result in altered ATPase

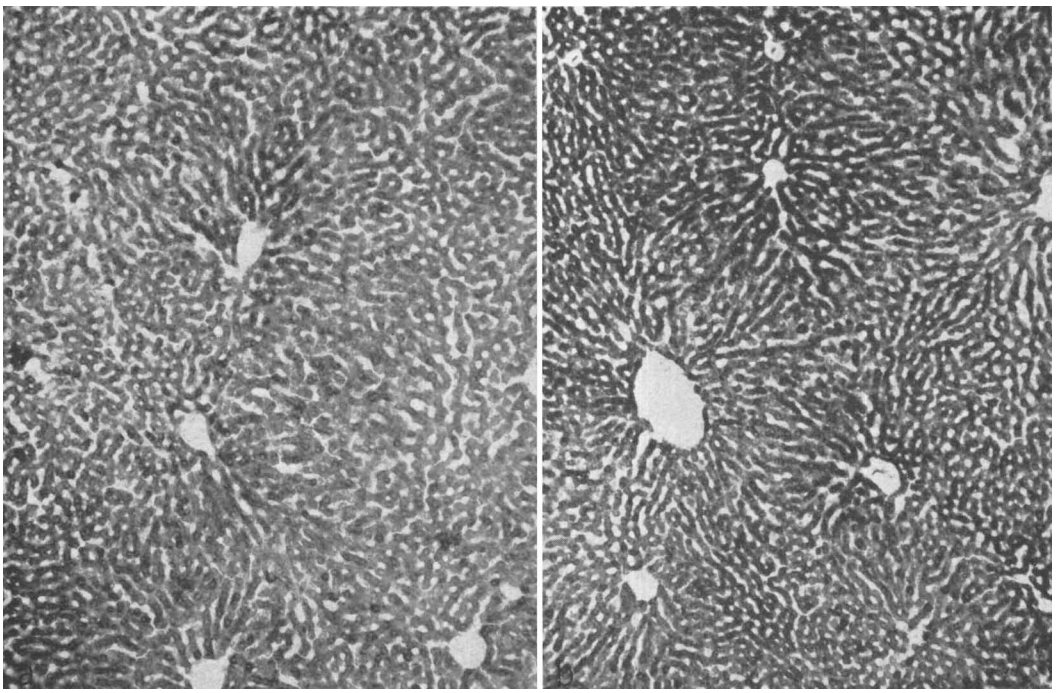

FIG. 2. a. Rat liver, G-6-Pase reaction 65 ×. Normal lobular distribution of glucose-6-phosphatase activity. b. Rat liver 90 min hypovolemia. G-6-Pase reaction 65 ×. Note increase in staining indicative of G-6-Pase activity.

reactivity, rather than reflecting an actual increase in membrane localized ATPase.

In spite of these limitations of histochemical methods, increased hepatic ATPase and G-6-Pase biochemical activity in response to hemorrhage must be considered. The increase in enzyme might occur either by increased enzyme synthesis or by conversion of the enzyme from an inactive form in the animals after prolonged shock. Chiquione (19), using histochemical techniques, observed an increase in G-6-Pase activity in livers of rats starved for 72 hours. It is well established that within 60–90 min after the inception of hypovolemia hepatic glycogen becomes depleted (1) as in starvation. Biochemical study demonstrated an increased hepatic glucose-6-Pase activity in response to prolonged cold stress (20). Also, certain dietary treatments may cause an increase in hepatic glucose-6-Pase activity in the rat within 24 hours (21). Feeding, in turn, influences the ability of rats to tolerate the shock procedure (22). Biochemical investigation of these relationships in shock will provide verification of histochemical leads.

It is noteworthy that increased membrane localized ATPase activity is seen at a time in hypovolemia when there is dynamic alteration of fluid flux across membranes (23), and that the increase in ATPase activity is most intense in the metabolically active centrilobular zones of the liver. The apparently increased G-6-Pase activity occurs at a time when there is rapid glycogenolysis, increased glucose uptake by muscle and decreased glucose transport by the gut (24). While apparent increase in the activity of these enzymes relates logically to known alterations of carbohydrate metabolism and fluid dynamics in shock, biochemical verification of these findings is required before an actual increase in hepatic enzyme activity can be postulated.

Summary. Twelve rats were subjected to hemorrhagic shock. Histochemical study of the liver revealed increased adenosine triphosphatase and glucose-6-phosphatase activity when compared to controls. The reason for these alterations may be either an actual increase in enzymes in the parenchymal cells

or altered histochemical reactivity due to cell necrosis, changes in membrane permeability, or deranged intra and extracellular ionic concentrations as a result of hypovolemia. The increased enzyme activities relate logically to altered carbohydrate metabolism and fluid dynamics in shock.

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Dose Response Relationships for the "Early" and "Late" Response to Erythropoietin* (32879)

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The response to erythropoietin (EP) in fasted rats measured 24 hours after injection ("early" response) varies markedly with the state of the animal at the moment of injection: the response measured 48 hours after ("late") does not (1).

In order to further characterize the "early" and "late" response to EP a dose response study was carried out.

Methods. A × C male rats 160–180 gm, were used. Ten rats were injected intravenously 1 day after start of fasting with 1, 2, 4, 8, and 16 units of (St A) Armour erythropoietin preparation Lot AL 038b kindly supplied by N.I.H. Hematology study section. In half of the rats a ^{59}Fe distribution study (1) was carried out 24 hours after EP injection: "early" response; and in other half, 48 hours after: "late" response.

Results. In the case of the 48-hour response (Fig. 1a) there is a highly significant ($p<0.001$) linear correlation between the

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