phyrins were noted in hepatic tissue of Sedormid-treated animals by Schwartz and Ikeda (4). Accumulation of these compounds in Sedormid porphyria and their absence in porphyrin induced by "collidine derivative" is further evidence of dissimilar mechanisms of the action of these two agents. Examination of sections of mouse liver (stained with H & E) from animals receiving "collidine derivative" for 7 days revealed a marked proliferation of bile ducts and periportal fibrosis. The portal areas were infiltrated with inflammatory cells. This picture of liver injury was associated with increased hepatic coproporphyrin III levels. No coproporphyrin I could be detected in livers of treated animals.

Mild oxidation of "collidine derivative" yields diethyl-2,4,6-trimethylpyridine-3,5-dicarboxylate. The latter compound has no effect on the porphyrin content of mouse liver. Williams(6) states that pyridine is oxidized *in vivo* to methylpyridinium hydroxide and excreted as an ethereal sulphate and a conjugated glucuronide. Similarly, picoline is oxidized *in vivo* to picolinic acid and excreted as the glycine conjugate, pyridinuric acid. The metabolism of "collidine derivative" has not been described; however, its activity may be related to an inability of liver to convert it to the oxidized, inactive compound: diethyl-2,4,6-trimethylpyridine-3,5-dicarboxylate.

Conclusions. Parenteral administration of 5 mg/mouse/day of diethyl-1, 4-dihydro-2, 4,6-trimethylpyridine-3,5-dicarboxylate ("collidine derivative") in corn oil causes increased hepatic and biliary proto- and coproporphyrin levels. Hepatic coproporphyrin was found to be entirely Type III isomer. No green porphyrins are found in livers of animals receiving "collidine derivative." Oxidation products of "collidine derivative" did not produce abnormalities in porphyrin metabolism within a 10-day observation period.

1. Figge, F. H. J., *J. Histochem. Cytochem.*, 1959, v7, 257.

2. Solomon, H. M., Figge, F. H. J., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 583.

3. Schwartz, S., Wikoff, H. J., *J. Biol. Chem.*, 1952, v194, 564.

4. Schwartz, S., Ikeda, K., Ciba Foundation Symposium Porphyrin Biosynthesis and Metabolism. Boston, Little, Brown and Co., 1955, p. 209.

5. Hantzch, A., *Annalen*, 1882, v215, 21.

6. Williams, R. T., *Detoxication Mechanisms*. New York, John Wiley & Sons, Inc., 1949, p. 199.

Received August 29, 1960. P.S.E.B.M., 1960, v105.

Glucose-6-Phosphatase Activity in Various Sheep Tissues.* (26150)

FIORA RAGGI, D. S. KRONFELD AND MAX KLEIBER
College of Agriculture and School of Veterinary Medicine, University of California, Davis

The significance of carbohydrate in ruminant metabolism differs in some ways from that in other mammals(1). Of special interest are the chemical precursors and sites of formation of glucose, because little glucose appears to be absorbed from the ruminal digestive tract(1). Arteriovenous differences have suggested that glucose may be contributed to the blood of sheep not only by the liver but also by extra-hepatic tissues(2). A similar result on ruminant-like marsupials has been reported(3). It is usually considered that tissues which can release glucose into the blood have glucose-6-phosphatase activity, and in the rat this is largely restricted to liver, kidney and intestine(4). To investigate the possibility that ruminants differ in this respect from rats we have estimated the glucose-6-phosphatase activity of various sheep tissues.

Methods. The technic has been described by Hers and de Duve(4,5). We repeated their work on rats, then continued with adult sheep. About 5 g samples of liver, kidney, intestine, salivary gland, brain, muscle, rumen epithelium and mammary gland were

* This research was supported in part by a grant from U. S. Atomic Energy Comm.

TABLE I. Glucose-6-Phosphatase Activity of Tissue Homogenates Expressed as mg P Liberated per Gram of Tissue from 4.2×10^{-3}M G-6-P at pH 6.5 and 37°C for 10 Min.

Tissue	Rat*	Sheep†
Liver	2.08	1.37 ± .16 (7)
Kidney	1.47	1.60 ± .20 (7)
Intestinal wall	1.08	.13 ± .05 (5)
" mucosa	—	.21 ± .10 (3)
Salivary gland	—	.10 ± .02 (9)
Muscle	trace	trace
Brain	—	"
Rumen epithelium	—	"
Mammary gland	—	"

* Mean of 2 observations.
† Mean ± stand. error (No. of observations).

taken from sheep killed by cutting the carotid and jugular vessels. Aqueous homogenates were prepared in the cold, the supernatants were incubated with glucose-6-phosphate (G-6-P), protein was precipitated with trichloracetic acid, and phosphorus in the filtrates was estimated by the Fiske and Subbarow method (for details see 4.5).

Results are shown in Table 1. On the whole our results with rat tissues confirmed those of Hers and de Duve(4), although we found rather less glucose-6-phosphatase activity in the liver and more in kidney and intestine, but observed the same order between organs, liver having the highest activity. The glucose-6-phosphatase activity of ovine intestinal wall and mucosa was much smaller than that of kidney and liver. It was also smaller than the activity of intestinal wall tissue from the rat. The enzyme activity was detectable also in salivary gland homogenates but was negligible in the other tissues.

Discussion. The low glucose-6-phosphatase activity of sheep intestine is in accord with other types of observations (recently reviewed by Lindsay)(1), which suggest that little glucose enters the portal blood from the digestive tract of sheep. The high phosphatase activity of ovine kidney suggests that the renal contribution of glucose to the blood, which has been estimated at 4-13% of the hepatic contribution in the dog(6), may be even greater in sheep. The slight phosphatase activity in the salivary gland leaves open the possibility that a little glucose might also be added to the blood by these glands (cp. 2).

Summary. Glucose-6-phosphatase activity was estimated in various sheep tissues. It was appreciable in liver and kidney, detectable in intestine and salivary gland, but negligible in brain, muscle, stomach, rumen epithelium and mammary gland. The results suggest the relative importance of these tissues in contributing glucose to the blood in sheep. They indicate particularly the relatively small potential role of sheep intestinal wall as a source of blood glucose.

1. Lindsay, D. B., *Vet. Rev. Annot.*, 1959, v5, 101.
2. Kronfeld, D. S., *Austral. J. Exp. Biol.*, 1957, v35, 257.
3. Moir, R. J., Somers, M., Waring, H., *Austral. J. Biol. Sci.*, 1956, v9, 293.
4. Hers, H. G., de Duve, Chr., *Bull. Soc. Chim. Biol.*, 1950, v32, 20.
5. de Duve, Chr., Berthet, J., Hers, H. G., Dupret, L., *ibid.*, 1949, v31, 1242.
6. McCann, W. P., Jude, J. R., *Bull. Johns Hopkins Hosp.*, 1958, v103, 77.

Received August 4, 1960. P.S.E.B.M., 1960, v105.

A Biological Assay for Ribonuclease with Sub-millimicrogram Sensitivity (26151)

J. POLATNICK AND H. L. BACHRACH
Plum Island Animal Disease Laboratory, Animal Disease and Parasite Research Division, Agricultural Research Service, U. S. Dept. of Agric., Greenport, L. I.

Ribonuclease (RNase) activity isolated from different sources can be assayed by chemical(1,2), manometric(3), spectrophotometric(4), turbidimetric(5), and isotopic(6) methods. The substrate in these methods is usually yeast ribonucleic acid (RNA) and measurements are made of its disappearance or hydrolysis products.