8. Hansen, P. W., Dam, H., *Acta Chemica. Scand.*, 1957, v11, 1658.
9. Zlatkis, A., Zak, B., Boyle, A., *J. Lab. and Clin. Med.*, 1953, v41, 486.
10. Bollman, J. L., Cain, J. C., Grindlay, J. H., *ibid.*, 1948, v33, 1349.
11. Wycoff, H. D., Parsons, J., *Science*, 1957, v125, 347.
12. Trinder, P., *Analyst*, 1952, v77, 321.
13. Wilcoxon, F., *Biometric Bull.*, 1945, v1, 80.
14. Chapman, D. D., Chaikoff, I. L., *J. Biol. Chem.*, 1959, v234, 273.
15. Blomstrand, R., Ahrens, E. H., Jr., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 802.
16. Bergstrom, S., Blomstrand, R., *Acta Physiol. Scand.*, 1956, v38, 166.

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Pressures in the Common Hepatic Duct of the Rat. (25360)

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The first measurements of pressure in the common hepatic duct in the rat were reported by Mann and Foster(1), and the sphincteric action of the intraduodenal portion of the duct in animals including the rat was later investigated by Mann(2). Reports on pressure in the extrahepatic bile ducts of other animals followed(3-6), but measurements of the secretory pressure of bile in albino rats were not reported until Friedman, Byers and Michaelis (7) included observations on a small series as part of a study on production and secretion of cholesterol in mammals. In these studies pressures were measured with the water manometer. Kelsey and Beard(8) measured pressures in the common bile duct of human beings with a strain-gauge manometer. Since this manometer is more convenient for continuous recording than the water manometer, we repeated some of previous studies of hepatic duct pressures in the normal rat and studied also the pressure after certain induced hepatic injuries.

Methods. Adult albino rats of Sprague-Dawley strain were used. Rats weighed between 200 and 400 g. The rats selected for study were of 3 types: (a) rats with normal livers, (b) rats with hepatic cirrhosis induced by inhalation, in a closed chamber, of 25 mg of carbon tetrachloride/minute in 50 liters of air, for 6 hours, 3 times a week for 6 weeks and once a week for additional 4 weeks and (c) rats with passive venous congestion of the liver induced by application of a cellophane band around the inferior vena cava above the liver 4 weeks previously. The rats were fasted routinely for 16 hours before any observation of pressure was made. Under ether anesthesia the common hepatic duct of each rat in the study was isolated and ligated at its midpoint. Two lengths of polyethylene tubing (P.E. 50) with internal diameter of 0.58 mm were introduced into the duct; the first was directed toward the liver and the second toward, but not entering, the opening of duct into the duodenum. The 2 tubes were brought out through the skin. Free flow of bile into the duodenum could be restored by joining the ends of the tubes with a cuff of polyethylene tubing. After operation the rats were immobilized in a Bollman cage(9) and initial recordings of pressure were made at time intervals varying from a half hour to 16 hours after operation. During the intervening period the animals were allowed free access to drinking water. The pressure in the common hepatic duct was recorded by means of a strain-gauge manometer (Statham P6-4G-250, 0-4 P.S.I.) equipped with a 3-way stopcock. This system was filled with water, and all air excluded from the system before the test. The ends of the polyethylene tubes leading from hepatic duct, were connected to the stopcock, and the strain-gauge output was recorded on a photokymograph. The pressure transducer was referred to the level of the exit of the tubes through the skin of the animal, which closely approximated the openings

TABLE I. Mean Pressures in Common Hepatic Ducts of Albino Rats.

Liver	Flow pressure		Duodenal-end pressure		Secretory pressure	
	No. observed	Mean pressures, cm H_2O	No. observed	Mean pressures, cm H_2O	No. observed	Mean pressures, cm H_2O
Normal	21	11.7 ± .8*	14	6.4 ± .5	27	24.0 ± .7
Cirrhotic	9	14.3 ± 1.6	8	8.2 ± 1.5	9	29.2 ± 2.3
Congested	3	12.4 ± 1.6	3	9.1 ± .8	4	23.7 ± 2.5

* ± stand. error of mean.

of the tubes in the bile duct. The pressure recording system was calibrated at intervals by introduction of known levels of static pressure from a water manometer. By use of the 3-way stopcock the pressure in the tube directed toward the liver (secretory pressure), the pressure in the whole system (flow pressure) and the pressure in the tube directed toward the duodenum in the lower portion of the hepatic duct (duodenal-end pressure) could be measured. Recordings of each pressure were made for at least 15 minutes at each observation. As indicated by Kelsey and Beard(8), because of the small volume needed to register pressure changes in the strain-gauge manometer, the need for perfusion of the hepatic duct with saline solution is obviated and the pressures recorded represent, as far as possible, the pressures determined by secretory pressure of the liver and resistance of the bile duct. The liver and duodenal wall in the region of ductal orifice were examined histologically at conclusion of most studies. Five aspects of the problem were studied. Additional preparation of animals needed for some studies will be mentioned with results.

Results. Measurements of flow, duodenal-end and secretory pressures. Measurements of these pressures were made on rats with normal livers, with cirrhosis and with chronic passive venous congestion. Number of observations and mean pressures obtained are given in Table I. The secretory pressure was higher in cirrhotic rats than in rats with normal livers or with chronic passive congestion of the liver, and duodenal-end pressure was higher in rats with passive congestion of the liver.

Respiratory movements and duodenal muscular activity were transmitted by tubes in the hepatic duct and marked pressure changes were induced by movement or exaggerated respiratory excursions in the rat.

On histologic examination at conclusion of these studies, the normal group showed no abnormalities; cirrhosis and chronic passive venous congestion were the respective abnormal findings in livers of the other 2 groups of animals. Gross congestion of duodenal mucosa was found in animals with chronic passive venous congestion of the liver, but not in cirrhotic and normal rats.

Simultaneous recording of biliary pressure and gastric and duodenal motility. For this aspect of the study we used 3 rats with normal livers and 3 with cirrhosis. To record gastric and duodenal motility we introduced, at time of operation, polyethylene tubes (P.E. 160), of internal diameter 1.14 mm, into the gastric antrum and second part of the duodenum. The tips of tubes were covered by small latex balloons, and these and the tubes themselves were filled with water. The tubes were introduced into the stomach and duodenum through a small incision in the greater curvature of the stomach which was subsequently closed with a purse-string suture. The gastric and duodenal tubes were connected to strain gauges similar to those used for recording biliary pressure. The gastric and duodenal motility was recorded simultaneously with the biliary pressure by means of the photokymograph.

Although some fluctuations in recordings of biliary pressure could be correlated directly with bodily movement, respiration or gastric or duodenal activity, it was impossible to conclude that all variations were related to these factors (Fig.). No differences were noted in motility recordings between normal and cirrhotic rats.

Biliary pressure after obstruction to the common hepatic duct. All 3 types of rats were used for this aspect of the study. From each rat a base-line recording of secretory pressure was made for 2 hours. Subsequent to this the polyethylene tubing leading from upper por-

TABLE II. Mean Secretory Pressures in Common Hepatic Ducts of Rats after Complete Obstruction to Bile Flow.

Liver	Before obstruction		Obstruction for:							
			15-24 hr		40-48 hr		65-72 hr		92-137 hr	
	No. observed	Mean pressures, cm H_2O	No. observed	Mean pressures, cm H_2O	No. observed	Mean pressures, cm H_2O	No. observed	Mean pressures, cm H_2O	No. observed	Mean pressures, cm H_2O
Normal	10	25.8 ± 1.3*	11	23.9 ± 1.1	8	25.8 ± 1.6	3	24.7 ± 5.1	3	16.0 ± 6.0
Cirrhotic	4	27.8 ± .9	3	27.3 ± 2.6	2	24.4 ± 1.6	1	19.8	2	19.1 ± 7.2
Congested	4	24.5 ± 2.7	4	19.2 ± 3.3	3	19.9 ± 4.8	1	22.5	3	8.3 ± 1.8

* ± stand. error of mean.

FIG. 1. Simultaneous recording of flow pressure in common hepatic duct and of movements of stomach and duodenum.

tion of common hepatic duct, was occluded by a clip to produce complete obstruction of outflow of bile, since in the preparation of the animal a ligature had already been applied to the midpart of the duct. Further recordings of secretory pressure were made at intervals after the occlusion. The 3-way stopcock obviated release of obstruction at any time. Each recording was of 15 to 20 minutes' duration. Sixty-two observations of secretory pressure were made on this group, 18 before obstruction and remainder at intervals from 15 to 137 hours after obstruction was begun. Eight were made during obstruction of 92 to 137 hours. These secretory pressures and the number of observations are summarized in Table II. Secretory pressures of animals tested showed no statistically significant alterations during obstruction to the common hepatic duct.

All rats became deeply jaundiced, however, and at necropsy gross dilatation of the extrahepatic bile ducts above the ligature was found. In no instance could leakage of bile into the abdominal cavity be demonstrated. On histologic examination extensive focal cellular necrosis of the liver was seen in all animals, but gross evidence of cholestasis was found only in cirrhotic livers.

Biliary pressure after inhalation of carbon tetrachloride. After control pressure readings were obtained, 6 rats with normal livers were placed in a closed chamber for 6 hours where they would inhale 25 mg of carbon tetrachloride/minute in 50 liters of air. Secretory pressure was recorded immediately and 24 hours after removal from the chamber. Two rats survived so that recordings could be made immediately after a second exposure for 6 hours to carbon tetrachloride. Each recording lasted half an hour. Free flow of bile into the duo-

TABLE III. Mean Secretory Pressures in Common Hepatic Ducts of Rats after Inhalation of Carbon Tetrachloride.

	No. observed	Mean pressure, cm H_2O
Control	6	23.8 ± 1.1*
After inhalation of carbon tetrachloride		
Immediately	6	21.7 ± 1.1
24 hr later	5	20.4 ± .7
Immediately after 2nd exposure	2	21.5 ± 1.4

* ± stand. error of mean.

denum was permitted during periods of inhalation and between recordings. Secretory pressure was slightly lower after inhalation of carbon tetrachloride. The pressure findings are summarized in Table III.

Biliary pressure after intravenous administration of chlorpromazine. Five rats with normal livers were used. Initial pressure readings were made and then 10 mg of chlorpromazine hydrochloride (thorazine)/kg of body weight was injected *via* tail vein. Continuous recordings were made for 3 hours after injection. For 2 rats, injection of thorazine was repeated at the end of 3 hours, and further pressure recordings were made for a similar period. Intravenous injection of thorazine was followed in all 5 rats by diminution in duodenal activity as shown by transmitted movement recorded from the tubes in the hepatic duct. This commenced within 5 minutes of injection and its duration was approximately 1 hour. All rats became drowsy within 5 minutes, and one animal had a generalized epileptiform convulsion lasting 2 to 3 seconds immediately following injection.

No effects on secretory, flow, or duodenal-end pressures were found in any animal in the 3-hour period following first injection or in the 2 rats given second doses. All livers were examined histologically at conclusion of experiments, and no changes could be found.

Comment. The mean level of secretory pressure of bile in the fasting rat with normal liver in this series, is broadly in agreement with that recorded by Mann and Foster(1) and by other investigators(7,10,11). Increase in secretory pressure in the cirrhotic rats over that in animals with normal livers and in those with chronic passive congestion of the liver is statistically significant (Table I). The mean flow pressure of 11.7 ± 0.8 cm of water in rats with normal livers lies within the range given by Kelsey and Beard(8) who, in the human subject, noted that this pressure varied from 7 to 22 cm of water. No previous recordings of flow pressure of bile in the rat are available for comparison.

From simultaneous recordings of biliary ductal pressure and gastric and duodenal motility, it was concluded that, in the albino rat, some sphincteric action is exerted by the lower end of the common hepatic duct and that, in normal and cirrhotic rats, the duodenal-end pressure can be interpreted as that sustained by the resistance of bile duct and the sphincteric action of its intraduodenal portion. This concept was suggested by lack of direct correlation between all fluctuations in recordings of ductal pressure and recordings of duodenal activity. If the duodenal-end pressure simply reflected intraduodenal pressure such a correlation would be expected. Existence of an anatomic sphincter has been observed in the rat(2). The higher duodenal-end pressure found in animals with chronic passive venous congestion of the liver, probably is related to congestion of the duodenal tissues near the intraduodenal portion of the common hepatic duct in these rats.

The apparent fall in mean secretory pressure in the 8 rats in which obstruction to the common hepatic duct had been maintained for 92 to 137 hours is considered to reflect inanition of these nearly moribund animals.

The fall in secretory pressure in rats on inhalation of carbon tetrachloride vapor was significant on statistical analysis. No previous findings in the rat are available for comparison.

Absence of any change in flow or duodenal-end pressures in the group which received thorazine by intravenous injection is in contrast to those of Menguy, Grindlay, and Cain (12) who found a rise in ductal pressure in cholecystectomized dogs after intravenous injection of the drug. No change in ductal pressure, however, was noted by Kelsey and Beard(8) in human subjects who were given thorazine by mouth.

Summary. 1) A method for recording pres-

sure within the common hepatic duct of the rat with a strain-gauge manometer is described. Measurements of pressure in the common hepatic duct were made on a series of fasting rats with normal livers, cirrhosis or passive venous congestion of the liver, and these were analyzed statistically. The higher mean secretory pressure in the rat with cirrhosis over that in the rat with normal liver and with passive venous congestion is statistically significant. 2) Secretory pressure in rats after inhalation of carbon tetrachloride vapor fell slightly but significantly. 3) The duodenal-end pressure in the common hepatic duct was related to findings on simultaneous recordings of biliary pressure and gastric and duodenal motility. This pressure was significantly higher in rats with chronic venous congestion of the liver than in normal or cirrhotic animals. The lower end of the common hepatic duct probably has some sphincteric action in the rat. 4) Intravenous injection of chlorpromazine hydrochloride (thorazine) produced no effects on pressure in the common hepatic duct in normal rats.

1. Mann, F. C., Foster, J. P., *Am. J. Physiol.,* 1918, v47, 278.
2. Mann, F. C., *J. Lab. and Clin. Med.,* 1919, v5, 107.
3. Elman, Robert, McMaster, P. D., *J. Exp. Med.,* 1926, v44, 151.
4. Whitaker, L. R., Am. Medical Assn., Atlantic City, N. J., May 29, 1925.
5. Potter, J. C., Mann, F. C., *Am. J. M. Sc.,* 1926, v171, 202.
6. Lueth, H. C., *Am. J. Physiol.,* 1931, v99, 237.
7. Friedman, Meyer, Byers, S. O., Michaelis, Fred, *Am. J. Physiol.,* 1950, v162, 575.
8. Kelsey, J. R., Beard, E. F., *Gastroenterology,* 1957, v32, 1122.
9. Bollman, J. L., *J. Lab. and Clin. Med.,* 1948, v33, 1348.
10. Sobotka, Harry, *Physiological Chemistry of the Bile,* 1937, Williams and Wilkins Co., Baltimore.
11. Brauer, R. W., Leong, G. F., Holloway, R. J., *Am. J. Physiol.,* 1954, v177, 103.
12. Menguy, R. B., Grindlay, J. H., Cain, J. C., *Gastroenterology,* 1956, v30, 752.

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Studies on Erythropoietin. II. Production of High-Titer Plasma in Sheep.* (25361)

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Most investigators used rabbits for production of erythropoietin-containing plasma, although the literature suggests that ability to respond to various erythropoietic stimuli is widespread among mammals. At start of our program aiming toward isolation and clinical testing of the pure factor, it became clear that an animal considerably larger than the rabbit would be desirable for economical production of amounts of material needed. After careful consideration of the price and availability of various domestic animals, we selected cull breeder sheep as most suitable for our needs.

Methods and materials. Cull breeder sheep, mostly ewes, in age range of 3 to 7 years, and weighing over 100 lb. (45.5 kg) were used. Hay constituted the bulk of diet during experiments, and water was supplied *ad lib.* Anemia was induced by subcutaneous injection of phenylhydrazine† above shoulder, alternating sides, on days 1, 3, and 5. Blood was harvested by exsanguination on day 7, using the following procedure: the animal was shackled by hind feet and lowered into a plastic bag containing an atmosphere of CO_2 supplied by dry ice. When struggling ceased (approximately 20 seconds), an incision through the skin was made with sharp knife starting from chin and extending to breast

* This article is based on work done under contract with Atomic Energy Comm.

† For injection, neutralized phenylhydrazine hydrochloride (Fisher Certified Reagent) was used at a concentration of 24 mg/ml.