DO SPECIES LACKING A GALL BLADDER POSSESS ITS FUNCTIONAL EQUIVALENT?

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It is a singular fact that certain closely related species of animals have, some of them, a gall bladder, while others do not. What can be the essential significance of the organ and the reason for this difference?

Embryologically, the vesica fellea arises secondarily as a cul-de-sac from the same anlage that forms the liver and bile ducts, and it is considered by many as an almost purposeless diverticulum. The erroneous nature of this view is sufficiently shown by the several functions now demonstrated for the organ. The matter has been discussed in a previous paper from this laboratory.[1] The fact that the gall bladder is a highly specialized organ renders all the more noteworthy its irregular distribution in both high and low forms of life. Thus among the higher animals it is present in the cow and sheep, while it is absent in the horse, present in the goat, and absent in the closely related deer—to be found in the hog and wild boar but not in the peccary of South America. Among birds, the hawk and owl possess it, while doves do not; and among the rodents the mouse is found with the organ, the rat without. One species of gopher,[2] the pocket gopher (*Geomys bursarius*), is without a gall bladder, while another (*Spermophilus tredecimlineatus*) the striped gopher, possesses it. Woods Hutchinson[3] is authority for the statement that in the giraffe it is at times present and again not.

[1] Rous, P., and McMaster, P. D., *J. Exp. Med.*, 1921, xxxiv, 47.

[2] Mann, F. C., *J. Lab. and Clin. Med.*, 1919–20, v, 107.

[3] Hutchinson, Woods, *Med. Rec.*, 1903, lxiii, 770.

The disposition of the bile ducts or the position of the pancreas throws no light on the riddle of the gall bladder. If we consider only animals without the organ, the horse has separate pancreatic and common bile ducts emptying into the duodenum through a common opening. The deer, rat, and pocket gopher (*Geomys bursarius*) show the pancreatic duct opening directly into the common bile duct well above the ampulla, this it also does in both rats and white mice, the former being without a gall bladder, the latter with it. No inference can be drawn from the high opening of the pancreatic duct, for this is as inconstant[4] as the presence of the gall bladder itself.

The vesica fellea has, as already indicated, functions sharply different from those of the ducts. Its rôle as a reservoir may on occasion, as after cholecystectomy, be partially taken over by the ducts.[5] Not so, however, with its modifying influences upon the bile. The bladder acts to concentrate the secretion greatly and to thicken it with mucus, whereas the ducts by contrast tend to dilute it, though to a negligible degree, with a thin product of their own.[6] Is it possible that in animals lacking the gall bladder, the concentrating function is lodged somewhere in the duct wall? This possibility can readily be tested out by ligation experiments on rats and mice.

If the gall bladder has the same activities in the mouse as in the dog, cat, and monkey,[1] then the bile submitted to its influence after ligation of the common duct should become concentrated by the action of the gall bladder. Granting that this really occurs,—and the findings now to be described show that it does,—will a concentration of bile also go on in the obstructed duct system of the rat, an animal devoid of a gall bladder, or will this bile be diluted as is the case with bile pent in the ducts of other animals which have been deprived of their connection with the bladder? The close relationship of the species and their similarity in habits give a special value to the test.

[4] Mann, F. C., Foster, J. P., and Brimhall, S. D., *J. Lab. and Clin. Med.*, 1919–20, v, 203.

[5] Haberer, H., and Clairmont, P., *Verhandl. deutsch. Ges. Chir.*, 1904, xxxiii, pt. 2, 81. Rost, F., *Mitt. Grenzgeb. Med. u. Chir.*, 1913, xxvi, 710.

[6] Rous, P., and McMaster, P. D., *J. Exp. Med.*, 1921, xxxiv, 75.

Method.

The pigment content of the bile was taken as the criterion of extrahepatic changes in concentration. Its utility in this connection has already been shown.[1] The range of the normal pigment content of mouse and rat bile was first determined with the animals on their ordinary ration (bread and milk, and grain).

From rats the bile was obtained into small rubber bags connected with fine cannulas inserted in the common duct. The bags were left within the animal's peritoneal cavity for 24 hours or more. Considerable amounts of secretion, ample for pigment analyses, were readily obtained in this way. The small size of the mouse, however, made necessary a different method, which will later be described.

All operations were carried out under ether, with aseptic precautions. To produce stasis, a triple ligation of the duct was done, the ligatures being placed just below the junction of the lowest hepatic duct with the common duct and well above the pancreatic duct entrance, and the common duct was then cut between the middle and lowest ties. The animals were allowed to live thereafter for periods of 1 to 16 days on a mixed diet of barley and oats, with bread and milk. Recovery from the operative procedure was rapid and the animals ate well. They were killed at intervals, the stasis bile was collected into a pipette, and its pigment content determined. In no instance had the continuity of the duct been reestablished. Cultures of the bile and liver tissue were regularly taken.

The Quantitation of Pigment.

Different methods were used to determine the pigment content of bile according to the amount available. When more than 0.3 or 0.5 cc. of rat bile was had, a reading was carried out according to the colorimetric method of Hooper and Whipple.[7] To a measured quantity of bile is added a known quantity of acid alcohol reagent. For a standard, instead of a wedge of artificial constitution, a chloroform solution of pure bilirubin (Schuchardt) was used, which was mixed with the reagent, as was the bile, and read against it in a Duboscq colorimeter.

When the quantity of rat bile was very small, the mixture of it with the acid alcohol reagent was made in a Miescher pipette such as is used in the Fleischl-Miescher hemoglobinometer and read against the standard bilirubin solution as ordinarily, but in a micro colorimeter.

[7] Hooper, C. W., and Whipple, G. H., *Am. J. Physiol.*, 1916, xl, 332.

The pigment content of normal mouse bile in the small quantities obtained was always insufficient to yield, with the reagent, a green color that could be read in the colorimeter. It was quantitated directly against a potassium bichromate scale, a method used as well with rat biles. For the purpose, glass tubes with a bore of 0.75 mm. and walls so thick (2 mm.) as to result in a magnification, were filled with bile and with the scale solutions. The tint of bile and standard matched very closely. A permanent series of tubes was made containing dilutions of potassium bichromate ranging from 6.0 to 0.05 per cent; and tests were made to determine whether the color intensity of mouse bile varied on dilution like that of a weak bichromate solution. Specimens of bile which corresponded in color with bichromate solutions of known percentage were diluted and compared again with the scale. They now agreed with a percentage bichromate solution corresponding to their dilution. Thus a bile solution corresponding in color with a 1 per cent bichromate solution, when diluted with equal parts of water, had the color strength of a 0.5 per cent bichromate solution—when with ten parts of water it corresponded with a 0.1 per cent solution, and so on. In order to standardize the scale, a number of normal rat biles of known pigment content, taken at random, were compared with the bichromate tubes. The results of this comparison have been assembled in Table I. There three subdivisions have been made according to the pigment strength of the bile employed. The bilirubin content in milligrams per 100 cc., as determined by the acid alcohol reagent, is given in Column A, and its reading in percentage of bichromate solution, as determined by comparison with the small glass tubes, is given in Column B. If the relative amount of pigment can be read as truly on the bichromate scale as by the Hooper and Whipple method, then $\frac{A}{B}$ should yield a constant, which constant incidentally is the expression in milligrams of bilirubin per 100 cc. of bile, of the colorimetric value of a 0.1 per cent bichromate solution, or for a 1 per cent solution, when multiplied by 10. It will be seen that the constant was only approximately yielded by the individual bile specimens, but the variation is much the same throughout the groups, and the average for the constant was 13.26, 13.51, and 13.57 for biles of

TABLE I.

Standardization of the Potassium Bichromate Scale.

Rat No.	A Actual amount of bilirubin per 100 cc.	B Per cent strength of bichromate solution corresponding in color to the bile.	$\frac{A}{B} \times 10$	Averages.	
	mg.				
8	8.9	0.7	12.7		
37	11.7	0.8	14.6	Pigment less than 15 mg. per 100 cc. of bile.	
38	12.5	0.9	13.77		
9	14.38	1.2	11.98	Average 13.26	
7	15.43	1.4	11.02		
6	16.8	1.4	12.0	Pigment amounts 15 to 20 mg. per 100 cc. of bile.	
14	17.85	1.2	14.88		
15	19.1	1.2	15.91	Average 13.51	13.45
10	19.23	1.4	13.75		
5	20.16	1.8	11.2		
16	20.83	1.5	13.88	Pigment amounts above 20 mg. per 100 cc. of bile.	
13	22.32	1.6	13.9		
11	22.72	1.5	15.15	Average 13.57	
12	24.51	1.8	13.6		

All bile collections were made by the bag-cannula method.

Rat biles collected by the bag-cannula method were employed. In Column A, the actual milligrams of pigment per 100 cc. of bile, as obtained by the acid alcohol method, are given. Column B gives the pigment strength of the bile in per cent of potassium bichromate solution as obtained by direct color comparison. If this latter comparison is a true one, then $\frac{A}{B}$ should yield an expression in milligrams of bilirubin per 100 cc. of bile of the colorimetric value of a 0.1 per cent bichromate solution, as determined from that particular specimen of rat bile. This multiplied by 10 should give the figure for a 1 per cent solution ($\frac{A}{B} \times 10$), and should be a constant. The readings are separated into three groups according to whether the pigment content of the bile fell below 15 mg. per 100 cc., between 15 and 20 mg., and over 20 mg. per 100 cc. It will be seen that the same approximate constant ($\frac{A}{B} \times 10$) is yielded by all three groups.

low, medium, and high pigment content respectively, the general average being 13.45. As the table shows, a bile which reads on the bichromate scale at 1 per cent will contain about 13.45 mg. per 100 cc. Thus a specimen of rat or mouse bile obtained from a duct could be placed in one of the small bore tubes, compared with the bichromate scale, and its pigment content quantitatively estimated.

The Pigment in Normal Mouse Bile.

The delicacy of the common duct in mice prohibited the use of the bag and cannula method which was successful with rats. Instead, bile was collected from animals under ether, through extremely fine long cannulas, with the aid of suction.

To obtain cannulas of sufficient flexibility, hard glass tubing was drawn out into capillary pipettes with an external diameter of about $\frac{1}{3}$ mm. and a length of 40 to 60 cm., with the small end carefully rounded to avoid laceration of the duct. The larger end of the tube was curved and the bend in it formed a sort of basin into which the bile from the capillary collected. Mild suction was maintained at this end, when desired, through a connection with a rubber tube and bulb filled with water, which could be lowered at will.

The larger end of the cannula was rigidly attached to a mechanical stage which permitted accurate movement in two dimensions of space and its flexible shaft was passed through three fixed wire loops, so arranged as to give it a marked downward curvature. All of the loops could be raised or lowered at will, thus increasing or decreasing the curvature of the shaft and correspondingly deflecting the open end of the cannula, so that it could be made to take the exact direction of the bile duct in the individual mouse. This adjustment was essential to prevent kinking and obstruction of the duct.

Under ether, the bile duct was exposed close to its entrance into the duodenum. By the use of the mechanical stage the cannula was then introduced either through a slit in the duct or by ligating the duodenum to either side of the papilla of Vater, opening it and thrusting the cannula through the papilla into the duct. A magnifying glass placed above the field of operation was found of service. The cannula once in the duct was always advanced to a point well above the entrance of the channel and in many instances tied in place, thus blocking off all pancreatic secretion. Often it plugged the duct so tightly that this was unnecessary. Mild suction was produced on the capillary pipette and the animal kept under ether for several hours while the flow of bile went on.

By this arrangement the gall bladder was left in direct connection with the cannula and *a priori* one would suppose that its contents

formed part of the normal bile collected through this latter. However, on first opening the abdomen to insert the cannula, the gall bladder was usually noted to be empty. Even when full, little of its contents was yielded to the cannula on suction.

Collections were successfully made by this method for periods as long as $6\frac{1}{2}$ hours. Body warmth was kept up by electric lights placed near the animal, and injury was combated with moist sponges. The bile obtained was very uniform in character, exceedingly abundant, clear, light amber-yellow but, as already said, too weak in pigment, in the small quantities obtainable, to yield a notable blue-green color with the acid alcohol reagent.

Table II shows the result of readings against the bichromate scale with the estimated amounts of bilirubin per 100 cc. of bile. An average from ten cases gives a pigment content of 2.17 mg. of bilirubin per 100 cc. of bile. The quantity of bile secreted per 100 gm. of body weight in an hour was about 0.2 cc., or, per kilo of body weight per day (24 hours), 34.0 cc. A gram of liver tissue of the mouse secreted about 0.03 cc. of bile per hour.

Results of Obstruction in Mice.

The bile was studied from many mice in which obstruction had been produced by ligation of the common duct. Obstruction lasting 1 to 3 days did not result in a marked dilatation of the ducts but the gall bladder was always found full of a bile much darker and more mucinous than normal mouse bile. It had early a clear, dark amber color, and the longer the obstruction the darker the bile. After 3 days, the ducts became dilated, though the gall bladder did not greatly increase in size. Both now contained abnormally dark bile in greater and greater amount from day to day. The actual quantity was difficult to measure, since in puncturing the ducts some was almost always lost.

In accordance with expectations based on the findings in larger animals with gall bladders,[1] the stasis bile always contained much more than the normal amount of bilirubin (Table III). When stasis had existed for more than 24 hours, the gall bladder bile had sometimes a dark greenish tint. This change of some of the pigment to biliverdin caused difficulty in the comparison with the bichromate

scale. Fortunately, the pigment amount was so great as to yield, even in the small quantities of bile at hand, the acid alcohol reaction of Hooper and Whipple whereby biliverdin and bilirubin are quantitated together.

Table III shows that the bile obtained after 1 day of stasis ranged in color strength, as expressed in terms of the bichromate scale, from 1.5 per cent bichromate to 4 per cent, with an average of 2.25 per

TABLE II.

Pigment Content of Normal Mouse Biles.

Mouse No.	Mouse weight.	Liver weight.	Period of collection.	Total bile.	Quantity of bile per 100 gm. of body weight per hr.	Quantity of bile estimated per kilo of body weight per day.	Quantity of bile per gm. of liver per hr.	Color strength in per cent of bichromate solution.	Amount of bile pigment per 100 cc. estimated from reading of bichromate scale.
	gm.	*gm.*		*cc.*	*cc.*	*cc.*	*cc.*		*mg.*
1	23.0	1.15	2 hrs., 45 min.	0.1+	0.158	38.0	0.032	—	—
2	21.6	1.4	4 " 15 "	0.06 (incomplete).	0.065	15.6	0.011	0.1	1.35
3	20.0	1.4	1 hr., 25 "	0.15	0.53	12.7	0.075	0.1	1.35
4	24.5	1.9	4 hrs., 20 "	0.29	0.273	65.5	0.035	0.1	1.35
5	23.3	1.2	5 " 5 "	0.14	0.118	28.3	0.023	0.15	2.02
6	24.0	1.2	2 " 50 "	0.04	0.059	14.2	0.012	0.3	4.03
7	27.0	1.9	2 " 50 "	0.12	0.157	37.6	0.022	0.2	2.69
8	24.0	1.8	5 " 20 "	0.32	0.25	60.0	0.033	0.1	1.35
9	—	—	5 " 40 "	0.18	—	—	—	0.25	3.36
10	—	—	3 " 10 "	0.05 (incomplete).	—	—	—	0.15	2.02
Average					0.20	34.0	0.03	0.16	2.17

cent. In normal animals, by contrast, (Table II) the range was from 0.1 to 0.3, and averaged 0.16 per cent. The stasis bile, then, was fourteen times as strong in pigment as the normal. After 1 day of stasis, no perceptible conversion of bilirubin to biliverdin had occurred.

In a number of cases bile found in the gall bladder was compared with that collected from the ducts of the same animal. The pigment strength of the bladder and duct biles were compared with the aid of the bichromate scale (Table IV).

TABLE III.

Pigment Content of Mouse Biles from Obstructed Ducts.

Mouse No.	Mouse weight.	Liver weight.	Period of obstruction.	Color strength in per cent of bichromate solution.	Amount of bile pigment per 100 cc. estimated from reading of bichromate scale.	Actual amount of bilirubin per 100 cc. of bile (acid alcohol method).
	gm.	*gm.*	*days*		*mg.*	*mg.*
11	27.8	2.25	1	2.0	26.89	—
12	23.0	1.7	1	4.0	53.78	52.08
13	19.5	1.2	1	2.0	26.89	—
14	24.2	2.2	1	1.5	20.17	—
15	21.5	1.35	1	2.2	29.58	26.04
16	27.75	1.55	1	—	—	82.6
17	—	—	1	—	—	47.7
18	25.4	2.1	1	2.0	26.89	27.77
19	25.5	1.85	2	—	—	—
20	23.0	1.2	2	—	—	52.1
21	25.0	1.9	2	—	—	38.1
22	—	—	13	—	—	32.0
23	—	—	14	—	—	35.0
Average.....				2.25 (after 1 day of obstruction only).		

Average pigment content of bile after 1 day of obstruction is 30.7 mg. per 100 cc.

TABLE IV.

Comparison of the Pigment Strength of the Bladder and Duct Biles from Mice at the Close of the Bile Collection.

Mouse No.	Results.
4	Gall bladder bile showed 7 times the concentration of duct bile after a 4 hrs. collection.
5	" " " " 4 " " " " " " " " 5 " "
6	" " " " 3 " " " " " " " " 3 " "
7	" " " " 5 " " " " " " " " 3 " "
8	" " " " 5 " " " " " " " " $5\frac{1}{3}$ " "
9	" " " " 4 " " " " " " " " $5\frac{3}{4}$ " "
Average.	" " " " 4.66 " " " " " " " " 4.3 " "

Normal and Stasis Biles of the Rat.

The rubber bags used to obtain normal bile from rats were made out of finger cots and attached to glass cannulas of capillary diameter, with the ends well rounded and the shaft curved to avoid torsion on the duct. The cannula was fastened in the duct with ligatures above the entrance of the pancreatic duct, and in some instances the pancreatic duct as well was tied. Such fat necrosis as sometimes followed this latter procedure seemed not to affect the animal materially in the short period of bile collection. The rats ate well after the operation. At the end of 18 to 24 hours they were killed with chloroform.

To produce obstruction, ligature and severance of the common duct was employed. Bile leaks into the peritoneal cavity seldom were found and animals in which they occurred were discarded. Tissue icterus always appeared in 24 hours and bile pigment was plentiful in the urine. The twenty operated animals were killed at intervals ranging from 1 to 16 days after obstruction.

At autopsy the degree of dilation of the obstructed bile ducts was noted and the approximate amount of bile present within them. Very occasionally positive cultures were obtained from bile or liver tissue of the rats into which bags had been inserted, but, probably owing to the short period of collection, such biles seemed otherwise normal. The not infrequent infected cases among the animals with obstruction were ruled out. The collecting bags gave an abundant yield of bile—never less than 1.5 cc. and as much as 6.5 cc. per 100 gm. of body weight in 24 hours.

From Table V it will be seen that the pigment content of normal rat bile as determined in sixteen animals was 17.05 mg. of bilirubin per 100 cc., with a variation of 8.9 to 22.3 mg. It is of interest to note that the bulk of bile secreted per gram of liver tissue per hour in rats and mice is almost identical, but the proportion of liver weight to body weight in rats, 1 to 21.7, is considerably smaller than that of mice, 1 to 14.6. The quantity of bile secreted per 100 gm. of body weight was, per hour, 0.151 cc., or per kilo of body weight per day (24 hours) 36.3 cc.; and of bile per gram of liver per hour 0.034 cc., or per day 0.82 cc.

The normal rat bile was always a clear amber fluid as viewed in a test-tube, quite transparent, and a brilliant light yellow when placed in one of the reading tubes for comparison with the bichromatic scale. It showed remarkably little variation in pigment concentration, but had as a rule relatively less color when the amount of bile

TABLE V.

Normal and Stasis Biles of the Rat.

Pigment content of normal biles.											Pigment content of biles from obstructed ducts.						
Rat No.	Rat weight.	Liver weight.	Period of collection.	Total bile.	Quantity of bile per 100 gm. of body weight per hr.	Quantity of bile estimated per kilo of body weight per day.	Quantity of bile per gm. of liver per hr.	Color strength in per cent of bichromate solution.	Amount of bile pigment per 100 cc. estimated from reading of bichromate scale.	Actual amount of bilirubin per 100 cc. of bile (acid alcohol method).	Rat No.	Rat weight.	Liver weight.	Period of obstruction.	Color strength in per cent of bichromate solution.	Amount of bile pigment per 100 cc. estimated from reading of bichromate scale.	Actual amount of bilirubin per 100 cc. of bile (acid alcohol method).
	gm.	*gm.*	*hrs.*	*cc.*	*cc.*	*cc.*	*cc.*		*mg.*	*mg.*		*gm.*	*gm.*	*days*		*mg.*	*mg.*
1	166	8.7	25	3.6	0.087	20.8	0.017	—	—	13.87	17	109.0	6.0	1	1.3	17.48	—
2	158	7.5	25	5.7	0.144	34.5	0.030	—	—	12.33	18	99.0	5.0	1	1.2	16.14	—
3	122	7.0	22½	7.1	0.258	62.0	0.045	—	—	13.55	19	88.7	4.4	1	1.6	21.51	—
4	160	9.5	22	6.2	0.176	42.6	0.030	—	—	13.15	20	92.5	4.2	1	1.4	18.82	—
5	—	—	19	2.4	—	—	—	1.8	24.20	20.16	21	76.5	3.7	2	1.2	16.14	—
6	92	5.8	—	—	—	—	—	1.4	18.82	16.8	22	77.5	5.5	2	1.3	17.8	—
7	178	7.2	18	2.5	0.078	18.7	0.019	1.4	18.82	15.43	23	92.2	5.5	3	1.2	16.14	—
8	163	7.7	22	7.8	0.218	52.2	0.046	0.7	9.41	8.93	24	80.8	5.1	3	1.3	17.8	—
9	158	6.1	22	—	—	—	—	1.2	16.13	11.98	25	—	—	4	1.8	24.2	—
10	163	6.5	18	4.8	0.164	39.4	0.041	1.4	18.82	19.23	26	93.2	4.7	5	1.8	24.2	25.75
11	170	6.6	18	2.9	0.095	22.8	0.024	1.5	20.17	22.72	27	—	—	5	—	—	14.0
12	158	5.8	18	1.8	0.063	15.1	0.017	1.8	24.20	24.51	28	—	—	5	—	—	15.4
13	153	6.5	18	3.1	0.113	27.1	0.027	1.6	21.51	22.32	29	—	—	5	—	—	10.68
14	107	4.2	18	5.2	0.271	64.9	0.068	1.2	16.14	17.85	30	93.9	5.8	7	2.0	26.89	26.31
15	167	6.9	18	4.1	0.136	32.8	0.033	1.2	16.14	19.1	31	170.0	8.1	10	1.5	20.17	17.86
16	158	6.2	18	4.6	0.162	38.9	0.041	1.5	20.17	20.83	32	—	—	10	1.5	20.17	14.2
											33	166.2	8.0	12	0.8	10.76	13.88
											34	150.7	7.8	12	0.8	10.76	12.5
											35	—	—	12	—	—	18.4
											36	—	—	16	—	—	18.45
Average.	151.3	6.95			0.151	36.3	0.034		18.71	17.05						18.6	17.02

Proportion of liver weight to body weight in the rat is 1 to 21.7. For this average only the data of normal bile collection rats were used.

secreted was great. The stasis bile was closely similar. No change from yellow to green took place such as occurs in the dog and cat, and occasionally in mouse bile after long obstruction. Though the dilatation of the ducts became greater each day, the pigment strength of their contents was never more than that of normal bile, and as a matter of fact as time passed the concentration of pigment approximated the lowest normal figures, probably as a result of dilution with fluid elaborated by the duct wall, as in the case of dogs and cats.[6] In contrast to these results, stasis bile of the mouse always underwent a marked concentration, as will be recalled.

To find out whether there might be an early concentration of bile in the duct, followed by a later dilution as indicated, the sequence of changes in the bile after obstruction was carefully followed by means of animals killed on successive days. After the 5th day of obstruction, sufficient bile could be collected in the duct for a quantitative reading with acid alcohol, but in all cases the bichromate scale was used as with the animals killed earlier. The results in this sequence of animals are distributed amidst the earlier ones in Table V, with which they agree completely. The final average of 17.02 mg. of bilirubin per 100 cc. of bile in obstructed instances, as determined by the alcohol reagent, is remarkably similar to the figure obtained for normal bile—17.05 mg. The bichromate reading of 18.6 mg. per 100 cc. of bile also compares with the result found in non-obstructed cases of 18.7 per 100 cc. It is thus seen that rat bile undergoes no concentration after leaving the liver, even during long stasis. The secretion is about eight times as strong in pigment as mouse bile obtained from the ducts.

From these findings it is clear that there resides in the rat ducts no ability to concentrate bile such as resides in the gall bladder of the mouse. The dilatation of the rat ducts was, relatively speaking, enormous, and progressed with such regularity that one could almost state from it the number of days of obstruction. In several instances the common duct reached a diameter of 15 mm. in its broadest portion and it contained in one case more than 2.8 cc. of bile which, though still of a clear pale amber and a normal pigment content, contained more mucus than normal bile. In some animals the intrahepatic dilatation of the ducts was so great that the parenchyma had become

merely a sort of covering upon them. The liver tissue showed marked cirrhosis and was clay-yellow. Histologically the condition seen was similar to that found in dogs after long obstruction—a perilobular cirrhosis.

Influence of Diet.

The amount of bile secreted by the rat (36.3 cc. per kilo of body weight per day) and by the mouse (48.3 cc.) is not nearly as great as is put out by the rabbit and guinea pig, but is more than the output of the dog and cat. Thus, according to Quincke and Hoppe-Seyler[8] the rabbit secretes 136.8 gm. of bile per kilo of body weight a day and the guinea pig 175.8 gm., while the dog yields but 20.0 gm. and the cat 14.5 gm. per kilo of body weight. The large bile output of the Herbivora has usually been ascribed to their diet; and our animals were on a vegetable ration. To determine the importance of this factor, several rats were placed on a meat diet for a period of 17 days, and bag collections were then made as in normal grain-fed animals. But the bile obtained was not unusual either in amount or character.

SUMMARY.

In a previous paper the point has been brought out that the influence of the gall bladder upon the bile differs entirely from that of the ducts, the one organ acting to concentrate the secretion markedly and the other to dilute it slightly. The question arises, in species lacking a gall bladder, whether the concentrating function of this organ will be found lodged in the ducts. To test the point, observations have been made upon the mouse and rat, since these animals though so nearly related have, the mouse, a gall bladder and the rat, none.

The normal bile was first studied. Both animals were found to secrete larger quantities than do cats and dogs, but less than the guinea pig and rabbit. Methods were worked out for the quantitation of the pigment which was used as the index to changes in concentration.

[8] Quincke, H., and Hoppe-Seyler, G., in Nothnagel, H., Specielle Pathologie und Therapie, Vienna, 1899, xviii, 59.

Bladder bile of the mouse was regularly found to be more concentrated than that collected from the common duct of the same animal. The bile collecting during stasis regularly showed a great increase in pigment content, such as in other species is brought about by the action of the gall bladder. In the rat, on the other hand, stasis bile never became more concentrated in pigment than the normal.

The gall bladder, then, is not only absent from the rat in form, but in one at least of its important functions. That its other obvious function—that of a reservoir—cannot be assumed in the rat by the ducts would seem to be indicated, not only by the small size of these channels, but by the recent observation of Mann[2] that the tonus of the sphincter of Oddi is almost negligible in the rat, in contradistinction to animals which possess a gall bladder.

It is an interesting fact that the bile of the rat, which as has been said, undergoes no condensation of bulk after leaving the liver, contains on the average eight times as much pigment as does the liver bile of the mouse which is submitted to concentration. Whether it is correspondingly strong in substances useful for digestion, and therefore *ab initio* requires no concentration, is a matter upon which little can be said at present. However, in this connection the fact that the bulk of bile secreted per gram of liver weight is identical in both animals may be significant. Although this output is the same, the mouse liver when compared with the body weight (1 to 14.6) is relatively larger than that of the rat (1 to 21.7), so that the mouse secretes somewhat more bile per 100 gm. of body weight. This bile as it comes from the liver is but one-eighth as strong at least in pigment as rat bile, but the concentrating activity of the gall bladder is so great that the products yielded to the intestine may become not dissimilar.