FATE OF BILIRUBIN IN THE SMALL INTESTINE*

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The question of the existence of an enterohepatic circulation of bile pigment has been a subject debated for the last 18 years. The main proponents of the existence of an enterohepatic circulation of bile pigment are Broun, McMaster and Rous (1) and McMaster and Elman (2), while on the other hand Whipple (3) has consistently expressed his disbelief in such a mechanism. In 1923 Broun, McMaster and Rous (4) published data from which they concluded that such a circulation existed. Two years later McMaster and Elman (2) published additional data to further substantiate this theory. The latter authors reported that bilirubin or urobilin either in pure solution or in bile, when introduced into the duodenum of bile fistula dogs, caused an increase of the total bilirubin excretion by the liver.

Whipple (3) doubted the existence of an enterohepatic circulation of bile pigment on the basis of insufficient evidence showing that either bilirubin or urobilin was actually absorbed from the intestine.

The opportunity presented itself of investigating the fate of bilirubin in segments of intestine in animals with modified Thiry (5) loops. In order to compare results from a segment of a physiological unit with the entire unit, the fate of bilirubin in the intestine of the unanesthetized dog has also been studied. Since the chief objection to the acceptance of the concept of an enterohepatic circulation lies in the lack of quantitative data showing that bilirubin is absorbed from the intestine, it seemed of importance to carry out studies to determine this point.

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Method

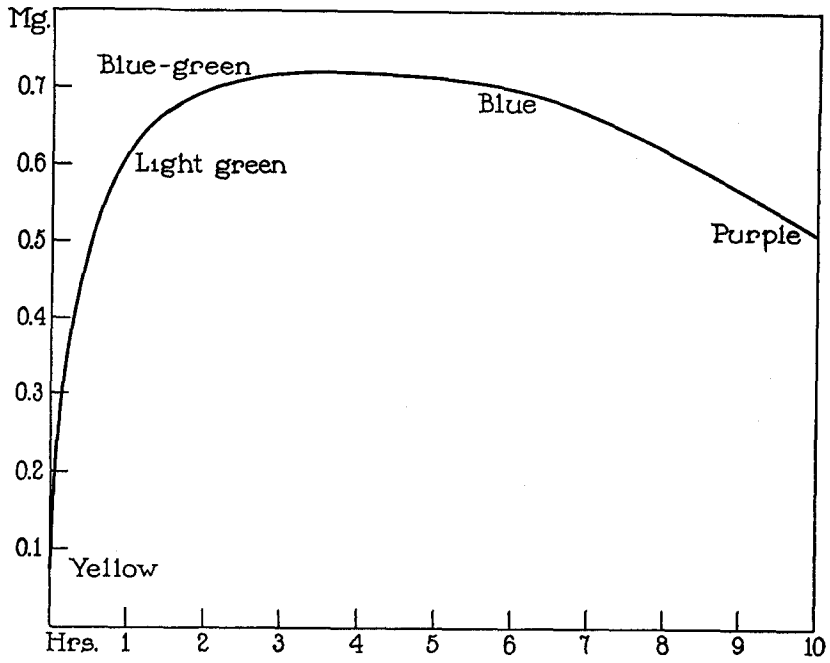
The animals used in these experiments were healthy dogs in whom a modified Thiry (5) loop had been prepared after the method of Johnston (6) some months previous to the experiment. This method permits of quantitative studies in unanesthetized animals. The nerve, blood and lymphatic supplies of these loops were intact and frequent studies of the rate of absorption of glucose indicated that the loops were functioning in a constant manner. It was only necessary to introduce a known amount of pigment into the loop and after a period of time had elapsed to remove all the fluid remaining and determine the pigment which was recovered. The loops were never sterile, but repeated irrigation results in a loop which is free from the usual organisms normally found in the intestine. Before any material was introduced into the isolated loop the latter was thoroughly cleansed. The material introduced into the loops for study was prevented from escaping by a special balloon catheter devised by Johnston (6). No anesthesia was necessary at any time.

The animal whose entire small intestine was to be utilized for absorption was operated upon under ether a week before the absorption experiment was to take place. Two catheters were introduced into the common duct, one of which was directed toward the liver and the other was directed toward the duodenum. By this procedure it was possible to collect all the bile and thus have the small intestine free from any bile pigment. In order to further insure that no pigment remained the animal was given a dose of magnesium sulfate 2 days before the experiment.

Food was withheld for 24 hours before the experiment began although the dog was allowed to have all the fluid desired. Known quantities of liver bile were introduced into the duodenum of the animals through the catheter which was directed toward the duodenum. The bile was allowed to remain in the intestine for a period of 1 hour at which time the animal was sacrificed. The intestinal tract was then rapidly removed from the animal and flushed first with water and then alcohol. These washings were immediately analyzed for bilirubin. The contents of the stomach were tested for bile pigment in order to ascertain whether any regurgitation of bile took place. If regurgitation had occurred the experiment was discarded. By this method it was possible to obtain almost total recovery (mean 97.5 per cent) of pigment immediately after the introduction of the bile.

The bile used was obtained from bile fistula dogs intubated by the double intubation method of Elman and McMaster (7). By this method it was possible to keep the animal in excellent condition since bile was obtained only at intervals and for a long period was rediverted into the duodenum. Daily bacteriological studies were made on the bile to ascertain whether it was sterile before being used. Bile placed in unsterile containers was labeled as contaminated bile. The infecting organisms were the streptococcus and staphylococcus. When grossly infected bile was desired normal saline solution containing colon bacilli was injected into the catheter directed toward the liver.

The Estimation of Bile Pigment.—The bilirubin was quantitatively determined by a modification of the Hooper and Whipple (8) method. A slightly stronger solution of acid-alcohol (0.40 cc. concentrated nitric acid and 6.00 cc. concentrated hydrochloride in 100 cc. 95 per cent ethyl alcohol) was used for the oxidation of the bilirubin. The increase in the acid concentration increased the speed of oxidation and this is further enhanced by keeping the solution at 37.5°C. instead of at room temperature which was the method outlined by Hooper and Whipple (8). By this procedure a greenish blue color develops which may then be compared with a standard color solution in a Duboscq colorimeter. The standard



GRAPH 1. Rate of oxidation in bilirubin.

was prepared by the addition of 0.75 cc. of a 1 per cent potassium dichromatic solution to 100 cc. of a 10 per cent copper sulfate solution. This standard was found to represent the color developed by 0.181 mg. bilirubin per cc. Various dilutions of the standard were utilized.

In the determination of bilirubin by this method, readings were made hourly after mixing the specimen of bile and acid-alcohol together until a maximum color concentration was reached. The maximum color usually developed within 4 hours. Once this color was reached it remained constant for several hours and then the deep greenish blue color changed to blue, purple and finally to black. Graph 1 indicates the rate of color change as compared with the standard solution.

In order to ascertain the experimental error of this method a weighed amount of bilirubin (Eastman Kodak Company) was dissolved in a known amount of 0.05 per cent sodium hydroxide solution. The concentration of bilirubin per cubic centimeter of solution was then calculated. A definite volume of the solution was then oxidized until the greenish blue color was reached and colorimetric determination made. The error of the method did not exceed 10 per cent.

RESULTS

Effect of Intestinal Juice on Bile Pigment in Vitro.—Before beginning the experiments on the absorption of bile pigment from the

TABLE I

Absorption of Bilirubin from Sterile Bile in Entire Intestinal Tract 1 Hour's Duration

Experiment No.	Amount in		Amount out	Loss	
	cc.	*mg.*	*mg.*	*mg.*	*per cent*
1	40.00	42.80	15.20	27.60	64.5
2	40.00	42.80	15.74	27.06	63.2
3	40.00	36.40	12.87	14.34	39.3
4	40.00	25.87	25.87	10.53	28.9
5	40.00	32.00	18.06	13.94	43.6
6	40.00	32.00	16.09	15.91	49.7
7	37.00	13.22	8.60	4.61	34.9
8	50.00	88.00	56.32	31.68	36.0
9	50.00	88.00	66.72	21.28	24.2
10	50.00	88.00	44.79	43.21	49.1
11	40.00	51.60	34.12	17.48	33.9
12	50.00	64.50	27.59	36.91	57.2
Average					43.7

intestinal loop it seemed important to know whether the intestinal juice secreted into the loop had any effect *per se* upon the bile pigment which would prevent good recoveries.

A known quantity of bile was mixed with some intestinal juice obtained from the loop. This solution was placed in a large test tube and kept at a temperature of 37.5°C. for a period of 2 hours. In one instance 1 cc. of the bile used for these studies contained 0.53 mg. of bilirubin before being mixed with the intestinal juice. After the 2 hour period 0.53 mg. of bilirubin was recovered. In none of these

TABLE II*a*

Absorption of Bilirubin from Sterile Bile in Intestinal Loops
2 Hours Duration

Experiment No.	Amount in		Amount out		Loss	
	cc.	*mg.*	*cc.*	*mg.*	*mg.*	*per cent*
1	25.00	19.00	53.00	20.93	+1.93	+10.1
2	25.00	19.00	39.00	21.25	+2.25	+11.7
3	25.00	19.00	130.00	18.98	0.02	0.1
4	25.00	19.00	68.00	20.06	+1.06	5.5
5	25.00	19.00	76.00	15.65	3.35	17.6
6	25.00	19.00	225.00	18.90	0.10	0.5
7	20.00	16.00	58.00	17.98	+1.98	+12.4
8	20.00	16.00	40.00	17.20	+1.20	+7.5
9	20.00	16.00	180.00	15.48	0.52	3.3
10	25.00	27.00	65.00	24.05	2.95	10.9
11	25.00	27.00	58.00	25.24	1.76	6.5
12	25.00	27.00	190.00	28.12	+1.12	+4.1
13	20.00	27.00	70.00	26.60	1.20	4.3
14	20.00	27.00	185.00	28.49	+0.69	+2.5
15	15.00	12.00	150.00	13.50	+1.50	+12.5
16	20.00	18.60	26.00	18.59	0.01	0.0
17	20.00	18.60	50.00	18.75	+0.15	+0.8
18	18.00	31.68	105.00	29.82	1.86	5.8
19	25.00	44.00	74.00	41.44	2.56	5.8
20	25.00	44.00	36.00	39.60	4.40	10.0
21	25.00	44.00	170.00	36.38	7.62	17.3
22	20.00	58.00	300.00	51.00	7.00	12.0
23	10.00	36.30	200.00	32.40	3.90	10.7
24	25.00	90.75	250.00	96.25	+5.50	+6.6
25	30.00	21.60	114.00	19.59	2.01	9.3
26	35.00	30.63	155.00	31.11	+0.48	+1.5
27	30.00	29.70	100.00	26.71	2.99	10.0
28	20.00	10.01	125.00	9.46	0.55	5.4
29	50.00	44.75	95.00	40.61	4.14	9.2
30	50.00	44.75	98.00	38.47	6.25	14.0

TABLE II*b*

Absorption of Bilirubin from Sterile Bile in Intestinal Loops
3 Hours Duration

Experiment No.	Amount in		Amount out		Loss	
	cc.	*mg.*	*cc.*	*mg.*	*mg.*	*per cent*
1	50.00	49.00	144.00	46.51	3.49	7.1
2	30.00	52.20	148.00	45.37	6.83	13.0
3	40.00	56.00	125.00	60.04	+4.04	+7.2

TABLE IIc

Absorption of Bilirubin from Sterile Bile in Intestinal Loops
5 Hours Duration

Experiment No.	Amount in		Amount out		Loss	
	cc.	*mg.*	*cc.*	*mg.*	*mg.*	*per cent*
1	50.00	44.00	420.00	40.99	3.01	6.8
2	50.00	44.00	90.00	47.93	+3.93	+8.9
3	50.00	44.00	150.00	45.00	+1.00	+2.2
4	40.00	30.20	395.00	32.19	+1.99	+6.6
5	40.00	30.20	107.00	31.03	+0.83	+2.7

experiments did the loss or gain in the pigment exceed the experimental error of the method. In a similar manner the secretion of the entire small intestine was used instead of that of the loop. Again quantitative recoveries were possible.

Fate of Bilirubin in Sterile Bile When Placed in the Intact Small Intestine.—As explained under methods, sterile bile was introduced into the duodenum of an unanesthetized dog by means of the common duct catheter. This bile remained in the small intestine for 1 hour. The animal was then sacrificed and the entire small intestine removed. The stomach was also removed and the contents tested qualitatively for any regurgitated bile. If a positive test for pigment was obtained the experiment was discarded. No bile was ever found in the large bowel if the animal was sacrificed 1 hour after the bile was introduced into the duodenum.

In twelve experiments the loss of bilirubin varied from 24.2 per cent to 64.5 per cent. The mean loss of bilirubin for this series was 43.7 per cent. In all experiments the amount of fluid removed was always less than the amount introduced (Table I).

Fate of Bilirubin in Sterile Bile When Placed in Intestinal Loops.—Sterile bile did not show any marked bilirubin loss at the end of 2 hours after it had been placed in the intestinal loop. In the majority of instances the bilirubin lost did not exceed the experimental error (Table II). It was thought that perhaps sufficient time was not allowed for the absorption of pigment in the intestinal loop. Therefore, eight other experiments were done, this time allowing the bile in three instances to remain in the loop for 3 hours and in five experiments

for 5 hours. It can be seen that in the majority of instances the loss of bile pigment did not vary markedly from the error of the method.

Fate of Bilirubin in Contaminated Bile When Placed in Intestinal Loops.—In a similar manner contaminated bile was placed in the

TABLE III

Absorption of Bilirubin from Contaminated Bile in Intestinal Loops 2 Hours Duration

Experiment No.	Amount in		Amount out		Loss	
	cc.	*mg.*	*cc.*	*mg.*	*mg.*	*per cent*
1	20.00	12.00	222.00	10.88	1.12	9.3
2	20.00	12.00	95.00	11.40	0.60	5.0
3	25.00	12.00	125.00	10.75	1.25	10.4
4	18.00	10.80	160.00	10.40	0.40	3.7
5	18.00	10.80	90.00	10.75	0.05	0.4
6	18.00	10.80	89.00	8.90	1.90	15.3
7	60.00	52.68	200.00	50.63	2.05	3.7
8	25.00	28.50	218.00	30.74	+2.24	+8.5
9	25.00	28.50	89.00	28.30	0.20	0.7
10*	35.00	61.60	174.00	60.39	1.21	1.9

* 3 hours.

TABLE IV

Absorption of Bilirubin from Infected Bile in Intestinal Loops 1 Hour's Duration

Experiment No.	Amount in		Amount out		Loss	
	cc.	*mg.*	*cc.*	*mg.*	*mg.*	*per cent*
1	30.00	14.40	83.00	13.45	0.95	6.6
2	30.00	14.40	68.00	12.78	1.62	11.2
3	30.00	14.40	125.00	13.50	0.90	6.2
4	30.00	10.65	75.00	9.50	1.15	10.7
5	30.00	10.65	50.00	9.00	1.65	15.4
6	30.00	8.47	65.00	7.45	0.93	10.8
7	30.00	8.47	95.00	8.36	0.11	1.3
Average						8.8

intestinal loop. The organisms found in this bile were usually the streptococcus and staphylococcus. In nine experiments the bile was allowed to remain in the loop for a period of 2 hours and in one

experiment for 3 hours. In only one instance did the recovery show a loss greater than the limit of the experimental error (Table III).

Fate of Bilirubin in Infected Bile When Placed in Intestinal Loops.—The bile used for these experiments was grossly infected with colon organisms. Also, the liver of the animals secreting this bile was damaged by the previous injection of a solution of colon bacilli into the biliary tract. Often the bile collected from these animals had an orange color and a putrid odor. When this was mixed with the acid-alcohol solution instead of obtaining the characteristic color changes which bilirubin normally undergoes, they were changed immediately to a deep red color. It was, therefore, usually impossible to estimate the bile pigment of the bile by this method. However, it was sometimes possible to obtain bile from these animals which was not so markedly affected and thus bile pigment could be determined. Using this type of bile seven experiments were carried out, the bile remaining in the loop for 1 hour. In three of these the amount lost was within the experimental error of the method while in another three the loss was very close to the limit of error (Table IV).

DISCUSSION

The results of these experiments indicate that there is a loss of bilirubin from sterile bile when the entire small intestine is utilized for the experiments. The question arises as to whether the bilirubin has been absorbed, or whether it has been changed to some substance in the lumen of the intestine that can no longer be estimated as bilirubin. Since it was found that complete recovery of bilirubin was possible when bile was incubated at body temperature with jejunal loop secretions for 2 hours, it can be concluded that such juice has no effect upon the bile pigment even after 2 hours contact. This was also true for juice from the entire small intestine. It is a fact, however, that urobilin is formed in the intact intestine.

The question naturally arises as to whether the pigment is absorbed as bilirubin or is converted to urobilin to be absorbed or excreted. The data presented cannot answer this question. McMaster and Elman (2) were able to show that urobilin is absorbed from the intestine. They found an increase in pigment output through bile fistulae after the introduction of urobilin into the intestine. Although

Blankenhorn (9) showed that after introduction of bile into the small intestine, there was little or no increase of bilirubin content of the blood in the portal vein as compared to that of the jugular vein, there was a marked increase of urobilin in blood of the portal vein. There was also an increase of urobilin in the lymph of the lymphatic vessels which led from the intestine. The experiments of Blankenhorn strongly suggest that most of the bile pigment absorbed from the intestine is carried away as urobilin. A consideration of the work of Blankenhorn and this present study would indicate that the intestinal contents and mucosa are capable of changing bilirubin to urobilin. Bollman, Sheard and Mann (10) were unable to find any increase in bilirubin content in the blood of the mesenteric veins after the introduction of bile into the intestine. These investigators, however, did not estimate the urobilin content of the blood.

The results obtained when either sterile, contaminated or grossly infected bile was placed in the modified Thiry loops strongly indicates that under the conditions of the experiments little or no pigment was absorbed. The difference between the loop experiments and those in which the intestinal tract was utilized as a physiological unit emphasizes the care which must be exercised in making generalizations from data obtained from only one portion of a physiological unit.

SUMMARY AND CONCLUSIONS

Since there was no loss of bilirubin from the jejunal loop, and no loss of bilirubin when pigment was incubated with juice from the loop segment, or juice from the entire small intestine, it may be concluded that the intestinal juice *per se* has no effect in converting bilirubin to urobilin in a 2 hour period, and that in the jejunal loop there was no absorption of pigment or no conversion to urobilin. The experiments showing loss of pigment in the entire intestinal tract suggest that in some place other than the jejunal portion of the intestine the combined activity of intestinal contents and intestinal cells does affect the bilirubin in the intestine. Whether the loss of bile pigment under such circumstances is due entirely to conversion, or to conversion and absorption, or to absorption of bilirubin as such, remains to be answered by subsequent investigations.

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