ON THE ABSORPTION OF BILE PIGMENTS FROM THE INTESTINE.

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Clinical and experimental studies of the distribution and elimination of bile pigments in man have furnished valuable information as to the state of the liver and the function of bile ducts. Like studies aimed at an understanding of the condition of the blood with special reference to hemoglobin economy have not been so successful although it is well understood that there is an essential relationship between bile pigment and blood pigment (1). That blood destruction finds expression in bile pigment formation (2) and that true bile pigment, as distinct from that of extraneous origin, has no other source than hemoglobin (3) are facts now generally accepted; but as yet no certain quantitative relationship between the two has been established. The chemical process, taking place within the body, which leads to a conversion of one pigment into the other is entirely unknown and other factors less fundamental in nature interfere with our using bile pigment, or any of its derivatives, as a means to measure blood destruction. Among the most important of these factors is the possible reabsorption of bile pigments from the intestine, a reabsorption which might change the bile output or add to the store of hemoglobin. There is much clinical and some direct experimental (4) evidence that such reabsorption does occur. But the final proof, namely a demonstration of the presence of bile pigments in the blood or lymph coming away from the intestine and its removal by the liver, has been wanting. To supply this evidence has been the object of the present investigation.

The blood of the portal vein has been examined frequently for bile pigments, but always under the complex conditions which result from opening the abdomen; and no bile pigments have been found by any observer.

The method I have employed here is new and makes the portal vein accessible to puncture almost at will, weeks after the healing of the operative wound[1] necessary for the introduction of the puncture apparatus. To render the portal vein thus accessible a hollow silver tube is placed within the abdomen, one end being fixed to the vein while the other end lies beneath the skin of the belly wall where it is easily found on palpation prior to introducing a needle. The needle is thrust through the skin, through the tube directly into the vein. The complete details of this method are given in another paper.

In the present study seven dogs were successfully operated upon and furnished nineteen different specimens of portal vein blood to be tested for bile pigments. Six of the animals were also operated for "altercursive intubation" of the common bile duct after the manner of Rous and McMaster (5), so that the pigment content of the intestine could be controlled at will. Both operations were done in each instance at a single sitting.

When the operated animals were well recovered and appeared to be healthy, they were bled from jugular vein and portal vein at the same occasion and the specimens examined for bile pigments. The animals equipped with "altercursive intubation" were, after preliminary bleeding, adjusted for bile collection so that no bile entered the intestine. Stools and urine were collected daily and examined for bile pigment. When stools were entirely acholic, the animal was again bled from portal vein and jugular vein. Following this bleeding, bile was permitted to return to intestine and when stools showed the return of pigment bleeding was done again. In accord with this plan portal vein blood and jugular vein blood were compared as to their bile pigment content under conditions of choice in so far as there was or was not bile within the intestine. In but one dog were we able to get portal blood of all three phases in the same animal.

Blood specimens were tested for bilirubin by the diazo reaction, but quantitative tests were not possible on account of the minute quantities found. Urobilin was tested by the Schlessinger fluorescence method and quantitative determinations made according to a plan

[1] Ether anesthesia was used in every instance where an animal was operated upon, and anesthesia continued throughout the operation.

proposed by Elman and McMaster (6) and changed somewhat to deal with very small amounts as found in blood and lymph.

In brief these animals at no time showed any bilirubin in portal vein blood or jugular blood, nor even any yellow coloring of the plasma. On the other hand urobilin was always present in portal vein blood whenever there was bile in the intestine and always absent from portal vein blood when bile was excluded from intestine. Urobilin was found occasionally in jugular blood but only when also in portal blood and always in less amounts. The amount of urobilin in portal blood averaged .008 mg. per cc., and that of the jugular blood .0006 mg. per cc.

The protocol given herewith is of the animal from which portal blood in all three phases was obtained; that is to say, portal blood when there was bile in the intestine, portal blood when there was no bile in the intestine, and portal blood when bile had been returned again to the intestine.

Protocol.

Dog 4, dachshund, 8 kilos.

October 22, 1925. Ether anesthesia. Operation for portal vein sampling and for "altercursive intubation" of common duct.

1st day after operation. Dog active, wound clean.

4th day after operation. Tapped portal vein for 30 cc. Portal blood was negative for bilirubin, positive for urobilin (.0036 mg. per cc.). Jugular blood negative for bilirubin, positive for urobilin (.00054 mg. per cc.). Clamp put on for bile collection.

27th day after operation. Stool and urine negative for urobilin and bilirubin. Bile urobilin-free. Tapped portal vein, 10 cc., negative for bilirubin and urobilin. Jugular vein blood negative for bilirubin and urobilin. Clamp put on for return of bile to intestine.

29th day after operation. Stools contain much urobilin. Urine small amount. Tapped portal vein, 6 cc., with considerable difficulty. Bilirubin negative, urobilin positive (.0018 mg. per cc.). Jugular blood negative for bilirubin, negative for urobilin.

Lymph Absorption.

Absorption by way of the lymph channels was next investigated and lymph obtained by intubating the thoracic duct in the neck was examined for bilirubin and urobilin under various conditions of

intestinal absorption. In every instance dogs were used, the animals being under ether anesthesia throughout the operation and the period of collection. Animals that had been fasting, and hence presumably not digesting and absorbing, animals that were liberally fed, and animals with enteric injections of bile and chyme containing bile were examined. Lymph specimens were tested for bilirubin and urobilin and compared with specimens of jugular vein blood in the same manner as was portal vein blood in the experiments above.

Eleven dogs that had fasted from 1 to 3 days but had abundance of water were examined. The lymph in five animals contained bilirubin and in six gave no test. Meanwhile the jugular blood in no instance gave a test for bilirubin. This same lymph tested for urobilin was positive in ten instances (average .011 mg. per cc.) while the blood was positive for urobilin in nine specimens (average .003 mg. per cc. or about $\frac{1}{4}$ as much).

Twelve dogs liberally fed and watered 2 to 5 hours previous to the experiment were tested in the same manner. Five of these animals were fed a meal composed of cooked beef lungs, bread, and 200 gm. of lard containing 20 mg. of pure bilirubin dissolved in the lard. Lard has been found to be a good solvent for bilirubin and in the above proportion is intensely yellow so that it can be diluted many times and still give a positive diazo test. Furthermore such lard can be emulsified without losing its bilirubin if kept in a neutral or slightly acid medium. It was thought that bilirubin thus protected from gastric or intestinal digestion might be absorbed into the lacteals after the manner of other fat-soluble dyes. Seven of the twelve dogs showed tests for bilirubin in lymph and five were negative. The positive tests were of the same order of magnitude as in the fasted animals, that is not enough to be measured. At the same time no animal showed bilirubin in jugular blood. The lymph of these twelve animals in every instance contained urobilin (average .0585 mg. per cc.), while the blood was eleven times positive, averaging .00531 mg. per cc. From this it can be seen that feeding had increased very materially the amount of urobilin of lymph and blood, but had practically no effect on bilirubin of either.

Six of the fasting animals tested for absorption by way of lymph were injected with bile containing mixtures directly into the duo-

denum, either by a previously established "altercursive intubation" or by syringe and needle into the exposed gut. Two of these animals received bile-stained chyme removed from another dog supposedly at the height of digestion.

This procedure had little or no effect on the bilirubin content of jugular vein blood for whereas all six animals had no bilirubinemia prior to injection only one showed any bilirubinemia after the injection and that one in amounts too small to be measured. Breast lymph and mesenteric lymph (collected in a manner shown below) also showed little or no change, being practically free of bilirubin at all times.

The injection did, however, cause urobilin to increase, and the jugular vein blood of six animals which, before injection, were three negative and three positive, after injection became five positive and one negative. Breast lymph was similarly affected as shown by Table I.

These experiments to investigate the absorption of pigment by way of the lymph (twenty-three in all) showed very clearly that when intestinal absorption was active urobilin was present in lymph in amounts greater than in systemic blood, and furthermore that when the concentration of pigment in the intestine was increased by injection, urobilin of the lymph was also increased.

It must be noted, however, that bilirubin was present in the lymph in twelve out of twenty-three tests, occurring slightly oftener in fed animals and injected animals than in fasting animals as shown by Table I. This does not indicate absorption of pigment, for such amounts of bilirubin might come directly from the liver by way of the liver lymphatics. The anesthesia did not cause bilirubin to appear in lymph for the general tendency was in all cases towards lesser amounts of pigment as the ether was prolonged. The observation that injections of bilirubin into the intestine did not increase the bilirubin content of the lymph argues very strongly that bilirubin of the lymph was not absorbed bilirubin.

To test the matter of the source of this pigment more critically five animals were operated in a manner to expose the lymphatics of the small intestine in the mesentery just as they enter the receptaculum chyli.

TABLE I.

A. Absorption by Portal Vein. Comparison of Bile Pigment Content of Portal Vein Blood and Jugular Vein Blood in Healthy Animals.

B. Comparison of Bile Pigment Content of Breast Lymph and Jugular Vein Blood of Eleven Fasting Dogs.

C. Comparison of Pigment Content of Breast Lymph, Mesenteric Lymph, and Jugular Vein Blood.

	A		B		C		
	Seven healthy animals		Eleven fasting dogs		Twelve fed dogs		
	Portal vein	Jugular vein	Breast lymph	Jugular vein	Breast lymph	Mesenteric lymph	Jugular vein
Bilirubin positive	0	0	5	0	7	3	0
Bilirubin negative	19	19	6	11	5	2	12
Urobilin positive	9 (Average .008 mg. per cc.)	8 (Average .0006 mg. per cc.)	10 (Average .011 mg. per cc.)	9 (Average .003 mg. per cc.)	12 (Average .0585 mg. per cc.)	5	11 (Average .0053 mg. per cc.)
Urobilin negative	*3	**4	1	1	0	0	1

* In these three instances the animal was acholic on account of bile diversion by way of "altercursive intubation."
** In three instances the animal was acholic on account of bile diversion.

D. Absorption by Way of Lymph. Comparison of Lymph with Jugular Vein Blood before and after Injection of Bile into Intestine.

	Six injected dogs					
	Before injection			After injection		
	Jugular vein	Breast lymph	Mesenteric lymph	Jugular vein	Breast lymph	Mesenteric lymph
Bilirubin positive	0	2	2	1	3	2
Bilirubin negative	6	3	0	5	2	0
Urobilin positive	3	3	2	5	4	2
Urobilin negative	3	3	0	1	1	0

This was done under ether anesthesia which was continued the entire time of the experiment. The intestines were protected from drying by large sheets of rubber dam and all bleeding avoided as carefully as possible. To make a good exposure the abdominal walls were cut down transversely between heavy wire ligatures and all bleeding points tied or clamped. A large lymph channel was then cannulated with a fine glass tube bent at a convenient angle and with a bulb blown in the stem to hold about 3 cc. When the cannula was in place suction applied by a rubber tube caused the cannula and its bulb to fill with lymph. The full bulb was then emptied with a Wright's pipette and in this manner as much as 30 cc. was collected for comparison with blood from the jugular vein.

Of five such specimens three gave positive diazo tests for bilirubin (the greatest being .02 of the van den Bergh unit). While the blood from jugular vein was negative.

All five contained urobilin and averaged .085 mg. per cc. or about the same order of magnitude found in thoracic duct lymph.

This evidence is very suggestive that small amounts of bilirubin were absorbed, for lymph collected in this manner could come from nowhere save the intestine. There remains, however, the possibility that this small amount of bilirubin could have been formed by the peritoneal membranes of the intestine from hemoglobin free in the belly for it is impossible to say that there was no bleeding and the duration of the experiment—*i.e.*, several hours—might possibly be occasion for bilirubin to be formed. The evidence as to urobilin on the other hand is conclusive that this pigment is absorbed from the intestine into the lymphatics.

COMMENT.

The experiments reported here require little comment by way of explanation and even less in interpretation. The observations on the portal vein blood and the jugular vein blood of the seven normal dogs are conclusive—that bilirubin is not and that urobilin is absorbed from the intestines. The proposition that absorbed pigment is in part or altogether removed by the liver from the blood is in fact a corollary to the proof that urobilin is absorbed at all. Such a function of the liver is further suggested by the fact that portal vein blood—as well as lymph from the intestine—always contains more urobilin than does blood obtained elsewhere—that is to say distal to the liver as located in reference to intestine. It is not vital to this paper to know what

becomes of biliary pigments once they are absorbed from the intestine. It is interesting to note that blood that has been through the liver contains much less of this pigment than do blood and lymph coming direct from the intestine. In none of these experiments was anything observed at variance to the idea that urobilin absorbed from the intestine appears in urine and in bile.

Table I is appended to show in condensed form all the data mentioned in the text as well as many other details omitted above for the sake of brevity. The table is self-explanatory.

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CONCLUSIONS.

Bilirubin as such is not reabsorbed from the intestine by way of the portal vein in healthy animals. Bilirubin may be absorbed from the intestine by the lymphatics but only in minute amounts.

Urobilin is reabsorbed from the intestine by way of the portal vein and by way of the lymphatics.

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