STUDIES ON THE TOTAL BILE.

IV. THE ENTEROHEPATIC CIRCULATION OF BILE PIGMENT.

By G. O. BROUN, M.D., PHILIP D. MCMASTER, M.D., AND PEYTON ROUS, M.D.

(From the Laboratories of The Rockefeller Institute for Medical Research.)

(Received for publication, December 26, 1922.)

According to accepted opinion the pigment of the bile represents waste from the hemoglobin molecule, and as such is eliminated from the organism. Yet the belief is well-nigh general that this waste, after undergoing a change to urobilin, is reabsorbed in part from the intestine. Why should the reabsorption take place? Does it indeed occur? Is any unchanged bile pigment reabsorbed? These are questions which have led to much work and more speculation. The present paper is concerned with but one of them, namely with the reabsorption of bile pigment, irrespective of its form.

Whipple, the most recent investigator of the theme, states that: "There is no evidence that bile pigment or stercobilin is absorbed from the intestine." And yet there are phenomena on record which might be construed as pointing to such an occurrence. It is true that Hooper and Whipple could find no indication in the bilirubin yield from fistula dogs that any pigment from bile given by mouth was absorbed. But they dealt with the yield of 8 hours only in each 24. Baldi noted as far back as 1883 that following the administration to fistula dogs of beef bile green with biliverdin, the dog bile changed from brown to green; and Schiff observed that the urine of animals so treated gave Gmelin's reaction. Stadelmann presents figures which show that when his fistula dogs were prevented from licking up the bile secreted during 12 hours of each 24 the output of pigment during the remaining period lessened somewhat, though not greatly. Since Wertheimer's work there has been no doubt of the fact that the normal

5 Wertheimer, E., *Arch. physiol. norm. et path.*, 1891, iii, series 5, 724; 1892, iv, series 5, 577.

699
liver will rapidly remove bile pigment from the blood stream and resecrete it into the bile.

Our experiments were carried out with dogs permanently intubated for the collection of the total bile. The methods employed in the care of the animals and the quantitation of the bile constituents have already been described. Often the secretion remained sterile during many weeks of collection, until the animal was killed. Such infections as occurred were frequently limited to the collecting apparatus; and the organisms were always non-pathogenic, and failed to effect quantitative changes in the bile pigment, as proven by comparing specimens of the infected bile incubated in vitro with others kept in the ice box, and by similar tests with biles previously sterile, and now infected in the test-tube with the organisms. The specimens compared were treated with acid alcohol according to Hooper and Whipple’s method, and viewed in the Dubosq colorimeter.

The observations of Baldi received an indirect confirmation early in the work. When our dogs were fed cooked liver of the sheep or ox, the bile for several days thereafter was noted to be green instead of the previous yellow-brown. But as Wertheimer remarks of Baldi’s finding, such a change may be the result merely of an oxidation of the bilirubin of the dog bile itself to biliverdin, and in any event it is essentially non-specific. Whenever air was present in the collecting balloons we employed there occurred some conversion of bilirubin to biliverdin. But this source of error was avoidable. Perhaps our best evidence for a significant relationship between liver feeding and the color change is to be found in the fact that the association of the two attracted our attention prior to knowledge of Baldi’s report.

Wertheimer tested for an enterohepatic circulation of pigment by administering sheep bile to dogs and searching the dog bile afterwards for cholohehmatin, present in the bile of the sheep and recognizable by spectroscopic means. He made collections directly from the common duct of etherized animals and had no difficulty in finding cholohehmatin after the injection of sheep bile into the circulation. But his experiments to determine a resorption of it from the intestine

---

during the few hours time that the conditions permitted yielded negative results. Our own endeavors, with unanesthetized and vigorous dogs given sheep bile by mouth, have been more successful.

**Experiment 1.**—A large number of unemptied gall bladders of the sheep were obtained from a slaughter house. The bile in some was green and in others a pale yellow. These latter were discarded and the green secretion was pooled. When given as such in quantity to dogs it nearly always induced vomiting, but when somewhat concentrated on the water bath it proved tolerable. Chloroform extracts of the material, evaporated and dissolved in ether, according to the method of MacMunn, showed with the spectroscope the four bands characteristic of cholohematin, namely one in the red about the line C, well marked and broad, another in the yellow near D, and two in the green. Portions of the material were fed by stomach tube to two dogs, the bile of which in chloroform extract had been spectroscopically examined for some days previously.

Dog 19, a male bull terrier weighing 13 kilos, yielded on the 37th to 40th days after intubation a bile the extracts of which showed with the spectroscope a narrow, pale band in the yellow-green as well as the usual obscuration of both ends of the spectrum. Early in the 41st day of period of collection two attempts were made to feed sheep bile, as such, by stomach tube. The entire quantity was vomited practically at once, and the spectrum for the period did not differ from that of the preceding days. Early in the 42nd period 35 cc. of sheep bile concentrated from an original bulk of 93 cc. was given by tube, and slightly more than an hour later 40 cc., representing 100 cc. of the secretion. Practically all was retained despite some retching. Extracts of the dog bile for the 24 hour period showed plainly the spectrum characteristic of cholohematin. It was much stronger in the next specimen of the 43rd collecting period, was still well defined in that of the 44th, and gradually disappeared thereafter, with no trace in the 47th specimen.

Dog 25, a rough haired terrier of 15½ kilos. Extracts of the bile obtained from this animal, during the preliminary observations of the 36th to 40th collecting periods inclusive, yielded the spectrum already described for the control specimens from Dog 19. At the beginning of the 41st period of collection 30 cc. of concentrated sheep bile, corresponding to 80 cc. of the original material, was given by tube, and followed 75 minutes later by 45 cc. more, equalling 120 cc. of the unchanged secretion. The same material was used as in the case of Dog 19, and just as in this animal the spectrum of cholohematin appeared and persisted for 5 days.

We have had abundant opportunity to confirm Wertheimer's statement that, under ordinary conditions, the bile of fistula dogs does not contain cholohematin in quantity sufficient to show strongly in the spectrum. In the cases of the two dogs cited, where the bile contained the substance, the experiments were repeated with the same material, and the results were essentially the same. No material that has been subjected to the same treatment has ever yielded any spectrum similar to that characteristic of cholohematin. We have had abundant opportunity to confirm Wertheimer's statement that, under ordinary conditions, the bile of fistula dogs does not contain cholohematin in quantity sufficient to show strongly in the spectrum.

---

not yield a spectrum which can be confused with that of cholohematin. The temporary appearance of the spectrum of the pigment after feeding it to dogs, in the form of sheep bile, would seem to prove conclusively that it is absorbed from the intestinal tract and resecreted by the liver.

The sheep bile used was lightly pigmented with biliverdin. Following its administration there was frequently a change in the color of the dog bile from yellow to green, as after feedings of the liver tissue of the herbivora, although the total pigment content of the dog bile underwent no appreciable alteration. But extremely little biliverdin is required to render bile green.

Experiments were now begun to test whether the giving of bilirubin by mouth in the form of dog bile would cause a rise in the output of bilirubin by the liver.

Since bilirubin is altered by weak acids, and in this instance would be submitted to the acid of the gastric juice, it seemed best to give large quantities in order to provide opportunities for some at least to reach the intestine in unchanged form. The material consisted of pooled 24 hour specimens derived, not infrequently, from the animal to be tested. The precise amount of bilirubin so administered was determined by the same method employed to quantitate the daily yield from the animal. The bile was given by stomach tube at the beginning of a collecting period, with, sometimes, a second portion several hours later. It was well tolerated. All the dogs were in good condition except one (No. 23). Shortly after the initial gavage they were fed as usual. The character of the diet, which was always poor in fat, and the amount taken were recorded, but the data have no importance in the present connection, since, as has been shown in a previous paper, diets such as we employed are void of effect on the yield of bilirubin from day to day. Nevertheless, a standard ration was fed throughout the experimental and control periods, save in special instances. The hemoglobin percentage of the blood was closely followed, because marked variations in it are usually accompanied by similar variations in the bilirubin output.

The extreme cholorrhea induced by bile feedings was occasionally associated with a curious color change in the pigment when submitted to acid alcohol prior to readings in the colorimeter. The ordinary change is through a transient green to a more enduring blue-green with which latter hue the inorganic standard corresponds. But in a few of our specimens the abnormally profuse bile turned instead to a brilliant purple. Urobilin was never present. The bile fed gave always the ordinary color reaction. We have had occasion to note that normal gall bladder bile,—which is sterile,—frequently changes under the influence of
acid alcohol to a purple hue, possibly from the presence in it of bilicyanin. Whatever the cause for the color peculiarity it renders the quantitation of pigment difficult. Occasionally this could be carried out only by a comparison of water-diluted specimens with similarly treated samples of bile of known pigment content.

The charts present the essential data on bile quantity and bilirubin yield for the entire series of thirteen experiments (Text-figs. 1 to 5).

In six of the ten experiments in which bile was fed on a single day or on two successive ones (Text-figs. 1 and 5) there occurred a marked rise in the bilirubin output followed by nearly as abrupt a drop to the previous level. The changes were too great and abrupt to be referable to intercurrent variations. In the remaining four instances (Text-fig. 2) the pigment output either underwent no alteration or the increase was insignificant. In one of them (Dog 24), as in one of the series giving positive results (Dog 20), the hemoglobin percentage of

---

9 Rous, P., and McMaster, P. D., J. Exp. Med., 1921, xxxiv, 47.
the blood varied considerably during the observations, and the bilirubin output underwent corresponding changes.

In three animals bile was administered by stomach tube on each of 6 to 10 successive days. In one such instance the bilirubin output altered but little (Text-fig. 3); in another it underwent occasional increases, more abrupt, and one of them far greater, than any observed during control periods (Text-fig. 4); while in a third (Text-fig. 5) the pigment yield doubled in quantity almost immediately that the feedings were begun, and increased till toward the end of them it had more than quadrupled. This dog (No. 23) had long been ailing, owing, as autopsy showed, to a high intestinal obstruction; and the administration of the bile was followed by a remarkable change for the better in its condition. The protocol has such interest that it will be summarized.
TEXT-FIG. 3. Effects of repeated bile feedings.

TEXT-FIG. 4. Effects of repeated bile feedings.
Dog 23, a brown spaniel, was found pregnant at the operation to insert a bile-collecting apparatus, and a hysterectomy was done. Immediately afterwards the animal weighed 15½ kilos. For some weeks it remained in good condition, save for a gradually developing secondary anemia of greater severity than that ordinarily following total loss of the bile. There was loss of weight, and a slight purulent discharge where the collecting tube emerged from the abdomen. The bile itself remained sterile until just before the animal was killed, after nearly 3 months of observation. 6 weeks from the time of the operation the animal was sluggish and its appetite was noted to be capricious. It now fell ill of a severe, intercurrent bronchitis that greatly depleted it. After 65 days in all, when it had recovered, it weighed only 11 kilos, ate almost nothing, and lay languidly in its cage. A variety of diets poor in fat failed to tempt it, and death seemed imminent. In two successive 24 hour periods only 9.1 and 9.8 cc. of bile respectively was secreted, instead of the 125 to 240 cc. given earlier. Yet
these small portions held the usual 24 hour quota of bilirubin. Bile feedings were now begun, of 200 cc. by gavage twice each day. Within 3 days the animal was lively, eating food of a sort previously refused, and gaining in weight. But after bile had been administered in large quantity for 7 days and the condition of the animal had become good, a watery, bile-stained diarrhea developed, accompanied by anorexia, and by the 10th and last day of bile feeding these disturbances had become severe. Immediately that the feedings were stopped the diarrhea ceased, the appetite returned, and during the next few days the dog appeared better than for some weeks. But soon pronounced symptoms of intestinal obstruction manifested themselves, rendering it necessary to sacrifice the animal,—on the 85th day. Autopsy showed that a loop of small bowel had become included in an adhesion near the exit wound for the collecting tube, with result in practically complete obstruction about 4 feet beyond the pylorus. Above this point the intestine was greatly distended, and full of a clear, nearly colorless fluid, while below it was empty. The liver and bile ducts were normal.

DISCUSSION.

The evidence here presented for the absorption of bile pigment from the intestine is of unequal value. The change in color of dog bile after the feeding of the green bile or the liver tissue of herbivora counts as no more than suggestive in this connection. The appearance in the bile of cholohematin, after administration of the pigment by mouth, shows conclusively that a substance nearly related to bilirubin undergoes enteral absorption and excretion by the liver. But as a matter of fact, recently proven, this is shown sufficiently by the presence of the substance in the bile of sheep and cattle. For cholohematin, or bilipurpurin, as it has also been called, is no true constituent of the bile of the herbivora, such as MacMunn and Wertheimer supposed it to be, but is of extrinsic origin, being a derivative of the chlorophyl of the food and identical with phylloerythrin. Only when the fodder contains chlorophyl is it found in the bile.

The results of the feeding of dog bile leave no doubt that the bilirubin output was increased thereby in many instances. But why was it not increased in all? One reason is that circumstances were frequently unfavorable to absorption from the intestinal tract. For bile in quantity by mouth often acts as a purgative and in consequence may itself be so rapidly hurried through the bowel as to appear unchanged.

in the stools. This took place in several of our animals; but unfortunately it did not attract attention until late in the work, so that the protocols cover the occurrence insufficiently. In contrast to such instances is that of Dog 23 in which the fed bile was probably retained longer than usual in the upper part of the intestinal tract, owing to partial intestinal obstruction. The enormous increase in the output of bilirubin may in this way be explained.

Is the pigment increase necessarily to be attributed to intestinal resorption? It is certainly not due to flushing out by the quickened bile stream. The independence of the rates of bile flow and of bilirubin secretion has impressed practically all workers; and we have recently recorded some striking instances illustrating it. One is mentioned in the history of Dog 23 here given. Furthermore, a flushing out of pigment could scarcely endure for days; and in any case there should follow a drop in the bilirubin output below the previous level until the substance has again accumulated in the organism. No such drop was to be seen.

A relatively small liberation of hemoglobin by blood destruction would account for the pigment increase. It was in this way that Stadelmann explained such increase as he observed after the feeding of large quantities of bile salts to fistula dogs. He fed no greater amounts than our dogs may have received in the form of bile, so his view deserves attentive consideration in connection with the present findings. If it be assumed that 1 gm. of bilirubin in the bile represents approximately 1 gm. of hematin from blood, that the hemoglobin molecule yields 4 per cent of hematin by weight, and that 100 cc. of dog blood with 100 per cent of hemoglobin by our Newcomer standard contains 13.8 gm. of hemoglobin,—as special determinations showed to be the fact,—then the destruction of but a few extra cubic centimeters of blood would provide for the added output of bilirubin after bile feedings. In Dog 29, for example, only 0.105 gm. of additional pigment was eliminated in the 3 days following the feedings. The breaking down of 19 cc. of blood with 100 per cent of hemoglobin, such as was possessed by the animal, would account for this. So small a destruction need bring about no appreciable change in the

hemoglobin per cent of a dog weighing 12 kilos. In the extreme instance of Dog 23 an average of approximately 0.072 gm. of extra pigment appeared in the bile on each of 11 successive days, which an extra destruction daily of 16.3 cc. of blood with 80 per cent hemoglobin would suffice to explain. The state of the animal was so abnormal that the loss might have occurred without evidence of it in the routine blood examination.

As against a destruction of corpuscles by cholates, the remarkable properties of the plasma to prevent hemolysis by such salts\textsuperscript{13} should be recalled. This had not been recognized at the time when Stadelmann worked. In an experiment described by Sellards\textsuperscript{14} the presence of blood serum in a 1 to 30 dilution sufficed to protect a few red cells from the hemolyzing influence of 1 mg. of sodium taurocholate, the salt of dog bile, in a total fluid mixture of 1.5 cc. It seems scarcely possible that under the conditions of intestinal absorption any comparable concentrations of taurocholate could have occurred. In 24 hour specimens from dogs given large amounts of the cholate of dog bile, Wisner and Whipple found no increase of pigment\textsuperscript{15} nor was one observed by Hooper\textsuperscript{16} in 6 hour specimens. Stadelmann studied the bile of 12 hours in each 24. It is possible that the pigment increases which he observed were not the result of a greater total output of the substance but merely of recurring temporary alterations in the rate of elimination, such as follow the administration of carbohydrates.\textsuperscript{17}

As the case stands at present there is every reason to believe that the increased bilirubin output after bile feeding is the result of an entero-hepatic circulation. Why indeed should not bilirubin be reabsorbed and resecreted if this happens with chlohematin, as is assuredly the case?

The proportion of bilirubin presumably reabsorbed in our experiments was sometimes negligible, and again great. In Dog 23 it


\textsuperscript{14}Sellards, A. W., \textit{J. Exp. Med.}, 1909, xi, 786.

\textsuperscript{15}Wisner, F. P., and Whipple, G. H., \textit{Am. J. Physiol.}, 1922, lx, 119.

\textsuperscript{16}Hooper, C. W., \textit{Am. J. Physiol.}, 1916–17, xlii, 280.

\textsuperscript{17}Rous, P., Broun, G. O., and McMaster, P. D., \textit{J. Exp. Med.}, 1923, xxxvii, 421.
amounted, as above mentioned, to 72 mg. of the 172 mg. administered each day, or 42 per cent. The giving of bile in bulk on one or two occasions would seem far less favorable to reabsorption than the intermittent delivery into the intestine of smaller quantities, as happens under normal circumstances. Quite possibly a part of the pigment in duodenal specimens obtained from human beings is representative, not of a final elimination of the substance by the organism but of an enterohepatic circulation of it. A great deal of bilirubin might thus circulate under pathological conditions.

That bile feeding is of benefit to man and other animals losing the secretion by a fistula has long been acknowledged. But many acknowledged principles are not sufficiently acted upon. According to a qualified authority, "Human beings who are losing all their bile through a fistula not only have no appetite but can hardly be forced to take food, and emaciate very rapidly. Intensely jaundiced patients may live years, but if, following an operation upon one of these cases, all the bile escapes by the fistula the loss of weight is very rapid and death may soon result if the patient is old." It is in this general relationship that the astonishing recovery of Dog 23 on bile feeding has seemed worth recording.

SUMMARY.

In dogs fed the green bile or the liver tissue of herbivora, the bile later secreted frequently becomes green, changing from the previous yellow-brown. When they are fed sheep bile that contains cholo-hematin, their bile comes to contain this pigment. When they are fed dog bile in quantity a well marked increase in the output of bilirubin by the liver frequently follows. Taken together, these facts indicate the existence of an enterohepatic circulation of bile pigment.

18 Extract from a letter to one of the authors by Dr. W. S. Halsted.