EFFECT OF THE INJECTION OF BILE ON THE CIRCULATION.[1]

By JOHN H. KING, M.D.

Instructor in Pathological Physiology, Johns Hopkins Medical School.

AND

HUGH A. STEWART, M.D.

Assistant in Pathological Physiology, Johns Hopkins Medical School.

(*From the Hunterian Laboratory of the Johns Hopkins Medical School.*)

Since the days of Majendie this subject has received no small amount of attention, for it has long been known that circulatory disturbances are frequently associated with obstructive jaundice, and endeavors have repeatedly been made to study these disturbances by the intravenous injection of bile into animals.

The first to make a systematic study of the bradycardia of jaundice was Röhrig (1), in 1863, and to his teaching, especially, is accredited the oft quoted statement "that the dominant action of the bile on the circulation is due to its bile salts—taurocholate and glycocholate." Subsequent observers have in general concurred with Röhrig that the bile salts are responsible for the bradycardia and low blood pressure of jaundice, but there is much diversity of opinion as to how the bile salts exercise this function. Three different views are held regarding the toxic action of bile on the heart. One is that the bile constituents act as a poison to the intracardiac ganglia and by paralyzing their action, impair the force of the heart beat and lower its frequency. Among those who hold this view are Röhrig (1), Legg (2), Ewald (3), Grob (4), Riegel (5), Laveran and Teisser (6). On the other hand, it is held by Rancke (7), Schack (8) and Löwit (9) that the bile salts possess a toxic action on all striated muscle, and that the effects on the heart fall

[1] Received for publication June 21, 1909.

in line with this general action—that the heart muscle is directly poisoned. The most recent workers on the subject—Spalitta (10), Weintraud (11), Brandenburg (12), and Meltzer and Salant (13), are inclined to the view that the slowing in heart rate is the result of a stimulation or increased tone of the cardio-inhibitory apparatus. While there is no unanimity among these authors as to the mechanism of bile poisoning, they are all, with the exception of Meltzer and Salant, agreed as to the constituent of the bile to which the toxic action is due, *i. e.*, the bile salts. Meltzer and Salant are non-committal, but suggest that there is either a mass action of the different bile constituents, or else some undiscovered constituent, to which the toxicity is due. The first authors to give evidence that the bile pigment possesses toxic properties were Bouchard (14) and de Bruin. Having injected bile into a rabbit's ear to determine the lethal dose, they found that removal of the pigments by charcoal lowered the toxicity by one-half. According to these authors the bile pigments possess a greater toxic property than the bile salts.

It seemed to us that in those researches which were undertaken to determine the toxic constituent of bile the discordant results have been due in a large measure to the methods employed. It is surprising how little attention has been paid to the concentration of the constituents investigated, as compared to their concentration in the normal bile. Empirical standards had been established and concentrations used far in excess of what is present in the bile or without any reference to the possibilities which might arise, even during the course of the disease. Thus, for instance, Traube (15) used a 33 per cent. solution of sodium cholate, whereas in no analysis of the bile of any animal has more than 5 per cent. been found.

In the experiments which we have carried out we used throughout pig's bile and injected this intravenously into dogs. The bile was obtained from the gall bladder immediately after slaughter of the animal, and to obviate the possibility of individual variations which might arise from the feeding of the animals, a mixed sample from at least twenty pigs, was used in every experiment. A first series of experiments was conducted for the purpose of ascertaining the dose of bile injected at a uniform rate which would produce a distinct

slowing of the heart rate. Special attention was given to the rate at which the bile was introduced, as variations in the rapidity of injection materially influence the dose which will produce cardiac effects. As an illustration of this point, in one experiment we found that 5 c.c. of bile injected into the jugular vein in one minute produced a drop in heart rate from 120 to 70 per minute, and a fall in blood pressure from 120 mm. Hg to 50 mm. Hg, whereas, if the same amount of bile be injected at a much slower rate of 0.5 c.c. per minute, it will produce no effect whatsoever, except a slight rise in pressure. In ten experiments we found that an average of 16 c.c. of whole bile, injected intravenously at the rate of 0.5 c.c. per minute, will cause a distinct slowing in rate in a dog of 14 pounds weight, and from 34 to 37 c.c. will stop the heart in diastole. The figure 36 c.c. we take as the lethal dose of pig's bile for dogs. This lethal dose is probably in excess of what would after a few hours kill the animal, but for our purpose it was necessary for the sake of comparison to know the amount of bile necessary to kill the animal on the table.

Effect of the Injection of Whole Bile.—In the experiment carried out in this research all the injections were made in the same manner. The bile was introduced from a burette connected with a jugular vein, usually the left, and the rate of inflow controlled by a screw clamp. Blood pressure was read off from a mercury manometer attached to the right common carotid artery.

Table I gives the figures for six experiments made with the injection of whole bile.

TABLE I.

No.	Blood pressure at commencement of experiment.	Preliminary rise and c.c. of bile to produce it.		Number of c.c. to produce distinct slowing.		Lethal dose in c.c.	Weight of animal.
		B. P.	c.c.	c.c.	B. P.		
1	125	10 mm.	7.5	18	90 mm.	46	15 lbs.
2	127	None		16	75 mm.	35	14
3	130	15 mm.	5	15	75 mm.	36	13
4	120	10 mm.	6	17	100 mm.	37	14
5	140	10 mm.	4.5	10	95 mm.	96	16
6	100	12 mm.	5.5	16	100 mm.	34	14½

There is almost invariably a preliminary rise of pressure amounting to about 10 c.c. following the injection of the first 10 c.c. of bile. This is more apt to occur when the bile is introduced at a greater rate than we have adopted. As will be pointed out later a similar rise in pressure may take place with the injection of pure sodium taurocholate in aqueous solution and it seems probable that to this constituent must be attributed the slight rise in pressure which occurs with the injection of pure bile. Following the preliminary rise of pressure there is a progressive and concurrent lowering of the blood pressure with the further injection of bile. It is not until from 10 to 18 c.c. have been introduced that the heart rate shows a distinct slowing and we have found that after the blood pressure has fallen 30 to 40 mm. Hg from its original height there is a definite drop in the heart rate. The fact that the blood pressure is affected as soon as the bile is introduced while the rate of the heart beat is not altered until a quantity of bile varying from 10 to 18 c.c. have been injected, would tend of itself to show that these phenomena are not the result of the same action. The lowering of the blood pressure must have a different physiological causation than the slowing of heart rate, and this is suggestive of the possibility that different constituents of the complex bile are responsible for these two effects. The lowering of blood pressure must, unquestionably, be due either to a weakening of the ventricular contraction by direct poisoning of the muscle fibers or to paralysis of the intracardiac ganglia—as to which of these possibilities is the correct one, is not of essential importance. The lowering of blood pressure is not due to vaso-dilation, as was found from the study of a plethysmographic record of a dog's hind limbs during the injection of the whole bile. The limb did not show an increase in volume.

Meltzer and Salant pointed out that the introduction of bile causes an increase of the vagus tone. They determined the smallest strength of low current necessary to produce cardiac effects on stimulation of the vagus in the animal before injection of bile, and compared this with the strength of current required to produce the same effect after the introduction of a certain quantity of bile. A smaller strength of current was necessary for the latter. In several

of our experiments we observed that section of the vagi relieved immediately the bradycardia and arythmia produced by bile injection. In one instance the heart had stopped for about two minutes, and on cutting the vagi it resumed its normal rate and rhythm. In the same way the injection of atropine will relieve, more or less completely, the bradycardia produced by bile. Weintraud has reported a case of obstructive jaundice where the injection of .0012 gm. atropine increased the heart rate from 60 to 120 per minute. In addition to the slowing in rate there is a slight delay in conduction tone between auricle and ventricle amounting in our experiments from 2/100 to 5/100 seconds.

It would appear then that there is ample experimental proof that the bradycardia of jaundice is the result of a heightened tone or direct stimulation of the vagus fibers in the heart, and that the lowering of blood pressure is caused by a direct poisoning action on the heart muscle or intracardiac ganglia, and it is in no way associated with the change in rate.

Effect of Injection of Separate Constituents of the Bile.—Having thus established the lethal dose of bile for dogs at a uniform rate of injection, and the effects, in their sequence, which follow the introduction of whole bile, the separate constituents were then injected in their normal concentration. The bile of the pig, like that of the rabbit and hare, contains almost exclusively glycocholic acid, and this is present in the concentration of 1.9 parts per 100. A solution somewhat in excess of this strength of sodium glycocholate namely, 3.2 parts per 100, was accordingly made up and injected into three animals.

TABLE II.

Experiment.	Blood pressure before.	Rate before.	Blood pressure after.	Rate after.	Bile in c.c.
13	140	140	145	142	45
18	125	176	125	126	40
17	135	216	165	202	40

These three experiments are of interest since they show that no harmful effects are produced on the heart or circulation by the amount of glycocholate present in a lethal dose of bile. In these three experiments the blood pressure either remained constant throughout the injection or else underwent a slight elevation

amounting in one experiment to 30 mm. Hg. Similarly the heart rate underwent no marked change. In Experiment No. 18 a drop in rate from 176 to 126 is recorded, but this is not of sufficient amount to justify its being called a bradycardia.

In a similar way the action of taurocholate was investigated. Although pig's bile contains only a trace of this substance, we injected a solution of sodium taurocholate in water, in the same concentration as was used with the glycocholate, of 3.2 per cent. It was found that the salts are identical in their action. They cause, in the strength in which they are present in normal pig's bile, neither a fall of blood pressure nor a showing of the heart rate when injected into dogs.

This result is not in conformity with the work previously done on the action of the bile salts on the circulation. Previous observations on this subject have been made without any reference to the concentration of the salts in the bile. In large enough doses and in great enough concentration the bile salts will undoubtedly lower the blood pressure, and also slow the heart, as was demonstrated by Legg, Rohrig and others. This, however, must be regarded as a purely toxic effect, from excessive dose, and is analogous to that which may occur with the injection of practically any salt—a small dose may be without effect, a large dose fatal.

Cholesterin.—A recent article, by Danilewsky (16), on the action of the products of metabolism, contains the statement that cholesterin is a direct stimulant to the muscular fibers of the heart. This author found that cholesterin is slightly soluble in Ringer's solution to the extent of only .005 per cent., and that even in this concentration it will cause a distinct augmentation of systole. We have not repeated Danilewsky's experiment, but the results we have obtained by the injection of an alcoholic solution of cholesterin are essentially different from what he has reported. Cholesterin, of which 0.16 parts per 100 are present in pig's bile, can readily be removed by repeated shaking of the bile with ether in a separating funnel. If the bile from which the cholesterin has been removed in this manner be injected it will be found that the lethal dose is in no way altered, *i. e.,* it still remains between 30 and 40 c.c. of bile.

This would tend to show that cholesterin does not possess very toxic properties, but we have found that when it is injected in alcoholic solution toxic effects are produced by the amount present in a lethal dose of bile. Table III shows the effect of the injection of cholesterin in alcoholic solution.

TABLE III.

Experiment.	Concentration.	Lethal dose.	Amount of cholesterin in lethal dose.	Weight of animal.
21	3.2 per cent.	7.9 c.c.	0.025 gm.	16 lbs.
22	3.2 per cent.	4.6 c.c.	0.014 gm.	9 lbs.
27	3.2 per cent.	5.5 c.c.	0.176 gm.	12 lbs.

It is evident from these experiments that cholesterin in alcoholic solution has a greater toxic effect than it does in the bile. Since 0.16 per cent. of cholesterin is present in normal pig's bile, and the lethal dose of bile for dogs is 40 c.c. even assuming that the toxicity did entirely depend on this substance, it will be seen that an amount less than 0.064 grams of cholesterin in bile is non-lethal. In alcoholic solution, however, the toxic dose of cholesterin as given in the table above is from 0.014 to 0.025 grams. It is difficult at first sight to correlate the results of these experiments with those obtained by the removal of cholesterin by ether. It is possible that this disagreement is the result of an experimental difficulty. Cholesterin is soluble only in warm alcohol, and with a slow injection it is difficult to prevent small quantities of cholesterin from precipitating just as the solution enters the veins. The precipitated cholesterin is thus carried as small emboli, which in reaching the heart act as violent poisons. Cholesterin must be regarded, however, as contributing in some measure to the toxicity of bile injected intravenously, but it is unlikely that it plays any part in the circulatory disturbances of jaundice, since it is now known that cholesterin does not circulate as do the bile salts and pigments.

Bile Pigments.—We wish especially to draw attention to the results we have obtained with the injection of the bile pigments, bilirubin and biliverdin. Pig's bile contains the two pigments bilirubin and biliverdin, the latter being a higher oxidation product of the former.

As it is exceedingly hard to obtain bilirubin in the pure state, and as the cost precluded us from making any extensive observations

with this pigment, we studied more especially biliverdin, which we were able to prepare for ourselves in the laboratory.

If the bile be treated with a weak salt of calcium, as a 5 per cent. solution of calcium lactate, the precipitate collected and the filtrate injected into an animal, it will be found that the filtrate is non-toxic, the animal being able to withstand the injection of many times the amount contained in a lethal dose of bile.

Table IV gives the results of three such injections.

TABLE IV.

Experiment.	Bile filtrate.	Blood pressure before.	Blood pressure after.	Corresponding amount of bile.
11	50 c.c.	150 mm. Hg.	150 mm. Hg.	100 c.c.
12	43 c.c.	170 mm. Hg.	110 mm. Hg.	86 c.c.
32	100 c.c.	140 mm. Hg.	145 mm. Hg.	166 c.c.

These experiments prove that the toxic elements have been removed from the bile by the addition of a calcium salt. As has been emphasized by Gamgee (17), the bile pigments act as weak acids and the precipitate formed by the addition of a soluble calcium salt to the bile consists almost entirely of the calcium salt in these instances. The experiments are of differential nature in the determination of the toxicity of the different bile constituents. The filtrate contains all the elements of the bile except the pigments, *i. e.*, the bile salts, cholesterin, lecithin, etc., yet the injection of an amount equal to several lethal doses of whole bile gives rise to no disturbance of pulse rate or blood pressure.

It would be natural to expect that the compound precipitate of calcium contains all the toxic elements of the bile. This compound is only slightly soluble in water, but completely so in alcohol. If an alcoholic solution be injected into an animal it will be found, contrary to expectation, that the amount of pigment obtained from several lethal doses of bile can be injected with practically no toxic effect.

TABLE V.

Experiment.	Amount of pigment in	Blood pressure before.	Rate before.	Blood pressure after.	Rate after.
33	75 c.c. bile	135 mm. Hg.	198	145 mm. Hg.	170
May 17	60 c.c. bile	175 mm. Hg.		125 mm. Hg.	
May 8	20 c.c. bile	155 mm. Hg.		155 mm. Hg.	

The only explanation which suggested itself to us was that the pigment had lost its toxicity by its union with calcium. We therefore proceeded to put this hypothesis to experimental test. The calcium biliverdin precipitate was accordingly treated with a 10 per cent. solution of hydrochloric acid and the precipitate washed with water until the washings were neutral to litmus. By this procedure the calcium was split off from its union with the pigment to form salts, leaving behind in a pasty mass the uncombined biliverdin. The biliverdin was then dissolved in alcohol and injected intravenously. The results showed in two experiments that the injection of pigment reproduced all the effects of the injection of whole bile, except the initial rise of blood pressure. Further the lethal dose of uncombined pigment thus injected corresponded almost exactly to the amount of pigment contained in a lethal dose of whole bile.

The following is a protocol of a typical experiment:

March 17, 1909.—Fox terrier; male; weight 12 lbs. Injection of alcoholic solution of biliverdin prepared from calcium precipitate of 200 c.c. of bile, freed of cholesterin, lecithin, fats, mucus and bile salts.

Pigment dissolved in 35 c.c. 95 per cent. alcohol.

Burette.	Heart blood pressure.	Rate.	Time.
26.7	135.0	156	11.10
27.6	128.5	134	
29.5	125.0	126	
30.9	120.0	122	
32.5	98.0	112	
33.7	75.0	100	
34.4	60.0	64	
34.7	10.0	Complete stoppage	11.30
8.0 c.c.	Dog died after 8.0 c.c. had been injected.		

The lethal dose of uncombined pigment in six experiments is given in Table VI.

TABLE VI.

Experiment.		
16	Pigment in 20 c.c. bile lethal.	Weight of animal 15 lbs.
24	Pigment in 30 c.c. bile lethal.	Weight of animal 15 lbs.
29	Pigment in 45 c.c. bile lethal.	
31	Pigment in 28 c.c. bile lethal.	Weight of animal 31 lbs.
May 18	Pigment in 22 c.c. bile lethal.	Weight of animal 11½ lbs.
May 19	Pigment in 30 c.c. bile lethal.	Weight of animal 24 lbs.

Average 30 c.c. approximately.

In order to render conclusive the observations upon the toxity of uncombined pigment and the relative non-toxicity of calcium pigment, we made three experiments from the same sample of bile, first to obtain the lethal dose of this special sample, second to obtain the lethal dose of the uncombined pigments obtained from it, and third to obtain the amount of combined pigment which the animal would stand without showing toxic symptoms. The whole bile which had been filtered was injected at the uniform rate of 0.5 c.c. per minute into the jugular vein of an animal weighing 14 pounds. After 16 c.c. had been introduced the blood pressure fell from its original height of 123 mm. Hg to 70 mm. Hg and the pulse rate dropped from 160 to 120 per minute. Thereafter the blood pressure fell steadily to zero and the heart stopped in diastole when 35 c.c. of bile had been injected. This gives a lethal dose of 2.5 c.c. per pound weight.

Having thus established the lethal dose of the sample of bile with which we were experimenting, we then precipitated the pigment from 200 c.c. of bile. The precipitate was dried and powdered and divided into two equal portions. One portion was treated with 10 per cent. hydrochloric acid to remove the calcium, washed and dissolved in 25 c.c. of 95 per cent. alcohol. The other portion of the precipitate which consisted of the calcium salt of the pigment was dissolved in the same amount of alcohol without previous treatment with hydrochloric acid. It was found that 7.1 c.c. of the first alcoholic solution was lethal for a dog of 24 pounds, and this in terms of whole bile gives a lethal dose of 1.4 c.c. of bile per pound of weight. In marked contrast to this we found that 15 c.c. of the alcoholic solution of calcium biliverdin, injected under the same conditions as the uncombined pigment, caused only a slight fall in the pressure in an animal weighing 22 pounds, and the animal survived the injection without untoward effects. This amount of pigment corresponded to that present in 60 c.c. of bile.[2]

In addition to calcium, the bile pigments form compounds with

[2] Two experiments were made with injection of alcohol intravenously into dogs. It was found that alcohol (95 per cent.) up to 17 c.c. injected at the same rate as that used for the rest of experiments, did not lower the blood pressure or produce bradycardia, and in our experiments we never used as much alcohol as this.

sodium and these substances also are relatively non-toxic. It seems to us that this property of the bile pigments of combining with calcium and sodium is responsible for the frequently expressed view that the pigments have no toxic effect upon the heart. Several investigators in studying this subject used as solvents for the pigments weak solutions of sodium hydroxide, but they apparently lost sight of the fact that there is in addition to the physical solution a definite chemical interaction with the formation of a sodium compound, and as above stated this compound does not possess the toxicity of uncombined pigment.

In order further to study the rôle of calcium in the action of pigment, and especially to determine whether in obstructive jaundice the pigments circulating in the blood unite with all the available calcium in order to render themselves non-toxic, we tied the common bile duct in three dogs and made a careful analysis of the calcium content of the organs and tissues.

Normal.	*Jaundiced.*	
	BLOOD.	
No. I 0.050 gm. Ca.	No. I 0.060 gm. Ca.	20 per cent. increase.
No. II 0.045 gm. Ca.	No. II 0.053 gm. Ca.	16 per cent. increase.
	No. III 0.060 gm. Ca.	20 per cent. increase.
	MUSCLE.	
No. I 0.030 gm. Ca.	No. I 0.025 gm. Ca.	17 per cent. decrease.
	No. II 0.024 gm. Ca.	20 per cent. decrease.
	LIVER.	
No. I 0.030 gm. Ca.	No. I 0.015 gm. Ca.	50 per cent. decrease.
No. II	No. II	
	BRAIN.	
No. I 0.073 gm. Ca.	No. I 0.069 gm. Ca.	6 per cent. decrease.
	No. II	

It will be noticed that throughout there is a definite increase in the calcium content of the blood and a definite diminution in all the organs and tissues analyzed. In view of what has already been said with regard to the readiness with which the pigments unite with calcium to form a non-toxic substance, these results in the opinion of the writers can be interpreted in the following way: The bile

pigments circulating in the blood in obstructive jaundice gradually absorb the available calcium of the organs and tissues to form the calcium compounds, in this way increasing the calcium content of the blood and producing a corresponding diminution in that of the tissues. It is possibly a protective mechanism, and from another aspect brings into prominence the importance of calcium as a regulator in disturbances of the body mineral and organic metabolism.

CONCLUSION.

1. We have confirmed previous work which shows that the injection of bile increases the tone of the vagus nerve, and that this action can be abolished after the administration of atropine.

2. We have found that the amount of bile salts in a lethal dose of pig's bile for dogs will, if inejcted alone, produce neither a fall in blood pressure nor a slowing in rate.

3. We have found that the amount of pigment in a lethal dose of the bile will, if injected alone, cause death with slowing of the heart and lowering of blood pressure.

4. We have found that the bile pigment in combination with calcium or sodium is less toxic than uncombined pigment.

5. We have found that in experimentally produced jaundice the calcium content of the blood is increased, while that of the liver, muscle and brain are decreased.

6. We are of the opinion that increase in calcium in the blood is a protective mechanism against the circulating pigments of obstructive jaundice.

BIBLIOGRAPHY.

1. Röhrig, *Arch. d. Heilkunde,* 1863, iv, 385.
2. Legg, *Proc. of the Royal Soc.,* 1875–6, xxiv, 442.
3. Ewald, Realencyklopädie v. Eulenberg, 1886, viii, 213. 2nd edition.
4. Grob, *Deut. Arch. f. klin. Med.,* 1888, xlii, 608.
5. Riegel, *Zeit. f. klin. Med.,* 1890, xvii, 252.
6. Laveran and Teisser, Nouveau Elements de la Pathologie, 1879, ii, 738.
7. Rancke, *Arch. f. Anat. u. Physiol.,* 1864, p. 340.
8. Schack, Inaug. Diss., Giessen, 1864.
9. Löwit, *Zeit. f. Heil.,* 1881, ii, 459.
10. Spalitta, Untersuche zur Naturlehre, 1894, xiv, 144.
11. Weintraud, *Archiv. f. exper. Path. u. Pharm.,* 1894, xxxiv, 37.

12. Brandenburg, *Berliner klin. Woch.*, 1903, xl, 865.
13. Meltzer and Salant, Studies on the toxicity of Bile, *Jour. of Exper. Med.*, 1905, viii, 280.
14. Bouchard, Leçons sur les autointoxication dans les Maladies, 1887.
15. Traube, Ueber den Einflus der Gallensaure auf die Hertztätigkeit, *Berliner klin. Woch.*, 1864, i, 85.
16. Danilewsky, Untersuchen über die physiologische Activität der Stoffwechselproducte, *Pflügers Archiv.*, 1907, cxx, 181.
17. Gamgee, Physiological Chemistry of the Animal Body, 1893, ii, 315 and 324.