NOTE ON HAEMOLYTIC AND HAEMOSOZIC SERUMS.

BY

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THE authors of this paper¹ were the first to obtain a haemosozic serum by injecting rabbits with ox-bile. This serum, when added in certain proportions to ox-bile, prevented the haemolytic action of this latter fluid on bovine red blood corpuscles. The same observers noted that if more bile was added to a quantity of bile neutralized by haemosozic serum, haemolysis took place whenever the amount of serum was increased correspondingly. In some cases, however, the serum of rabbits injected with bile was not haemosozic but haemolytic; and, occasionally, a mixture of such haemolytic serum and haemolytic bile was less active in a concentrated than in a dilute solution.

Later,² the same observers showed that the same serum might be both haemosozic and haemolytic. Discussing their first results,³ they endeavoured to explain these contradictory phenomena by the supposition that one or more of the haemolysins present in bile may give rise to haemosozin when injected into the blood, while the others have no such effect. Conversely, the haemosozin so produced may have a specific action on one or more haemolysins, and not on the others, and such a serum, mixed with bile, would neutralize one or more of the haemolysins present, without acting on the others at all. If this hypothesis be correct, it follows that haemosozic serum would not prevent haemolysis when once the bile exceeded a certain quantity, because the bile would then contain a certain amount of unneutralized haemolysin sufficient to dissolve the red corpuscles.

With reference to the fact that the injection of bile occasionally produced a haemolytic serum, they suggested the following explanation:

We may assume that just as the haemolysin present in bile gives rise to haemosozin in the serum, so does the haemosozin of bile cause the formation of haemolysin, and that under certain conditions (for example, increased amount of haemosozin in bile, idiosyncrasy of animal, etc.) the formation of haemolysins exceeds the normal, or may even exceed that of haemosozins, with the final result that the serum becomes haemolytic.

In order to demonstrate the truth of these theories it is necessary to show:

1. The presence of two or more haemolysins in bile, of which one or more produce a haemosozic serum when injected into rabbits, whereas one or more does not do so.

2. The presence in haemosozic serum of a haemosozin acting on one or more haemolysins and not on the other haemolysin or haemolysins.

3. The production of a haemolytic serum by the injection of haemosozin.

The following experiments were made to elucidate these questions (space compels us to give but a few illustrative experiments):

HAEMOLYTIC SERUM PRODUCED BY THE INJECTION OF CERTAIN BILE-CONSTITUENTS.

A certain quantity of fresh ox-bile (200 to 300 c.cm.) is precipitated with three times its quantity of absolute alcohol and then filtered. The precipitate is suspended during one night (in the ice-box) in distilled water, containing 2 per cent. chloroform. This is now filtered, and the filtrate again precipitated with three times its volume of absolute alcohol. The precipitate after filtration is dried over sulphuric acid *in vacuo*. This precipitate (in future called "haemosozic precipitate") is now dissolved in physiological salt solution, so that each cubic centimetre contains 2 mg. of precipitate, and this solution is then injected in varying doses into rabbits.

EXPERIMENT I.—Rabbit 63 received 0.14 of Haemosozic Precipitate in Three Injections in 23 Days.

	Serum 69	Physiological Salt Solution.	Haemosozic Precipitate.	Bile.	Blood.	Result
Tube 1 ..	5	35	—	—	2	††
" 2 ..	10	30	—	—	2	†††
" 3 ..	5	25	10	—	2	∞
" 4 ..	10	20	10	—	2	∞
" 5 ..	—	25	10	5	2	†††
" 6 ..	—	35	—	5	2	†††

N.B.—The experiments were all conducted in the way we have repeatedly described (see BRITISH MEDICAL JOURNAL, June 18th, 1904, p. 1618). The bovine red blood corpuscles were washed twice in normal salt solu-

tion and centrifugalized. The numbers refer to $\frac{1}{10}$ c.cm. Thus, 5=∞^{0.5}; †††=complete haemolysis; †=slight haemolysis; †† and †††=an intermediate stage; ∞=no haemolysis.

Exactly the same results were obtained with other serums prepared in the same way. It was ascertained also that a temperature of 56° C., acting for 30 minutes, rendered the haemosozic precipitate inactive.

These experiments show that (1) the haemosozic precipitate injected into a rabbit renders the serum of that animal haemolytic. (2) The haemosozic precipitate prevents the haemolysis of the serum so produced, but does not neutralize all the haemolysins of bile.

In our first paper we had stated already that the injection of bile occasionally produces a haemolytic serum. The action of the haemosozic precipitate on such a serum is shown in the following table:

EXPERIMENT.—Serum 23 is a Haemolytic Serum from a Rabbit Inoculated with Bile.

	Serum 23.	Physiological Salt Solution.	Haemosozic Precipitate.	Red Blood Corpuscles.	Result.
Tube 1 ..	10	30	—	2	†††
" 2 ..	10	20	10	2	∞
" 3 ..	5	25	10	2	∞
" 4 ..	5	35	—	2	†††

The haemosozic precipitate, therefore, although it has no action on bile, acts powerfully on the haemolytic serum of an animal injected with bile.

HAEMOSOZIC SERUM PRODUCED BY THE INJECTION OF CERTAIN BILE CONSTITUENTS.

Fresh bile is precipitated with three times its volume of absolute alcohol and filtered. The precipitate remaining on the filter is suspended for one night (in the ice-box) in distilled water containing 2 per cent. of chloroform and then filtered. The undissolved part remaining on the filter dissolves slowly in 10 per cent. chloride of sodium. This solution (called in future "biliary haemolysin No. 1") was then used for the injection of animals. (N.B.—One volume of solution corresponds to one volume of bile.)

EXPERIMENT.—Rabbit 54 received 3 c.cm. of Biliary Haemolysin No. 1 in a Period extending over 18 Days.

	Serum 54	Physiological Salt Solution	Bile.	Biliary H. No. 1.	Red Blood Corpuscle.	Result.
Tube 1	5	35	—	—	2	∞
" 2	—	30	10	—	2	†††
" 3	—	35	5	—	2	†††
" 4	10	20	10	—	2	†††
" 5	10	25	5	—	2	†††
" 6	—	30	—	10	—	†††
" 7	—	35	—	5	2	††
" 8	—	25	—	5	2	∞
" 9	10	30	—	10	2	†

The results with the same serum and other serums prepared in the same manner were always the same. By the injection of biliary haemolysin No. 1, therefore, a haemosozic serum is produced which has a slight action on bile, but a marked neutralizing action on biliary haemolysin No. 1.

NEUTRAL BILIARY SERUM PREPARED BY INJECTION OF CERTAIN BILE CONSTITUENTS.

After the precipitation of the haemosozic precipitate (see above) the alcohol is evaporated *in vacuo* over sulphuric acid. The dried residue is then dissolved in a quantity of physiological salt solution corresponding to the quantity of bile precipitated with alcohol. This solution (called "biliary haemolysin No. 2") is distinctly though not so powerfully haemolytic as the original bile, and was used for injection into rabbits,

EXPERIMENT.—Rabbit 9 received 12 c.cm. of Alcoholic Extract in Five Injections during a Period extending over 35 Days.

	Serum 9.	Physiological Salt Solution	Bile.	Biliary H. No. 2.	Red Blood Corpuscle.	Result.
Tube 1	5	35	—	—	2	co
" 2	—	35	—	5	2	††
" 3	5	30	—	5	2	††
" 4	—	30	—	10	2	†††
" 5	15	25	—	5	2	††
" 6	—	35	5	—	2	††††
" 7	—	30	10	—	2	††††
" 8	5	30	5	—	2	††††
" 9	10	25	5	—	2	††††

Although the alcoholic extract, therefore, is haemolytic, its injection into rabbits confers no haemolytic or haemosozic properties on the serum of animals so treated.

Further other experiments showed that the haemosozic precipitate from bile did not neutralize the biliary haemolysin No. 2, and the serum of an animal injected with these haemolysins had no effect on a biliary haemolytic serum.

CONCLUSIONS.

1. Bile contains at least two haemolysins or groups of haemolysins: (1) Biliary haemolysin No. 1 (insoluble in alcohol), which produces a haemosozic serum when injected into rabbits; and (2) biliary haemolysin No. 2, which produces no haemosozic serum when injected.
2. Bile contains a haemosozic precipitate which, when injected into rabbits, produces a haemolytic serum.
3. The haemosozic precipitate added to bile never prevents completely the haemolysis produced by bile.
4. The haemosozic precipitate neutralizes the haemolysin insoluble in alcohol (haemolysin No. 1), but not the haemolysins soluble in alcohol (haemolysin No. 2).
5. The haemosozic precipitate prevents the haemolytic action of a serum produced by the injection of bile.

REMARKS.

Since the chemical composition of bile varies greatly even in the same animal, probably therefore the amount of haemolysin and haemosozin in it varies also. We have seen that as biliary haemolysins and haemosozins have very different physiological effects, the injection of bile which contains both necessarily produces very complex changes in the serum of an animal—changes all the more complex as the chemical composition of bile varies considerably. A serum of an animal injected with bile may have the most varied effect on the red corpuscles; and, as we have seen, although as a rule such a serum is haemosozic, sometimes it is haemolytic, sometimes both haemosozic and haemolytic, and sometimes neither.

It appears to us that these experiments throw light on some of the facts which have been observed concerning the serums of animals injected with lower micro-organisms or their products. A culture, whether filtered or unfiltered, whether heated or not, most probably contains, like bile, substances antagonistic to one another; in consequence, the serum of an animal injected with such complex substances may contain several new substances, some of which may inhibit or predispose to infection, some may neutralize and some increase the effects of bacterial poisons, some, again, may have no appreciable specific action whatever. Such a hypothesis may account for the impossibility of increasing the antitoxic power of certain specific serums, or of obtaining a serum neutralizing *in vivo* more than the minimum fatal dose of certain pathogenic bacteria.

REFERENCES.

- ¹ BRITISH MEDICAL JOURNAL, July 18th, 1903, p. 130. ² Ibid., 1904, i, p. 658. ³ Journal of Pathology and Bacteriology, March, 1904.

A CASE OF EMPYEMA OF THE GALL BLADDER, WITH UNUSUAL SYMPTOMS.

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THE following case is, I think, worth recording, owing to the great difficulty in diagnosis resulting from the unusual physical signs which were present.

In most textbooks it is definitely stated that the dullness

caused by a tumour of the gall bladder is continuous with the liver dullness, and that the intestine is never situated in front of an enlarged gall bladder.

In this case there was an empyema of the gall bladder, the result of the obstruction of the commencement of the cystic duct by a calculus. Owing to the fact that the liver was enlarged, so that the anterior edge, which was very thin, reached almost to the level of the umbilicus, though a dull note on percussion was only obtained for $1\frac{1}{2}$ in. below the costal margin, the tumour was situated below the level of the umbilicus. In addition to this, the transverse colon was adherent to the under surface of the liver between its anterior sharp margin and the fundus of the gall bladder, causing a resonant note to be obtained on percussion between the hepatic dullness above and the gall bladder dullness below. Moynihan, in his book, says: "In one case I have found the colon to be adherent to the edge of the liver above the gall bladder, so that on inflation the swelling was recognized as being below the colon, and made less easily palpable. Lawson Tait and Lücke have described cases in which the small intestine was adherent in this position."

As a result of these two factors—low position of the tumour and resonance above it—a wrong diagnosis of appendicitis was made.

The following is the history of the case:

Mrs. B., aged 40, was admitted to the Royal City of Dublin Hospital on Friday, June 9th, 1905. The previous Monday she was suddenly seized with pain in the right side; she vomited everything she took, and her temperature on two occasions during the week was 100° and 101° respectively. Her bowels were confined until Thursday, when they were moved as the result of purgatives and enemata; she had been kept more or less under the influence of opiates during the week owing to the severe pain which was situated in McBurney's spot. On admission her pulse was 60 and her temperature 99°. There was a large oval swelling situated in the right side of the abdomen just below the level of the umbilicus and close to the outer border of the rectus: the liver dullness extended for $1\frac{1}{2}$ in. below the costal margin, but there was a broad band of resonance between this and the tumour; the tumour itself was dull on gentle percussion, but resonant on deep percussion. Though there was no cutaneous hyperaesthesia, the patient complained of so much pain and tenderness over the tumour that it was very difficult to make a thorough examination. The abdomen was not distended, but the muscles were rigid over the tumour, though there was no general abdominal rigidity. The patient was ordered an enema, and hot turpentine stupes were applied to the abdomen; all food was withheld except small quantities of milk and soda water. The bowels were well moved and the patient was better in the evening, the temperature 100° and the pulse 68, and the pain was less severe.

In the morning the patient appeared worse, the temperature 100.8° and the pulse 68, and the respirations 30 to the minute; the abdomen was moving freely with respiration, which did not cause any movement of the tumour; the pain was very severe and paroxysmal, entirely localized to the region of the tumour; there had been no vomiting since admission. A leucocyte count was made, with the following result: Total leucocytes, 22,666 per c.mm.; neutrophils, 82 per cent.; eosinophiles, 1 per cent.; lymphocytes, 9 per cent.; hyaline, 8 per cent. There was a history of an attack of pain (not so severe as this one) lasting some days eleven years ago, accompanied by jaundice, and disappearing after the passage of gall stones; this, however, was not elicited till after the operation. During the present attack there was at no time any jaundice.

A diagnosis of appendicitis with abscess formation was made, and immediate operation was decided on.

The abdomen was opened over the tumour by an oblique incision passing through the muscles and the outer border of the sheath of the right rectus muscle. The parietal peritoneum was not adherent to the tumour, which was covered by the transverse mesocolon. The caecum and appendix were normal. The anterior edge of the liver was found projecting down to the level of the umbilicus, and underneath it a large tense tumour, covered by the transverse mesocolon, and with the transverse colon adherent to the under surface of the liver, in front of it; the colon and mesocolon, with a layer of lymph, were peeled off the under surface of the liver and the tumour; while doing this some thick pus welled up from the depths of the wound, the intestines were packed off with gauze wipes, and about 4 oz. of pus and mucus were swabbed up. A perforation was then found in the neck of the gall bladder, which was temporarily closed by forceps. The gall bladder was then separated from the under surface of the liver and drawn out of the wound, the neck of the gall bladder and cystic duct were freed, ligated, and divided, some strips of gauze were passed down to the bottom of the wound and the abdominal incision closed except for 1 in. in its upper part, and the patient was sent back to bed.

Everything went well for the first week; the temperature came down to normal on the third day, the gauze was removed on the fourth day, and the stitches were taken out on the ninth day, the wound being perfectly healed, except where the gauze drain had been, and there being scarcely any discharge. That evening the patient's temperature went up to 100° and she complained of cramps in her legs, followed by great pain and oppression of the chest, a great sinking feeling, and a cold perspiration all over the body; the pulse was very weak and difficult to count, about 126. She was given a hypodermic of strychnine