AN ACTION OF ARSENIC ON THE RED BLOOD CORPUSCLES, AND A THEORY OF THE BLOOD DEFECT IN PERNICIOUS ANAEMIA.

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ACTION OF ARSENIC ON RED BLOOD CORPUSCLES.

CLINICAL experience has defined certain therapeutic uses of arsenic, which may be called empirical in so far as they are imperfectly explained by pharmacological experiments. This is notably the case in regard to the action of arsenic on the blood, especially in pernicious anaemia and in malaria.

Since 1880, Sir Thomas R. Fraser, as a result of numerous clinical observations, has pointed out in his systematic and clinical lectures that arsenic has no appreciable influence in increasing red blood cells or haemoglobin in simple anaemia or in health.

In 1898 Stockman and Greig, from experiments on healthy animals came to the conclusion that "arsenic does not increase the number of red or white corpuscles, nor the amount of haemoglobin," and that "as regards the action as a haematinic in pernicious anaemia and other morbid conditions, our experiments point very conclusively to a stimulation of the bone marrow. In these cases, however, although it may increase the number of red blood corpuscles, it does not, so far as we can see, affect the real cause of the disease and therefore must be considered a purely symptomatic method of treatment."¹

Later, however, Stockman and Charteris found that lead, phosphorus, mercury, and other substances produce effects on the bone marrow similar to those produced by arsenic; and other observers showed that nucleic acid, peptones, phosphorus, and other bodies are capable of producing the same kind of change as arsenic does. Under all these influences the increase in the cellular element of the bone marrow is of the "leucoblastic" type. "These changes do not indicate increased activity in the direction of the formation of red blood corpuscles, but rather point to an increased production of the finely-granular leucocytes of the blood." "It corresponds to an attempt to deal with the poison by means of an increased flow of leucocytes into the blood."

They concluded, therefore, that "arsenic has no direct effect in increasing the production of red blood corpuscles by the bone marrow. The 'haematinic' action of arsenic in pernicious anaemia, malaria, lymphadenoma, leukaemia, and some other diseases probably results from a specific action on the parasites which cause these diseases, and not from any direct action on blood formation."²

This process of reasoning, which is not confined to these authors, and which virtually maintains that arsenic, because it has no action on the blood-forming organs, must therefore act on the parasites of those diseases in which it proves of benefit, involves an important fallacy of omission, for arsenic may act neither on the blood-forming organs nor on the often presumed parasite, but on the formed blood corpuscles.

Indeed, in regard to malaria at least there is not wanting an opposite opinion, for Cushny states that "it is very questionable whether it (arsenic) acts on the malarial organism, for its efficacy often seems due rather to its improving the general nutrition and lessening the cachexia and wasting."³

I have recently performed a series of experiments with regard to this third possible factor in explaining the haematinic value of arsenic, namely, its action on the formed red blood corpuscles, with the result that arsenic has been found to exert on them *in vitro* a pronounced protective influence against the haemolytic action of one of the most commonly used haemolytic agents, distilled water.

I hope soon to investigate the action of other compounds of arsenic on the resistance of the red blood corpuscles, and against other haemolytic agents.

The following series of experiments illustrates the methods adopted and the results obtained. The saline solution used in all experiments was 0.85 per cent. sodium chloride in distilled water. Blood was obtained from a

prick of the finger. The haemolytic tubes were all of the same size.

EXPERIMENT I. *Arsenious Acid 1 in 10,000.*

A blood suspension was made by adding 60 c.mm. of blood to 1.5 c.cm. of saline solution, and 0.5 c.cm. of this suspension was put into each of three tubes, A, B, and C.

To A was added 0.5 c.cm. of a solution of arsenious acid, 1 in 5,000, obtained by dissolving 0.01 gram of arsenious acid in 50 c.cm. of saline solution, the blood corpuscles being thus subjected to a solution of 1 in 10,000. To B was added 0.5 c.cm. saline solution, and to C 1.5 c.cm. saline solution.

The tubes were allowed to stand for one hour at room temperature, after which 1 c.cm. of distilled water was added to A and to B. The addition of the distilled water immediately caused almost complete haemolysis in B, but only very slight haemolysis in A. Thus print held behind Tube B could easily be read through the tube, whereas through Tube A scarcely any difference could be made between print and page.

It could further be seen, by comparing A with C (in which the total bulk of fluid and the number of contained corpuscles were the same), that the former was slightly the less opaque, showing that some haemolysis had taken place in it.

The three tubes were then allowed to stand for fifteen hours at room temperature. At the end of that time it was found that the supernatant fluid in A was slightly coloured, in B strongly coloured, and in C colourless. In A and C a mass of corpuscles of approximately equal size had collected at the bottom of the tubes, and in B this was represented by a faint greyish sediment. When the solutions were shaken up, and a drop of each examined under the microscope, it was found that A and C abounded with coloured corpuscles, while in B these were very sparse.

It is therefore apparent that the immersion of red blood corpuscles for one hour in a solution of arsenious acid 1 in 10,000 induces in these corpuscles a condition, in which they manifest an increased resistance to the haemolytic action of a hypotonic solution, as compared with corpuscles which have been subjected to exactly the same conditions apart from the influence of arsenic.

EXPERIMENT II.

The effect produced in one hour by this strength of solution was so pronounced that it was interesting to know if a similar effect could be produced in a shorter time. The foregoing experiment was therefore repeated with only a time difference.

A blood suspension was made by adding 60 c.mm. of blood to 1.5 c.cm. of saline solution, and 0.5 c.cm. of this suspension was put into each of three tubes, A, B, and C. To A was added 0.5 c.cm. solution of arsenious acid (1 in 5,000 saline); to B 0.5 c.cm. saline; and to C 1.5 c.cm. saline.

After the lapse of five minutes 1 c.cm. of distilled water was added to A and to B, and there resulted a slight amount of haemolysis in A, and almost complete haemolysis in B. C was again used as a control to show absence of haemolysis. The tubes were then allowed to stand for fifteen hours at room temperature, and at the end of that time no further haemolysis had occurred in A.

EXPERIMENT III.

This experiment was performed in order to determine whether this action of arsenious acid is an action on the red blood corpuscles alone, and independent of the presence of the serum. 60 c.mm. of blood was added to 5 c.cm. saline, and centrifuged for twelve minutes. The supernatant fluid was then pipetted off, and 5 c.cm. saline again added to the remaining corpuscles and the tube centrifuged. This was repeated a third time, and to the sediment of corpuscles was finally added 1.5 c.cm. of saline. This was now used as the blood suspension, and 0.5 c.cm. of it was put into each of three tubes A, B, and C.

As in previous experiments, to A was added 0.5 c.cm. of 1 in 5,000 solution of arsenious acid, to B 0.5 c.cm. saline, and to C 1.5 c.cm. saline. A and B were then centrifuged for fifteen minutes, after which the supernatant fluid in each was pipetted off. To each tube was then added 2 c.cm. saline, and they were again centrifuged for twelve minutes. This latter process was repeated. To the sediment of corpuscles in each tube was then added 1 c.cm. of saline. One c.cm. of distilled water was then added to A and B. This induced a partial haemolysis in both, but the haemolysis was markedly more complete in B than in A. In this experiment the corpuscles were first washed free of serum by thrice centrifuging with saline solution. Arsenious acid was then allowed to act on the washed corpuscles in one tube for fifteen minutes, during which time the tubes were being centrifuged. The arsenic solution was then removed by thrice centrifuging with saline.

From the fact that the addition of distilled water now produced considerably less haemolysis in those corpuscles which had been subjected to arsenic than in those which had gone through the same processes minus arsenic, three conclusions may be drawn:

1. That arsenious acid is fixed to the red blood corpuscles.
2. That this process takes place very rapidly.
3. That it protects these corpuscles against the haemolytic action of distilled water.

Other experiments have been performed with weaker solutions of arsenious acid, so that the corpuscles have been subjected to a strength of 1 in 50,000, 1 in 100,000,

1 in 200,000, 1 in 400,000, and 1 in 500,000. The protective action of arsenic on the red blood corpuscles was progressively less as the strength of solution diminished, but was still perceptible even in so great dilution as 1 in 400,000.

The question now arises as to whether there could be this strength of solution in the blood during the therapeutic administration of arsenic. A single maximal pharmacopoeial dose of arsenious acid, namely, 5 mg., if all absorbed, would allow the possibility of there being in the blood of a man weighing 70 kilograms, a concentration of 1 in 1,000,000, assuming the blood to be one-fourteenth of the total body weight.

The fact that an antihæmolytic action of arsenic was found in my experiments only with solutions not less dilute than 1 in 400,000 does not discount such an effect being produced during the therapeutic administration of arsenic—in the first place, because arsenic is frequently given in larger doses; secondly, because from the slowness of its elimination it may easily be found in the blood in more concentrated solution than 1 in 1,000,000 when pharmacopoeial doses are repeated; and, finally, because it seems to attach itself so rapidly and firmly to the red blood corpuscles that during repetition of its administration the absorbed arsenic may be largely taken up by these corpuscles and not proceed any farther.

In connexion with this action of arsenic on the blood it is important to note that "arsenic is found in largest quantity in the liver after absorption," and that "in the blood most of the arsenic is said to be contained in the blood cells, comparatively little being free in the plasma."⁴

Assuming, then, that arsenic is found in the blood corpuscles after absorption, and that it exerts on the red blood corpuscles a protective action against hæmolysis at least by distilled water, can one assign to this action any part in explaining the as yet imperfectly explained benefit which results from the administration of arsenic in blood diseases? I venture to suggest that this action of arsenic on the formed red blood corpuscles may play at least some part in producing its beneficial effect in the treatment in the first place of pernicious anaemia, for it may protect the erythrocytes against destruction, whether by the normal physiological processes, or by an abnormal pathological process; and secondly of malaria, not by acting on the parasite, for "it is questionable whether it acts on the malarial organism," but by an action on the red blood corpuscles in the direction of rendering them less permeable by the plasmodium.

If this is partly its action in blood diseases we can agree with Stockman and Greig that "it does not affect the real cause of the disease, and, therefore, must be considered a purely symptomatic method of treatment," and this fact may explain why in regard to pernicious anaemia the benefit produced by arsenic is so often a temporary one.

A THEORY OF THE BLOOD DEFECT IN PERNICIOUS ANAEMIA.

The etiology of idiopathic or pernicious anaemia is still obscure, and therapeutic measures are correspondingly inadequate to combat the disease. In these circumstances any reasonable suggestion bearing on its pathology or treatment may have some value.

Pernicious anaemia in regard to blood abnormality is characterized especially by a great diminution in the number, with generally a high individual hæmoglobin value of the red blood corpuscles. It presents in this respect a striking contrast to simple anaemia, in which disease, while the number of the red blood corpuscles is not conspicuously lessened, each individual erythrocyte is markedly poorer in hæmoglobin than are the red cells of healthy blood.

In the case of simple anaemia, though the ultimate etiology of it may be debated, this at least is generally accepted, that there is for some reason a deficient supply to the blood-forming organs of material available for making hæmoglobin, and that this defect of material can be made good by the therapeutic administration of iron.

The facts that in pernicious anaemia there is found abundance of iron in the liver and in other organs, and that iron is useless as a remedy, would seem to show that in this disease the defect does not lie in the hæmoglobin

factor of the corpuscles. Moreover, each erythrocyte contains in many cases a quantity of hæmoglobin in excess of that which is found in the erythrocytes of healthy blood.

May the defect not lie in the other constituent of the red cell—the stroma? There may be supplied to the blood-forming organs in a patient suffering from pernicious anaemia a deficient available quantity of those materials which go to form the stroma of the red blood corpuscles. There is present, as it were, in the body hæmoglobin enough, but not enough material for stroma in which the hæmoglobin may find place.

The stroma of the red blood corpuscles consists largely of lecithin and cholesterolin. The condition known as pernicious anaemia may be due to a deficiency in the body of lecithin or cholesterolin, and the administration of these substances may be of some use as a remedy.

Certain facts go to show that there may be some defect in the stroma of the red cells in this disease. For example, they do not run into rouleaux in normal fashion. It has been shown that bodies suspended in a watery solution run into rouleaux only when possessed of a fatty covering. The absence of this phenomenon in pernicious anaemia may be due to a defect in the stroma of the red blood corpuscles, in the lecithin and cholesterolin elements, which though not true fats are yet of a fatty nature. The condition of poikilocytosis and abnormal staining of the red cells, sometimes present in pernicious anaemia, may be due partly to the same defect.

On such a hypothesis of the blood defect in pernicious anaemia, the results of its somewhat empirical treatment may possibly be explained. The giving of bone marrow recommended by Fraser may be merely a method of supplying to the blood-forming organs these constituents of stromae which they lack. Lecithin is certainly known to exist naturally in the bone marrow. Arsenic, which, since its introduction by Byrom Bramwell in this disease, has been found to benefit a certain number of cases, may act on the stroma of the red blood corpuscles in such a way as to remedy this defect and increase their resistance. Indeed, lecithin has been found to increase the number of the red cells, and some experiments which I have made with regard to the action of arsenic on these cells have gone to show that it increases their resisting power.

Nor is there any inherent reason, if a deficiency of iron, for example, or of calcium can cause morbid conditions, why disease may not also be set up by a lack of such necessary constituents as lecithin or cholesterolin.

REFERENCES.

- ¹ Stockman and Greig, *Journ. Physiol.*, 1898, vol. xxiii, p. 376. ² Stockman and Charteris, *Journ. Path. and Bacteriol.*, 1903, vol. viii, p. 443. ³ Cushny, *Textbook of Pharmacology*, third edition, p. 621. ⁴ Cushny, *ibid.*, p. 618.

Memoranda : MEDICAL, SURGICAL, OBSTETRICAL.

STINGING FISH.

THE following are two cases of poisoning, due to the sting of a fish, which came under my treatment quite recently in Catalan Bay :

CASE I.—On the evening of April 11th, 1908, I was called to see M. G., aged 58, a fisherman, who had been stung on the base of the second finger of his right hand by a well-known poisonous fish called the Arrània (spider, in Spanish); the scientific name I have not yet been able to find out. The first symptom was intense pain, followed in a few seconds by swelling of the right hand, travelling up to the elbow in less than fifteen minutes, and within one hour the swelling had reached the upper arm. The patient was crying with agony, and was rapidly passing into a condition of collapse. I applied a ligature to the upper arm and strong solution of ammonia to the wound, but I did not see the case until an hour after the patient had been stung. Afterwards I administered brandy and water by the mouth, as the patient was very feeble and almost pulseless. The skin soon assumed a dusky-red colour. The ligature was removed and the arm well covered with poultices made of boracic lint and boracic wool. The next morning the glands in the axilla were very much enlarged and the whole arm enormously swollen and oedematous. The usual febrile symptoms were present, and suppuration threatened. I made several incisions about an inch deep into the arm and the dorsum of the hand, and collected at least one pint of clear straw-coloured fluid, alkaline in reaction, which became solid on boiling, due to the large amount of albumen it contained. After the punctures had