BIOCHEMICAL CHANGES IN THE BLOOD OF MONKEYS DEVELOPING MALARIAL HÆMOGLOBINURIA AND THEIR SIGNIFICANCE IN THE ÆTIOLOGY AND TREATMENT OF HUMAN BLACKWATER FEVER.

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In a large number of diseased states important biochemical changes are induced in the animal body and a careful study of these changes furnishes data of value in diagnosis and prognosis of the disease. Even when one is dealing with a disease of unknown ætiology, a study of the blood chemistry sometimes affords suggestive information on the probable nature of the causative agent and indicates the lines of treatment that might be profitably employed. The study of the biochemical changes taking place in the blood of hæmoglobinuric monkeys suggested that the sudden severe hæmolysis leading to hæmoglobinuria in human blackwater fever may be the direct outcome of the biochemical alterations induced in man as a result of chronic malarial infection. Our detailed investigations on the biochemical changes taking place in the blood of hæmoglobinuric and non-hæmoglobinuric monkeys during the different stages of the disease, with special reference to those chemical constituents that are known to be associated with the phenomenon of hæmolysis, such as cholesterol, inorganic and organic phosphorus and glucose, brought out the fact that in the pre-hæmoglobinuric stage the following changes are conspicuous: (*a*) a fall in glucose and a rise in inorganic phosphorus; (*b*) a rise in cholesterol esters, and a fall in total cholesterol in a fair number of cases and a marked fall in free cholesterol in all cases; and (*c*) a rise in organic phosphorus. The interpretation and significance of these variations are discussed below.

Fall in Glucose and Rise in Inorganic Phosphorus.

This was a constant feature in all the hæmoglobinuric monkeys studied. The fall in blood sugar got more pronounced as the infection in the animal got more intense. There are three possible ways of explaining this depletion of glucose: (*a*) that it is due to the utilization of the sugar by the parasite; (*b*) that it is due to the burning up of the sugar by the body to a greater extent; and (*c*) that it is due to an upset in the glycogenolysis and also in the mobilization of sugar. The fact that a rise in inorganic phosphorus is associated with this fall in glucose in monkeys shows that the animal is not

utilizing the sugar in the normal way. The parasites need sugar for their multiplication and growth and they probably obtain this from the hexose-phosphates of the red cells. The inorganic phosphorus arising out of this hydrolysis of the hexose-phosphate probably accounts for the increase in this substance. When insulin and sugar are administered to these monkeys it is found that this rise in the inorganic phosphorus does not take place. This shows that the accumulation of the inorganic phosphorus is essentially due to the upset in carbohydrate metabolism. The bearing of this upset on the hæmolytic process is not clear. In hæmoglobinuric monkeys it is possible to maintain normal levels for glucose and inorganic phosphorus through injection of glucose and insulin but by doing this the hæmolytic process is not inhibited, showing that the observed changes in these constituents, whatever be their underlying causes, have no direct bearing on the hæmolytic process.

In human blackwater fever, although a similar depletion of glucose has not been observed, all studies indicate that a rise in inorganic phosphorus does occur in a certain percentage of cases. This rise has however been explained as being due to renal dysfunction. Even if the change was due to an upset in carbohydrate metabolism there is no evidence to show that it plays a direct part in the hæmolytic process.

Fall in Free Cholesterol, Rise in Ester Cholesterol and Fall in Total Cholesterol in a certain percentage of cases.

Fall in free cholesterol was a constant feature in all monkeys developing hæmoglobinuria. In view of the property of free cholesterol to inhibit hæmolysis caused by saponin, etc., this observation is of particular importance. The constancy with which the hæmoglobinuria is associated with a marked fall in free cholesterol suggests that the hæmolysin is probably one whose activity can be inhibited by cholesterol. This is supported by the observations that in non-hæmoglobinuric monkeys free cholesterol was invariably high or normal. This shows that if mobilization and synthesis of free cholesterol kept pace with the increased production of fatty acids no hæmolysis or hæmoglobinuria would result.

The marked rise in ester cholesterol suggests that prior to the onset of hæmoglobinuria there is an increased production of fatty acids and that these acids are being transported in the form of esters to the tissues. If this is correct then the fatty acids may be considered to be the hæmolytic agents that act when free cholesterol is not present in sufficient amount. Thus the synthesis and mobilization of free cholesterol appear to be of greatest importance in the prevention of hæmolysis and hæmoglobinuria.

In human blackwater fever all previous workers have estimated only total cholesterol and that too after the onset of hæmoglobinuria. The results have therefore not only been variable but also inconclusive. We have in 8 cases of human blackwater fever estimated the blood cholesterol as free

and ester cholesterol separately and the results obtained are in conformity with the findings in monkeys. The results suggest that in human blackwater fever the hæmolytic agent is a fatty acid that acts when free cholesterol is not present in sufficient amount.

Now the question arises as to why there should be an increased production of fatty acids in malaria and in hæmoglobinuria; several explanations are possible: (1) Increase in fatty acids may be due to the growth and multiplication of the parasite. (2) Increase in fatty acids may be due to an increased demand for purposes of repair and regeneration of damaged tissues. (3) Increase in fatty acids is the result of an upset in carbohydrate metabolism and fat metabolism. (4) Increase in fatty acids is due to an increased demand of the substance for the purpose of disposal of the inorganic phosphorus that is accumulating and for converting this into the organic form. (5) Increase in fatty acids may be the result of damage to the liver. There is some evidence to substantiate a few of these possibilities in monkeys but the evidence in favour of liver damage being the cause of fatty acid production is overwhelming. In the case of man, where blackwater fever is not associated with an intense parasitization as in the monkey and, where there is no evidence of a deranged carbohydrate metabolism, the only possible explanation is a damaged liver. Pathologically also there is evidence to show that there is such a damage. This damaged state of the liver can very well account for an abnormal fatty acid production in human blackwater fever as well.

The next question is as to why the synthesis and mobilization of free cholesterol should fail. Here again a study of the pathology of the liver and adrenals in man and monkeys has shown that the most striking change is a fatty degeneration and focal necrosis of the liver and degeneration of the adrenal cortex. As these two organs are recognized to be closely associated with cholesterol metabolism it can readily be understood why cholesterologenesis and mobilization should fail. Another possible explanation for the depletion of cholesterol is the associated damage to the reticulo-endothelial system which is an important storehouse for cholesterol in the body.

Rise in Organic Phosphorus.

The rise in organic phosphorus as lecithin, namely the alcohol ether soluble fraction, was noted in both hæmoglobinuric and non-hæmoglobinuric monkeys towards the later stages of the infection. Such rise in organic phosphorus may be explained in any of the following three ways: (1) that it is derived from the destroyed R.B.C.; (2) that it indicates a method of disposal of the excess of fatty acids and inorganic phosphorus that is being produced; and (3) that it indicates an upset in fat metabolism due to liver injury. Of these, there is ample confirmation of the last explanation. As regards human blackwater fever, the alcohol ether soluble phosphorus compounds, namely lecithin and

other allied derivatives, have not been previously estimated. In the 8 cases studied by us we noted a rise in this constituent as in monkeys.

The increase in organic phosphorus raises a very important question regarding the nature of the hæmolysin involved and the rôle of lecithin in the hæmolysis of blackwater fever. As this increase of organic phosphorus in monkeys was noted in both the hæmoglobinuric and non-hæmoglobinuric groups it looks at first sight that the increase in this constituent may have nothing to do with the hæmolysis. But the fact that in the former the rise in lecithin is associated with a marked fall in free cholesterol suggests that the hæmolysin is one that is not only inhibited by excess of free cholesterol but also activated by the presence of excess of lecithin.

From the above discussion it will be seen that the phenomenon of hæmoglobinuria both in man and in monkeys is determined by the manner in which the host reacts to the stimulus of malarial infection. The production of the hæmolytic agent, as well as the conditions that favour its action, seem to be the result of altered metabolism due principally to liver injury. Whatever the true nature of the hæmolysin may be it is clear that its action is definitely inhibited by excess of *free cholesterol*. So in the treatment and prevention of blackwater fever we should aim at the following : (1) preventing liver injury or correcting the consequent alterations in fat metabolism ; (2) stimulating the cholesterologenetic centres in a manner such that the synthesis and mobilization of free cholesterol will keep pace with the increased demand ; and (3) stimulating the reticulo-endothelial system so that phagocytosis and the cholesterol stores of the body may be maintained. Our preliminary studies show that all these can be accomplished to a certain extent by injections of glucose, ascorbic acid and cortin.