Hæmmaglutination by horse serum and jaundice in mice / C.C. Okell and G.I. Steed.

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HÆMAGGLUTINATION BY HORSE SERUM AND JAUNDICE IN MICE.

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While testing about 500 specimens of therapeutic horse sera for their toxicity to mice, it was found that one serum injected intravenously constantly gave rise to jaundice which in the majority of cases was fatal within twenty-four hours in a dose of 0·5 c.c. The horse from which this sample had been obtained had at no time had an injection of mouse cells or tissue of any kind. On further investigation this jaundice was found to depend upon the marked agglutinative power of the serum in question on the red cells of mice. The serum had practically no hæmolytic power *in vitro* for mouse cells when either guinea-pig or mouse serum was used as complement: faint trace of hæmolysis was noted when mouse complement was employed and this only when the highest relative amounts of serum to cells were used. The hæmagglutinative power of the serum was however high, at least twice as high as that of the majority of samples of horse sera. Serum of moderate agglutinative power can be concentrated by the usual ammonium sulphate method so that it gives rise to jaundice. A search was made and a sample of serum from another horse was found which agglutinated mouse cells *in vitro* to a high titre. It could be correctly predicted that, like the original serum, this would cause jaundice in mice.

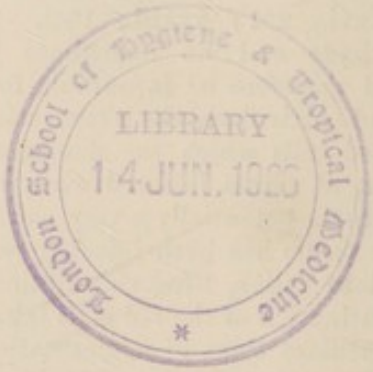
Mouse cells which had been previously agglutinated *in vitro*, washed three times, shaken and injected into mice produced jaundice but usually no immediate embolic effects. On the other hand injection of even large amounts of mouse hæmoglobin did not produce jaundice.

The phenomenon we were observing was clearly a hæmolytic jaundice due to the effect of agglutinin on mouse cells and though produced by a converse method seemed to be essentially the same as that occurring after transfusion of human patients with the blood of unsuitable donors. So far we have not been able to find records of hæmolytic jaundice produced experimentally as a pure hæmagglutinative effect (but see R. Bieling and S. Isaac *Zeit. ges. exp. Med.* 1921, xxv., 1; 1922, xxvi., 251; xxviii., 154). There appears to be no damage to any other tissue and no immediate intravascular lysis of red cells. Conditions should therefore be favourable for studying the mechanism of hæmolytic jaundice and particularly the phagocytic activities of the reticulo-endothelial system on red cells which are damaged but are still capable of circulating. The amount of serum injected when mixed with the circulating cells of the mouse constitutes potentially a very powerful agglutinative system. A few minutes after the serum has been injected one may remove a sample of blood from the mouse into saline, Ringer's solution, citrate-saline or isotonic glucose and massive agglutination of the red cells takes place. When withdrawn the blood shows no immediate signs of agglutination to the naked eye and if withdrawn into mouse plasma it takes several minutes to show obvious agglutination. For a time we thought that no agglutination proper was taking place in the vessels of the mouse and that it was only after withdrawal and interference with the normal balance of the blood that it occurred. But careful observation of the circulation of the blood in the mesenteric capillaries and veins after injection of the agglutinating serum convinced us that agglutination occurs but that the inhibitory effect of the whole blood, aided no doubt by the commotion of the circulation, prevented agglutination from giving rise to immediate

embolic accidents. Agglutinated masses break up easily at the bifurcation of small vessels and individual cells find their way along the finest capillaries; indeed intermittence in the procession of the cells was more marked in the smaller veins than in the capillaries themselves. Death immediately after injection was usually produced only when a very large dose of serum was used.

As in jaundice following human transfusion, hæmoglobinuria was a constant sign and came on rapidly after the injection of serum, sometimes after as short an interval as ten minutes. The urine contained no detectable bile salts. The agglutinated cells are removed by the cells of the endothelial system and the red cell count falls very rapidly during the first six hours after injection until it reaches about one-eighth of the normal count. It tends to remain at this level until death takes place. It was found difficult to make serial blood counts in mice by puncture of the tail veins and one of us (G.I.S.) found that samples of 0·1 to 0·7 c.c. of blood can be withdrawn by intracardiac puncture with a hypodermic needle. A series of several specimens, *e.g.* six in an hour, can be obtained from a mouse without influencing appreciably its normal count or causing any apparent harm to the animal. The operation can be performed single-handed in a few seconds.

A few minutes after the injection the spleen becomes enlarged and congested with red cells, and we thought that it took a predominating part in the removal and destruction of the sensitised or agglutinated cells. Extirpation of the spleen had however little effect either on the destruction of the blood as followed by blood counts or on the development of jaundice. In the majority of experiments the serum was injected twenty-four hours after splenectomy so as to minimise as far as possible compensatory effects. Microscopically the spleen liver and lungs were much congested and the endothelial cells in these localities showed active phagocytosis of red cells; the Kupffer cells of the liver seem to be particularly active in this respect. There is of course nothing unexpected in these observations which are consonant with the teaching of Aschoff, McNee and their school on the function of the endothelial cells in relation to hæmolytic jaundice.



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