INHERITED MACROCYTIC ANAEMIAS OF THE HOUSE MOUSE

IV. THE ALLEVIATING EFFECT OF BLOOD INJECTIONS

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(With One Text-figure)

Both allelomorphs of dominant spotting in the mouse, **W** and $\mathbf{W^v}$, give rise to a macrocytic anaemia when homozygous. The lethal anaemia of **W/W** animals was first observed by Detlefsen (1923) and studied by de Aberle (1927). She found that the erythrocyte count was only 14% of normal at birth, though leucocyte numbers were less reduced. Lethal anaemics are often stillborn; those surviving birth rapidly lose weight and die in a very few days. The anaemia of $\mathbf{W^v/W^v}$ mice is less severe (Little & Cloudman, 1937). The haematology of **W** and $\mathbf{W^v}$ homozygotes, heterozygotes and compound has been investigated in detail by Grüneberg (1939, 1942) and Attfield (1951) and described in previous papers in this series.

Subcutaneous injections of liver extracts into $\mathbf{W^v/W^v}$ mice by Grüneberg (1939) gave no positive improvements in the blood picture. Liver administered orally was found to be of no therapeutic value to **W/W** anaemics by Gowen & Gay (1932). But these authors had more success when they injected blood from normal mice into the peritoneal cavity of the lethal anaemics. Of eighteen so injected, eleven had a longer life than untreated controls and three became adult, turning out to be black-eyed whites, like $\mathbf{W^v}$ homozygotes. The blood injection had to be repeated frequently, or a rapid deterioration in condition took place. Gowen & Gay were unable to decide whether the injected blood was simply having the effect of a transfusion, or some other less direct influence. Grüneberg (1947) has discussed this problem and suggested methods of solving it.

This paper describes experiments designed to show the way in which injected blood acts to improve the condition of **W/W** anaemics.

Material and methods

The **W** stock used to produce anaemics had recently been outcrossed to the pure line C57 Black. Heterozygotes were very fertile, with large litters. But 43% of the anaemics were stillborn. The mean birth weight of those living was 1·24 g.; that of normals was 1·58 g.

Blood for injection was obtained from adult normals of the same stock. These were chloroformed and blood was drawn from the inferior vena cava immediately after death. All equipment was carefully sterilized.

The first aim was to repeat Gowen & Gay's experiments. Newborn anaemics were injected intraperitoneally with about 0·05 ml. of whole blood, immediately after its collection from the donor. This process was repeated every one or two days on survivors and every 3 or 4 days after the second week of survival.

Results

Although many anaemics died soon after injection some definite successes were obtained, though not so high a proportion as Gowen & Gay had. Of twenty-seven injected, seven lived for over a week and showed signs of growth and development, such as the freeing of ear-flaps and the appearance of hair. Of sixteen injected and weighed, three more than doubled their weight. The longest-lived animal survived for 27 days, and its weight increased from 1·45 to 8·10 g. Untreated animals in this stock seldom live for more than 2 days and have never been known to live for more than a week. They have shown no signs of development or of weight increase.

Blood from the tails of some of the injected anaemics was used to make blood-films, which were stained in Biebrich scarlet, to show the erythrocytes clearly. An examination of these under high power showed the presence of two distinct populations of cells of

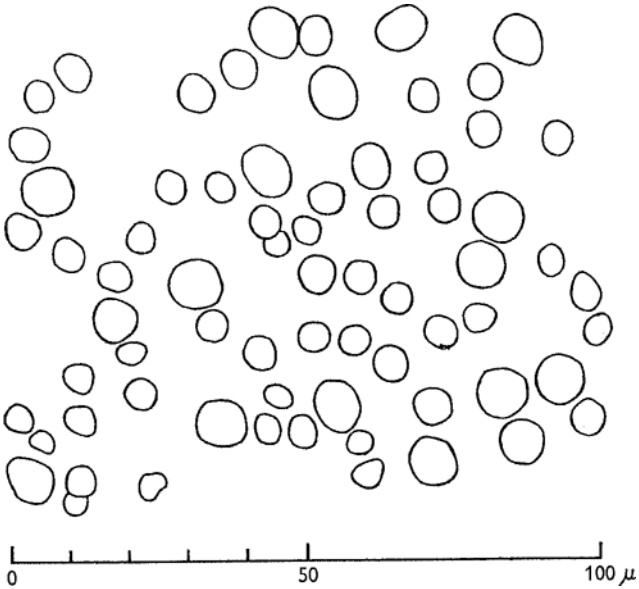

Fig. 1. Blood-film from **W/W** anaemic, injected two days earlier with whole blood from adult normal.

different sizes, with no appreciable overlap in cell diameters. A camera lucida drawing of a group of these cells is shown in Fig. 1.

The smaller erythrocytes are those of the injected adult blood, contrasting with the macrocytic cells of the newborn anaemic. The mean diameter of red blood cells of newborn **W/W** mice is 8·6 μ, while that of adult normals is about 6·4 μ (Attfield, 1951).

This blood-picture meant that injected blood cells could pass intact out of the peritoneal cavity into the general circulation. Therefore it seemed probable that the alleviation of the condition of the anaemics was simply a transfusion effect. But it was still possible that the action of injected whole blood was mainly due to something in the injected normal plasma.

To test this possibility, the anaemics were given virtually plasma-free blood. A suspension of whole blood in isotonic saline (0·93% sodium chloride for mouse blood—N. K. Pannikar, unpublished) was centrifuged at 3000 r.p.m. until the cells were loosely packed at the bottom. The supernatant fluid was decanted, more saline was added and

the process repeated five times in all. The washed cells in an approximately equal volume of saline were then injected into the anaemic. As with whole blood, this process was repeated if the animal survived. The volume injected could not be gauged exactly; owing to the low viscosity of the mixture not all stayed in the body-cavity. Microscopic examination of the centrifuged blood showed some distortion and fragmentation. This was reduced by slowing the speed of centrifuging in later experiments and by using Alsever's solution (Wintrobe, 1946), containing dextrose and sodium citrate as well as sodium chloride, instead of isotonic saline.

Since the plasma was initially diluted with at least six times its volume of isotonic fluid, and at least three-quarters of the fluid was removed after each spinning, the final concentration of plasma in the fluid injected should not have been more than $\frac{1}{6} \times (\frac{1}{4})^4$, or $\frac{1}{1536}$.

Of twenty-seven anaemics injected with washed cells, nine increased in weight. One of these doubled its weight, living for ten days and starting to grow hair. Four of those which eventually gained weight had initially lost weight. Blood-films from treated anaemics showed the same general picture as with whole blood injections.

Of five newborn anaemics injected with heparinised plasma alone, none showed any signs of an improvement in condition. One lived for 3 days, but its weight dropped steadily, though it was reinjected each day.

DISCUSSION

Seven out of twenty-seven anaemics injected with whole blood lived for over a week; one out of twenty-seven injected with washed cells lived for over a week. Constructing a 2×2 table from these data and testing for significance by Fisher's 'exact method' gives the significant value of $P = 0{\cdot}025$. This suggests that though washed cells can prolong the life of **W/W** anaemics they are less efficient in their action than whole blood. This might be expected on *a priori* grounds, owing to the action of the following factors:

(*a*) physiological unbalance resulting from the injection of blood-cells in a medium other than their normal one,

(*b*) damage to the red cells due to the repeated washing,

(*c*) no nutritive aid from the plasma.

The effect of these is probably sufficient to explain the less efficient action of washed cells. Plasma alone seemed to have no action and there was no indication of increased haemopoiesis when blood-smears made after several injections were examined. There is, therefore, no reason to postulate that whole blood has any other effect than that of a transfusion.

The passage of blood corpuscles and other small particles from the peritoneal cavity into the circulation has been demonstrated in other animals besides the mouse. Hayem (1884) used contrast in corpuscle size to prove that dog's blood injected into the peritoneal cavity of the rabbit could later be found in the general circulation. Siperstein & Sansby (1923) studied the intraperitoneal transfusion of citrated blood in the rabbit. Injected erythrocytes entered the blood-stream rapidly and, in both anaemics and normals, led to a sharp temporary rise in blood-values, followed by a more permanent increase lasting several days. The injection acted as a true transfusion and not as the absorption of nutrient material. The authors recommended the intraperitoneal route for blood transfusion as a useful therapeutic method in man, especially for infants.

The way in which injected particles reach the general circulation has been studied by Allen (1936) and Simer (1948). Both agree that the main route is via the diaphragm and its lymph plexus, draining into the anterior mediastinal lymph-nodes. In the mouse, Allen has described 'peritoneal stomata' over lymphatic lacunae, in which injected frog erythrocytes were seen caught, on their way through into the lymphatics. In rats, Simer found that injected frog erythrocytes (diameter 11 μ) did not pass through into the lymphatic plexus, though yeast-cells (diameter 4–5 μ) did. He therefore considered that the potential openings in the mouse peritoneum were larger than in the rat.

The evidence of previous work on intraperitoneal injection of blood appears, therefore, to support strongly the view that blood thus injected into a **W/W** anaemic acts as a transfusion, alleviating the anaemia by direct action and thereby prolonging life. It must be emphasized that this response to blood injection is a general one; therefore it can tell us nothing about the particular cause of this anaemia.

SUMMARY

1. Experiments on the intraperitoneal injection of normal blood into **W/W** lethal anaemics show that the red blood cells pass through the peritoneum into the general circulation.

2. The blood has the effect of a transfusion, alleviating the severity of the anaemia by its direct action and thereby prolonging life.

I am indebted to Dr H. Grüneberg, who suggested this investigation, for his helpful advice and criticism.

This work was carried out during the tenure of an Agricultural Research Council research studentship.

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