SOME RECENT ADVANCES IN HAEMATOLOGY*

By D. E. Smith, M.R.A.C.P., D.C.P.

Tonight I propose to speak on two aspects of haematology in which certain recent work of great general interest has been done. The first subject is the problem of bone-marrow transplantation.

BONE MARROW TRANSPLANTATION

Physicians and haematologists have for many years been painfully aware of the poor results obtained in the treatment of such diseases as the leukaemias, the hypoplastic and aplastic anaemias and the severe haemolytic anaemias due to genetically determined abnormalities of red cells. In all these diseases there is an abnormality of bone marrow cells, either inherited or acquired, which is permanent. In certain leukaemias, the acute lymphoblastic leukaemias, a reversion of the abnormal cells to cells which are morphologically and functionally normal can be brought about in many cases by corticosteroid therapy. However, these remissions are certainly not permanent and a reversion to abnormal leukaemic cells always occurs.

Faced with this permanent cellular abnormality the only possible way to return these patients to normality would be to destroy the abnormal cells and then repopulate the bone marrow with normal homologous bone marrow cells. Thus we have two problems:

(a) The destruction of abnormal bone marrow cells, and

(b) The repopulation of the marrow by normal bone marrow cells.

With regard to (a), complete destruction of haemopoietic bone marrow cells can be achieved by total body X-ray irradiation. However, as many other cell types are severely damaged by the dosages of X-ray used, particularly such tissues as gastro-intestinal tract epithelium, it is unlikely that this method will ever be used in man.

It is much more probable that folic acid antagonists, purine antagonists or similar chemical agents will be used to destroy the abnormal bone marrow cells. It is known that folic acid antagonists and purine antagonists can cause complete bone marrow aplasia in man, with relatively little damage to other cell types. However, little work has yet been done on this aspect of the problem, most of the investigators having been engaged upon the problems of bone marrow transplantation.

With regard to this second problem, that of bone marrow transplantation, the first work of significance was performed in 1951 by Jacobson et al[1] who demonstrated that lethally irradiated mice had their lives greatly prolonged by either spleen shielding from the irradiation or post-irradiation homologous splenic implantation (intraperitoneal). Apparent bone marrow regeneration occurred in these lethally irradiated mice with either of these added procedures. Shortly after this work was done Congdon et al[2] in 1952 showed that mice and guinea pigs who had been subjected to lethal total body X-ray irradiation, could have their lives considerably prolonged by the intravenous administration of homologous haemopoietic bone marrow. Both these two independent groups of workers showed that bone marrow regeneration occurred in the treated animals, whereas the control irradiated animals died within fourteen days of irradiation showing complete aplasia of bone marrow. Those irradiated animals who had been given bone marrow suspensions intravenously lived more than thirty days and all eventually died, but death in these animals was not due to irradiation aplasia of bone marrow but was due to the effects of irradiation on other tissues.

At that time it was generally thought[3] that the effect of intravenously administered bone marrow was due to humoral factors present in normal bone marrow cells which in some way stimulated the irradiation damaged marrow cells to proliferate normally. Some support was given to this humoral theory by the work of Congdon and Lorenz[4], who demonstrated that rat bone marrow, given intravenously to irradiated mice, would prolong their lives and cause bone marrow regeneration in much the same way as intravenous homologous bone marrow. It seemed unlikely that homotransplantation and heterotransplantation of bone marrow cells were the reasons for the apparent bone marrow regeneration and so the humoral theory held sway for a short period.

However, Dr. J. F. Loutit and his co-workers had never been satisfied with this humoral

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hypothesis and considered that it was unlikely that the injected bone marrow cells were actually repopulating the irradiated marrow. In 1956 Ford, Loutit el al[5] produced irrefutable proof that experimental animals given intravenously injected bone marrow after irradiation showed repopulation of their bone marrow by the injected bone marrow cells. These workers used a strain of mice which have an atypical "markner" chromosome. This markner chromosome was easily recognisable when the mouse cells were in mitosis. Lethally irradiated mice were given intravenous injections of mouse marrow from mice with the markner chromosome. The irradiated mice showed bone marrow regeneration and prolonged survival time and examination of the "regenerated" bone marrow showed that all the cells in mitosis showed the markner chromosome. This finding indicated that bone marrow transplantation had occurred, that the transplanted cells proliferated to produce a normal bone marrow and that regeneration of the irradiated bone marrow cells did not occur. In other words these experiments showed that the reformation of the bone marrow was due entirely to proliferation of the transplanted cells. These workers, in the same publication, reported that in another group of experiments bone marrow from Wistar rats was injected into irradiated mice and study of the chromosome pattern of the "regenerated" bone marrow showed once again that all the cells of the bone marrow originated from the transplant — in this case, an example of heterotransplantation.

At about the same time Nowell et al[6] reached the same conclusion using a different technique. The mature granulocytes of rats are strongly positive for alkaline phosphatase, while the mature granulocytes of mice are completely negative for alkaline phosphatase. These workers injected normal rat bone marrow into lethally irradiated mice. Not only was there evidence of repopulation of the mouse bone marrow by the alkaline phosphatase positive rat cells, but after two to four weeks virtually the entire population of bone marrow granulocytes was made up of alkaline phosphatase positive cells. Normal non-irradiated mice injected with rat bone marrow showed no evidence of such repopulation. Although the above findings of Nowell et al are open to other interpretations, it is highly probable that the results of their experiments indicate that heterotransplantation of marrow cells had actually occurred.

From the experimental work quoted above it is now obvious that homotransplantation and heterotransplantation of bone marrow is possible. Certain facts have also emerged from this experimental work:—

(1) It was necessary to destroy by irradiation the "natural" immune defence against homologous and heterologous cells in order that bone marrow transplantation be successful. Non-irradiated animals showed no evidence of bone marrow transplantation when injected intravenously with bone marrow cells. I will return to this problem of natural immunity shortly.

(2) Bone marrow cells injected into an animal intravenously seek out normal bone marrow sites and repopulate these sites. The so-called pulmonary barrier does not apparently hold back these cells.

Some other experimental work on the above lines is of interest. Certain mice suffer from a genetically determined microcytic hypochromic anaemia which is very similar to thalassaemia in man. Russell et al [7] subjected these mice to moderate irradiation (200r) and then injected normal mouse marrow intravenously. The bone marrow was repopulated by the normal mouse cells and at 147 days after the injection 80% of the circulating red cells were of the normal type. Apparently some bone marrow had survived this moderate irradiation and contributed 20% of the red cells present in the circulation at 147 days. The haemoglobin levels of these treated mice was only slightly below that found in normal mice. Thus, in mice, a genetically determined anaemia has, for practical purposes, been cured by bone marrow transplantation. As in the other experimental work non-irradiated mice suffering from this anaemia and then injected with normal mouse marrow showed no evidence of survival of this bone marrow as shown by the failure of any normal mouse red cells to appear in the peripheral blood. Certain mice suffer from a leukaemia which is transmissible by intravenous injection of blood from one mouse suffering from this leukaemia into another normal mouse. This normal mouse subsequently develops leukaemia. Barnes, Loutit et al[8] gave these leukaemic mice lethal doses of X-ray irradiation, sufficient to destroy both the leukaemic cells and the normal marrow cells. These mice were then given homologous bone marrow intravenously. Most of these mice not only survived the "lethal" irradiation but also showed no evidence of leukaemia. Here we have a leukaemia in an experimental animal being apparently effectively treated by bone marrow transplantation.

I would now like to say a few words about natural immunity as destruction of natural immunity is necessary in order that transplantation of bone marrow be successful. Natural immunity may be considered to be the property of an organism which prevents proliferation in that organism of foreign cells, certain bacteria and certain viruses to which the organism has not been previously exposed. In mammals this property of natural immunity develops early in foetal life and is well established when the foetus is fully developed. The first proof of this absence of natural immunity in embryonic life was demonstrated by Owen[9]. He showed that many bovine twins had in their blood stream two different types of red cells, each type having their own distinct antigenic structure. Chorionic vascular anastomoses are quite common in non-identical bovine twins and during embryonic life transfer of erythroblasts from one twin to the other occurs. Because of lack of natural immunity at this stage the foreign erythroblasts proliferate in the "host" embryo and so a true red cell chimaera is produced. True chimaeras have also been produced experimentally in mice and chickens by the injection of foreign cells during embryonic life[10]. The first true red cell chimaera has recently been described in man by Dunsford et alii". This was a woman who had a non-identical twin. Her red cells were of two distinct antigenic types.

Certain facts of importance have been determined from the work on naturally occurring and experimentally produced chimaeras. Firstly it is apparent that after embryonic life chimaera formation does not occur unless total body X-ray irradiation is used to destroy natural immunity. Secondly it is apparent that the newly forming system of immunity in the embryo or the reforming system of immunity after irradiation is tolerant of the foreign antigens present in the foreign cells and antibodies to these foreign antigens do not develop subsequently. An example of this is seen in experimentally produced chimaeras as these chimaeras have been shown to be tolerant of skin grafts of the same antigenic structure as those cells which were injected into the embryo to produce the chimaera state. Main and Prehu[12] have similarly shown that irradiated mice of one strain injected with bone marrow from a second strain of mice are subsequently tolerant of skin grafts from the second strain to which the first strain of mice is normally intolerant.

From all the above work it is evident that homotransplantation and heterotransplantation are possible during embryonic life and after destruction of natural immunity by total body X-ray irradiation. However total body X-ray irradiation in the dosage used has such serious effects on so many cell types that it is necessary to look to some other means of depressing natural immunity. It has been well known for some time that human plasma has certain natural immune characteristics e.g. normal human plasma has certain anti-bacterial actions and also certain virus neutralising properties. These are natural properties of human plasma and do not represent "acquired" immunity. It has been considered that natural immunity in man to foreign cells is due to some factor in human plasma similar to the factor or factors responsible for the bactericidal and virus-neutralising properties of human plasma. Pillemer et al[13] have claimed to have isolated a new serum protein, Properdin, which they consider is responsible for the natural immune characteristics of plasma. Certain properties of Properdin are of interest:

(1) Natural bactericidal activity. Properdin free plasma has practically no bactericidal action on Shigella dysenteriae whereas when purified Properdin is added to Properdin deficient plasma the normal bactericidal action of plasma on this organism is seen. Other organisms used in assessing natural immunity are affected similarly by Properdin.

(2) Heat labile factor of plasma which combines with and inactivates certain viruses (e.g. influenza, mumps, Newcastle disease viruses). Apparently Properdin is responsible for this action of normal plasma.

(3) Total body X-ray irradiation reduces plasma Properdin levels to very low levels.

(4) Zymosan, a complex yeast polysaccharide, specifically neutralises Properdin both in vivo and in vitro. Given intravenously Zymosan causes a marked fall of plasma Properdin levels[14].

(5) Properdin requires the presence of complement and magnesium for its actions.

From the above it can be seen that there is some evidence that natural immunity may be due to Properdin, however, the matter is still not settled and the Properdin theory of natural immunity is still a theory. However if Properdin is responsible for natural immunity then reduction of plasma Properdin levels by Zymosan injections may be the way in which natural immunity can be depressed safely so as to allow successful bone marrow transplantation to take

place. This, as far as I know, has not been attempted as yet but Herbut and Kraemer[15] showed that Zymosan given intravenously increased markedly the number of successful takes in weanling Wistar rats of a human colonic carcinoma HR132. Weanling Wistar rats are normally fairly resistant to transplantation of this tumour. Whether the action of Zymosan in this work was due to depression of plasma Properdin levels or due to some other effect of Zymosan is of course not known.

Finally I would like to say that experimental work has shown that bone marrow transplantation can be successfully formed. Further work to determine a safe method of depressing natural immunity is necessary before bone-marrow transplantation could be used as a therapeutic agent in man.

ADVANCES IN RESEARCH INTO LEUKAEMIA

The second aspect of recent haematological research on which I intend to speak concerns the relationship of the thymus to certain leukaemic states.

The thymus is an endocrine gland, the functions of which are for practical purposes completely unknown. Although the normal function of the thymus is unknown there is growing evidence that the thymus plays a prominent part in the induction and persistence of certain leukaemic states.

There is firstly certain indirect evidence of thymic participation in the leukaemic state. When cortisone first became available for clinical use, it was inevitable that it should be tried in acute leukaemias. It soon became evident that approximately 30% of cases of acute leukaemia responded dramatically both clinically and morphologically to cortisone therapy. Several investigators then decided to find out if these cases which responded to cortisone therapy were in any way different morphologically from those acute leukaemias which did not respond to cortisone. Wintrobe et al[16] found that in a retrospective analysis of fifty cases of acute leukaemia complete remissions had occurred in 68% of the cases of acute lymphoblastic leukaemia and in none of those falling into the myeloblastic or monocytic categories. Other investigators have also found that in acute leukaemias it is only the lymphoblastic type which will show remissions with cortisone. I have been struck by the remarkable return to normal of bone marrow and peripheral blood in the cases I have seen of acute lymphoblastic leukaemia remitting under cortisone. These remissions are in no way permanent and almost all cases show relapses within twelve months. Now one of the actions of cortisone (or hydrocortisone or ACTH) is that it is powerfully thymolytic. That is it induces rapid and marked atrophy of the thymus. Whilst the action of cortisone in these cases may not be due to its thymolytic action, I think you will agree when you have heard other evidence to come associating the thymus with lymphatic leukaemias that it is highly likely that the remissions induced in acute lymphoblastic leukaemia by cortisone are due to its thymolytic action. It is of interest to note at this stage that cortisone also occasionally is of great benefit in chronic lymphatic leukaemia whereas there is no evidence of benefit in chronic myeloid leukaemia.

Certain direct evidence linking the thymus with lympatic leukaemias will now be presented. Several strains of mice show a high incidence of lymphatic leukaemia and lymphosarcoma. If these strains of mice are subjected to thymectomy then there is marked fall in the incidence of spontaneous lymphatic leukaemia and lymphosarcoma[17]. Re-implantation of the thymus into these thymectomised mice causes the incidence of lymphatic leukaemia and lymphosarcoma to return to the usual high level[18].

While the above work gives evidence of a direct link between the thymus and lymphatic leukaemias, I think the following work of Dr. Donald Metcalfe at the Walter and Eliza Hall Institute, Melbourne represents a remarkable contribution to our knowledge of the leukaemias and indicates again a definite link between the thymus and lymphatic leukaemias. Dr. Metcalfe found that the serum of human patients suffering from certain types of lymphatic leukaemia and related conditions produced a lymphocytosis when injected by the intracerebral route into young mice. The factor present in the serum of these patients may be called "lymphocytosis stimulating factor" or L.S.F. It was shown that L.S.F. was present in high titre in these patients and was not detectable in normal serum or in the serum of patients suffering from a variety of other diseases. L.S.F. has all the properties of a globulin and is either a globulin or is closely associated with the globulin fraction of serum. Dr. Metcalfe then decided to try to determine the site of production of L.S.F. and in order to do this extracts of practically all normal human tissues were made and tested on mice for their lymphocytosis producing effect. The only normal human

tissue which when submitted to extraction could produce a lymphocytosis in mice was the thymus. The thymic extracts had an identical action with that of L.S.F. found in the serum of patients suffering from certain types of lymphatic leukaemia and related conditions. For practical purposes Dr. Metcalfe has shown that L.S.F. is present in the normal thymus and absent from normal serum and is present in the serum of the patients referred to above in high concentration. This work leaves no doubt in my mind of the important part the thymus plays in lymphatic leukaemia.

After completing this work in Melbourne, Dr. Metcalfe went to Boston to continue his experimental work in the leukaemia research unit of Dr. G. Farber who is one of the foremost workers in leukaemia in the United States.

It is interesting to theorize on the problem of the mode of action of the thymus in lymphatic leukaemias. It is possible that these leukaemias represent a state of endocrine imbalance where the thymus produces excessive amounts of hormone (L.S.F.) causing the undue proliferation of lymphoid tissue seen in these diseases. I am sure you will agree that Dr. Metcalfe's work represents one of the most significant advances in our knowledge of leukaemia and I hope that his continued experiments will reap more knowledge concerning the relation of that mysterious organ, the thymus, to lymphatic leukaemias.

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