STUDIES ON ANEMIA IN F_1 HYBRID MICE INJECTED WITH PARENTAL STRAIN LYMPHOID CELLS*

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It is now well established that animals given lethal doses of x-radiation can be protected from the immediate effects by injections of homologous or heterologous bone marrow or spleen but later develop a fatal disease, called "secondary disease" by Barnes *et al.* (1) and "homologous disease" by Trentin (24), which is characterized by emaciation, lymphoid atrophy, and anemia (8, 2, 24, 29). A similar syndrome, called "wasting disease" by Kaplan and Rosston (15), has been observed in sublethally irradiated F_1 hybrid mice injected with tissue from the bone marrow, spleen, thymus, leukocytes, or blood of mice of either parental strain (19, 27, 6, 13). Both secondary disease and wasting disease have been interpreted as reactions of the injected lymphoid tissue against the host, resulting in destruction of the host's tissues by the graft (27, 28, 23–26, 5). The immunological nature of this reaction led Kaplan and Rosston (15) to suggest that the anemia might be an experimental counterpart of the Coombs-positive hemolytic anemias frequently seen in patients with malignant lymphomas. They thought it likely that the anemia resulted from a destruction of host erythrocyte precursors by the injected cells, leading to a decrease in erythrocyte production, and an accelerated destruction of circulating red cells.

In the work to be reported here, wasting disease was produced in F_1 hybrid mice by sublethal irradiation and transplantation of lymphoid tissue from one of the parental strains. The survival of ^{51}Cr-labeled erythrocytes of either donor or host origin was studied in wasted animals and in several types of controls. Donor lymphoid cells were derived from either lymph node and thymus or from the spleen, which also contained active erythropoietic elements. Anemia was assessed by following changes in blood hemoglobin concentration.

Materials and Methods

The animals used in these experiments were inbred C_{57}BL/Ka and BALB/c mice, and the F_1 hybrid produced by mating animals of these strains. The inbred lines have been propa-

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gated in this laboratory since 1948 by random brother-sister mating. Recipient animals were F_1 hybrid mice of either sex, 30 to 40 days old at the start of an experiment. They were injected with lymphoid cells either from donor animals of the $C_{57}BL$ strain or from control donors of the same F_1 hybrid combination. Cells from donors of one sex were always injected into recipients of the same sex.

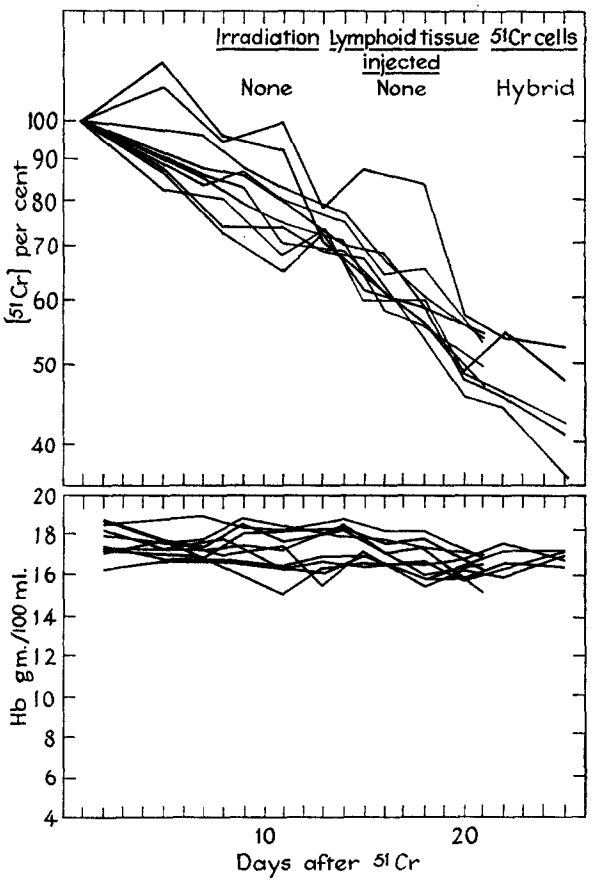

FIG. 1. Hemoglobin concentration and survival of hybrid red cells in normal F_1 hybrid mice.

Red cells from F_1 hybrid mice or from $C_{57}BL$ mice were labeled with ^{51}Cr *in vitro* (14) as follows. To 2 ml. heparinized, whole blood, 1 ml. of an acid–citrate–dextrose solution (Abbott) and 1 ml. of an isotonic saline solution containing 400 to 500 μc. ^{51}Cr (specific activity 400 μc./μg.) as sodium chromate were added with mixing. The amount of chromium added (0.5 μg. per ml. blood) was well below the level at which damage to the red cells has been observed (22). The mixture was allowed to stand for 45 minutes at room temperature, after which it was gently centrifuged, the supernatant removed together with the buffy coat, and the cells washed once with isotonic saline. The cells were then resuspended in isotonic saline and the

volume readjusted to 2 ml. An aliquot (0.1 ml.) of the cell suspension was injected intravenously into each animal. Subsequently, blood samples of 5 μl. were taken from the cut tail and the red cells lysed in 3 ml. of a dilute potassium ferricyanide solution for hemoglobin measurement. Hemoglobin concentration was estimated colorimetrically as cyanmethemoglobin using a Beckman DU spectrophotometer. The samples were retained for assay of ^{51}Cr

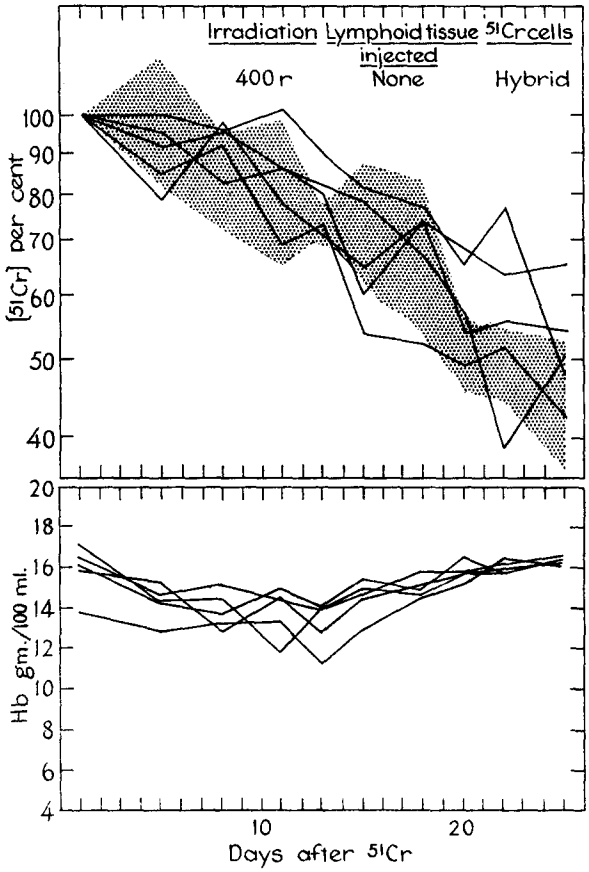

FIG. 2. Effect of irradiation on hemoglobin concentration and survival of hybrid red cells in normal hybrid mice.

content in a well-type scintillation counter. The first sample was taken 1 or more days following the injection of labeled cells, when any cells damaged during the labeling process may be assumed to have been removed from the circulation; serial samples were taken from each animal at intervals of 2 or 3 days thereafter. All samples from an individual animal were counted at the same time, thus avoiding the need to correct for physical decay of ^{51}Cr. The total ^{51}Cr content of the circulating blood was calculated for each time of sampling (assuming the blood volume to be 7 per cent of the body weight) and expressed as a percentage of that for the initial sample. Cumulative errors due to the small amount of ^{51}Cr removed during sampling are small and no correction was made.

Irradiations were carried out using 120 kp x-rays filtered by 0.25 mm. Cu + 1 mm. Al at a distance of 30 cm., the dosage rate being 24 r/minute. The total body dose given to all irradiated mice was 400 r (tissue dose). Irradiations were performed 2 to 3 hours after the first blood sample for ^{51}Cr assay had been taken, and lymphoid cells were injected within 4 hours of the irradiation.

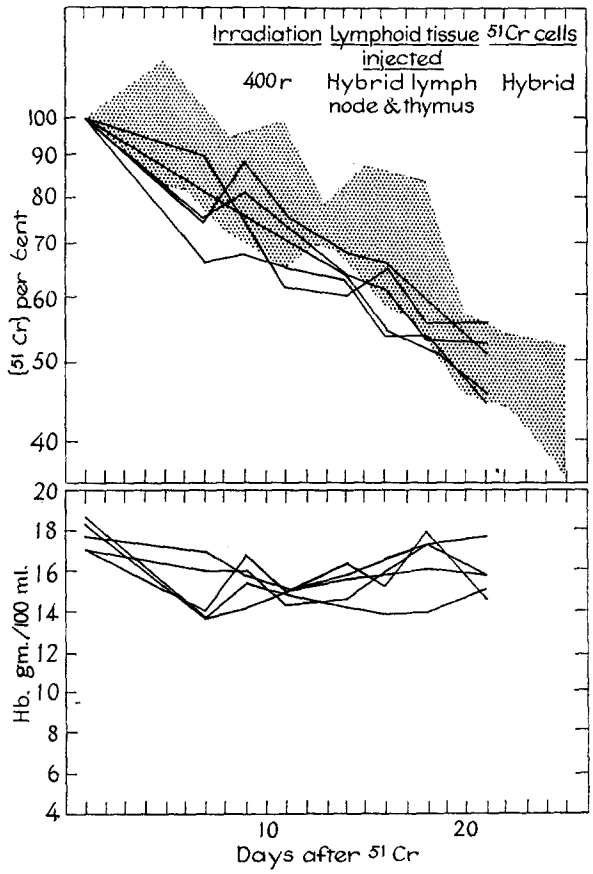

FIG. 3. Survival of hybrid red cells after irradiation and injection of hybrid lymphoid cells.

Purely lymphoid cells were derived from the lymph nodes (axillary, inguinal, and mesenteric) and thymus of donor mice. In some experiments, spleen cells consisting of mixed lymphoid, myeloid, and erythroid elements were used. The cells were suspended in saline and a volume of 0.5 ml. or less of suspension containing 150 to 300 $\times$ 10^6 cells was injected into each recipient.

Experiments were performed on groups of five mice. The groups were caged identically with free access to Purina laboratory chow and water, and animals were weighed three to four times each week.

RESULTS

The survival of ^{51}Cr-labeled hybrid red cells in ten normal isologous F_1 hybrid mice is shown in Fig. 1. The total ^{51}Cr content of the circulating blood

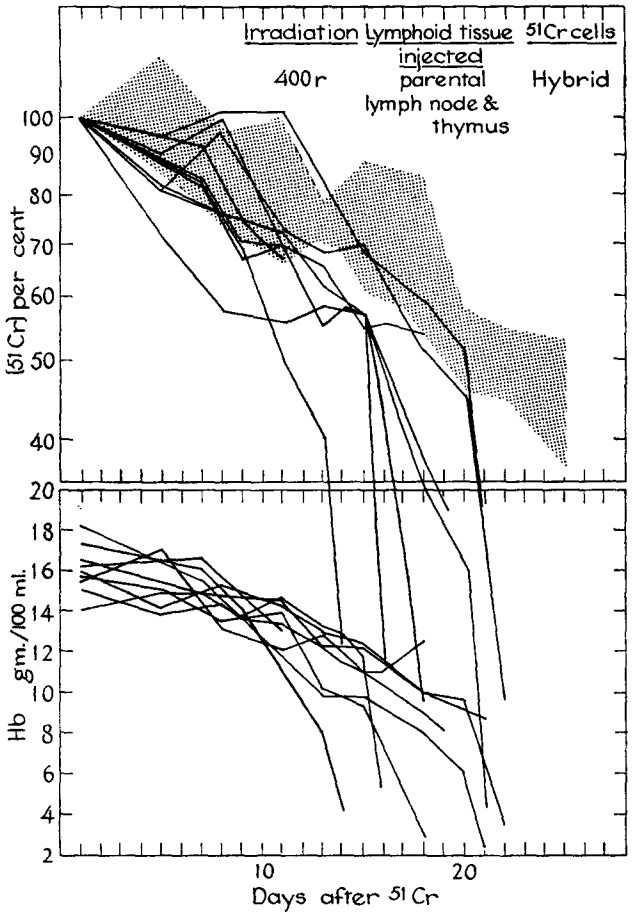

FIG. 4. Survival of hybrid red cells after irradiation and injection of parental lymphoid cells.

at each time of sampling has been expressed as a percentage of that for the initial sample; the hemoglobin concentration of the recipient animals is also shown. Fig. 2 shows similar results in mice given 400r x-radiation immediately after the first blood sample was taken. This dose of radiation caused a slight fall in hemoglobin level with subsequent recovery but did not affect the survival of ^{51}Cr-labeled isologous erythrocytes already in the circulation. A similar fall

in hemoglobin was observed (Fig. 3) in mice given a subsequent injection of lymph node and thymus cells from isologous F_1 hybrid mice; again, the survival of ^{51}Cr-labeled isologous erythrocytes was unaffected. By contrast, irradiated mice given a subsequent injection of parental lymph node and thymus cells

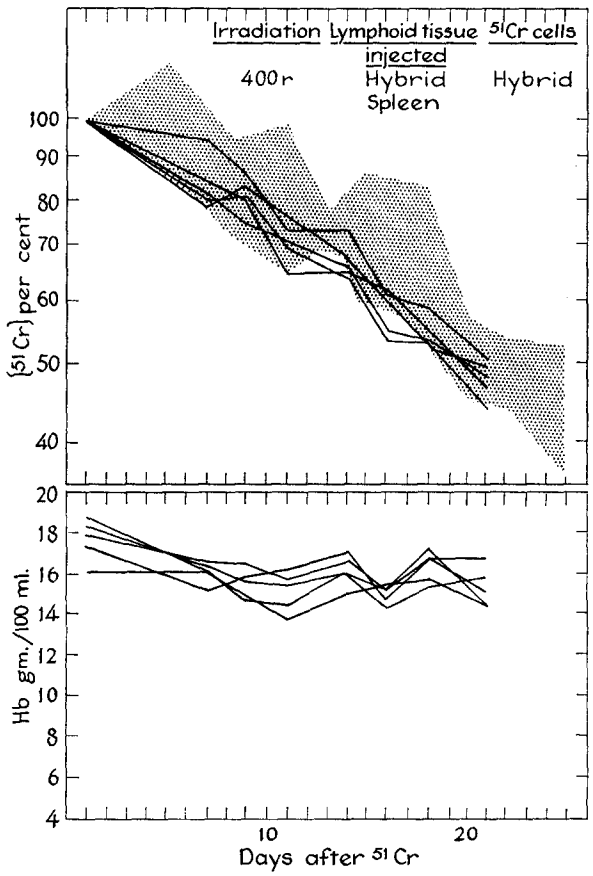

FIG. 5. Survival of hybrid red cells after irradiation and injection of hybrid splenic cells.

(Fig. 4) from $C_{57}BL$ mice suffered a profound fall in hemoglobin concentration between 2 and 3 weeks after treatment. At the same time that the anemia appeared, the number of ^{51}Cr-labeled isologous hybrid erythrocytes surviving in the circulation showed a very marked decrease. All mice in this group died within 22 days of the injection of lymphoid cells.

A similar difference was seen between groups of mice injected with isologous hybrid or parental spleen cells. In mice given hybrid spleen cells (Fig. 5), the loss of ^{51}Cr-labeled hybrid erythrocytes from the blood was similar to that

seen in normal mice or in mice given irradiation only. However, if parental spleen cells were injected (Fig. 6), there was a sudden fall in hemoglobin to very low levels and a concomitant rapid decrease in the number of ^{51}Cr-labeled hybrid erythrocytes surviving in the circulation. The anemia occurred 9 to 11

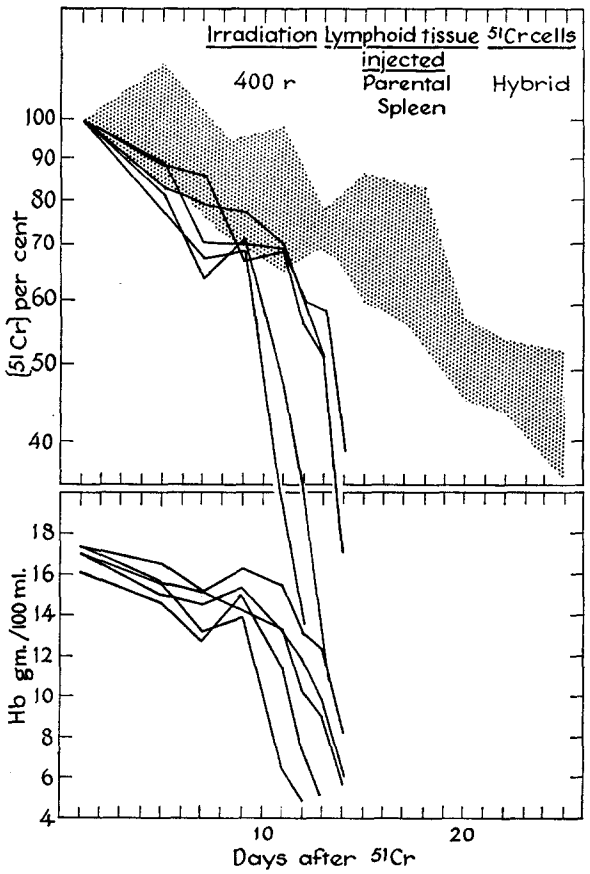

FIG. 6. Survival of hybrid red cells after irradiation and injection of parental splenic cells.

days after injection of parental spleen cells, somewhat earlier than was observed in mice given purely lymphoid parental cells (*cf.* Fig. 4). All mice given parental spleen after irradiation died within 2 weeks of treatment or were killed when moribund. At postmortem examination two of ten mice were found to have suffered extensive hemorrhage into the peritoneum or intestinal wall.

The survival of ^{51}Cr-labeled parental erythrocytes in F_1 hybrid mice given irradiation only (Fig. 7) or irradiation followed by an injection of hybrid lymph node and thymus cells (Fig. 8) was similar to the survival of hybrid

erythrocytes (*cf.* Figs. 2 and 3). However, if parental lymph node and thymus cells were injected (Fig. 9), the number of ^{51}Cr-labeled parental erythrocytes in the circulation showed a sudden decrease when anemia occurred. It was unfortunate that in this group several animals died before the anemia appeared.

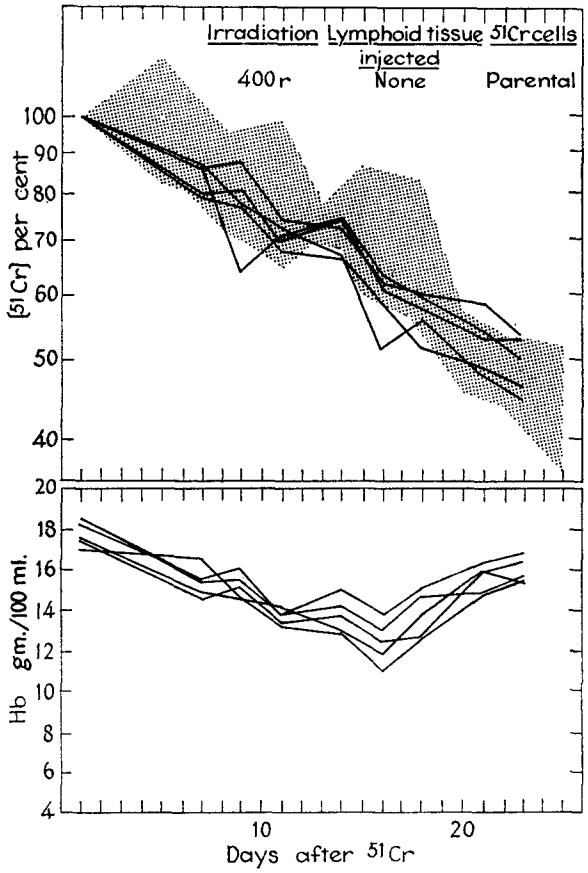

FIG. 7. Effect of irradiation on hemoglobin concentration and survival of parental red cells in normal hybrid mice.

The survival of parental ^{51}Cr-labeled erythrocytes in F_1 hybrid mice injected with parental spleen cells is shown in Fig. 10. Although these became anemic at about the same time after injection as similarly treated mice (Fig. 6), two of the group maintained their hemoglobin level at about 10 gm./100 ml. blood, and eventually began to recover. Both these mice died, however, before their hemoglobin levels had returned to their original values. In the mice which did not maintain their hemoglobin levels, the same sudden loss of ^{51}Cr-labeled

erythrocytes was observed as in Fig. 6. This sudden loss was not seen in the mice which began to recover from the anemia.

The results of the experiments shown in Figs. 9 and 10 were not considered sufficiently conclusive to decide whether or not parental erythrocytes behave

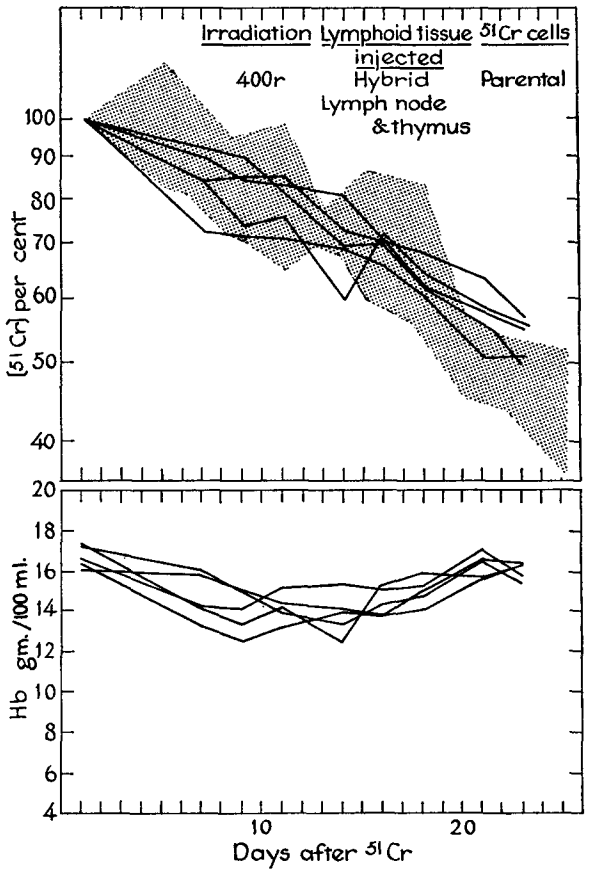

FIG. 8. Survival of parental red cells after irradiation and injection of hybrid lymphoid cells.

differently from hybrid erythrocytes in mice in which wasting disease had been produced by injection of parental lymphoid cells. The experiments were accordingly repeated, and the results are shown in Figs. 11 to 14. Here it appears (Figs. 11 and 13) that parental ^{51}Cr-labeled erythrocytes survived no longer than hybrid ^{51}Cr-labeled erythrocytes in mice injected with cells from parental lymph nodes and thymus. Of the animals given parental spleen cells (Figs. 12 and 14), some again began to recover from their anemia; in these

animals the sudden loss of ^{51}Cr-labeled erythrocytes either did not occur or was checked at the time when the hemoglobin level began to recover. It appears that both parental and hybrid ^{51}Cr-labeled erythrocytes suffer the same fate as the animals' own erythrocytes.

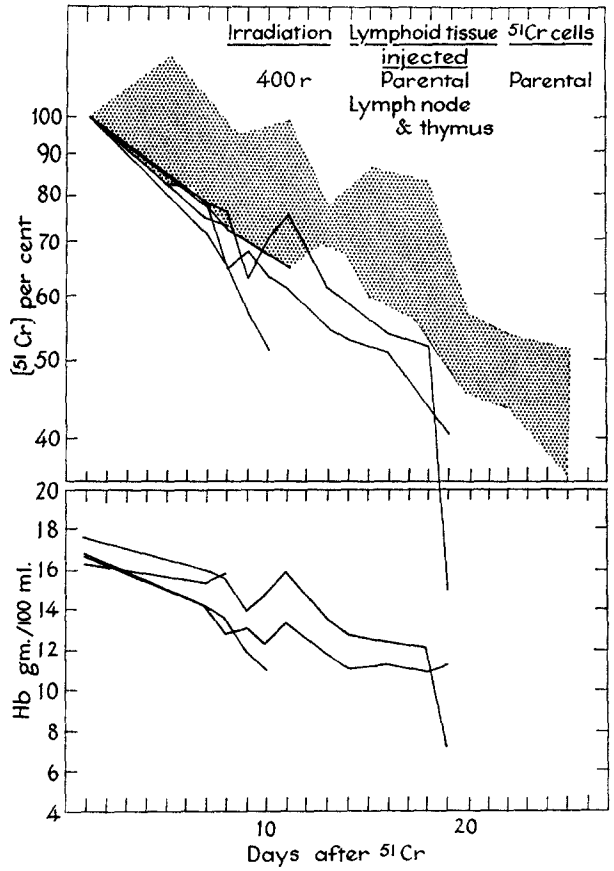

FIG. 9. Survival of parental red cells after irradiation and injection of parental lymphoid cells.

DISCUSSION

These results confirm previous observations that in F_1 hybrid wasting disease, a profound and often fatal anemia occurs 2 to 3 weeks after whole body irradiation and lymphoid tissue transplantation. Such anemia is more severe than that observed after irradiation alone (Fig. 2) or irradiation followed by an injection of isologous hybrid lymphoid cells (Fig. 3 and 5), in which

cases the slight and transient anemia can be accounted for by a lack of production of new red cells due to the effect of irradiation on erythropoietic tissues. In animals with wasting disease, there must, therefore, be in addition some destruction of circulating red cells, due to hemolysis or erythrophagocytosis

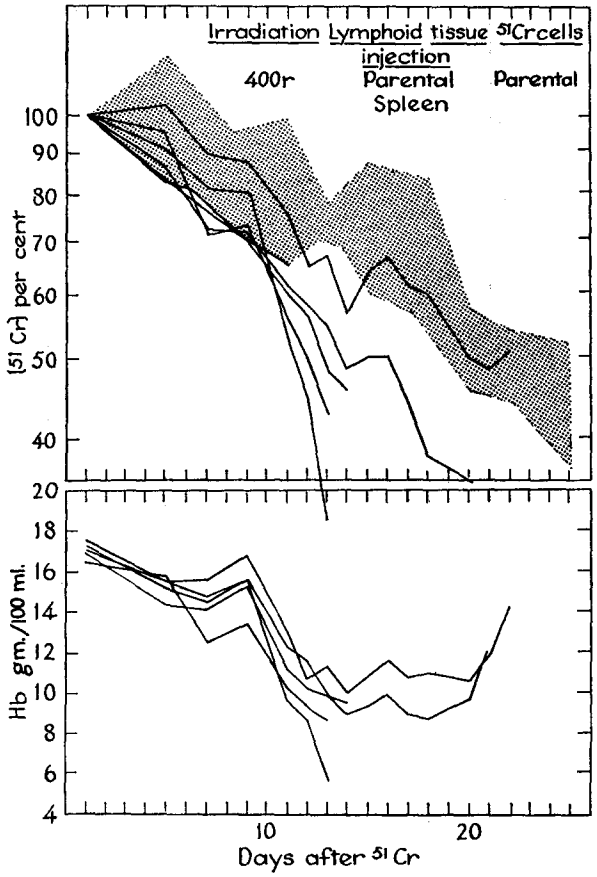

FIG. 10. Survival of parental red cells after irradiation and injection of parental splenic cells.

(as suggested by Kaplan and Rosston, 15) or to hemorrhage, or to any combination of these mechanisms. Our most striking finding is that this sudden severe fall in circulating erythrocytes in wasting disease is associated with the loss of not only F_1 hybrid erythrocytes but also of transfused parental strain erythrocytes (Figs. 9, 10, 13, 14). On the hypothesis that wasting disease is due to a graft *versus* host reaction, the latter finding is at first sight entirely unexpected.

Porter (18) has made similar studies of the survival of ^{51}Cr-labeled erythro-

cytes in rabbits protected from lethal doses of radiation by the injection of homologous bone marrow. He found that in rabbits which developed secondary disease, ^{51}Cr-labeled autologous erythrocytes disappeared completely from the circulation whereas labeled erythrocytes from the homologous marrow donor

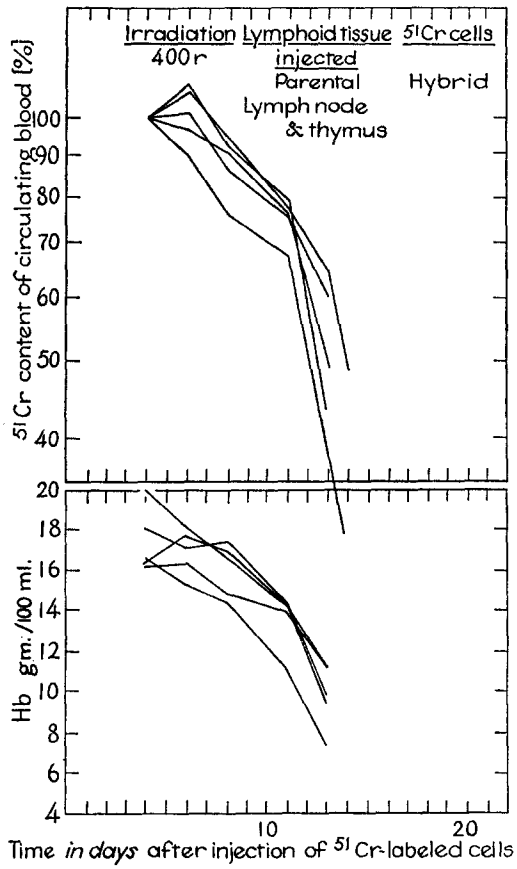

FIG. 11. Survival of hybrid red cells after irradiation and injection of parental lymphoid cells.

did not. From this result and from the evidence of positive direct Coombs tests in all rabbits with secondary disease he concluded that selective destruction of the host erythrocytes occurred, due to immune hemolysis.

It is difficult to reconcile these results with our own, for it appears that if an immune hemolysis occurs in mice, it is not specific for the host erythrocyte but affects the donor erythrocytes as well.

Several possible explanations for our findings may be considered:

1. Heterozygosity of Mouse Strains.—The injected cells, whether from hybrid

or parental donors, were always taken from pooled samples from a number of mice. If, due to genetic drift, these animals were no longer homozygous, there would exist the possibility that a single hybrid host would be genetically incompatible with an indeterminate number of the injected cells, antibodies against which might be produced which could react with those parental red

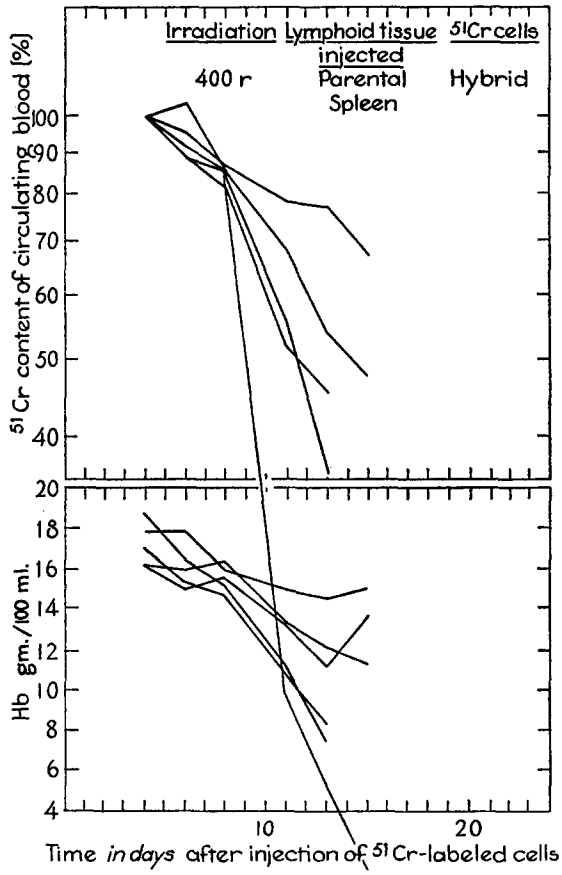

FIG. 12. Survival of hybrid red cells after irradiation and injection of parental splenic cells.

cells bearing the foreign antigen. The increased destruction of hybrid host red cells would be explained as a consequence of a graft *versus* host reaction by those grafted parental cells which *were* genetically compatible with the host. Although complete homozygosity cannot be guaranteed by the breeding system used, we consider the above explanation to be most unlikely for the following reasons. First, neither in this study nor in the previous study from this laboratory (15), using the same strain of animals, did wasting disease ever occur in F_1 hybrids injected with isologous cells; such animals never

developed profound anemia and handled ^{51}Cr-labeled parental or isologous erythrocytes in entirely normal fashion (Figs. 3, 5, 8). Furthermore, since increased destruction of injected parental red cells occurred in four different experiments whenever parental lymphoid tissue had also been given, one

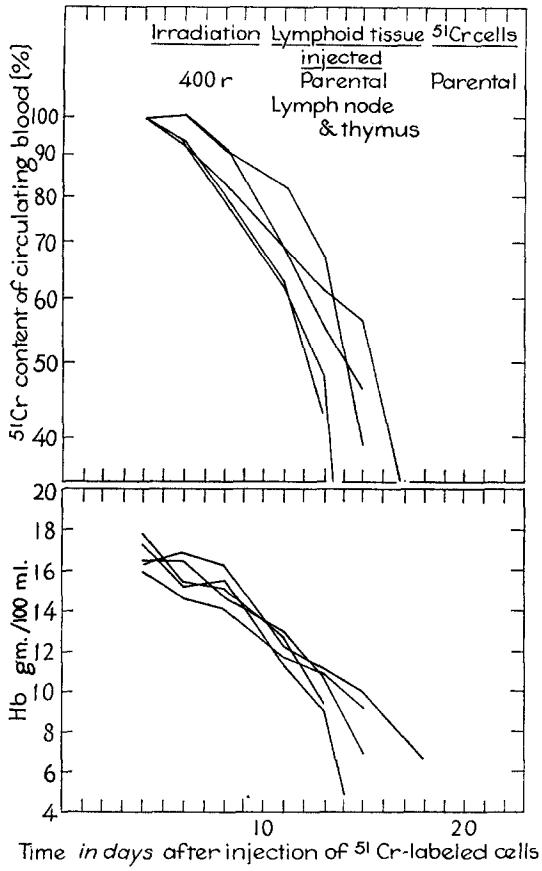

FIG. 13. Survival of parental red cells after irradiation and injection of parental lymphoid cells.

would have to postulate a serious breeding error rather than a chance error in selection of a donor animal of a foreign strain. Although exchange skin grafting has not been done between our $C_{57}BL$ mice and their F_1 hybrids, equally good evidence of homozygosity of the parental strain is provided by the fact that when isologous thymic grafts were implanted in thymectomized, irradiated $C_{57}BL$ mice (the parent strain used in these experiments), cortical regeneration in the thymic graft occurred in 87 per cent of 105 grafts examined (4). Experi-

ence with thymic grafts since that time has not indicated any appreciable change.

2. "Non-Specificity" of Antibody.—Antibodies against the host produced by the graft and released into the circulation might be adsorbed on to the surface

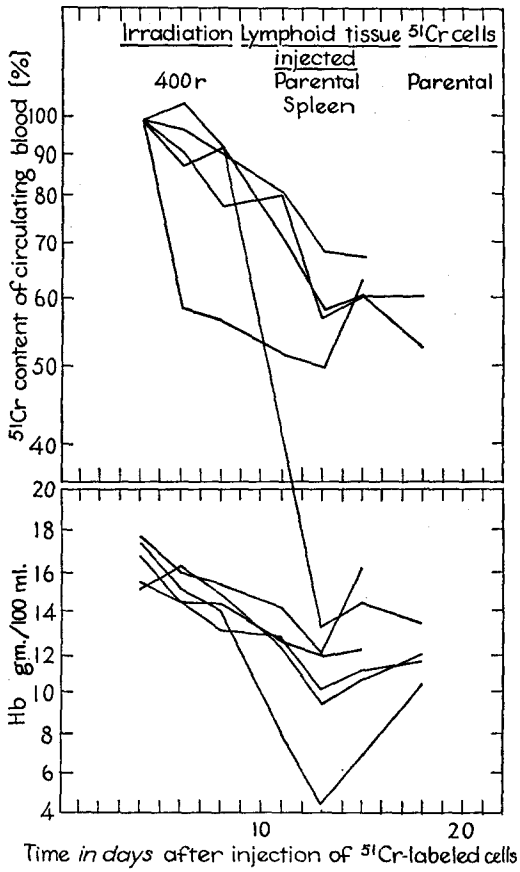

FIG. 14. Survival of parental red cells after irradiation and injection of parental splenic cells.

of all red cells, whether host or parental. Such adsorbed antibody might in some way render the cell susceptible to destruction, agglutination, or erythrophagocytosis. If this explanation is correct, once antibody has been formed, labeled red cells obtained from any strain of mouse might be expected to show the same decreased survival.

It is of interest to note that the antibody produced in the wasted animals is similar in its relative non-specificity to antibodies produced in such human

autoimmune diseases as acquired hemolytic anemia (10) and systemic lupus erythematosus (12).

3. Antibody Production in Response to Altered Parental Material.—Campbell (3) has suggested that antigenicity is often and perhaps always, intimately associated with incomplete intracellular fragmentation of antigenic material. In autoimmune hemolytic disease, an antigenic, possibly denatured, protein fragment from some source (*e.g.* parental lymphoid cells) may be adsorbed to cell surfaces or be associated intracellularly with RNA. If this fragment, derived from parental tissue, in its altered state now constitutes an antigen for host tissue (and consequently, for unaltered parental tissue), one may expect the production of specific antibodies against it. If these antibodies in turn were capable of cross-reacting with unaltered tissue genetically very similar to the fragment, one may envisage an autoimmune reaction against both parental and hybrid tissue. Circulating erythrocytes, parental or host, would be destroyed by an autoimmune hemolysis. It would also be necessary that lymphoid tissue of both host and parent be likewise destroyed by the same mechanism as that affecting the erythrocytes. The simultaneous destruction of parental and host cells has, in fact, been suggested by Kaplan and Rosston (15), although they viewed the reaction more as a cell *versus* cell attack. The same authors noted that animals may occasionally recover from wasting disease. In the rare instances available for study, such recovered animals, when again challenged with parental lymphoid tissue, have again developed wasting disease (Kaplan, unpublished data). One possible explanation of this is that the originally injected parental lymphoid tissue had been destroyed, and that the lymphoid tissues of such recovered mice had been repopulated by their own (hybrid) cells. Death of both target cells and parental lymphocytes occurred when target cells were mixed with preimmunized homologous spleen cells and the mixture placed in cell-impermeable diffusion chambers in hosts isologous for the target cells (30). If one provisionally accepts the thesis that antigenic fragments elicit the response described above, one must also inquire why the injection of *hybrid* lymphoid cells does not likewise lead to the persistence of such fragments. To account for this, we must postulate that the hybrid host, with its individual enzyme systems, is capable of disposing of, or degrading hybrid lymphoid cells or protein more effectively than it can handle similar tissue derived from the parent. Although such a view would appear to stand in contradiction to the concept which states that an F_1 hybrid cannot distinguish parental tissue from its own, it should not be rejected solely on these grounds. The hypothesis presented in this paragraph might be tested by comparing the survival of hybrid red cells, parental red cells of the strain from which the injected lymphocytes were taken, and red cells from the *other* parent, injected when the wasting syndrome is just beginning. A normal survival of red cells from the *other* parent, and decreased survival of host and "donor"

parental red cells, would indicate that a graft *versus* host reaction was not involved in the anemia of wasting disease.

4. Erythrocyte Sequestration and Destruction by the Spleen.—The decreased survival of red cells may have little to do with the production of autoantibodies, but may be due to abnormal splenic trapping and destruction of red cells, as a manifestation of splenic involvement in the wasting syndrome. It is conceivable that coating of red cells with antibody, as mentioned in a previous paragraph, might predispose to splenic removal of red cells. Red cells from any strain would be similarly affected. The postulate could be tested by repeating the experiment with splenectomized hybrid hosts.

5. Hemorrhage.—In some instances immune hemolysis may be masked by a more rapidly acting mechanism such as hemorrhage. Since parental lymphoid tissue causes atrophy of all hematopoietic elements in irradiated F_1 hybrid mice (19, 9, 15, 7), host megakaryocytes will be affected and the production of platelets by megakaryocytes will be greatly reduced. This immunological effect would be added to a depression of megakaryocyte activity already caused by irradiation (20). The consequent thrombocytopenia could lead to loss of blood by hemorrhage. Hemorrhage into the peritoneum was noted in some mice dying of wasting disease in the experiments reported here, and has previously been observed by Kaplan and Rosston (15).

The partial recovery from anemia seen in some of the mice in which wasting disease was produced by injection of parental spleen cells as opposed to the non-recovery in mice injected with purely lymphoid cells is interesting in this connection. It has been established that in animals given lethal doses of x-radiation, a protective effect follows injection of homologous or heterologous bone marrow or spleen owing to repopulation of hematopoietic tissues of the host by the injected tissue, resulting in a radiation chimera (16, 17, 11). It is probable that similar repopulation of hematopoietic tissue occurs in sublethally irradiated F_1 hybrid mice given parental spleen cells. Kaplan and Rosston (15) observed that in mice with wasting disease the morphology of the red pulp of the spleen varied with the source of parental lymphoid cells. In mice given purely lymphoid cells from parental lymph nodes and thymus there was profound depletion of all nucleated hematopoietic elements, whereas in mice given parental spleen cells the red pulp often contained actively proliferating hematopoietic elements. Regeneration of hematopoiesis in the splenic pulp in F_1 hybrid mice given parental spleen cells was also observed by Cole and Garver (7). Such repopulation of the splenic tissue would result in renewed production of platelets as well as erythrocytes, with a concomitant cessation of hemorrhage, enabling the transplanted erythropoietic tissue to restore erythrocyte numbers to normal levels. Smith, Makinodan, and Congdon (21) have shown that in mice protected from lethal doses of x-radiation by injections of rat bone marrow, the platelets in the recipients' circulation on the 14th

day after injection and thereafter were all of rat origin. Platelet counts in mice with wasting disease might help to determine whether or not hemorrhage is a controlling factor in the course of the anemia.

SUMMARY

The survival of ^{51}Cr-labeled erythrocytes has been studied in F_1 hybrid mice in which wasting disease was produced by injection of parental lymphoid cells taken either from lymph nodes and thymus or from the spleen. Coincident with the development of the disease syndrome, there occurred a severe anemia accompanied by a sudden loss of circulating labeled erythrocytes, whether host or parental. This finding suggests that the anemia is not due solely to specific immunologic reaction of donor tissue against host erythrocytes.

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