

## EFFECT OF NEW-BORN RABBIT AND MOUSE LIVER SUSPENSIONS ON X-IRRADIATED RABBITS\*

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PIONEER work by Jacobson (1952) and Lorenz, Uphoff, Reid and Shelton (1951) established that homologous or heterologous cell suspensions of blood-forming tissues when administered to lethally X-irradiated rodents can induce rapid haemopoietic recovery and increase survival.

Early evidence tended to favour the hypothesis that these beneficial effects were due to "humoral" stimulation by some unidentified factor causing accelerated regeneration of the host marrow (Jacobson, 1952). However, the possibility that "cellular repopulation" of the depleted marrow by the injected donor cells might be the mechanism was repeatedly stated by Loutit (1954). Recently several reports have appeared giving direct evidence of such survival and proliferation of injected marrow and spleen cells in irradiated recipients (Ford, Hamerton, Barnes and Loutit, 1956; Nowell, Cole, Habermeyer and Roan, 1956; Lindsley, Odell and Tausche, 1955; Makinodan, 1956; Merwin and Congdon, 1956; Mitchison, 1956; Vos, Davids, Weyzen and Van Bekkum, 1956; Porter, 1957).

With the general acceptance of cellular repopulation as the main mechanism by which haemopoietic suspensions bring about their therapeutic effects, it was considered worthwhile to reinvestigate the reported beneficial effects on X-irradiated rabbits of liver cell suspensions from new-born rabbits (Jacobson, Marks and Gaston, 1956*a*) and mice (Jacobson, Marks and Gaston, 1956*b*).

In the experiments reported here it is shown that whereas rabbit liver causes some increase in the 56-day survival rate of X-irradiated rabbits, mouse liver does not. Further by using the sex difference in rabbit heterophils as a "label" (Porter, 1957) it is shown that the introduced blood-forming cells from new-born rabbit liver survive and repopulate the host's depleted haemopoietic tissue. Successful transplantation does not occur following treatment by mouse liver.

### MATERIALS AND METHODS

Young adult male albino rabbits, which were not inbred in the genetic sense, weighing 2.8-3.2 kg. were used as recipient animals.

X-rays were produced in the first experiment by a 250 kvp. Phillips machine, in the second by a 250 kvp. Westinghouse machine, both operating at 15 mA, and with 0.5 mm. Cu and 1 mm. Al filters giving a half value layer in Cu of the filtered beam of 1.7 mm. The exposure rate was 93 r/min. at 50 cm. centre of animal to target, measurement being made in air with a Victoreen ionisation chamber, each animal receiving 900 r rotational total body irradiation.

The liver suspensions for post-irradiation injection were obtained as follows.

*Homologous 1-3-day-old female rabbits.*—After killing the donors by ether the liver was quickly removed and suspended gently in 10 ml. of cold saline. The number of nucleated cells per cu. mm. was determined by making a dilution with 2 per cent acetic acid in a white

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cell counting pipette and counting the cells on a standard haemocytometer. Such suspensions contained on the average  $500 (\times 10^6)$  nucleated cells, one liver being used to treat each rabbit.

*Heterologous 1-3-day-old CF No. 1 mice of both sexes.*—The donors were killed by decapitation and the livers removed and suspended in 10 ml. of saline. Several livers were used to treat each rabbit, the cell suspension being adjusted so that each 10 ml. contained approximately  $500 (\times 10^6)$  nucleated cells.

Injections of these cell suspensions were given within 30 min. of the death of the donor and were administered intravenously into the marginal ear vein of the recipient rabbit one to three hours following irradiation. Controls each received 10 ml. of saline intravenously.

Total leucocyte counts and microhaematocrits were determined on the irradiated animals every 2 days for the first 30 days and once per week thereafter. Blood films were taken from the ear at the same time intervals and in the rabbit liver experiment stained by Wright's method and the heterophils examined for nuclear "drumsticks", as described previously (Porter, 1957). The number of such cells seen in examining 300 heterophils was recorded as percentage of the heterophil count. In the mouse liver experiment two films were taken from each rabbit, one being stained by Wright's method, the other by Gomori's method (1941) for alkaline phosphatase. Heterophils showing the characteristics of mouse neutrophils, i.e. faintly staining inconspicuous granules and a negative reaction for alkaline phosphatase, were then sought. (Rabbit "neutrophils" have conspicuous eosinophilic granules and are strongly positive for alkaline phosphatase.)

The rabbits were weighed daily for the first month, then at weekly intervals. All animals dying were autopsied, but the brain was not examined. Thymus, spleen, mesenteric lymph node and appendix were taken, fixed in formol-saline and routinely stained with haematoxylin and eosin. Marrow from the mid-point of the shaft of the right femur was taken from each animal, fixed in Helly's fluid and stained with haematoxylin and eosin. Marrow smears were prepared by the method of Berenbaum (1956) and treated with Wright's stain. In the mouse liver experiment additional samples of marrow were stained for alkaline phosphatase, the smears by Gomori's method, the sections by Nowell's technique (1957).

#### *Experimental procedure*

A total of 111 male New Zealand white rabbits were exposed to a single dose of 900 r whole body X-irradiation. Of these 36 (30 per cent) died in shock shortly after the irradiation. The surviving 80 animals were divided into 2 groups (Table I).

TABLE I.—*Effect of Neonatal Mouse and Rabbit Liver Treatment on survival of Rabbits Receiving 900 r Whole Body X-irradiation*

Group	Treatment	No. of animals	Alive at 14 days		Alive at 56 days	
			No.	Per cent	No.	Per cent
I	Saline	20	1	5	1	5
	Rabbit liver	20	10	50	5	25
II	Saline	20	2	10	1	5
	Mouse liver	20	3	15	2	10

*Group I* consisted of 40 animals, 20 of which received saline after irradiation whilst the other 20 were given female neonatal rabbit liver.

*Group II* consisted of a further 40 animals, 20 of which were controls and received saline after irradiation, the other 20 received baby mouse liver.

## RESULTS

### *Group I*

*Those treated with neonatal rabbit liver.*—The results for this group of 20 male rabbits are summarised in Table II.

A successful haemopoietic transplant was shown by 10 animals (50 per cent) as indicated by rapid restoration of the total leucocyte count to normal and the appearance in the peripheral blood of significant numbers of female heterophils

TABLE II.—*Analysis of Effects on 20 Male Rabbits of 900 r Whole Body X-irradiation followed by Intravenous Neonatal Rabbit Liver Suspension*

Outcome	No.	Per cent
Failed to show a successful haemopoietic transplant—		
Died from gastric perforation	6	50
Died from infection	3	
Regenerated own bone marrow and survived	1	
Died from gastric perforation	3	50
Died at 19 and 25 days as result of early rejection of transplant by host	2	
Died from infection at 44th day with transplant intact	1	
Remained alive and well with haemopoietic transplant intact and functioning for 56 days	4	

bearing characteristic nuclear “drumsticks” (Fig. 1). Two of these animals subsequently died as the result of early rejection of the haemopoietic transplant by the host ; one died at the 44th day following progressive wasting, diarrhoea

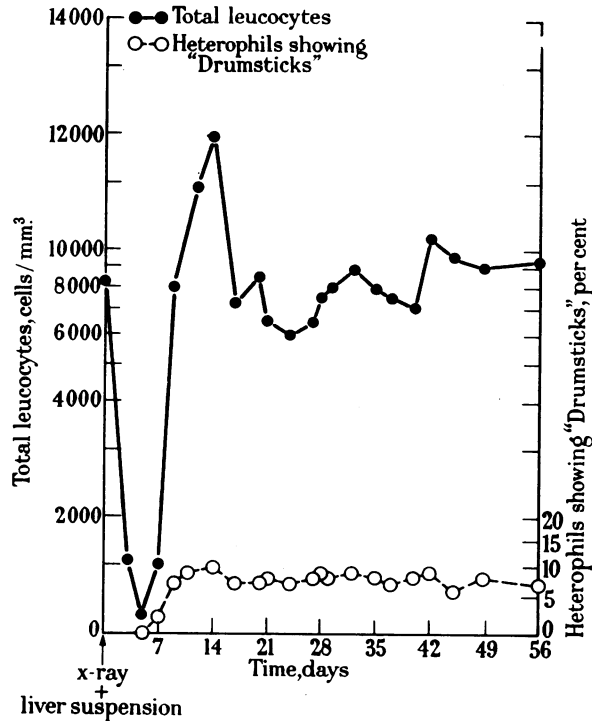
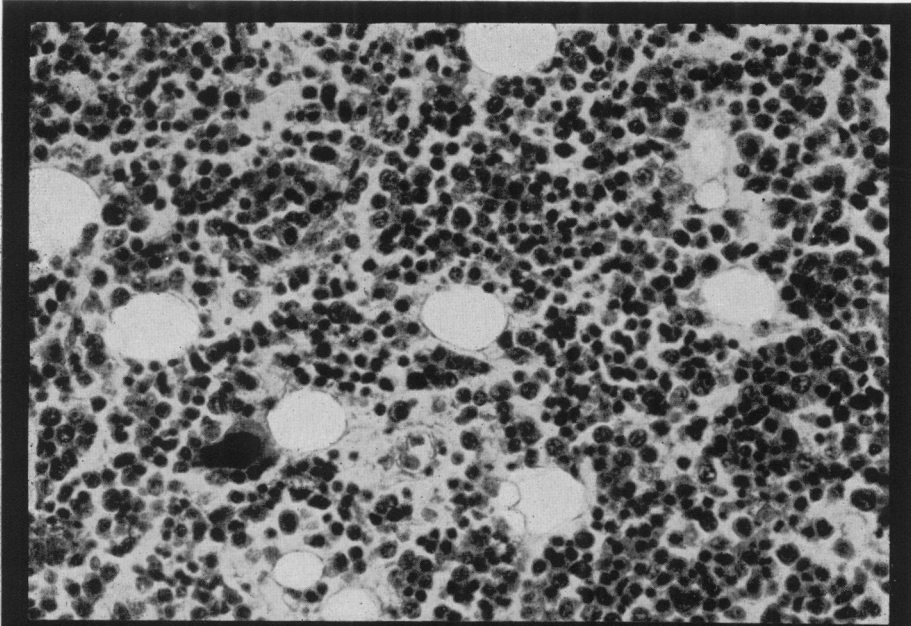


FIG. 1.—Appearance of female heterophils in peripheral blood of male irradiated rabbit after treatment with neonatal female rabbit liver suspension.

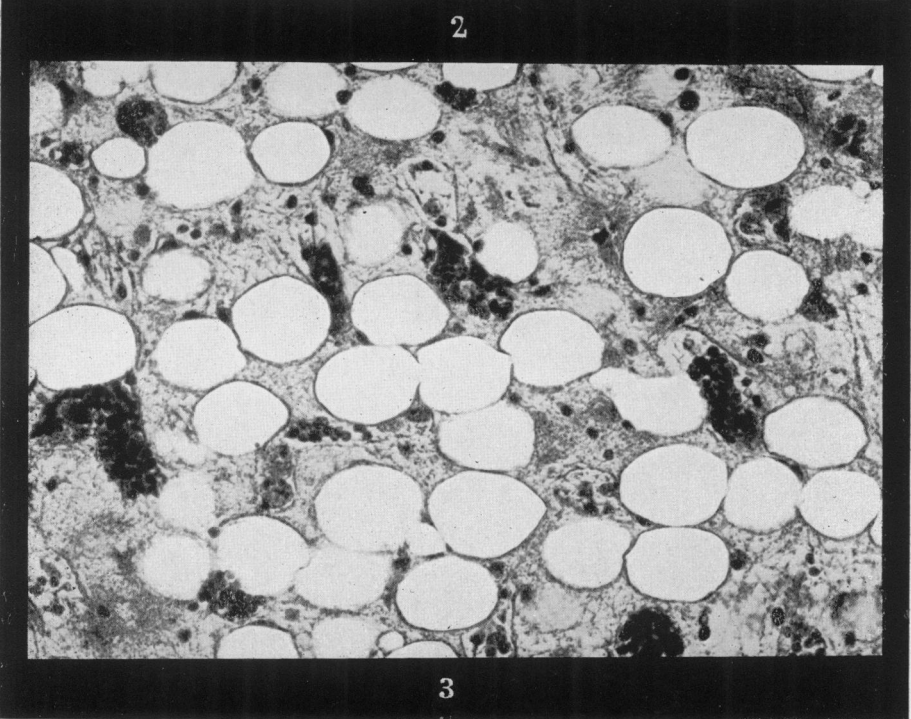
EXPLANATION OF PLATE

FIG. 2.—Femoral bone marrow from neonatal rabbit liver treated animal dying nine days after irradiation from perforation of a gastric ulcer. Haemopoietic transplantation had occurred as shown by the presence of female heterophils in the peripheral circulation and in marrow smears. Haematoxylin and eosin.  $\times 400$ .

FIG. 3.—Aplastic femoral bone marrow from irradiated mouse liver treated animal dying ten days after irradiation from perforation of a gastric ulcer. The venous sinuses are distended with red cells and there is no haemopoietic regeneration. Haematoxylin and eosin.  $\times 400$ .



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3

and leucocytosis, with marked lymphoid atrophy, but with an intact and functioning marrow transplant, probably due to an immune response of the graft against the host (Uphoff, 1957); three died from gastric ulcer perforation; and four survived the experimental period with the haemopoietic transplant intact and still contributing female cells to the peripheral blood.

Autopsy of animals from this group at the time of death or sacrifice showed the bone marrow to be normal or hyperplastic (Fig. 2) with female cells demonstrable in smears. The two cases which died as the result of early rejection of the haemopoietic transplant by the host were an exception: there was marked bone marrow destruction with only a few tiny islands of surviving haemopoietic cells.

Of the 10 animals which failed to secure a successful transplant only one regenerated its own bone marrow and survived.

*Controls.*—In the control group one animal survived the experimental period after regenerating its own bone marrow. Of the remainder, eight died from gastric perforation, four from intestinal haemorrhage and seven from infection, mainly bronchopneumonia: all showed marrow aplasia at autopsy (Fig. 3).

### Group II

*Those treated with neonatal mouse liver.*—The results for this group did not differ materially from those obtained for the two control groups, only three animals surviving the experimental period after regenerating their own bone marrow. Of the remainder, six died from gastric perforation, three from intestinal haemorrhage and eight from infection. Marrow aplasia was present in all 17 at autopsy. In no instance were neutrophilic, alkaline phosphatase negative heterophils of mouse type seen in any of the peripheral blood smears obtained after irradiation, and it was concluded that heterotransplantation of mouse tissue had not occurred.

*Controls.*—In this group two animals survived after regenerating their own bone marrow, seven died from gastric perforation, four from intestinal haemorrhage, one from intussusception and six from infection.

Table I shows the numbers of animals surviving in each of these experimental groups at 14 and 56 days after treatment.

### DISCUSSION

The results of these experiments confirm the ability, noted recently by Jacobson and his associates (1956a), of neonatal rabbit liver to improve the early survival rate of X-irradiated rabbits. This beneficial activity is now shown to be due to the presence of blood forming cells in the liver suspension, which are capable of surviving and giving rise to progeny which repopulate the host's depleted haemopoietic tissues and lead to rapid haematological recovery. These findings supplement those of Ford *et al.* (1956) which have led to the general recognition that the "protective" activity of spleen and bone marrow after radiation is directly related to their content of primitive haemopoietic elements. There now seems little reason to believe that a humoral factor is of importance following the use of any of these suspensions.

Failure of mouse neonatal liver preparations to similarly modify the response of X-irradiated rabbits was associated with inability of the mouse cells to persist and proliferate even temporarily in the new host, presumably because of the degree of genetic dissimilarity. This observation is at variance with an earlier report

that baby mouse liver enhances the survival of irradiated rabbits (Jacobson *et al.*, 1956b).

Some evidence in mice has suggested that foetal homologous tissues may cause less delayed immunological reaction than adult homologous tissues (Congdon and Urso, 1957). But Dixon and Weigle (1957) have recently shown that although baby rabbits cannot make antibodies, splenic and thymic cells from these animals injected into X-irradiated adult recipients are capable of agglutinin production. With these observations in mind it is worth noting that one of our animals treated with neonatal rabbit liver died at the 44th day with the syndrome of diarrhoea and wasting now associated with an immune reaction on the part of the introduced foreign tissues against the host (Uphoff, 1957; Trentin, 1957).

The proneness of rabbits to death from initial shock and gastric ulceration and perforation has been noted previously (Porter, 1957) and in the present experiments resulted in a high mortality despite successful haemopoietic transplantation.

#### SUMMARY

Injection of liver cells from baby female rabbits favourably modifies the post-irradiation course of male rabbits exposed previously to 900 r whole body X-irradiation. This is accompanied by persistence and function of donor haemopoietic cells, as shown by rapid haematological recovery and the appearance of female heterophils in the peripheral circulation of the irradiated host.

Post-irradiation treatment with neonatal mouse liver cells fails to exert any beneficial effect on irradiated rabbits. No evidence of cellular transplantation is obtained in this latter instance.

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