action, reducing mitotic division abruptly in the hepatocytes of the regenerating liver and in the Kupffer cells of the intact mice. The proliferative activity of the Kupffer cells in the partially hepatectomized animals of all three groups was approximately equal and was significantly higher than the control level.

On the whole these results are evidence that, irrespective of the species of origin of the interferon, it led to a persistent decrease in the proliferative activity of the regenerating liver cells. The persistence of this effect is shown by the retarded gain in weight of the liver in the experimental animals, which was particularly clearly marked in mice receiving the highly purified human interferon.

The discovery of inhibition of regeneration of the liver in mice by preparations of human interferon may provide a convenient model with which to study the antiproliferative activity of such preparations *in vivo*.

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POTENTIATING THE ABILITY OF MOUSE LYMPHOCYTES TO TRANSFER "REGENERATION INFORMATION" BY REPEATED LIVER RESECTION

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KEY WORDS: lymphocytes; resection of the liver.

Lymphocytes of partially hepatectomized (resection of $^2/_3$ of the liver) and of unilaterally nephrectomized mice are known to have the property of potentiating proliferation and growth of the corresponding organs in intact syngeneic animals [1, 2, 5, 7, 8]. This phenomenon can conventionally be called the transmission of "regeneration information."

The object of the present investigation was to study the ability of lymphocytes to transfer "regeneration information" during repeated operations on the liver, and depending on the quantity of tissue removed.

EXPERIMENTAL METHOD

Three practically identical series of experiments (four groups of experiments in each series) were carried out on 250 sexually mature male CBA mice. The general scheme of the experiments was as follows. An injection of $7 \cdot 10^7$ lymphocytes, isolated from the spleen by the method used previously [1, 3], in medium 199 was given into the caudal vein of intact mice. The donors of the lymphocytes for recipients of group 1 were mice from which two lobes of the liver had been removed by the usual method in one stage [6].

The donors for the recipients of group 2 were mice from which these lobes had been resected in two stages. The second operation was performed 20 days after the first, which corresponded to a period of 2 weeks after restoration of the weight of the liver.

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TABLE 1. Mitotic Index (in °/₀₀) of Liver and Kidney Cells of Intact Mice Receiving Splenic Lymphocytes from Syngeneic Normal and Partially Hepatectomized Donors

Group of recipients	Character of operation on donor's liver	Number of recipients	Cells		
			hepatocytes	buffer cells of liver	tubular epithelium of kidney
1	Single stage removal of two lobes	27	0,32±0,08	2,4±0,3	0,25±0,07
2	Two-stage removal of two lobes	32	0,21±0,06	1,68±0,3	0,18±0,05
3	Removal of one lobe	37	0,01±0,006	1,10±0,23	0,35±0,08
4 (intact donors)	—	32	0,03±0,014	1,03±0,16	0,32±0,05

Only one lobe of the liver was resected in the donors for the recipients of group 3. Control animals (group 4) were given an injection of 7×10^7 lymphocytes isolated from the spleen of intact mice. The donors of all four groups were killed with chloroform vapor at the same time of day, and at intervals of 10-15 min, 17-18 h after the operation.

The recipients were killed 49 h after lymphocyte transfer. The liver and kidney of the recipients were fixed in Carnoy's fluid. Paraffin sections 5 μ thick were stained with hematoxylin and eosin. The number of dividing cells was determined and the results subjected to statistical analysis by the method described previously [1, 2].

EXPERIMENTAL RESULTS

It will be clear from Table 1, which gives the results of all three series of experiments, that the lymphocytes of animals after removal of one lobe of the liver (group 3) did not have the property of stimulating mitotic activity in the liver cells of intact recipients, irrespective of which lobe was removed (the smaller (central) or larger (left lateral)).

Removal of two lobes of the liver (group 1), on the other hand, was accompanied by manifestation of ability to transfer the proliferative stimulus. In other words, in the experiments with operation on the liver the same pattern was revealed as after injury to the kidney: The strength of the stimulation effect depended on the quantity of tissue removed [2] and was not determined by the presence of a wound surface, for damage to the parenchyma of the organ after the two variants of the operation was about equal.

Injection of lymphocytes of partially hepatectomized mice into the recipients caused no change in the mitotic index in the kidney cells compared with the control, evidence of the mainly organ specificity of the phenomenon.

After removal of two lobes of the liver in two states, lymphocytes from the hepatectomized animals potentiated proliferative activity in the hepatocytes and Kupffer cells of the recipients (group 2). An increase in mitotic index was recorded in all three series of experiments (within each series the increase was significant in two of the three series of experiments). Consequently, the second operation increased the ability of the lymphocytes to transfer "regeneration information."

It must be noted that at the second operation the weight of liver removed (one lobe) was the same as that which under ordinary conditions (without a preliminary first operation) does not lead to manifestation of this property of the lymphocytes.

The results thus showed that the ability of lymphocytes of partially hepatectomized donors to potentiate proliferative activity in the liver of intact recipients depends on the quantity of organ tissue removed and can be increased by a previous resection some time beforehand. These facts recall the well known phenomenon of immunologic memory and are in harmony with the hypothesis of the immunologic nature [1, 4] of the property of lymphocytes to transfer "regeneration information."

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EFFECT OF SPLENIC LYMPHOCYTES FROM DONORS POISONED WITH CARBON TETRACHLORIDE ON MITOTIC ACTIVITY AND α-FETOPROTEIN PRODUCTION OF LIVER CELLS IN SYNGENEIC RECIPIENTS

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Mitotic activity of mouse liver cells is increased after intravenous transplantation of splenic lymphocytes from partially hepatectomized syngeneic donors into the animals [2]. Mitotic activity of hepatocytes also is increased during regeneration of the liver after poisoning with carbon tetrachloride (CCl_4) [7]. This process is accompanied by the appearance of an embryo-specific protein — α-fetoprotein (AFP) — in the animal's blood serum [1].

It was interesting to discover whether lymphocytes of CCl_4-treated donors would affect mitotic activity and AFP production in the recipients' liver, and the investigation described below was carried out for this purpose.

EXPERIMENTAL METHOD

SWR mice aged 2-3 months, used as lymphocyte donors, were given one or 11 doses of an 8% solution of CCl_4 in sunflower oil (one dose was 0.1 ml of the preparation [1]) perorally. The CCl_4 was given twice a week. Cell suspensions in medium 199 were prepared from the spleens of the experimental and intact donors 16 h after administration of CCl_4. The washed cell suspension was injected into the caudal vein of intact recipient mice of the same strain in doses of $2 \cdot 10^7$ or $7 \cdot 10^7$ lymphocytes in 0.7 ml medium 199 (Table 1). Intact animals and mice receiving 0.7 ml of medium 199 intraperitoneally also were investigated.

The animals were decapitated 50 h after transfer of the lymphocytes. Colchicine solution was injected into the animals 4 h before sacrifice in a dose of 5 mg/kg body weight.

Pieces of liver 3-4 mm thick were fixed in a mixture of acetone-formalin—0.03 M phosphate buffer, pH 6.1-6.2 (9:5:6) and embedded in paraffin wax [3].

The mitotic index in the hepatocytes was determined in liver sections 3 μ thick, stained with hematoxylin, by counting from 2600 to 25,000 hepatocyte nuclei [5].

AFP was revealed in liver sections by an indirect immunoperoxidase method, using monospecific antibodies against AFP and a preparation of donkey antibodies against rabbit γ-globulin, conjugated with horseradish peroxidase [9].

Monospecific antibodies against AFP were obtained from rabbit antisera against mouse AFP on sepharose 4B—AFP sorbent. The specificity of staining was verified by means of an antibody preparation neutralized with the equivalent quantity of pure AFP. Treatment of a serial section with antiserum against mouse γ-globulin served as the control for nonspecific uptake of serum proteins by the hepatocytes [8].

The AFP concentration in the animals' blood sera was determined by double immunodiffusion in gel [4] with a standard test system.

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