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Inhibition of Foreign Spleen Reaction by Inactivation of Donor Cells with Recipient Antigen. (25305)

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Homologous lymphoid cells from adult mice implanted into neonatal(1) or irradiated adult (2,3) recipients cause a lethal reaction that has been ascribed to production of antibodies by the grafted cells against the tissues of the host. Since, however, the probability of death varies markedly with the number and immunologic reactivity of the cells inoculated, the recipient's age, and the dose of radiation (2), factors other than the incompatibility of donor and recipient influence the ability of the implanted cells to react lethally. We therefore postulated that the implanted cells are unable adequately to react against the recipient if exposed to too high a concentration of antigen and tested the hypothesis by heavily exposing the donor cells *in vitro* to recipient antigen prior to their implantation. Results of these experiments form the basis of this report.

Methods. Twelve-week-old female (C57BL $\times$ 101)F_1 mice were exposed to 500-600 r whole-body X radiation under the following conditions: 250 kvp, 30 ma, 3 mm of Al added filtration, 93.7 cm target-to-mouse distance, hvl 0.44 mm of Cu, 85 r per minute. Within one hour after irradiation, each mouse was injected intraperitoneally with a suspension of C57BL spleen cells in Tyrode's solution, receiving the equivalent of one donor spleen ($\sim 150 \times 10^6$ nucleated cells) in 0.5-2.0 ml. The spleen cell suspensions were prepared in a Potter-Elvehjem glass homogenizer from pooled intact spleens of young adult C57BL male donors. In some instances (Table I), the spleen cell suspensions were previously incubated with donor or recipient liver, as follows: to the suspended spleen cells was added a suspension of liver cells from young adult mice, prepared with a Potter-Elvehjem glass homogenizer, one spleen being added to one liver in the first, and six spleens to one liver in subsequent experiments. Benzathine penicillin G was also added, 300-600 units per ml.

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TABLE I. Effects of Pretreatment of Implanted C57BL Spleen Cells* on Survival of (C57BL × 101)F_1 Recipients.

Material inj.	X-ray dose (r)	Recipients: Fraction dying in 35 days	Recipients: Fraction with donor-type RBC	Recipients: Mean time of death (days)
1 C57BL spleen	600	32/32		19
+ 1 C57BL liver	500	5/5		16
+ 1/6 " "	600	32/32		19
+ 1 (C57BL × 101)F_1 liver	500	2/5	3/3	26
+ 1/6 " "	600	3/24	11/21	19
None	600	1/32		7

* For description of spleen cell suspensions see text.

The pooled liver-spleen suspensions were refrigerated at ~10°C for about 19 hours before injection. The suspension was mixed by gentle agitation; one-spleen-dose aliquots were withdrawn for each recipient animal and injected through a 22-gauge hypodermic needle into the peritoneal cavity. After inoculation, the recipients were observed until death or for periods up to 200 days. The animals were caged in groups of 5-10 and had free access to food and water at all times. At intervals after inoculation, the erythrocytes of randomly selected mice were tested by starch gel electrophoresis(4) and hemoglobin solubility characteristics(5) to determine whether they were of donor or recipient type.

Results. All recipients of spleen cells plus donor liver homogenate or spleen cells alone were dead within 35 days, whereas most recipients of spleen cells plus recipient liver homogenate survived indefinitely (Table I). The few deaths in the latter group occurred slightly later than those in the former groups. Pathologic changes in the mice that died resembled those described earlier(2,6). In the majority of survivors, erythrocytes of donor type appeared in the circulating blood in increasing numbers after the 15th day, indicating transplantation and proliferation of donor-type erythrocyte progenitors. Irradiation (600 r) alone killed only one of 32 mice exposed.

Discussion. Inhibition of the foreign spleen reaction by incubation of the grafted cells *in vitro* with recipient-type antigen cannot be ascribed to killing of all the grafted cells, since the majority of surviving recipients exhibited gradually increasing numbers of donor-type circulating erythrocytes. Furthermore, C57BL bone marrow incubated similarly with recipient liver caused 5 out of 5 (C57BL × 101) F_1 recipients to survive otherwise lethal X irradiation (900 r) and subsequently to show circulating donor-type erythrocytes (G. E. Cosgrove and R. A. Popp, 1959, unpublished data). It may be inferred, therefore, that incubation with recipient-type antigen *in vitro* decreased the ability of the implanted lymphoid cells to react immunologically against the recipient without decreasing the ability of stem cells to repopulate the marrow.

Although the inhibition of the graft's immunologic reactivity remains to be explained, inactivation of the grafted cells by a process like that postulated to account for the ontogenetic development of tolerance to self(7) is a conceivable mechanism. Other possibilities include changes in the host-graft relation, such as immunologic "paralysis," "adaptation," and "enhancement," which have been discussed previously in relation to bone marrow chimerism(8). That lymphoid cells may be destroyed by excess antigen has been suggested by Rich and Lewis(9) and Gorer and Boyse(6).

Summary. C57BL mouse spleen cells incubated with (C57BL × 101)F_1 liver homogenate killed only 17% of sublethally irradiated (C57BL × 101)F_1 recipients, whereas when incubated with C57BL liver homogenate or injected without incubation such cells killed 100% of sublethally irradiated (C57BL × 101)F_1 recipients within 35 days. Although the immunologically inactivated spleen cells showed a decreased ability to kill, circulating donor-type erythrocytes were found in surviving recipients, indicating repopulation of the bone marrow by surviving graft-derived stem cells. From this, it is inferred that immuno-

logic inactivation did not result from nonspecific destruction of all types of implanted cells.

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1. Billingham, R. E., *Ann. N. Y. Acad. Sci.*, 1958, v73, 782.

2. Schwartz, E. E., Upton, A. C., Congdon, C. C., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 797.

3. Cole, L. J., Ellis, M. E., *Science*, 1958, v128, 32.

4. Popp, R. A., Cosgrove, G. E., Jr., Owen, R. D., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 692.

5. Popp, R. A., Cosgrove, G. E., *ibid.*, 1959,

6. Gorer, P. A., Boyse, E. A., *Immunology*, 1959, v2, 182.

7. Burnet, F. M., in *Immunity and Virus Infection*, V. A. Najjar, ed., John Wiley & Sons, N. Y., 1959, p1.

8. Barnes, D. W. H., Ford, C. E., Ilbery, P. L. T., Koller, P. C., Loutit, J. F., *J. Cell. and Comp. Physiol.*, 1957, v50 (Sup.), 123.

9. Rich, A. R., Lewis, M. R., *Johns Hopkins Hosp. Bull.*, 1932, v50, 115.

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Effect of Epinephrine on Glucose Uptake and Glycerol Release by Adipose Tissue *in vitro*.* (25306)

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Available information suggests an inverse relationship between carbohydrate utilization and release of free fatty‖ acids by adipose tissue. Decreased glucose metabolism (fasting, diabetes mellitus) is generally accompanied by increased free fatty acid release, and conversely, increased glucose metabolism (glucose feeding, insulin administration) by decreased release(1-5). *In vitro* addition of epinephrine to adipose tissue, however, results in accelerated free fatty acid release concomitant with increased rate of glucose assimilation. In addition, there is increased release of free glycerol from adipose tissue under these conditions. This report demonstrates that carbohydrate utilization and free fatty acid release are not necessarily linked, and suggests that rate of lipolysis may be regulated independently.

Materials and methods. Adipose tissue fragments from epididymal fat pads of normal fed rats (Wistar strain obtained from Albino Farms or Harvard Biological Labs) were incubated in Krebs-Ringer bicarbonate buffer containing 4% human serum albumin (obtained from Protein Fn., through courtesy of Dr. R. J. Pennell). Glucose concentration was 5 mM/L and that of free fatty acids bound to the albumin approximately 0.8 mM/L. For analysis of hormonal effects, tissues from the same animal were always used as controls. Incubations were otherwise carried out as previously described(6,7,8). At the end of incubation, medium glucose was determined by method of Nelson(9), after precipitation of proteins with $ZnSO_4$ and $Ba(OH)_2$. For glycerol determinations, an aliquot of the supernatant fluid was oxidized with periodic acid(10), and the formaldehyde thus generated was determined with chromotropic acid(11). That the formaldehyde formed in excess of amount derived from glucose could be taken to measure glycerol was shown by submitting an aliquot of supernatant fluid to paper chromatography. Using n-butanol-acetic acid-water (4:1:5) and n-butanol-acetic acid-water-conc. HCl (20:5:25:1) as solvents and lead tetraacetate as location reagent, 3 spots could be identified, cor-

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‖ Free fatty acid = nonesterified fatty acid (NEFA) or unesterified fatty acid (UFA).