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Failure of RNA to Inhibit Rejection of Skin Homografts.* (29452)

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Ashley *et al*(1), Axelrod and Lowe(2) and Jolley, Hinshaw and Peterson(3) have reported that the systemic administration of ribonucleic acid (RNA) to experimental animals resulted in prolongation of skin homograft survival. Prior work from this laboratory(4) has demonstrated that incubation *in vitro* with lymphoid RNA may inhibit sensitized rabbit lymph node cells from manifesting transplantation immunity, as indicated by the inability of these cells to produce a "transfer reaction"(5).

It therefore appeared desirable to attempt to inhibit the rejection of skin homografts in rabbits by the infusion of lymphoid RNA *in vivo* and, in addition, to determine whether or not it was possible, in our laboratory, to confirm the work of Jolley and co-workers (3), who reported markedly prolonged survival of skin homografts in rabbits induced by repeated administration of homologous, liver RNA.

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Materials and methods. Purposely outbred New Zealand White rabbits were used as experimental animals. The rabbits weighed approximately 2 kg. The techniques of excision of spleen and lymph nodes and of full thickness skin grafting used in this laboratory have been described(4,6). In these experiments the skin grafts, approximately 1.5 cm square, were excised from and sewn in place on the rabbits' ears in all instances.

Extraction of RNA from rabbit tissue by the cold phenol method has also been described(4,6). The concentration of RNA in the preparations used in these experiments was determined by the optical density at 260 mμ in a Beckman model DU spectrophotometer.

The survival of skin homografts in the present experiments was considered to have ended when they showed unequivocal signs of rejection—eschar formation, infarction, loss of pliability, etc. Skin autografts were used as controls in all experiments. The autografts in all animals discussed below sur-

vived throughout the experimental period.

The assay employed for ribonuclease (RNase) activity was that described by Eichel(7). Commercial yeast RNA† was dialyzed against distilled water, lyophilized, redissolved in distilled water and stored in the frozen state until used as the substrate. Five times recrystallized bovine pancreatic ribonuclease‡ was used as the standard. The pH of the reaction mixture was maintained at 7.0 with Veronal acetate buffer.

Polyvinyl sulfate (PVS) was prepared by the method of Bernfeld *et al*(8) from polyvinyl alcohol§ refluxed at 70°C with chlorosulfonic acid and pyridine.

Results. Donor RNA: In this series of experiments New Zealand rabbits were studied in pairs. The spleen and the mesenteric and popliteal lymph nodes were excised from the donor animal of each pair and RNA was extracted from this tissue. The recipient of each pair then received a full thickness skin homograft from the donor and upon completion of the RNA extraction, the RNA preparation from the donor was immediately infused intravenously into the recipient. The survival of the donor skin homografts is listed in Table I. The mean survival was 7.0 ± 0.6 (S.D.) days. As reported elsewhere the mean survival time of skin homografts exchanged at random between New Zealand animals in this laboratory is 7.4 ± 1.2 (S.D.) days(9). Therefore, the survival of the skin homografts in this experimental group was not significantly different from that in control animals, $p>0.3$.

RNase inhibitors: In an attempt to prevent the breakdown of the intravenously administered RNA by the circulating RNase of the recipient animals, an attempt was made to inhibit the serum RNase activity in a series of rabbits with 4 known RNase inhibitors—heparin, citrate, versene and PVS. In 10 control animals the serum RNase activity was found to vary from 0.1 to 0.6 μg/ml. In a series of experiments performed in triplicate it was found that heparin at 1 and 2 mg/kg, sodium citrate at 25 and 50 mM/kg, sodium versenate at 250 mg/kg and PVS at 12.5 and 15 mg/kg had no detectable effect on serum RNase activity. All compounds were administered intravenously in saline in volumes of 5 to 10 ml and were tested both when infused rapidly and when administered slowly over the course of an hour with a Harvard infusion pump. In either case serum RNase activity was tested immediately after completion of the infusion.

TABLE I. Effect of Donor RNA.

Rabbit No.	RNA injected (mg)	Survival skin homografts (days)
1797	4.3	6
1991	3.0	7
1449	3.5	8
1154	2.8	7
1162	8.2	7
2104	3.0	7
		Mean = 7.0 ± .6 S.D.

Donor RNA + PVS: Because of the reported potency of PVS as an RNase inhibitor *in vitro*(10,11), it was decided to test the effect of infusion of donor lymphoid RNA and PVS on survival of skin homografts despite the fact that no effect of PVS on serum RNase activity could be detected, as noted above. New Zealand rabbits were again studied in pairs. RNA was extracted from the lymphoid tissues of the donor as before and, after addition of PVS, was infused intravenously into the recipient immediately after the application of a skin homograft from the donor. The results are listed in Table II. It appeared likely after 3 experiments that the combination of RNA and PVS had led to

TABLE II. Effect of Donor RNA + PVS.

Rabbit No.	RNA injected (mg)	PVS injected (mg)	Survival skin homografts (days)
2795	3.8	5	5
2053	3.6	5	6
2737	4.3	5	5
	PVS alone		
2034		15	5
2039		15	5
2578		30	4
2591		30	5
2029		15	6
			Mean = 5.0 ± .7 S.D.

† Schwarz BioResearch, Inc., Orangeburg, N. Y.

‡ Worthington Biochemicals, Freehold, N. J.

§ Elvanol, E. I. DuPont de Nemours & Co., Inc.

accelerated rejection of the donor skin homografts in 2 instances. This experimental series was therefore completed by testing the effect of intravenously administered PVS alone on survival of skin homografts in 5 additional rabbits (Table II). The skin homografts in these 5 animals survived an average of 5.0 ± 0.7 (S.D.) days, a time very significantly shorter than the survival of skin homografts in control New Zealand animals, $p<0.001$.

Liver RNA: In this final series of experiments an attempt was made to reproduce the work of Jolley *et al*(3). Rabbits were again studied in pairs. A skin homograft was taken from the donor of each pair and was then incubated *in vitro* for 20-30 minutes in RNA (concentration 1 mg/ml) extracted from the liver of a freshly sacrificed rabbit. Following incubation the skin homograft was sutured in place on the ear of the recipient as before. The recipient then immediately received an intravenous infusion of rabbit liver RNA (5 mg/kg). Repeat injections of liver RNA were given intravenously each day thereafter until the rejection of the skin homograft was complete. The results are listed in Table III. The mean survival time of the skin homografts was 6.8 ± 0.4 (S.D.) days, not significantly different from that in the control New Zealand animals, $p>0.1$.

Discussion. The data presented here indicate that under the present experimental conditions intravenous administration of either lymphoid or liver RNA had no detectable effect on survival of skin homografts in rabbits. This result is perhaps not surprising in view of prior work from this laboratory(4) which has demonstrated that inhibition of the ability of sensitized lymphoid cells to produce a transfer reaction, after incubation *in vitro* with homologous lymphoid RNA, was prevented by exposure of the RNA to small amounts of RNase. Certainly the serum and tissue fluids of mammals contain considerable RNase activity as demonstrated in the present experiments. Moreover the RNase inhibitors tested above apparently failed to diminish this activity when administered systemically.

TABLE III. Effect of Liver RNA.

Rabbit No.	Total RNA injected (mg)*	Survival skin homografts (days)
2138	50	6
2136	60	7
2109	60	7
2579	60	7
2038	60	7
		Mean = 6.8 ± .4 S.D.

* 10 mg RNA injected I.V. daily until unequivocal gross rejection of homograft occurred.

These results are at variance with those obtained by Ashley *et al*(1) and by Axelrod and Lowe(2) in the rat and by Jolley and co-workers(3) in the rabbit. It is of interest that Axelrod and Lowe(2) found that RNA from the homograft donor was effective in inhibiting rejection of skin homografts only in pyridoxine deficient rats. The effect of pyridoxine deficiency on serum RNase activity is unfortunately unknown.

The reason for the discrepancy between the present results and those of Jolley *et al* (3) is not readily apparent since, in the final series of experiments reported above, the protocol paralleled that used by these investigators in all important respects. However, the details of the procedure used by Jolley and co-workers for extraction of RNA have not, to our knowledge, been reported. Differences between the final product obtained by their method and that used in the present experiments might conceivably account for the differences in results.

An incidental finding in the present investigation was the accelerated rejection of skin homografts in rabbits treated with intravenous PVS. The mechanism of action of PVS in producing this result is unclear; however, it is likely that the PVS contributed an adjuvant-like effect and thus induced a heightened immune response in these animals.

Summary. 1. Intravenous infusion of donor lymphoid RNA and RNA extracted from rabbit liver had no detectable effect on survival of skin homografts in White New Zealand recipient rabbits. 2. Intravenous administration of heparin, sodium citrate, sodium versenate and polyvinyl sulfate did not detectably depress serum ribonuclease activity in rabbits. 3. Intravenous infusion of poly-

vinyl sulfate induced an accelerated rejection of test skin homografts.

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Prolonged Pancreatic Hypertrophy and Reversibility in Rats Fed Raw Soybean Meal. (29453)

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It was reported previously that 3 adverse effects were observed when raw soybean meal was fed to rats for 38 days as the sole source of dietary protein(1). These effects, namely, decreased growth rate, reduced feed efficiency, and pancreatic hypertrophy, were eliminated when heated soybean meal ($\approx$ 14% protein) replaced the raw soybean meal in the diet. The earlier report also showed that supplementation of the raw soybean meal diet with valine, tyrosine, methionine and threonine corrected the poor growth and improved the feed efficiency, but did not prevent pancreatic hypertrophy.

The importance and significance of pancreatic stimulation in the raw soybean meal syndrome was confirmed recently by Muelenaere(2). His findings indicated that the difference between the nutritive value of raw and autoclaved soybeans was due to an extra demand for protein made on the animal in order to keep up with the faster epithelial cell regeneration and the sharply increased digestive enzyme secretions.

The present work was undertaken to investigate the reversibility of pancreatic hypertrophy, and to determine whether continuous pancreatic stimulation for approximately one-quarter of the rat's lifespan would result in malignancy of the pancreas.

Materials and methods. The basal diet fed to control rats had the following percentage composition: dextrose 50.8, cornstarch 20.0, crude casein 17.2 (N $\times$ 6.25 = 14% protein), salts U.S.P. XIV 4.0, complete commercial vitamin mix 2.0, soybean oil 4.0, and powdered cellulose 2.0. Composition of the hexane-extracted raw soybean meal on a moisture-free basis was nitrogen 7.93% (protein 49.6%) and oil <0.5%. In the diet fed to experimental rats for 38 and 192 days, the raw soybean meal was substituted in the basal diet at a level of 28.4%, to provide 14% protein at the expense of 17.2% crude casein, 9.2% dextrose and 2.0% cellulose.

Albino rats from our colony were divided into uniform groups of 5 per dietary treatment and caged on wood shavings with free access to food and water. Weekly records of body weights and food intake were kept. At

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