TABLE II. Effects of Treatment on Parasite Densities in Liver of Mice. Infection intravascular with *Leishmania donovani*.

Animal groups	Dosage, mg/k	Trials with mice, parasite density in liver (as "total" parasites) 1	2
	Untreated controls		
One hr after inoc.		29	32
8 days *idem*		276	363
	Treated		
Pentostam	94	51	135
Fungizone	2.5	<3	
	1.25	<7	
	.675	31	
	.337		103
	.168		245
Nystatin	2		152*
	1		410*
	.2		337*
	.1		394*
Xerosin	100		120
	50		154
	25		149

For additional details, see footnote, Table I.

pound where these adhesions are minimal. In the mouse treated IV with Fungizone, no toxic effects were noted, even in the highly effective range of dosages.

Xerosin, as previously reported for virus infections(5), exerts a significant effect on parasite reproduction, but inhibition is not complete even close to the maximum tolerated dose. Fumagillin acts very much like xerosin. TWSb was active at levels near the maximum tolerated dose. Although unlike the antibiotics reported here, it is included in further evaluation of the 8-day method.

Nystatin was minimally active at the highest concentration used. As seen in Table II the numbers of parasites in the liver were not unequivocally significant from the control but the numbers of parasites in the spleen were significantly reduced over those found in the controls (1.5 *vs.* 5.6 "total" Lds, respectively; P = 0.001).

The activity of amphotericin B is especially noteworthy. At 5 mg/k it produced the same effect as 188 mg/k of Pentostam; namely, no visible parasites in the sample counts of spleen or liver at 8 days.

Summary. Using an eight-day method for screening compounds against experimental visceral leishmaniasis, the following compounds showed activity in hamster or mouse infection or in both: amphotericin B (and Fungizone), fumagillin, nystatin, Sb dimercaptosuccinate and xerosin. The activity of Fungizone is especially noteworthy.

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Effect of Prior Inoculation of Packed Erythrocytes on Survival of Skin Homografts in Rats.* (25081)

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Production of a "second-set" reaction to skin homografts in rabbits following primary inoculation of whole blood or one of its fractions has been ascribable solely to the leucocytes; no such reaction was detected in response to primary inoculations of erythrocytes(1). On the other hand, erythrocytes are capable of stimulating resistance to sarcoma homografts in mice(2). In the present study, a prior inoculation of erythrocytes

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TABLE I. Survival of Homografts on Non-Immunized and Immunized Random-Bred Rats as Determined by Epidermal Survival

Hosts† immunized with	Post-operative day of examination										
	6	7	8	9	10	11	12	13	14	15	16
—	2/2*	1/1	4/4	1/2	4/4	2/3	2/2	1/2	1/3	0/2	0/1
Skin homograft	1/3		2/6		1/8		0/2		0/2		0/1
Red cells	1/2		1/4		2/5	1/1	0/3	0/2	0/2	0/2	0/2

* No. of grafts with surviving epidermis/No. of grafts examined.
† Donors of antigens were same individuals that furnished test homografts.

apparently brought about an earlier rejection of skin homografts in rats.

Materials and methods. Experiments were first carried out with random-bred rats and later with 3 strains of inbred rats. Full thickness grafts were transplanted by a method similar to that described by Billingham and Medawar(3). Graft survival was primarily determined from histological sections of grafts cut at 5 or 6 vertical levels. A graft was recorded as having broken down when destruction of its epidermis was apparently complete. In 2 experiments, rats were "immunized" prior to grafting with packed erythrocytes prepared as follows: Six to 8 ml of blood were withdrawn from a single donor by cardiac puncture, defibrinated by gentle agitation in a flask containing glass beads, then placed in agglutination tube with internal dimensions of 10 x 105 mm and centrifuged at approximately 1900 x g for 12 minutes. Supernatant plasma was drawn off and 0.6 - 0.8 ml of packed cells carefully withdrawn from bottom of tube. This resulted in an approximate 10-fold reduction in number of leucocytes when compared to whole blood. Each rat received one subcutaneous injection of 0.1 ml, approximately equivalent to erythrocytes present in 0.2 ml of whole blood. *Experiments with random-bred rats.* A single line of random-bred albino rats was obtained from recently established commercial colony. Three experiments were performed. (a) *Non-immunized controls.* One donor furnished a "first-set" graft to each of several recipients. After interval ranging 6 through 16 days, the grafts were removed for microscopic study. (b) *Immunized controls.* One donor was used to supply first-set grafts as before. These were left in place and 21 days later, the same donor was used to supply a second homograft to a new graft bed in each recipient. The second-set grafts were later removed for sectioning. (c) *Rats immunized with erythrocytes.* Each animal received one subcutaneous injection of packed erythrocytes followed in 21 days by a skin homograft from the red cell donor. These grafts were subsequently removed and sectioned. Hereafter, animals treated as in (b) and (c) will be referred to as "skin-immune" and "red cell-immune" respectively. In addition to a single "test" homograft, a few animals in the immunized groups also received a homograft from animal not directly related to donor or host (these will be referred to as "unrelated" homografts) and an autograft, so that 3 grafts were linearly arranged in the same graft bed. In both red cell- and skin-immune hosts, graft survival time was reduced in comparison with non-immunized controls (Table I). No surviving grafts were found beyond 11 days in the red cell-immune and 10 days in skin-immune recipients. In homografts on untreated hosts, no surviving grafts were found beyond 14th day. One autograft failed, however, the others were apparently viable. Epidermal survival time of unrelated homografts on immunized hosts approximated that of control homografts on non-immunized hosts although it may have been slightly reduced. *Experiments with inbred rats.* The methods were essentially those described above with 2 exceptions. The time interval between immunization and test grafting was shortened from 21 to 10 days in view of collateral evidence in mice(4). Prior to sacrifice of each host presence or absence of blood circulation within grafts was determined with stereoscopic microscope(5). In doubtful cases, the host was given an intravenous injection of 30% India ink in physiological saline and

TABLE II. Survival of Buffalo Skin Homografts on Non-Immunized and Immunized Fischer Hosts as Determined by Epidermal Survival and Circulation.

Fischer hosts immunized with	Post-operative day of examination								
	3	4	5	6	7	8	9	10	11
—	4/4* (0/4)	4/4 (4/4)	4/4 (4/4)	5/5 (5/5)	4/4 (4/4)	5/5 (3/5)	5/5 (0/5)	2/4 (0/4)	0/4 (0/4)
Buffalo skin homograft	5/5 (0/5)	5/5 (0/5)	3/4 (0/4)	0/5 (0/5)	1/4 (0/4)	0/5 (0/5)	0/4 (0/4)	0/4 (0/4)	0/5 (0/5)
Buffalo red cells	4/4 (0/4)	4/4 (2/4)	4/4 (1/4)	4/4 (0/4)	4/5 (0/5)	2/4 (0/4)	0/5 (0/5)	0/5 (0/5)	0/4 (0/4)

* No. of grafts with surviving epidermis/No. of grafts examined.
Observations on circulation shown in parentheses.

the grafts reexamined. Animals were derived from inbred stocks of Fischer, Buffalo, and M520 rats in their 58th, 20th, and 60th inbred generations respectively.† Fischer rats were recipients of 3 grafts; a Buffalo skin homograft, an M520 skin homograft and an autograft. On day of sacrifice, the 3 grafts on each Fischer host were examined for the presence of circulation, then removed and fixed for subsequent histological study. On an average, 4 Fischer rats were sacrificed daily from 3 through 11 days inclusive in each of 3 experiments. (a) *Non-immunized controls.* These animals received 3 simultaneous grafts whose survivals were subsequently studied. (b) *Immunized controls.* Fischer rats received a single first-set Buffalo graft that was left in place. Ten days later, each received 3 grafts as outlined above in a new graft bed. Second-set grafts were taken from the first-set graft donor. (c) *Rats immunized with erythrocytes.* This experiment was similar to preceding except that Fischer hosts were immunized with Buffalo erythrocytes instead of a first-set skin homograft.

Results. The epidermal survival of Buffalo skin homografts on red cell-immune hosts was shortened in comparison with first-set homografts, but not to the extent observed in second-set homografts (Table II). Graft survival based on circulation yielded a similar result. Circulation was present in 3 of the Buffalo grafts on red cell-immune hosts in contrast to its absence in all second-set homografts. Circulation was present in all first-set homografts examined on days 4 through 7, and absent in all grafts subsequent to day 8. The epidermis of all autografts remained viable and circulation was present in grafts examined on days 4 through 11. On non-immunized hosts, survival of M520 grafts approximated that of Buffalo homografts. On immunized hosts, however, circulation and epidermal survival continued for about a day longer in the unrelated M520 grafts than in Buffalo homografts.

Discussion. The apparent reduction in survival time of skin homografts in animals receiving prior inoculation of packed erythrocytes resembles the reported resistance against tumor homografts following red cell inoculation(2). These findings appear to be contrary to the report that red cells are unable to elicit transplantation immunity(1), however, there is some uncertainty. Decreased survival of homografts reported here could be ascribed to leucocytes present in the inocula. This is possible, but appears unlikely in view of small numbers of leucocytes present and of studies on induced resistance to a transplantable tumor in mice where it was indicated that degree of resistance was more a function of number of cells than kind of cells injected(2,6). In addition, our experiments involved a different species. Furthermore, the donor-host strain combination may be an important factor and recent evidence from mice indicates that the erythrocyte may lack certain transplantation antigens(7).

The combination of unrelated homograft and a homograft from the donor of red cells or first-set skin graft was especially useful in random-bred rats where more variation in graft survival was encountered. An immune

† Breeding stocks of inbred rats were obtained from Dr. George Jay, N.I.H., Bethesda, Md.

response could usually be detected since epidermal destruction was usually more advanced in the homograft from the red cell or first-set skin graft donor. The somewhat reduced survival time of unrelated homografts on immunized hosts may have been the result of a relatively small number of antigenic factors shared in common between the homografts(8). Autografts remained viable and apparently were not influenced by adjacent degenerating homografts. This observation parallels that of Eichwald and his co-workers(9).

Summary. Skin homografts were made between individuals of a single line of random-bred and between 3 strains of inbred rats. Graft survival was determined from histological sections of graft epidermis, and presence or absence of circulation. Survival time of skin homografts was reduced in individuals that had received a prior inoculation of packed erythrocytes.

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Isolation of Coagulation Factors by Continuous Flow Electrophoresis.* (25082)

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Continuous flow paper electrophoresis, recently(1) applied to study of blood coagulation proteins, achieved partial separation and recovery of these factors. The present studies used this technic, confirmed and extended the findings of Lewis, *et al.*(1), and provided new data on the Stuart, proconvertin, and Hageman clotting factors.

Materials and methods. The principles of continuous flow paper electrophoresis are described by Block, Durrum, and Zweig(2). The Beckman/Spinco model CP was used with barbital buffer pH 8.6, ionic strength 0.02, and 14°C. 15-20 ml samples, previously dialyzed overnight against the buffer at 4°C were fractionated by constant current of 40 milliamps which developed 500 volts. 32 fractions were collected during the usual run of 16-20 hours. At the end of this time the curtain was removed, dried 30 minutes at 110°C, and dyed with bromphenol blue. Protein in each sample was determined by a biuret method. Fibrinogen, prothrombin, proconvertin, PTC, AcG, and AHF were measured by standard assays(3). The last 2 factors, found labile under the conditions in early experiments, were excluded from the later routine. Hageman factor was estimated using Hageman deficient substrate in partial thromboplastin time test(4). Stuart factor was assayed by specific assay(5) which used a substrate deficient only in Stuart factor. *Refined preparations.* In many instances, fraction collecting tubes could be selected which contained only Stuart, proconvertin, or Hageman factor. These selected fractions were concentrated by overnight dialysis against 10% gelatin, which removed 25-40% of water and buffer salt. The dialysate was lyophilized and stored at −20°C until reconstituted with distilled water. To refine Hageman factor, the pH was adjusted to 5.2 with dilute acetic or hy-

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