between amount of urokinase (or other activator) added and maximum rate of fibrinolysis for clots with a given amount of incorporated plasminogen (Fig. 4). The method was useful for quantitating activator in human urine, and may be useful for other body fluids.

Summary. The time course of fibrinolysis was observed by release of solubilized protein from washed clots. The slope of the resulting curve was proportional to the amount of plasminogen incorporated into the clots; plasminogen added after clotting did not alter the maximum rate of lysis. With a constant amount of plasminogen, the rate of fibrin lysis was a measure of the amount of activator added.

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Changes in Plasma Proteins of Mice During Rejection of Skin Homografts and Heterografts.* (27725)

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Hardin reported an increased concentration of alpha globulin in the serum of C3H mice which were rejecting full thickness skin homografts, which he interpreted as being a non-specific reaction to injury and inflammation(1). Because skin homografts produce circulating antibodies of several types and gamma globulins apparently are not increased during antibody formation, we have questioned whether increase in the alpha globulin fraction is not the result of specific antibody production. We have reasoned that, if increased alpha globulin is the result of antibody formation, repeated grafting should cause a continued rise in this fraction as the immune state is increased, and heterografts should produce a greater rise in alpha globulin than homografts of the same size. In addition, if, as Pressman has hypothesized, concentration of tissue rejection antibodies in the graft or graft bed is responsible for previous failures to demonstrate circulating antibodies, alpha globulins should rise following removal of the graft if tissue rejection antibodies are formed in this fraction of the plasma proteins (3). The following experiments were performed to determine whether an increasing state of foreign tissue immunity is accompanied by increased alpha globulin and whether excision of a foreign tissue graft has any effect upon the alpha globulin fraction.

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Material and methods. C57 mice were used for skin donors and C3H mice for recipients in the homograft experiments. Skin from the ear of New Zealand rabbits was transferred to C3H mice in the heterograft experiments. The same donor animal was used repeatedly when multiple grafts were transferred. The skin grafts were 2 × 2 cm full thickness grafts sutured into a similar size defect on the posterior thorax. Just before the skin grafts were performed, the tail was excised from the recipient animal and 1 cc of whole blood was allowed to drop into a microcentrifuge tube. At the conclusion of the experiment, the mice were beheaded and blood was collected from the carotid system in the same manner. After clot retraction had occurred and centrifugation had been performed, batches of serum were frozen and stored at 0°C. Paper electrophoresis of the serum proteins was determined with a Spiñco Model R paper electrophoresis apparatus; electrophoresis patterns were not affected by freezing, as was shown also by Hardin(1).

Exp. 1. Eighteen mice were autografted

TABLE I. Mean Percentages before and after Skin Graft.

		Single graft			Multiple grafts		Excision of grafts	
	Type of graft	Autograft	Homo-graft	Hetero-graft	Homo-graft	Hetero-graft	Homo-graft	Hetero-graft
	No. mice	18	8	17	9	14	10	18
Alb.	Initial	64.64	60.13	68.84	63.04	62.72	65.43	65.30
	Final	58.71	51.34	57.48	43.11	52.61	53.14	49.24
	Difference	5.93	8.79	11.36	19.33†	10.11	12.29	16.06†
	S.E. of mean diff.	3.44	7.13	1.97	3.83	2.17	4.39	3.23
	Variance of diff.	212.69	406.28	66.05	131.88	66.26	192.57	177.53
α_1	Initial	7.75	10.22	6.89	7.88	7.88	7.02	8.55
	Final	9.63	9.99	7.18	9.87	8.62	9.24	8.93
	Difference	−1.88	.23	−.29	−1.99	−.74	−2.22	−.38
	S.E. of mean diff.	.79	2.42	.42	.91	.54	1.43	.92
	Variance of diff.	11.42	46.86	3.04	7.51	4.03	20.42	15.11
α_2	Initial	7.08	5.67	4.73	4.83	5.89	7.37	6.21
	Final	5.44	7.60	7.15	9.62	7.73	5.71	7.86
	Difference	1.64	−1.93†	−2.42†	−4.79‡	−1.84†	1.66	−1.65
	S.E. of mean diff.	1.41	.82	.66	.32	.64	1.81	1.14
	Variance of diff.	35.86	5.36	7.58	9.45	5.77	32.83	23.43
β	Initial	15.27	18.53	14.22	16.69	16.61	14.18	14.63
	Final	19.15	26.90	24.51	28.22	24.45	24.43	27.87
	Difference	−3.88	−8.37	−10.29	−11.53*	−7.84	−10.25	−13.24*
	S.E. of mean diff.	3.97	4.29	1.18	1.74	1.37	1.78	1.83
	Variance of diff.	284.14	147.40	23.59	27.31	26.41	31.93	60.54
γ	Initial	5.25	5.41	5.32	7.57	6.91	6.01	5.17
	Final	7.06	4.17	3.68	9.23	6.59	7.47	6.09
	Difference	−1.81	1.24	1.64	−1.66	.32	−1.46	−.92
	S.E. of mean diff.	1.01	1.27	.64	1.27	.68	1.67	.85
	Variance of diff.	18.61	12.94	6.91	24.48	6.59	28.11	13.12

* Probability <.10
† Probability <.05
‡ Probability <.01
} Significantly different from autograft.

by excising a 2 × 2 cm skin graft and returning it immediately to its original site. Eight C3H mice were homografted with C57 skin and seventeen C3H mice were heterografted with rabbit skin. Blood was drawn just before application of the graft and 7 days following the grafting procedure.

Exp. 2. Seven days following rejection of a homograft, 9 mice received a second homograft from the same donor animal and 14 mice received a second heterograft from the same rabbit which donated the first graft. Blood was drawn just before application of the first graft and 7 days after application of the second graft.

Exp. 3. Ten mice were homografted twice and 18 mice were heterografted twice, as in Exp. 2; the second homograft (or heterograft), however, was excised 4 days after it was applied. In this experiment, blood was drawn prior to application of the first graft and 2 days following excision of the second graft.

Each recipient served as its own control in that both a pregraft and a postgraft serum sample were subjected to electrophoresis. The difference between the pregraft and the postgraft electrophortic pattern was analyzed rather than the actual value of either determination.

Results are shown in Table I. Although there is an apparent decrease in the albumin fraction following homografting and heterografting, the decrease is significantly different from changes following an autograft only following repeated homografts and just after removal of heterografts. A decrease in albumin appeared to correspond with a large increase in the alpha 2 globulin and was significant only when increase of alpha 2 globulin was maximum, such as following application of multiple homografts. There were no significant changes in the alpha 1 fraction following any grafting procedure. Significant increase in the alpha 2 fraction was observed in single and repeated homografts when com-

pared to autografts and could be reversed by excising the grafts prior to obtaining serum. In the case of homografts, the increase in alpha 2 fraction was greater following repeated grafts than after a single graft. Although a single heterograft caused a greater rise in alpha 2 globulin than did a single homograft, multiple homografts resulted in larger alpha 2 fractions than did multiple heterografts.

Changes in the beta fraction were found only in animals which had been homografted repeatedly and in mice which had had an excision of a heterograft 48 hours before withdrawal of blood. The possibility is not great that changes in the beta fraction were significant (p less than .1) and these data should be considered only as suggestive. Changes in the gamma fraction were not significant in any group.

Discussion. Although, as Hardin pointed out, increased levels of alpha globulin have been observed in many inflammatory and tissue destructive conditions, the progressive rise of alpha 2 globulins with repeated grafting, the greater increase of alpha 2 globulins in first set heterografts compared to homografts, and the decrease of elevated alpha 2 globulins following removal of homografts are suggestive of a cause and effect relationship between the development of foreign tissue immunity and rise in alpha 2 globulins(1). Failure to find a greater elevation of the alpha 2 globulins following application of second set heterografts does not necessarily negate a possible relationship between rising alpha globulins and progression of the immune state. Animals which have received multiple heterografts often are quite sick in a manner which is not understood clearly. In our experiments, heterografted mice did not eat normally, lost weight, showed a general change in coat texture, and often died before an experiment was complete. Excision of the heterograft, even before necrosis developed, reversed these findings and was accompanied uniformly by a decrease in the alpha 2 globulins.

The hypothesis that a foreign tissue transplant might be absorbing antibodies and that excision of the transplant would cause a rise in serum globulin was disproven; excision of the graft was followed by a decrease in alpha 2 globulins–presumably because of removal of the antigenic stimulus.

The production of hemagglutinins, as described by Mitchison and Dube and believed by them to be separate and not associated with tissue antibodies, could be responsible for the increase in alpha 2 globulins following transplantation of foreign tissue(2). The fact that hemagglutinins are produced later than tissue antibodies, however, and that they continue to be produced after the antigen has been removed strongly suggests that hemagglutinins are not responsible for a rise in alpha 2 globulins, which, in fact, decrease sharply following removal or rejection of the homograft.

Conclusions. Mice which have received homografts and heterografts respond by increasing the level of their alpha 2 globulins. A greater increase of alpha 2 globulins is observed following the application of second set grafts, and a decrease in alpha 2 globulins can be demonstrated following excision of foreign tissue grafts. Such findings suggest that the alpha 2 globulins are altered by foreign tissue antibody formation. That such antibodies are related to the specific destruction of foreign tissue implants, however, is conjectural.

Summary. Following application of a single homograft or heterograft of full thickness skin, albumin, alpha 1, beta, and gamma globulin fractions of the plasma proteins of C3H mice remain unchanged as compared to changes following autografting while the alpha 2 fraction increases significantly. Repeated grafting resulted in higher alpha 2 levels than did a single graft. A return to normal of the alpha 2 fraction could be produced by excising the homograft or heterograft before necrosis occurred.

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