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Serum Electrophoretic Changes Following Heart Allotransplantation.* (31593)

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Serum electrophoretic changes in animals receiving allotransplants have been observed by a number of investigators; however, the results are not uniform and depend upon the animal species and the type of graft used. In earlier studies using cornea and skin allografts(1,2), no consistent effect on serum electrophoretic patterns of the recipient animals was found. On the other hand, West and his co-workers(3) demonstrated a definite change in the level of alpha-2 globulin during the course of kidney allograft rejection. They explained this phenomenon as a response to the inflammatory reaction present in the rejected organ. Peacock and Biggers(4), using skin allograft and xenograft, found a progressive rise in alpha-2 globulin levels after repeated grafting and thought that this represent a cause-and-effect relationship between the development of foreign tissue immunity and a rise in this serum protein fraction. Paronetto and his associates(5), using liver allograft, reported an early increase in alpha-2 and a rise of gamma globulin fraction following rejection of the graft. They stated that this indicates the possibility of production of graft specific antibodies. Pressman(6) has suggested that even though such antibodies may be present, their immediate fixation to the grafted tissue may prevent any alteration in the protein fraction in which those antibodies reside. If this is true, the removal of the graft should trigger a rise in the level of that fraction.

In the present study, experiments were performed to determine changes in serum electrophoretic patterns following allotransplantation of the heart, and their possible modification by antimetabolite treatment; the effect of the removal of the graft on serum protein levels was also followed.

TABLE I. Experimental Design.

Group	Description	No. of animals
I	First set grafting	9
II	Second set grafting	8
III	First set grafting + azathioprine injection	4
IV	Azathioprine injection only	2
V	Sham operation	5

Material and methods. Twenty-eight adult mongrel dogs weighing from 10 to 20 kg were used. Animals were first observed for at least 3 weeks to confirm their general health. During the observation period, total serum protein, serum electrophoresis, and body weight were recorded once a week, and those showing an abnormality were eliminated. The animals were divided into 5 groups (Table I). Group I, 9 dogs, received first set allografts of the heart; Group II, 8 animals, received second set grafts; Group III, 4 animals, was subjected to first set grafting and received daily treatment with azathioprine (Imuran®) starting on the day of transplantation; Group IV, 2 dogs, was subjected to daily azathioprine injections alone; and Group V, 5 dogs, sham operations were performed.

Transplantation of the heart was performed by a modification of Mann's technique as described previously(7). Puppies weighing from 3 to 5 kg served as heart donors. The transplanted hearts were observed daily and rejection was considered complete when the

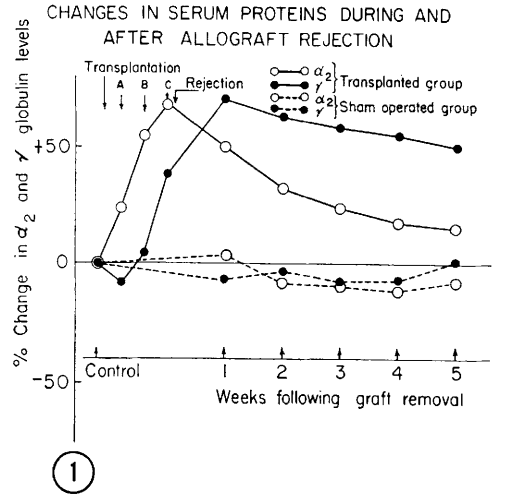
* Work supported by USPHS Grant HE 05043, Michigan Heart Assn., the John A. Hartford Foundation and the Burroughs Wellcome Fund.

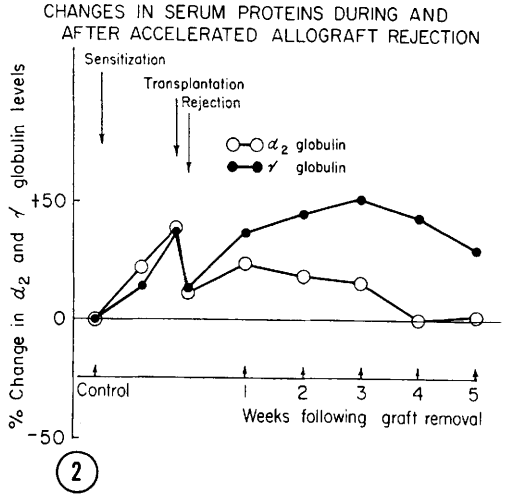
TABLE II. Changes in Serum Proteins After 1st Set Allotransplantation (Group I).

			Serum protein (alpha 2, gamma globulin) levels (g/100 ml)									
				Days after transplantation								
Exp No.	Survival of graft (days)	Serum protein fraction	Control (8 wk avg pre-transplantation)	A 2-3 days	B 4-6 days	C 7-9 days	Weeks after rejection of graft 1	2	3	4	5	8
165	8	α_2	.53	.69	.98	.94	.77	.71	.70	.63	.62	
		γ	.54	.49	.52	.72	.82	.92	.81	.96	.89	
206	11	α_2	.72	.87	.83	.94	.73	.59	.49	.51	.52	
		γ	.61	.63	.66	.84	1.03	.93	.79	.66	.72	
230	7	α_2	.40	.49	.73	.75	.71	.65	.51	.48	.49	.50
		γ	.70	.73	.90	1.08	1.59	1.55	1.32	1.38	1.30	1.15
231	11	α_2	.50	.58	.60	.73	.82	.77	.56	.57		
		γ	.70	.57	.58	.63	.97	.94	.62	.57		
238	7	α_2	.47	.48	.65	.70	.63	.47	.58	.50		
		γ	.67	.52	.61	.72	.96	.74	.85	.85		
243	9	α_2	.50	.68	.62	.71	.70	.63	.55	.56	.46	
		γ	.53	.45	.52	.94	.84	.73	.95	.80	.65	
245	4	α_2	.34	.40	.67		.37	.41	.46	.39	.44	
		γ	.73	.67	.73		1.19	1.40	1.40	1.38	1.00	
258	8	α_2	.33	.52	.74	.68	.78	.71	.77	.69	.61	.31
		γ	.45	.45	.56	.66	.83	.87	.99	1.06	1.03	.66
260	10	α_2	.37	.45	.55	.69	.68	.51	.55	.55	.54	.40
		γ	.46	.45	.63	.76	.92	.78	.80	.70	.70	.61
Mean ± S.E.		α_2	.46	.57	.71	.77	.69	.61	.57	.54	.53	
			±.04	±.05	±.04	±.04	±.04	±.04	±.03	±.03	±.03	
			p	<.05	<.001	<.001	<.001	<.005	<.05	<.10	<.20	
		γ	.60	.55	.63	.83	1.02	.98	.95	.93	.90	
			±.04	±.04	±.04	±.06	±.08	±.10	±.09	±.10	±.09	
			p	<.25	<.50	<.001	<.001	<.001	<.001	<.001	<.001	
% Change		α_2		+23.9	+54.3	+67.4	+50.0	+32.6	+23.9	+17.4	+15.2	
		γ		−8.3	+5.3	+38.3	+70.0	+63.3	+58.3	+55.0	+50.0	

heart stopped beating. The rejected grafts were removed immediately, and the animals were kept for further tests. Sensitization of the animals was performed by intraperitoneal injection with spleen homogenates from the prospective donor puppies 9 days prior to transplantation. Techniques for this procedure have been described(7). The dose schedule of azathioprine treatment was as follows: 5 mg/kg body weight on the day of transplantation and on the first postgrafting day the drug was reduced to 2 mg/kg body weight. Injections were continued at this level until sufficient prolongation of graft survival was obtained. The fourth group was injected with azathioprine at the same dose schedule for 3 weeks without receiving transplants. Peripheral white cell counts were performed twice a week on the azathioprine treated animals. Sham operation consisted of an incision of the neck, exposure of the carotid artery and the jugular vein, and ligation of both vessels. Blood samples were drawn daily in the first week, every other day in the second week, and twice a week thereafter. Animals in the first, second, and third group receiving transplants were observed for 5 weeks after rejection and removal of the graft, and the sham operated and azathioprine injected animals were also observed for 5 weeks after operation or the discontinuation of injection. Paper electrophoresis was performed in a Beckman Model R, Durrum type cell with veronal buffer at pH 8.6, ionic strength 0.075. Paper strips were stained with bromphenol blue dissolved in methanol then analyzed in a Spinco Analytrol with a 500 mu interference filter and a B-5 cam.

For comparative purpose, the periods from the time of grafting to complete rejection were





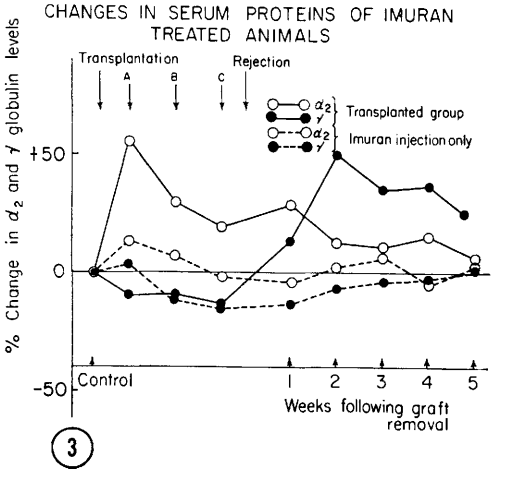


FIG. 1. Changes observed in serum protein levels during and after first set allograft rejection (Group I). Alpha-2 globulin increased soon after transplantation, while gamma globulin fraction rose and remained at a high level following rejection and removal of the graft. Sham operated animals (Group V) did not show any significant change. Control values are expressed as averages of alpha-2 and gamma globulin levels prior to transplantation over a period of 8 weeks.

FIG. 2. Changes seen in serum protein levels during and after second set allograft rejection (Group II). After rejection and removal of the graft, a significant and persistent increase in the gamma globulin fraction was seen, while the alpha-2 level returned to control values. Control values are expressed as averages of alpha-2 and gamma globulin levels prior to transplantation over a period of 8 weeks.

FIG. 3. Changes in serum protein levels during and after first set allograft rejection in animals treated with azathioprine (Group III). Increase in alpha-2 was observed following transplantation while gamma globulin level remained unchanged until the graft was rejected and removed. After this, gamma globulin level increased significantly. Control values are expressed as averages of alpha-2 and gamma globulin levels prior to transplantation over a period of 8 weeks.

divided as follows: an initial stage of 2 to 3 days following transplantation (A); a terminal stage of 2 to 3 days prior to removal of the graft (C); and the intermediate stage between the period immediately following transplantation and rejection (B).

Results. Group I (First set allotransplantation, Table II): The grafted hearts survived 4 to 11 days. Electrophoretic results showed two significant changes in the alpha-2 and gamma globulin fractions. As seen in Fig. 1, alpha-2 fraction rose almost immediately after transplantation, rising nearly 70% above the original level, then dropping after the rejected graft was removed. The gamma globulin fraction, on the other hand, remained at its original level or fell slightly until the end of rejection; following removal of the graft, this fraction increased. There occurred only a slight diminution of the level of gamma globulin during the 5 weeks following rejection. In 3 dogs, kept for an additional 3 weeks, gamma globulin levels failed to return to control values. The serum albumin level of these animals fell slightly after transplantation and remained low until 5 to 10 days after graft removal. There was no consistent change in alpha-0, alpha-1 and beta globulin fractions.

TABLE III. Changes in Serum Proteins After 2nd Set Allotransplantation (Group II).

Exp No.	Survival of graft (days)	Serum protein fraction	Serum protein (alpha 2, gamma globulin) levels (g/100 ml) Control (8 wk avg pre-sensitization)	At end of sensitization	At rejection	Weeks after rejection of graft 1	2	3	4	5
264	1	α_2	.53	.70	.62	.70	.61	.59	.61	.54
		γ	.54	.79	.53	.74	.63	.76	.65	.76
266	1	α_2	.40	.67	.55	.67	.54	.60	.45	
		γ	.58	.66	.63	.80	.81	.79	.83	
268	1	α_2	.51	.70	.51	.62	.70	.62	.54	.47
		γ	.80	1.28	.84	1.04	1.07	1.44	1.28	1.17
270	1	α_2	.57	.56	.64	.43	.48	.63	.63	.57
		γ	.67	.87	.68	.74	1.10	1.08	1.23	.90
272	1	α_2	.79	.92	.73	.95	.66	.57	.40	.47
		γ	.95	1.32	1.29	1.61	1.22	1.00	.99	.95
274	1	α_2	.54	.92	.57	.79	.77	.85	.55	.55
		γ	.45	.87	.74	.64	.79	.94	.69	.58
276	1	α_2	.54	.69	.65	.76	.84	.55	.66	.49
		γ	.59	.68	.51	.67	.78	.75	.73	.63
278	1	α_2	.48	.89	.61	.55	.54	.62	.55	.55
		γ	.59	.92	.58	.90	1.14	.98	1.03	.86
Mean ± S.E.		α_2	.55	.76	.61	.68	.65	.64	.55	.52
			±.04	±.05	±.02	±.06	±.04	±.03	±.03	±.04
			p	<.001	<.20	<.01	<.05	<.10		
		γ	.65	.90	.73	.89	.94	.98	.93	.84
			±.06	±.09	±.09	±.11	±.08	±.08	±.09	±.07
			p	<.005	<.25	<.005	<.001	<.001	<.005	<.02
% Change		α_2		+38.2	+10.9	+23.6	+18.2	+16.4	0	+1.8
		γ		+38.5	+12.3	+36.9	+44.6	+50.8	+43.1	+29.2

Group II (Second set allotransplantation, Table III): The sensitized animals rejected the transplants uniformly within 1 day. After sensitization, both alpha-2 and gamma globulin levels rose; at the time of graft rejection, both these fractions dropped considerably (Table III, Fig. 2). Following removal of rejected grafts, alpha-2 fraction increased slightly then returned to control level. In contrast, gamma globulin level showed a rapid and significant increase immediately after the removal of the rejected grafts (Fig. 2, Table III).

Group III (First set allograft treated with azathioprine, Table IV): Daily injections of azathioprine at a dose of 5 mg/kg for the first 2 days and subsequent doses of 2 mg/kg prolonged survival time of the graft. In 3 experiments, azathioprine administration was discontinued after 16 days, and the grafts were rejected within 3 to 5 days later. However, one animal receiving azathioprine treatment rejected the graft on the 13th postoperative day. Electrophoretic studies in these animals again revealed an early increase in alpha-2 fraction even when the rejection was delayed (Fig. 3). Gamma globulin level remained at the original or slightly lower level until rejection was complete (13 to 21 days after transplantation). Gamma globulin fraction rose immediately after removal of the graft and reached one and one-half times the control value. Duration of this elevation exceeded 5 weeks. Albumin level dropped slightly following transplantation; alpha-0, alpha-1 and beta globulin showed no consistent change.

Animals receiving azathioprine injections only (Group IV), and sham operated animals (Group V), revealed only a slight fluctuation in both alpha-2 and gamma globulin levels (Fig. 1. and 3).

Discussion. The results indicate that there were two major electrophoretic changes in serum proteins during and after rejection of an allotransplanted heart. These occurred in

TABLE IV. Changes in Serum Proteins After 1st Set Allografting with Azathioprine Treatment.

GROUP III

Serum protein (alpha 2, gamma globulin) levels (g/100 ml)

Exp No.	Survival of graft (days)	Serum protein fraction	Control (8 wk avg pre-transplantation)	Days after transplantation A 2-3 days	B 10-12 days	C 17-19 days	Weeks after rejection of graft 1	2	3	4	5
246	19	α_2	.51	.73	.62	.47	.48	.45	.42	.45	.47
		γ	.57	.51	.52	.45	.51	.77	.80	.81	.71
248	21	α_2	.47	.94	.66	.66	.60	.57	.54	.59	.48
		γ	.61	.56	.43	.42	.72	.88	.77	.77	.63
249	13	α_2	.40	.62	.52		.65	.53	.53	.53	.47
		γ	.60	.57	.59		.74	1.01	.82	.81	.72
250	20	α_2	.41	.52	.50	.57	.61	.50	.49	.50	.51
		γ	.60	.51	.61	.61	.73	.92	.85	.87	.92
Mean ± S.E.		α_2	.45	.70	.58	.54	.58	.51	.50	.52	.48
			±.03	±.09	±.04	±.04	±.04	±.03	±.08	±.03	±.10
			p	<.005	<.02	<.05	<.02	<.20	<.20	<.10	<.40
		γ	.60	.54	.54	.52	.68	.90	.81	.82	.75
			±.01	±.02	±.04	±.05	±.06	±.05	±.01	±.02	±.06
			p	<.005	<.005	<.001	<.001	<.001	<.001	<.001	<.001
% Change		α_2		+55.6	+28.9	+20.0	+28.9	+13.3	+11.1	+15.6	+6.7
		γ		−10.0	−10.0	−13.3	+13.3	+50.0	+35.0	+36.7	+25.0

GROUP IV

Azathioprine injection only

Exp No.	Survival of graft (days)	Serum protein fraction	Control (8 wk avg pre-azathioprine inj)	During azathioprine inj A 2-3 days	B 10-12 days	C 17-19 days	Weeks after discontinuation of azathioprine 1	2	3	4	5
252		α_2	.44	.38	.39	.37	.35	.38	.46	.42	.43
		γ	.52	.56	.50	.41	.48	.50	.50	.55	.62
253		α_2	.45	.61	.54	.49	.48	.52	.47	.42	.45
		γ	.63	.62	.50	.55	.50	.55	.60	.55	.56
Mean ± S.E.		α_2	.44	.50	.47	.43	.42	.45	.47	.42	.44
			±.01	±.12	±.07	±.06	±.07	±.07	±.01	±.01	±.10
		γ	.57	.59	.50	.48	.49	.53	.55	.55	.59
			±.06	±.03	±.01	±.70	±.01	±.02	±.05	±.01	±.03
% Change		α_2		+13.6	+6.8	−2.0	−4.5	+2.3	+6.8	−4.5	0
		γ		+3.5	−12.3	−15.8	−14.0	−7.0	−3.5	−3.5	+3.5

the alpha-2 globulin and in the gamma globulin fraction. Alpha-2 rose immediately after transplantation, reaching its highest point in stage C, and diminished to almost control level after the graft's removal. A similar pattern was obtained when the rejection was delayed by azathioprine. Elevation of alpha-2 globulin fraction, as Hardin pointed out(8), may be due to an inflammatory process, or it could be the result of noncytotoxic antibodies, such as hemagglutinins directed against a graft, as described by Mitchison and Dube (9). West and his associates(3), who also demonstrated an increase in alpha-2 fraction following kidney allotransplantation, believe it to result from inflammatory reaction or necrosis. Our sham operated animals did not show any significant alteration in this serum alpha-2 globulin levels rendering unlikely the relationship between surgical trauma and inflammation to the elevation in this globulin fraction. It is therefore more likely that the rise in alpha-2 globulin represents the formation of a non-cytotoxic graft specific anti-

TABLE V. Changes in Serum Proteins After Sham Operation (Group V).

		Serum protein (alpha 2, gamma globulin) levels (g/100 ml)					
			Weeks after sham operation				
Exp No.	Serum protein fraction	Control (8 wk avg pre-sham operation)	1	2	3	4	5
213	α_2	.56	.51	.50	.42	.50	.55
	γ	.77	.71	.77	.68	.65	.78
227	α_2	.55	.64	.59	.54	.55	.57
	γ	.51	.57	.54	.60	.60	.65
228	α_2	.44	.51	.34	.26	.30	.32
	γ	.78	.76	.69	.61	.65	.69
241	α_2	.38	.38	.31	.41	.37	.35
	γ	.80	.80	.87	.80	.86	.86
251	α_2	.63	.59	.59	.68	.55	.56
	γ	.63	.58	.55	.57	.56	.56
Mean ± S.E.	α_2	.51	.53	.47	.46	.45	.47
		±.05	±.04	±.06	±.07	±.05	±.06
		p	<.80	<.50	<.40	<.30	<.50
	γ	.70	.66	.68	.65	.66	.71
		±.06	±.04	±.06	±.04	±.05	±.05
		p	<.60	<.80	<.50	<.60	<.90
% Change	α_2		+3.9	−7.8	−9.8	−11.8	−7.8
	γ		−5.7	−2.9	−7.1	−5.7	+1.4

body. Gamma globulin levels were not altered significantly while the graft was functioning. However, this fraction rose immediately after removal of the graft, and in most cases the elevation was maintained for a prolonged period. When rejection was delayed by injection of azathioprine, gamma globulin levels failed to rise until rejection and removal of the graft. From these results, it appears that the change in gamma globulin fraction is related to the allograft rejection. Although the presence of graft specific cytotoxic antibodies in this fraction has not been shown, it seems likely that they are present but are removed or fixed by the grafted tissue. As the graft is removed, these antibodies are no longer fixed and their presence is then reflected by a rise in serum gamma globulin.

Summary. Serum electrophoretic changes in animals receiving allotransplantation of the heart were studied. Two major changes were observed: an early elevation in alpha-2 globulin and a late (post rejection) increase in gamma globulin fraction. The rise of alpha-2 globulins could not be correlated with cytotoxic changes leading to rejection. It is therefore felt that the initial rise in alpha-2 globulin represented a non-cytotoxic graft specific antibody. The change in gamma globulin seemed to be closely related to rejection and possibly represented cytotoxic graft specific antibody.

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