Studies on the Transplanted Heart. Role of Gamma-Globulin in Transplantation Immunity.* (28755)

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Previous studies from this laboratory(1) have suggested that homograft rejection results from the action of humoral antibodies since there was increased capillary permeability of the graft before cellular infiltration occurred. Similar results were obtained in experiments in which the isolated heart was perfused with cell-free sensitized plasma(1). Experiments performed by Najarian and Feldman(2,3) are further evidence for the importance of humoral antibodies. Their findings confirmed that physical contact with sensitized cells is not necessary to elicit accelerated rejection, and that a humoral agent liberated by cells is capable of homograft rejection. As indirect evidence, Good and his co-workers(4,5) found that skin homograft to patients with congenital agammaglobulinemia resulted in a prolonged survival. The present studies were designed to explore further the role of the plasma gamma-globulin fraction in homograft rejection.

Material and method. Mongrel dogs weighing from 15 to 30 kg and puppies weighing 3 to 5 kg were used. All transplantations of the heart were performed as second set homograft as previously described(6). Blood samples for serum electrophoresis were drawn from the recipient animal before and after grafting at intervals from 2 to 3 days. Paper electrophoresis was performed in a Beckman model R, Durrum type cell with veronal buffer at pH 8.6, ionic strength 0.075. Degammaglobulinization of the plasma for exchange transfusion was performed by the method of Pennell(7), which consists in precipitation of gamma-globulin by zinc diglycinate. Plasma thus obtained was mixed with an equal volume of stored red blood cells. Fresh solution of 6-mercaptopurine (6-MP) was prepared every 2 days by dissolving the powder in 1 N sodium hydroxide. Gross and histological studies were performed on all transplanted heart; when 6-MP was used, spleen, liver, bone marrow and lymph nodes of the recipient were also examined. The control group consisted of 10 animals sensitized with spleen cells from the donor 6 days prior to transplantation. In the second group, exchange transfusions using approximately 2000 cc of red blood cells suspended in degammaglobulinized plasma were performed on 6 sensitized animals one day prior to transplantation. Plasma infused into the animals contained about one-third of the normal gamma-globulin (0.23 g/100 cc). The third group consisted of 10 animals treated with 6-MP. The drug was given intramuscularly (0.5 mg per kg of body weight daily) starting on the day of sensitization with spleen homogenate.

Results. 1. Changes in serum gamma-globulin level in control animals. Changes in serum gamma-globulin level and survival time of the grafts are shown in Table I. At time of grafting, 6 days after sensitization, the gamma-globulin level had increased in 7 cases out of 10 (Table I). Fig. 1 illustrates that gamma-globulin levels continued to increase even after the grafts had been rejected and removed. Seven grafts were rejected within 24 hours after transplantation and histological studies showed an accelerated reaction consisting of early widespread necrosis, and intense polymorphonuclear cell infiltration. In 3 cases (#413, 422 and 436) the gamma-globulin level in serum failed to increase (Table I). Although one animal died on the fourth day of transplantation before the graft was rejected, 2 other grafts survived for 8 and 20 days respectively. Sec-

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TABLE I. Changes in Serum Gamma-globulin Following Sensitization.

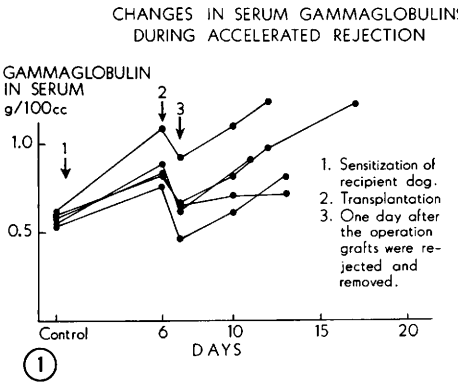
Exp #	Gamma-globulin level, g/100 cc Control	At grafting	Survival time, days
23	.46	.62	1
24	.93	1.38	1
377	.60	.82	1
378	.62	1.09	1
437	.55	.89	1
438	.41	.58	1
441	.58	.82	1
413	.45	.20	8
422	.48	.39	4*
436	.20	.22	20
Mean # 23-441	.59	.89	1
" #413-436	.38	.27	10.7

* Animal died before graft was rejected.

tions of both grafts showed almost intact myocardium with minimal cellular infiltration.

2. Effect of exchange transfusion on survival time of grafts. Results obtained on sensitized animals subsequently subjected to exchange transfusion are shown in Table II. As expected, the gamma-globulin level had increased 5 days after sensitization; after exchange transfusion, it was reduced to 50% of this value. However, survival time of the graft was not prolonged and histological studies showed the typical pattern of accelerated rejection as described previously.

3. Effect of 6-MP on plasma gamma-globulin levels and graft survival time. Following 6-MP administration, 6 grafts out of 10 survived more than 10 days. In spite of sensitization of the animals, the gamma-globulin level decreased to less than 59% of control value in 7 cases out of 10 (Table III). In cases #401, 402 and 408, low gamma-globulin levels were maintained beyond one month and the grafts survived for that period. Histology of these grafts showed almost intact myocardium with minimal cellu-



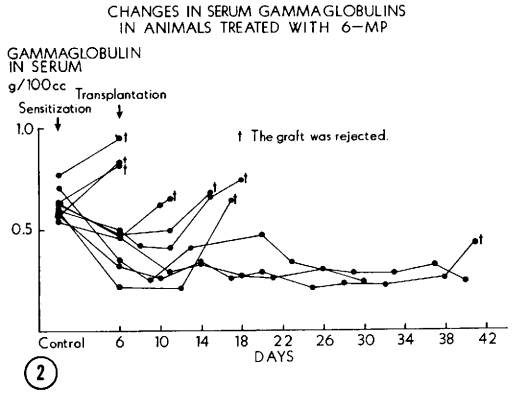


FIG. 1. Changes in serum gamma globulin during the second-set homograft rejection. Serum gamma globulin level increased following sensitization and continued to rise even after rejection of the graft.

FIG. 2. Changes in serum gamma globulin in the host animal treated with 6-mercaptopurine. In most cases serum gamma globulin level fell; in spite of sensitization of the animal the grafted heart survived as long as levels of serum gamma globulin remained low.

TABLE II. Changes in Serum Gamma-globulin Following Exchange Transfusion.

Exp #	Amt blood in exchange transfusion, cc	Gamma-globulin level, g/100 cc Control	Exchange transfusion Before*	After	Survival time, days
376	1900	.59	.62	.33	2
386	1900	.61	.67	.34	1
387	1900	.44	.56	.40	1
388	1850	.83	1.11	.50	1
389	1850	.67	.88	.41	1
390	2000	.78	1.04	.63	1
Mean	1900	.65	.81	.43	1

* Gamma-globulin level 5 days after sensitization and before exchange transfusion.

TABLE III. Changes in Serum Gamma-globulin in the Animal Treated with 6-Mercaptopurine (0.5 mg/kg).

	Gamma-globulin level, g/100 cc			
Exp #	Control	During survival of graft	At rejection	Survival time, days
380	.57	—	.83	1
392	.77	—	.95	1
393	.60	.49	.74	14
395	.63	.49	.68	10
401	.63	.29	—	40 *
402	.58	.26	.43	39
404	.64	—	.82	1
405	.54	.47	.65	5
406	.58	.26	.64	11
408	.71	.32	—	31 *
Mean	.63	.37	.72	15.3

* Animal died before graft was rejected.

lar infiltration. In the remaining 4 cases, gamma-globulin levels were lowered initially but increased several days later, and grafts were rejected soon after this (Fig. 2). Two animals died before rejection of the grafts probably because of respiratory infections. In cases #380, 392 and 404, the treatment with 6-MP was ineffective—gamma-globulin level increased markedly after the sensitization and grafts were rejected within 24 hours. Histological examination of these 3 grafts showed an accelerated rejection. Histological studies on the organs from the animals treated with 6-MP revealed striking atrophy of the lymphoid tissue in the spleen and lymph node, fatty change of the hepatic cells and biliary stasis in the liver, and atrophy with fatty replacement of the blood forming cells in the bone marrow.

Discussion. The results illustrate that sensitization of the recipient animal with spleen homogenate is followed by an increase in serum gamma-globulin level suggesting the development of humoral antibodies against cellular fractions derived from the spleen cells. The grafts in these animals showed accelerated rejection. Three cases in this group showed no appreciable increase in serum gamma-globulin level; this may be due to inadvertent extraperitoneal administration of spleen cells or to a defect in the immune response system. The grafts in these animals failed to show accelerated rejection. Treatment with 6-MP resulted in prolonged graft survival. There was a significant decrease in serum gamma-globulin levels, and the duration of hypogammaglobulinemia coincided with the survival time of the graft. The effect of 6-MP on antibody production and homograft survival is well known(8,9,10,11, 12).

Our results indicate a positive correlation between homograft survival and serum gamma-globulin level. Reduction of gamma-globulin in the sensitized animal by exchange transfusion failed to prolong survival time of the graft, although the procedure resulted in lowering of serum gamma-globulin (50%) to values as low as those observed during treatment with 6-MP. A possible explanation for this difference may be that the antibody production system remained intact, whereas it was depressed during treatment with 6-MP.

Summary. Sensitization of the recipient animal with spleen homogenate resulted in a significant increase in the serum gamma-globulin fraction and subsequent accelerated rejection of the graft. In exceptional cases in which serum gamma-globulin level did not increase, the accelerated rejection was absent. Treatment with 6-MP reduced the gamma-globulin level and the grafts in these animals showed prolonged survival. Reduction of gamma-globulin by exchange transfusion failed to prolong survival time of the graft. These results are in accord with the view that humoral factors present in the gamma-globulin fraction play an important role in homograft rejection.

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Effects of Drugs on the Electric Knife Fish, *Eigenmannia virescens.** (28756)

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Although fish have been used for bioassay of drugs(1,2,3), they have not been found generally useful, due in part to problems encountered in quantitating their responses. In previous reports from these laboratories(4,5), it was determined that the electric transparent knife fish, *Eigenmannia virescens,* responded quantitatively to neurotrophic polypeptides and to lysergic acid diethylamide. This paper presents an extension of studies with the electric transparent knife fish to observations on the effects of methylphenidate, pentylenetetrazole, phenobarbital, morphine, and nalorphine.

Methods and materials. Two days before each experiment, the fish was put in an individual 20 l aquarium. The pH of the water in the aquarium was maintained at 8.2 with Longlife Aqua-age buffer (Longlife Fish Food Product, Derville, N. J.) and temperature was kept at 25°C. Since these fish are sensitive to bivalent cations, the water was pre-treated with EDTA, 200 mg per 20 l. On the day of experiment, the aquarium was divided into 2 unequal compartments by means of a plastic grid. The fish was maintained in the smaller compartment. Two silver electrodes (E_1) were placed in opposite corners of the compartment containing the fish. Another pair (E_2) was placed in the larger compartment. Electrodes E_2 were placed approximately 1 cm from each other and as far from the fish as possible without jeopardizing recording because of distance or interference from extraneous signals. Because of their relative positions, electrodes E_1 recorded primarily movement, whereas E_2 tended to treat the fish-generator as a point source, and predominately recorded changes in amplitude. The dependence of E_1 on movement and the failure of movement to modify E_2 were checked when the electrodes were positioned and constituted the major criteria for electrode placement. The sides of the aquarium were made opaque with cardboard and the top was covered with red plastic to allow observation and minimize photic stimulation. The fish was permitted to acclimate to this new environment for a period of 2 hours. Electrical activity was amplified and the information from each electrode pair was recorded on a dual channel tape recorder for one hour. The test drug was then administered and the tape recorder was turned off, except in the case of pentylenetetrazole, in which instance recording was continuous. After an appropriate interval (see Results), recording was resumed. The electrical activity of the fish was also monitored by means of a cathode ray oscilloscope.

E. virescens produce a continuous electrical discharge of a frequency of approximately 300 cps. The frequency is constant for individual fish and was not modified by drugs. However, the amplitude of the signal

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