8. Battifora, H., Eisenstein, R., McCreary, P., Laing, G. H., Am. J. Path., 1966, v48, 421.
9. Condie, Good, Progress in Microbiology IV, The Biology of Myelin, Hoeber-Harper, 1959, Chap. XII.
10. Paterson, P. Y., Didakow, N. C., Proc. Soc. Exp. Biol. and Med., 1961, v108, 768.
11. Kabat, E. A., Wolf, A., Bezer, A., J. Immunol., 1952, v68, 265.
12. Paterson, P. Y., Beisaw, N. E., ibid., 1963, v90, 532.
13. Levine, L., Cowan, K., Osler, A., Mayer, M., ibid., 1953, v71, 359.
14. Lumsden, C. E., Brain, 1949, v72, 198.

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Effect of Large Doses of Subcellular Fractions on Skin Graft Survival in Mice. (30987)

T. TAMIMI, G. J. VOSIKA, AND J. B. AUST
Dept. of Surgery, Medical School, University of Minnesota, Minneapolis

Injection of large doses of disrupted cells has been successfully used for induction of tolerance to skin graft in mice across the weak sex histocompatibility barrier(1-5).

The distribution of transplantation antigens in the different subcellular fractions was tested by the ability of the latter to produce sensitivity to a subsequently placed skin graft (6). The purpose of this paper is to describe the distribution of transplantation antigens as manifested by their ability to induce tolerance to skin graft across the Y, H_3 and H_2 histocompatibility barriers. Additional pretreatment of the recipients with sublethal doses of irradiation and continuous administration of antigen were also investigated in some of these animals.

Materials and methods. Preparation of antigenic material. The donor animals were sacrificed by ether inhalation. The spleen and liver were immediately removed and put in a 0.25 M sucrose solution at pH 7.2 and 5°C in the ratio 1:4 by volume. The organs were homogenized in an Omni mixer at 16,000 r.p.m. The homogenate was then frozen and thawed 3 times in acetone-CO_2 snow. This treatment resulted in complete disruption of all the cells. The homogenate is then centrifuged in a Lord centrifuge at different speeds depending upon the preparation used. The refrigerated Spinco-ultracentrifuge was used to prepare the 100,000 *g* sediment and supernate. The material was stored at —20°C until used. The time of storage did not exceed one week.

Administration and dosage. In the experiments across the Y histocompatibility barrier (C_{57}BL male to female) the 2,000, 10,000, and 30,000 *g* sediments were given intraperitoneally in doses of 0.25 ml daily for 10 days which corresponded to 9, 3 and 10 mg of dry tissue weight respectively. The 30,000 *g* and 100,000 *g* supernate were administered intraperitoneally in doses of 1 ml daily for 10 days. The 100,000 *g* sediment was diluted with sucrose solution to its original volume and injected I.P. in doses of 1 ml daily for 10 days.

In experiments designed to cross the H_3 histocompatibility barrier difference, the 10,000 *g* and 30,000 *g* sediments were given together as 0.25 ml intravenously for the 1st dose and thereafter daily intraperitoneally for 15 days. The 10,000 *g* supernate which excludes the nuclear and mitochondrial fraction was given in a 1 ml dose intravenously followed by intraperitoneal daily injection of 1 ml for 15 days.

In experiments designed to overcome the H_2 histocompatibility barrier the antigen was injected in a similar manner. The number of daily injections was increased to 20 days.

X-ray irradiation. A dose of 300 r (measured in air) was delivered with a General Electric Maximar 220 X-ray machine under the following conditions: 15 MA, 220 KVP, 60 cm FSD, ¼ mm Cu + 1 mm AL filter. Dose rate under these circumstances was 47 r/min measured in air. Antigen injections

TABLE I. Effect of Large Doses of Subcellular Fractions on Male to Female C_{57}BL Skin Graft.

Treatment	No. tolerant	%	P value
None	5/61	8	
Male 2,000 G Sed	6/21	30	<.05
" 10,000 G "	19/34	56	<.005
" 30,000 G "	22/37	59	<.005
" 30,000 G Sup	12/52	23	<.1
" 100,000 G Sed	7/16	44	<.05
" 100,000 G Sup	5/15	30	<.05
Female 10,000 G Sed	0/3	0	
" 30,000 G "	2/10	20	
" 30,000 G Sup	1/10	10	

were started 24 hours following the irradiation.

Skin grafting. In all these experiments, skin grafts were placed the day following the last injection. The recipients were all 2 months old. Grafts were examined daily for signs of rejection. Tolerance was considered to be the survival of a healthy graft for 100 days in the case of C_{57}BL and 30 days in other strain combinations.

Results and discussion. It is evident from Table I that the transplantation antigens are widely dispersed throughout the cell with maximal concentration in the 10,000 *g* and 30,000 *g* sediments which correspond to the mitochondrial and microsomal fractions respectively. Of importance is the fact that the 100,000 *g* supernate still retained antigenic activity. This might be due to the presence of a soluble form of the antigen. Table II shows the effect of additional pretreatment with X-irradiation on the skin graft survival in Balb/c to DBA/2 mice. The effect of timing and continuation of the antigen injection are also included. It is obvious that there is a synergistic effect (75% tolerant) when X-irradiation and antigen injection were used together (Group 4). When antigen was given alone (Group 2) or sublethal doses of irradiation were given together with DBA/2 antigen (Group 7), the percentages of tolerant mice were 28 and 12.5% respectively.

The continuous administration of antigen after grafting (Group 5) resulted in 60% tolerance (thus simulating the injection of viable replicating cells). The grafts were ultimately rejected in spite of the continuous antigen injections. Mortality rate appears to be related to amount of manipulation, number of antigen injections and amount of X-irradiation. Although many animals showed gross evidence of peritonitis, no microscopic studies were performed and we cannot tell the cause or mechanism of death.

The use of the subcellular fractions across the H_2 histocompatibility barrier was unsuccessful in producing tolerance or in prolonging the skin homograft survival.

Summary. 1. The use of large doses of subcellular fractions can successfully induce tolerance across the weak sex-linked histocompatibility difference. The transplantation

TABLE II. Effect of Large Doses of Subcellular Fractions on the Skin Graft Survival in Male Balb/c to DBA/2 Mice.

Exp group	Antigen donor	Subcellular fraction	Daily dose (ml)	Time of antigen injection	X-irradiation	No. of grafts surviving 30 days	% of grafts surviving 30 days	% mortality
1	None				—	0/30	0	0
2	Balb/c	10,000 g & 30,000 g sed	.25	20 days before grafting	—	4/13 (P =.023)	28	13
3	"	10,000 g sup	1	*Idem*	—	3/13 (P —.019)	25	40
4	"	" "	1	*Idem*	+	3/4 (P <.001)	75	81
5	"	" "	1	20 days before grafting and continued till rejection	—	3/5	60	75
6	"	" "	1	after grafting till rejection	—	1/10	10	41
7	DBA/2	" "	1	before grafting	+	1/8	12.5	67

antigens are widely dispersed throughout the cell with maximal concentration in the 10,000 *g* and 30,000 *g* sediments. 2. A low incidence of tolerance was also noted when the H_3 histocompatibility difference was crossed. This was increased either by additional pretreatment with sublethal doses of irradiation or continuation of the antigenic injection after grafting, both of which resulted in a much higher mortality rate. The use of subcellular fractions in an attempt to produce tolerance across the H_2 histocompatibility difference was not possible in this study.

1. Medawar, P. B., Transplantation, 1963, v1, 21.
2. Martinez, C., Blease, M., Good, R. A., Nature, 1963, v198, 1319.
3. Kelly, W. D., Smith, J. M., Martinez, C., Good, R. A., Proc. Soc. Exp. Biol. and Med., 1964, v115, 8.
4. Kelly, W. D., Brow, I., Fed. Proc., 1964, v23, 452.
5. McKneally, M. F., Kelly, W. D., Resnick, J., Martinez, C., Surg. Forum, 1964, vXV, 131.
6. Monaco, P. A., Wood, L. M., Russell, S. P., ibid., 1964, vXV, 133.

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Ligation of Mouse Submandibular Glands and Its Effect on Components of Nerve Growth Factor.* (30988)

PEDDRICK WEIS AND ELMER D. BUEKER
Department of Anatomy, New York University, College of Dentistry and Graduate School of Arts and Science, New York City

A protein which enhances the growth of spinal and sympathetic ganglia and their processes has been found in various tissues and secretions of chicks, reptiles, and in several mammalian species(1,2). The richest source of this nerve growth factor (NGF) is the mouse submandibular gland(3). Fluorescent antibody studies have indicated that NGF is located in the serous tubule(4), a structure whose size is dependent upon androgens(5). It has been recently demonstrated that the diameter of the serous tubule (Tubular Index) and the log of the concentration of NGF in the mouse submandibular gland are directly proportional(6).

Mouse NGF has two components, both of which must be present for biological activity. As described by Schenkein and Bueker(7), these are: 1. a protein, "A," with a molecular weight of about 8,600 and 2. a dialyzable peptide, "C," with a molecular weight of about 3,500.

Junqueira reported on the effect of excretory duct ligation on the rat submandibular gland(8). Ligation caused complete atrophy of acinar cells, while the tubular cells, though diminished in size, retained their basic structure. Since this change was atrophy rather than degeneration, he called the ligated gland a "resting gland." To contribute additional observations on the mode of biosynthesis of NGF, we have investigated the concentrations of, and isotope-labeled amino acid incorporation into, "A" and "C" in mouse submandibular glands in the "resting" state which results from ligation. Of special interest was the possibility of correlating the fluorescent antibody localization of NGF in the serous tubules with the retention of only the tubules in the "resting" gland, along with the continued production of NGF in ligated glands.

Method. Adult male Swiss white mice were anaesthetized with intraperitoneal injections of pentobarbital-sodium. Their submandibular ducts were ligated with 4/0 silk sutures just at the site of leaving the glands, with individual sutures used on each side. The single midline incisions were closed with interrupted silk sutures. At 5-day intervals after the operations, 5 animals were injected intraperitoneally with ^{14}C-algal protein hydrolysate† for a total of 3.33×10^6 counts per

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