NPSH in both the deficient and the control groups.

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Antinuclear Factor in Sensitized Lymph and Serum from Skin Homografted Dogs.* (28516)

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The transfer of transplantation immunity with sensitized lymphoid cells has been considered as strong evidence that homograft rejection is a form of delayed-type or cellular hypersensitivity. However, recognition that transferred, sensitized cells are capable of producing humoral antibody(1,2) as well as the failure to observe apparently significant numbers of such cells in grafts undergoing rejection(3,4) disallows for absolute exclusion that the host *vs* graft reaction may be mediated by a humoral factor or factors. Najarian and Feldman noted accelerated rejection of skin homografts in mice(4) and guinea pigs (5) and Kretschmer and Perez-Tamayo(2) in rabbits when transferred, sensitized lymphoid cells were enclosed in cell-impenetrable millipore chambers. The former have also recently observed rejection of skin homografts with the transfer of the clear supernate obtained from sonicated, sensitized lymphoid cells to the graft site(6). The active material had many of the characteristics of a gamma globulin. This information as well as the recognized inconsistencies concerning the demonstration of transplantation antibodies in serum indicates the pertinence of investigating lymph of homografted animals for possible humoral factors which may play a role in homograft rejection.

The purpose of this report is to record the occurrence of an antinuclear factor as demonstrated by the indirect fluorescent antibody technic in the supernate of lymph in approximately 50% of dogs sensitized with a full-thickness skin graft.

Materials and methods. Mongrel dogs of both sexes weighing 13-23 kg were utilized in all experiments. Animals were maintained on Ken-L-Ration and water *ad lib.* Full-thickness homografts or autografts of skin were applied to the inner aspect of the right thigh with aseptic technic. Lymph fistula was produced 3-14 days following grafting in recipient and donor dogs anesthetized with pentobarbital sodium (24 mg/kg). Utilizing sterile surgical technic the muscles in the anterior cervical triangle were severed above the manubrium and the jugulo-subclavian junction exposed. A long piece of polyethylene tubing (PE 90-190) or silastic tubing (X-7002-062) was inserted into the thoracic

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TABLE I. Incidence of Antinuclear Factor in Recipient, Donor and Autografted Dog Lymph and Serum at Various Times.

	3d*	5d	7-9d	14d	Total	(%+)
Graft + recip. lymph	2/5†	6/11	3/6	2/2	13/24	54.2
" + recip. serum	0/5	0/9	0/4	0/2	0/20	0
" + donor lymph	0/5	0/9	1/6	0/2	1/22	4.5
" + donor serum	0/5	0/10	0/5	0/2	0/22	0
" + ACG (direct)	0/5	0/10	0/6	0/2	0/24	0
Donor + recip. lymph	2/5	2/10	3/8	2/2	9/25	36
" + recip. serum	0/5	0/8	3/6	0/2	3/21	14.3
" + donor lymph	0/5	0/9	0/8	0/2	0/24	0
" + donor serum	0/5	0/9	0/7	0/2	0/23	0
" + ACG (direct)	0/5	0/9	0/8	0/2	0/24	0
Host + recip. lymph	2/5	1/11	1/8	1/2	5/26	19.2
" + recip. serum	0/5	0/8	2/6	0/2	2/21	9.5
" + donor lymph	0/5	0/11	0/8	0/2	0/26	0
" + donor serum	0/5	0/9	0/7	0/2	0/23	0
" + ACG (direct)	0/5	0/11	0/8	0/2	0/26	0
Autograft + lymph			0/6		0/6	0
" + serum			0/6		0/6	0
" + ACG			0/6		0/6	0
Indiff. skin + positive recip. lymph		0/3				0

* Days after grafting.

† # positive/total examined.

duct and immobilized with tacking sutures. Accessory lymphatic channels were ligated and divided. The free end of the tube was brought through a separate skin incision and the neck incision closed. Division of the neck muscles minimized dislodgement of the tubing from the thoracic duct. Lymph was collected for 2-3 hours into a sterile beaker immersed in ice chips. Ten ml of blood was withdrawn at this time and the animals were sacrificed. The cell free supernate of lymph was obtained by centrifugation at 4°C, 2000 RPM and quickly frozen until used which was 24 hours later.

Portions of graft, host, donor and occasionally indifferent dog skin were removed and bisected. One-half was fixed in 10% formalin, dehydrated and imbedded in paraffin in the usual manner. Sections were cut and stained with hematoxylin and eosin. The other was quickly frozen on dry ice and stored at −20°C. Sections of these were prepared in a cryostat and stained with hematoxylin and eosin and the direct and indirect fluorescent antibody technic. The latter consisted of the application of recipient and donor lymph supernate and serum samples prior to staining with anti-canine gamma globulin (anti-CG) or anti-canine albumin (anti-CA) conjugated with fluorescein isothiocyanate. Outline of the staining sequences is presented in Table I. Anti-CG and anti-CA were prepared as described previously(7) as well as purchased commercially.† The purity of all conjugates was assessed prior to use by immunoelectrophoresis. In selected instances sections were treated with deoxyribonuclease (.01%, 37°C, 3 hr) or unconjugated canine gamma globulin prior to application of lymph or sera and conjugated anti-CG. In some instances conjugated anti-human or anti-rat gamma globulin were utilized instead of anti-CG in the staining sequence. Fluorescence microscopy was performed with an OSRAM HGO 200 mercury lamp and Zeiss exciter and barrier filters.

Proteins of test lymph and sera were analyzed by zone electrophoresis as described previously(8).

Results. As indicated in Table I preferential localization of gamma globulin was not observed by the direct fluorescent antibody technic with anti-CG in any section of skin examined. On the other hand, brilliant nuclear fluorescence was encountered in sections of approximately 50% of the homografts when they were pretreated with their respective samples of recipient lymph and subsequently anti-CG (Fig. 1A, B, C). In many instances fluorescence was confined to nuclei

† Antibodies, Inc., Davis, Calif.

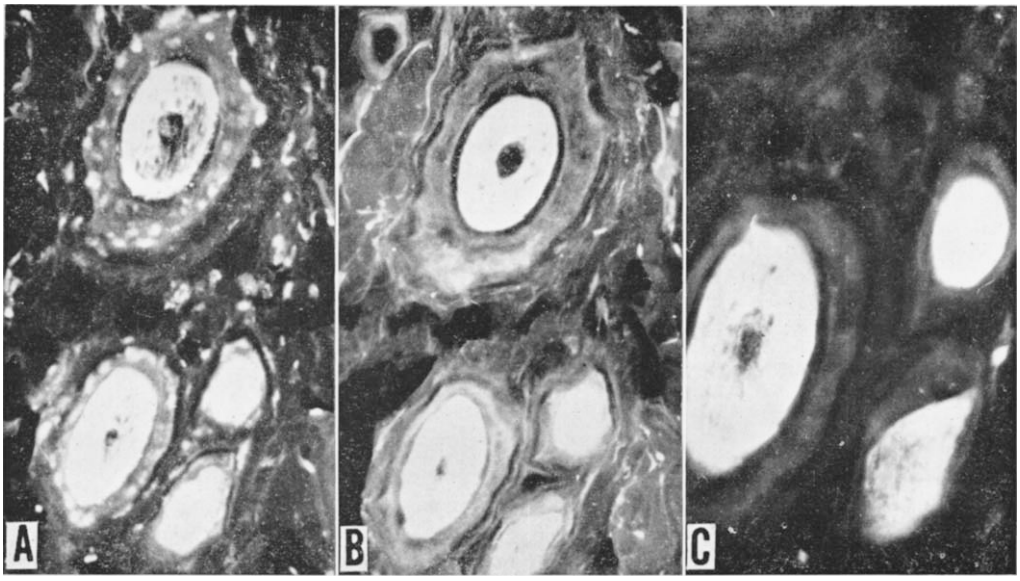

FIG. 1 A. Portion of 5 day homograft treated with its sensitized lymph stained with anti-CG. Nuclei of hair sheath and fibrocytes of corium exhibit specific fluorescence but that of hair shaft is due to non-specific staining (× 250). B. Serial section of skin depicted in A treated with donor lymph prior to staining. Nuclei are unreactive. Some non-specific fluorescence and autofluorescence are evident (× 250). C. Serial section of skin depicted in A & B but pretreated with recipient serum. Nuclei are unreactive (× 400).

of epithelium comprising the epidermis and skin appendages, although not infrequently nuclei of mesenchymal tissues also exhibited this reaction. The reaction was not abolished with pretreatment of the skin sections with deoxyribonuclease but nuclei were unreactive in those instances in which unconjugated anti-CG preceded use of the conjugated preparation and when conjugated anti-CA or anti-human or anti-rat gamma globulins were utilized as the marker. Approximately one-third of the donor and one-fifth of the normal host skins exhibited nuclear fluorescence with recipient lymph. In only one instance was a positive reaction with donor lymph encountered and that was with graft skin. Approximately 20% of donor skin and 10% of host skin revealed nuclear fluorescence when treated with recipient serum and conjugated anti-CG. All examples of autografts as well as indifferent skins pretreated with sensitized lymph yielding a positive reaction were unreactive.

A statistically significant increase in gamma globulin, total protein and albumin was observed in recipient or sensitized lymph as compared to that obtained from donor animals (Table II). Absolute correlation between degree of elevation and presence of antinuclear factor, however, was not observed. A decrease in total serum protein, albumin and beta globulin was noted in recipient serum. Elevation of total protein and albumin was noted in lymph from autografted dogs.

Discussion. The results indicate that the supernate of sensitized lymph often contains a demonstrable antinuclear factor in its gamma globulin fraction which reacts with epithelial and less frequently mesenchymal nuclei of their respective homograft and donor skin, and less often "normal" host skin. The nature of the reaction with the latter tissue is as yet unclear. One possibility in this regard is that it may represent a reaction to damaged or altered nuclei of the host's tissues at the graft site, although none of the lymph samples obtained from autografted animals contained antinuclear factor. Only one of 22 specimens of donor lymph contained an antinuclear factor which reacted with epithelial nuclei of one homograft. It seems unlikely that the nature of this false positive reaction is due to the release of

TABLE II. Lymph and Serum Proteins of Recipient, Donor and Autografted Dogs.

	#	T.P. (g%)	Alb. (g%)	α_1 (g%)	α_2 (g%)	β (g%)	γ (g%)
Recipient							
Lymph	18	3.96 ± .90*†	1.65 ± .72†	.32 ± .13	.44 ± .12	.61 ± .31	.83 ± .30†
Serum	12	4.92 ± .66†	1.98 ± .68†	.37 ± .13	.69 ± .19	.64 ± .14†	1.25 ± .38
Donor							
Lymph	13	2.69 ± .96	1.00 ± .40	.29 ± .13	.36 ± .15	.61 ± .28	.44 ± .35
Serum	18	6.30 ± 1.1	2.66 ± .65	.38 ± .16	.82 ± .31	1.29 ± .71	1.14 ± .34
Autografted							
Lymph	6	3.98 ± .53†	1.84 ± .60†	.27 ± .06	.47 ± .12	.87 ± .24	.53 ± .32
Serum	8	5.23 ± .70	1.99 ± .88	.34 ± .09	.78 ± .17	.89 ± .21	1.23 ± .30

* Standard deviation.

† P = .01 or <.01 as compared to donor.

DNA-bound antinuclear factor by such manipulation as freezing and thawing which has been demonstrated in some normal human sera(9) since all preparations were handled in an identical manner. The failure to observe antinuclear factor in all lymph specimens examined may be due to chronological variations in degree of sensitization produced by different homografts or actual alteration of nuclei during rejection rendering such structures unreactive. Although fewer samples contained antinuclear factor at 3 days following grafting and all exhibited nuclear binding at 14 days there appear to be too few examples studied at such time intervals to warrant conclusive statement. Also sections of graft at this latter time were more difficult to evaluate since rejection was advanced. Although the elevation of the gamma globulin fraction of sensitized lymph may reflect antinuclear or other humoral factors in this material no consistent correlation between the former and presence of antinuclear factor was evident. The presence of antinuclear factor in only a few serum samples and lack of change in serum gamma globulin also suggests that such antinuclear factor gains access into the general circulation but may in most instances be subject to dilution or other alteration disallowing for its demonstration by the technic employed.

Whether this antinuclear factor represents a non-specific reaction to damaged tissue as is suspected in some so-called collagen and autoimmune disorders or is of pathogenetic significance in homograft rejection is uncertain. The failure to observe such factor in the lymph of autografted animals might be considered evidence contrary to the former. Further, we have observed prolonged homograft survival in dogs with lymph fistula of short duration. Such animals did not exhibit apparent nutritional deficit which might otherwise account for prolonged survival. More significant, however, in this regard are preliminary observations which indicate that injections of sensitized, but not control lymph, into homograft sites accelerate their rate of rejection.

Summary. A factor reacting with epithelial and mesenchymal nuclei of 54% of skin homografts in dogs, 36% of donor and 19% of host's normal skin could be demonstrated in their respective host's, sensitized lymph supernate by the indirect fluorescent antibody technic. Recipient serum less frequently contained this factor and only against nuclei of donor (14%) and host's normal skin (9%). Pretreatment of skin sections with Dnase failed to alter this reaction. Lymph from homografted dogs exhibited elevation of total protein, albumin and gamma globulin. However, this latter could not be absolutely correlated with the occurrence of antinuclear factor. The precise role of antinuclear factor in homograft rejection remains to be demonstrated.

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Chronic Catheterization of the Coronary Sinus. (28517)

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The effects on the coronary circulation of naturally occurring and pathological stresses can now be quantitated by means of an electromagnetic flowmeter chronically implanted on the left coronary artery of the unanesthetized resting or active dog(1). It seemed desirable, therefore, to attempt chronic catheterization of the coronary sinus to provide an accessible pathway for sampling coronary sinus blood in combination with the left coronary flow so as to permit simultaneous studies of left ventricular metabolism(2).

Methods and materials. Catheters were prepared from a 30 cm segment of polyvinyl tubing (I.D. .042 in., O.D. .074 in.) and a 9 mm segment of surflene tubing† (I.D. .042 in., O.D. .062 in.). One end of the surflene tubing was flanged to a dimeter of 3 mm by gentle heating. The polyvinyl segment at one end was enlarged by stretching it over the tip of a sharp-pointed hemostat, and sleeved over the non-flanged end of the surflene tubing, a distance of approximately 5 mm, thus forming a 1 mm groove between the end of the polyvinyl tubing and the surflene flange.

Previously trained mongrel dogs weighing 16-18 kg were anesthetized with intravenous pentobarbital (30 mg/kg). Respiration was maintained through an endotracheal tube connected to a positive pressure respirator supplied with room air. Although catheterization of the coronary sinus could be performed with greater facility through the right chest, a left thoracotomy was generally used.

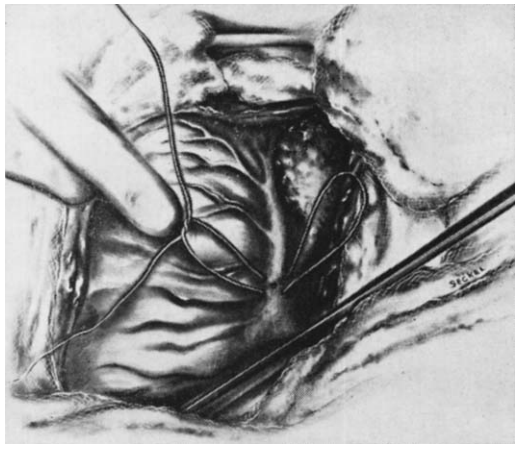

FIG. 1. Placement of horizontal mattress suture perpendicular to longitudinal axis of the coronary sinus.

This permitted simultaneous implantation of an aortic catheter for arterial pressure measurement, and of flow transducers on a coronary artery and the ascending aorta. The left chest was opened in the 4th intercostal space and the heart was suspended in a pericardial cradle. For clear visualization of the coronary sinus, the left ventricle was gently elevated and rotated anteriorly toward the right. A horizontal mattress suture (4-0) was placed superficially in the wall of the coronary sinus approximately 2.0 cm central to the coronary ostium (Fig. 1). An assistant made a preliminary hitch and held the free ends of the suture under slight tension while the operator held the opposing loop. This maneuver controlled and elevated the wall of the sinus, and thereby facilitated opening and cannulating the coronary sinus. A sharp-pointed 15-gauge needle was used to

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