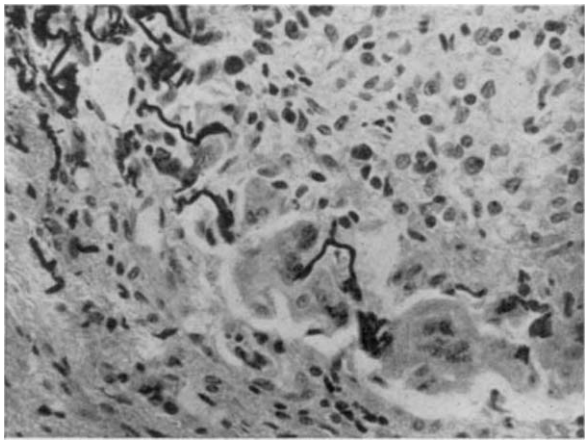

Fig. 2. Section of common carotid artery, twenty-one days after the same experiment as presented in Fig. 1. (Weigert's resorcin fuchsin and Van Gieson, × 492)

From these results, it is clear that elastase has an elastolytic activity *in vivo* and the fragments of elastic fibres exist as foreign bodies, and it is reasonable to presume that the following conditions are necessary for the occurrence of elasticophagic giant cells: (1) a specific destruction of elastic fibres to provoke giant cells—for example, hard and slightly soluble fragments of internal elastic lamina; (2) a mesenchymal activation brought about by some factors.

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[1] Shimizu, K., and Sano, K., *Rinsho Geka*, **3**, 377 (1948).
[2] Gilmour, J. R., *J. Path. Bact.*, **53**, 263 (1941).
[3] Kimmelstiel, P., *et al.*, *Arch. Path.*, **54**, 157 (1952).
[4] Baló, J., and Banga, I., *Nature*, **164**, 491 (1949).
[5] Hall, D. A., *Biochem. J.*, **59**, 459 (1955).
[6] Kunz, K., *Virchow's Arch.*, **332**, 469 (1959).

IMMUNOLOGY

Anti-elastin Antibodies in Normal and Pathological Human Sera

Soluble peptides obtained by alkaline–alcoholic degradation of bovine aorta-elastin were shown to be antigenic in rabbits[1]. Though these peptides (the so-called x-elastin[2]) are of relatively low molecular weight, passive haemagglutination titres up to about 1/1000 could be obtained[3].

Specific precipitation tests were then performed on normal and immunized rabbit sera, using ^{131}I-labelled x-elastin[4]. These experiments showed that even normal rabbit serum precipitated with labelled elastin peptides if incubated for 1 week in the cold. The antibody titres were quite low, and only 1–2 per cent of the labelled peptides were present in the precipitate[4]. These results were similar to those obtained by Grabar for antigelatine antibodies[5]. The experiments were then extended to normal and pathological (atherosclerotic) human sera in order to explore a possible relationship between anti-elastin antibodies and pathological modification of the vessel wall.

Rabbit red cells were coated with x-elastin prepared as described[1,2], using tetrazotized benzidine as a coupler, and the passive haemagglutination test[6] performed with serial dilutions of decomplemented sera. Controls for agglutination of uncoated red cells and for self-agglutination were included in every run. We report here the

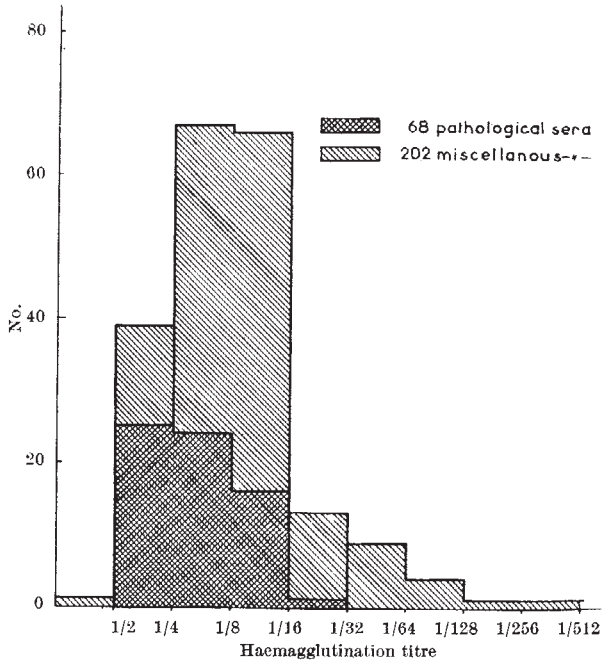

Fig. 1. Frequency distribution of anti-elastin titres in human sera

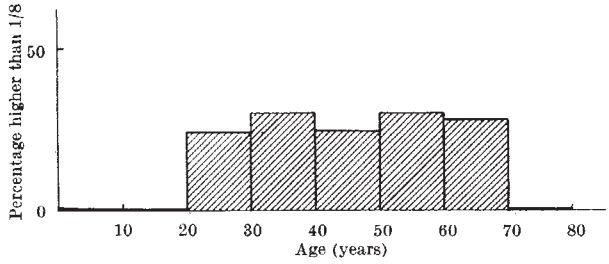

Fig. 2. Anti-elastin titres in 100 human sera as a function of age

results obtained on the first 200 sera examined. Fig. 1 shows the frequency-distribution curves of haemagglutination titres for 68 severe atheromatous patients and 134 miscellaneous sera ('normal' sera, or from patients without recognizable signs of atheromatosis). Though most of the sera studied showed a very low titre (1/2–1/16), about 15–20 per cent of the normal population exhibited significantly higher titres (1/32 to 1/512). In the atherosclerotic sera, the distribution frequency of the low titres was similar to that in the normal population, but titres higher than 1/32 were not encountered. Fig. 2 gives the age distribution of titres higher than 1/8 for the whole population studied. It can be seen that sera with titres higher than this arbitrarily chosen level belong to individuals between 20 and 70 years of age. Younger and older ones have titres between 1/2 and 1/8.

The specificity of anti-elastin antibodies was investigated by absorbing them to fibrous elastin (prepared by the alkaline extraction procedure[2] from bovine aorta), to insoluble collagen (bovine hide powder), or to bovine serum albumin (Armour). Twenty mg of each protein was added to 1 ml. of serum, incubated for 1 h at 37° C and overnight in the cold, then centrifuged. The supernate was tested for anti-elastin antibodies and the adsorption cycle repeated until constant titres were obtained. After 1–2 adsorptions of sera on fibrous elastin, the haemagglutination titre vanished (decreased to 1/2 or even below). Neither collagen nor BSA had any effect on the haemagglutination titres.

These results seem to indicate that anti-elastin antibodies are present in human sera. The low titres observed are probably due to the fact that only a fraction of the

peptides of κ-elastin carries the antigenic determinant. The absorption of these antibodies from the circulating blood to the elastic fibres in the vessel wall or elsewhere has also to be considered. We believe that desmosine and isodesmosine, the particular amino-acids involved in the cross-linking of elastin[7], may play an important part as antigenic determinants[1,3]. If this is so, wide cross-reaction between elastins of different species (carrying these same amino-acids) could be expected.

It seems probable that the anti-elastin antibodies occurring in human sera can be considered as auto-antibodies. It can be hypothesized that cathepsin-like enzymes slowly degrade elastin[8] and release soluble peptides into the circulation. These peptides would elicit the antibody response. As elastin is an insoluble structural protein, its soluble derivatives might not be recognized as 'self' by the competent cells. These antibodies would have a tendency to react with elastic fibres, mainly at places where the degradative process renders them more accessible. An antigen-antibody complex could be formed by such a mechanism in the vessel wall. Such complexes may very well play a part in the formation or spreading of a local tissue lesion. One example of such a reaction is, for example, the immune nephritis[9,10].

According to our hypothesis, the degradation of elastic tissue followed by antibody formation against the degradation products may be important in the pathological alteration of the vessel wall and especially in atheromatous plaque formation. This would explain the presence of anti-elastin antibodies in most of the human sera that were examined, as well as the absence of higher titres in the sera of severely atheromatous patients. In the latter, according to the hypothesis, the elimination of antibodies by adsorption to the eroded elastic structures would be more intensive than in normal individuals.

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[1] Robert, L., Parlebas, J., Poullain, N., and Robert, B., *Protides Biol. Fluids*, **11**, 109 (1963).
[2] Robert, L., and Poullain, N., *Bull. Soc. Chim. Biol.*, **45**, 1317 (1963).
[3] Robert, L., Stein, F., Pezess, M. P., and Poullain, N., *J. Atherosclerose* (in the press).
[4] (In preparation.)
[5] Grabar, P., *Ann. Inst. Pasteur*, **88**, 11 (1955).
[6] Halpern, B. N., Jacob, M., Binaghi, R., and Parlebas, J., *Rev. Fr. Allergie*, No. 4, 201 (1961).
[7] Thomas, J., Elsden, D. F., and Partridge, S. M., *Nature*, **200**, 651 (1963).
[8] Robert, L., and Robert, B., *Exposés Ann. Biochimie Médicale*, **24**, 269 (1963).
[9] Baxter, J. H., and Goodman, H. C., *J. Exp. Med.*, **104**, 467, 487 (1956).
[10] Lange, K., and Wenk, E. J., *Amer. J. Med. Sci.*, **228**, 448, 454 (1954).

Influencing the Survival of Skin Homografts by a Lymphatic Fistula

The lymphatic system plays an important part in a number of processes evoked by homografts. By an extirpation of the regional lymph nodes it is possible to prolong the survival of a skin graft[1-3]. A similar effect can be obtained by injuring the nodes by radioactive radiation or nitrogen mustard[2,4].

The influence of the extirpation of nodes is of relatively short duration because further nodes are then linked with the lymphatics and take over the task of regional nodes[5,6]. We therefore tried to develop a lymphatic fistula in the regional lymphatic node and to evoke an interruption of the lymph system lasting for a longer period.

Table 1. Influence of Lymphatic Fistula on Survival of Skin Homografts

Skin graft only (days)	Drainage (days)	Air-pump drainage (days)	Air-pump drainage and irrigation (days)
14	13	17	27
16	15	52	19
12	23	23	34
15	13	23	51
10	12	20	35
12	51	70	18
	16	18	23
	23	23	19
	23	49	
		12	
Mean 13·1	21·0	30·7	28·2

Chinchilla rabbits weighing 2,500–3,000 g were used. Full-thickness skin grafts were transplanted from black donors to the ear of the Chinchilla recipients which were of different colour. Fistulae were developed in such a way that half the regional lymphatic node was extirpated after ligation of the blood supply and with a patent afferent lymphatic vessel; a drain of an inner diameter of 7 mm was sutured to the area which was thus created.

The first group consisted of control animals without drainage of the lymph system. In the second group we left only animals with drainage. In the third group we connected the drainage to an air-pump of a constant pressure of $-0{\cdot}2$ kp/cm². In the fourth group we irrigated the injured surface of the node with a physiological solution containing heparin. After seven days the fistula was removed.

During the experiments all rabbits were kept in a special harness and injections of 'Heparin Retard Spofa' in doses of 4,000 units were administered subcutaneously twice daily.

Table 1 shows the results of the experiments. In the control group skin homografts on the ear of the rabbit survived an average of 13·1 days (10–16 days) until complete necrosis. In the second group the survival was 21·0 days on average (13–51 days). In the third and fourth groups the survival was practically the same, averaging 30·7 days (12–70 days) without irrigation and 28·2 days (18–51 days) with irrigation.

The results show that a long-term fistula with the possibility of lymph flow considerably prolongs the survival of grafts as compared with a control group as well as with a group without suction. We can therefore assume that in these two series the integrity of the lymph system is renewed much later as compared with experiments where only extirpation of the lymph nodes was performed.

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[1] Vrubel, J., Paper read at Ann. Conf. Inst. Clin. and Exp. Surgery, Prague (May, 1958).
[2] Vrubel, J., *Folia Biol.*, Prague, **7**, 103 (1961).
[3] Stark, R. B., Dwyer, E. M., and De Forest, M., *Ann. N.Y. Acad. Sci.*, **87**, 140 (1960).
[4] Vrubel, J., and Vavrejn, B., *Albertova sbirka* sv. 37, 162, SZdN (1961).
[5] Málek, P., and Kolc, J., *Rozhl. Chir.*, **38**, 441 (1959).
[6] Vrubel, J., and Vrubelová, H., *Nature*, **193**, 1094 (1962).

Chromosome Marker Studies in the Graft-versus-Host Reaction in the Chick Embryo

The production of splenomegaly and other lesions by the injection of adult avian blood into the chick embryo is due to an immunological reaction between donor blood cells and host embryo tissues[1,2]. Biggs and Payne[3] have shown that the reaction is accompanied by proliferation of donor cells within the host spleen. They were able to distinguish donor (male) cells from host (female) cells by