bines rapidly and stoichiometrically with purified C'1 esterase (*2*, *3*). The highly purified EI contains about 12 percent hexose and 17 percent *N*-acetylneuraminic acid (*4*). These properties, together with its sedimentation constant and absorbancy ($E^{1\%}_{1cm}$) were strikingly similar to those of α_2-neuraminoglycoprotein (α_2NGP) isolated by Schultze *et al.* from human plasma (*5*). This report presents immunologic and electrophoretic data which indicate that this inhibitor and the glycoprotein are identical.

The esterase inhibitor was isolated from serum in highly purified form (*6*), and α_2NGP was purified from human plasma (*5*). The α_2NGP preparation was 90 to 95 percent pure and contained a small amount of orosomucoid. Each protein was dissolved (4 mg/ml) in 0.005*M* sodium phosphate buffer, *p*H 6.7. Rabbit antiserum to EI was prepared by repeated injections of purified EI into rabbits; the antiserum was made monospecific by absorption with serum from a patient with hereditary angioneurotic edema, a disease in which there is little or no circulating EI (*7*). Horse antiserum to whole human serum was obtained from a commercial source.

The esterase inhibitor and α_2NGP reacted with rabbit antiserum to EI to form a line of complete identity (Fig. 1). A line of identity was also found with the horse antiserum to human serum. In addition, a second line was formed between α_2NGP and horse antiserum to human serum, indicating a small amount of impurity (orosomucoid) in the former.

Immunoelectrophoresis (*8*) of EI and α_2NGP against rabbit antiserum to EI and horse antiserum to whole serum is shown in Fig. 2. Both EI and α_2NGP produced a single arc in the α_2 position against monospecific rabbit antiserum to EI (Fig. 2a) and horse antiserum to whole human serum (Fig. 2b); further, a mixture of equal volumes of EI and α_2NGP also produced a single, somewhat less symmetrical arc in the same position (Fig. 2c). The EI concentration in normal serum is probably too low to form a precipitate arc against the antiserum used (Fig. 2a).

A comparison of the two protein preparations by analytical disc-gel electrophoresis (*9*) showed that, in 10 percent acrylamide gel, both EI and α_2GNP produced a similar broad band relatively close to the top of the separating gel; the presence of a trace amount of orosomucoid in the α_2NGP preparation was demonstrable as a faint, more anodic band. The broadness of the major bands increased in gels of lower acrylamide concentration.

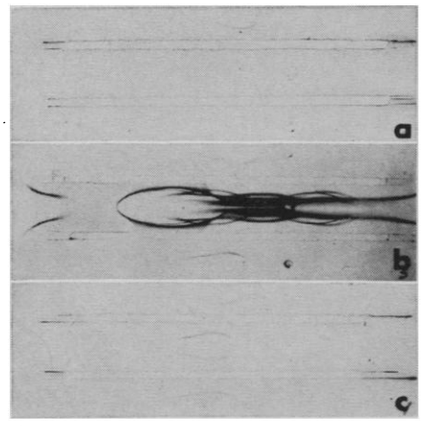

Fig. 2. Immunoelectrophoresis. (a) Top well, C'1 esterase inhibitor; center well, normal human serum; bottom well, α_2-neuraminoglycoprotein. Troughs contain rabbit antiserum to C'1 esterase inhibitor. (b) Top well, C'1 esterase inhibitor; center well, normal human serum; bottom well, α_2-neuraminoglycoprotein. Troughs contain horse antiserum to normal human serum. (c) Top well, C'1 esterase inhibitor; bottom well, α_2-neuraminoglycoprotein; center well, mixture of equal volumes of samples used in top and bottom wells. Troughs contain rabbit antiserum to C'1 esterase inhibitor. The anode is to the left.

Although a more rigorous chemical comparison is lacking, it appears from the immunochemical evidence that EI and α_2NGP are the same protein. The preparation of α_2NGP was inactive in inhibiting C'1 esterase because of the acid conditions used in its isolation; the ability of EI to inhibit C'1 esterase is also irreversibly destroyed below *p*H 5.5 (*2*).

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30 October 1968

Mechanism and Prevention of Fixed High Vascular Resistance in Autografted and Allografted Lungs

Abstract. *The fixed vascular resistance observed in transplanted lungs and attributed to denervation can be avoided by angioplastic widening of the pulmonary artery anastomosis. With distensible arterial anastomoses, autografted and allografted lungs vasodilate normally with increased flow and can sustain dogs whose contralateral pulmonary artery is ligated immediately after lung transplantation.*

Transplantation of a lung with simultaneous ligation of the opposite pulmonary artery has caused death of the recipient animal within a few days (*1*). Death has been attributed to fixed, increased intrinsic vascular resistance in the transplanted lung regarded as consequent to its denervation (*1*, *2*). Forcing the transplant to accept the entire pulmonary blood flow has been thought to cause either fatal right heart failure or pulmonary insufficiency secondary to damage to the pulmonary microvasculature. In view of the fact that human recipients of lung transplants will have vascular disease in the remaining lung, the ability of laboratory animals to survive transplantation of one lung and simultaneous ligation of the opposite pulmonary artery is important in determining the feasibility of therapeutic lung transplantation.

We studied five groups of six dogs each, before and after the right pulmonary artery (RPA) was ligated through a left thoracotomy. Group 1 comprised normal control animals. In animals of group 2, a snug-fitting but not constricting cloth band was placed around the left pulmonary artery (LPA) before ligation of the RPA. In animals of group 3, the LPA, the left mainstem bronchus, and a cuff of the left atrium containing the left pulmonary veins were transected. The lung was then removed from the chest, perfused with 300 ml of cold (4°C) Ringer's lactate solution (U.S.P.) at a pressure of 25 cm-H_2O, and replaced in the chest. Vascular and bronchial continuity was restored with fine, continuous, braided dacron sutures. Then the RPA was ligated. Lungs were ischemic for 50 to 70 minutes. In animals of group 4, the left lungs were removed and replaced like those in group 3 except that the diameter of the pulmonary artery at the site of suture was increased by a patch of jugular vein

Table 1. Response in each group to experimental procedure. Values represent means and standard errors. PAP, mean pulmonary artery pressure; PVR, total pulmonary vascular resistance; LLBF, left lung blood flow; LLVR, left lung vascular resistance; LPA, left pulmonary artery; RPA, right pulmonary artery; and LPAG, the mean pressure gradient across the anastomosis or band.

Group No.	Before manipulation				After operation and RPA ligation					
	PAP	PVR $\left(\frac{\text{dyne sec}}{\text{cm}^5}\right)$	LLBF (liter/min)	LLVR $\left(\frac{\text{dyne sec}}{\text{cm}^5}\right)$	Diameter LPA* (mm)	PAP* (mm-Hg)	LLBF (liter/min)	LLVR $\left(\frac{\text{dyne sec}}{\text{cm}^5}\right)$	Diameter LPA* (mm)	LPAG (mm-Hg)
1	14 ± 1	481 ± 46	1.1 ± .1	1068 ± 102	11.4 ± .6	22 ± 2	2.3 ± .2	765 ± 49	13.8 ± .7	0
2	17 ± 2	479 ± 50	1.3 ± .1	1064 ± 112	11.7 ± .6	30 ± 2†	2.0 ± .2	1323 ± 106†	15.0 ± .9	15 ± 1
3	15 ± 1	457 ± 44	1.2 ± .1	1012 ± 100	10.0 ± .2	30 ± 3†	1.5 ± .2	1589 ± 166‡	13.9 ± 1.9	16 ± 3
4	13 ± 1	470 ± 60	1.1 ± .1	1043 ± 133	11.2 ± .7	21 ± 2	1.9 ± .2	900 ± 101	13.2 ± 1.0	0
5	14 ± 2	474 ± 110	1.2 ± .2	1048 ± 241	11.8 ± .8	21 ± 2	2.1 ± .2	765 ± 99	13.9 ± .9	0

* Measured proximal to the site where an LPA anastomosis or band was or would be located. † Significantly different from comparable values in groups 1, 4, and 5 ($P < .05$). ‡ Significantly different from comparable values in groups 1, 4, and 5 ($P < .01$).

(Fig. 1a). In each animal in group 5, the left lung of an unrelated mongrel dog of equal size was implanted by the same methods except that a distensible pulmonary artery anastomosis was achieved by spatulation of the recipient and donor vessels (Fig. 1b).

In all groups, pressures in the pulmonary artery proximal and distal to the site of the anastomosis or band were measured with a pressure transducer connected to a silastic cardiac catheter. The position of the catheter tip was located by palpation. Total pulmonary blood flow and left lung blood flow (LLBF) after ligation of the RPA were measured by the dye dilution technique. Indocyanine green dye was injected through a catheter in the pulmonary artery, and blood was drawn through the densitometer from a catheter in the femoral artery by a continuous withdrawal pump. All measurements of pressure and flow were made within 15 minutes after manipulation. Pulmonary vascular resistance was calculated by the formula

$$VR = PA \times 80/F$$

where VR is pulmonary vascular resistance in dynes second centimeter^{-5}, PA is the mean pulmonary artery pressure in millimeters of Hg, and F is pulmonary blood flow in liters per minute. This simplified expression of pulmonary vascular resistance, in which left atrial pressure is disregarded, was used because measurement of accurate left atrial pressures in dogs receiving a transplant was technically difficult. In most animals, constancy of left atrial pressure before and after operation was confirmed by the constancy of the back pressure (wedge pressure) recorded when the pulmonary artery catheter was advanced to obstruct a branch artery. In calculating resistance of the left lung before ligation of the RPA, we assumed that 45 percent of total pulmonary blood flow was distributed to the left lung.

The hemodynamic changes that occurred in response to the experimental procedure within each group were consistent (Table 1). In normal animals (group 1), with occlusion of the RPA, the blood flow of the left lung increased, the vascular resistance decreased 27 ± 5 percent (mean ± S.E.), and the LPA increased 21 ± 3 percent in diameter and 46 ± 7 percent in cross-sectional area.

In animals of group 2, the LPA could not dilate in the region of the indistensible band. The failure of RPA occlusion to produce a decrease in the vascular resistance of the left lung in group 2 and its ability to do so in group 1 depended on the capacity of the LPA to dilate. In both groups, innervation was intact; thus, the high fixed vascular resistance in group 2 could not be due to denervation of the left lung. In animals with a denervated left lung but a normally distensible LPA and LPA anastomosis (groups 4 and 5), the vascular resistance of the left lung decreased normally after RPA occlusion, even though it often increased initially after transplantation and before the occlusion of the RPA (Table 2).

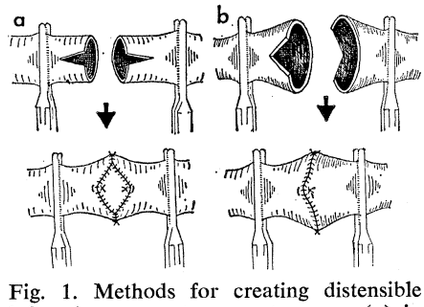

Fig. 1. Methods for creating distensible left pulmonary artery anastomoses: (a) in autografts (group 4); (b) in homografts (group 5).

The animals with a distensible LPA (groups 1, 4, and 5) contrasted with those in which distensibility of the LPA was limited by a standard anastomosis (group 3) or by a band (group 2). Despite differences in innervation between groups 2 and 3, the vascular resistance of the left lung in both failed to decrease after RPA occlusion, and pressure measurements in the LPA were comparable. The mean pressure gradient (0 to 2 mm-Hg) at the anastomosis or band before RPA ligation was insignificant, but a gradient of 10 to 22 mm-Hg developed after ligation. Thus 51 ± 5 percent of the fixed high vascular resistance of the left lung in these groups was located at the indistensible LPA band or anastomosis, which became the site of significant stenosis as the rest of the vessel dilated and flow

Table 2. Changes in left lung vascular resistance in a representative animal from each experimental group. PA, pulmonary artery.

Group No.	Left lung vascular resistance (dyne sec cm^{-5})				
	Before manipulation	After transplantation		After right PA occlusion	
		Proximal*	Distal†	Proximal*	Distal†
1	835			775	775
2	722			911	446
3	880	1048	920	1224	513
4	852	1023	1023	765	765
5	1117‡	1170	1170	759	759

* Calculated with pulmonary artery pressure measured proximal to site of PA band or anastomosis. † Calculated with pulmonary artery pressure measured distal to site of PA band or anastomosis. ‡ Measured in donor animal.

increased. Normal animals and those with a distensible LPA anastomosis (groups 1, 4, and 5) never developed a pressure gradient and had significantly lower mean pressures in the central pulmonary artery after RPA occlusion (Table 1).

Vascular resistance in all transplanted and nontransplanted lungs distal to any LPA narrowing was similar. In all cases, vascular resistance distal to the indistensible LPA decreased normally when flow to the left lung increased after RPA ligation (Table 2).

Thus, much of the fixed resistance noted in standard lung autotransplants can be attributed to the indistensible arterial anastomosis rather than to altered innervation. Denervation does not influence the capacity of the small pulmonary vessels to dilate in response to increased flows. Our results indicate that this form of pulmonary vasodilatation is largely a passive or mechanical phenomenon (*3*, *4*) which cannot occur without the normal distensibility of the large pulmonary arteries.

All animals in groups 2 and 3 died within 48 hours of operation. All the normal animals (group 1) and five animals in each group with a transplant and a distensible LPA anastomosis (groups 4 and 5) survived for more than 7 days after operation. The transplanted lung therefore did assume total pulmonary function while its vasculature carried the entire pulmonary blood flow.

Many (*1*, *2*, *5*) although not all (*6*) investigators have observed that transplanted lungs have a high vascular resistance. Ischemic injury to the pulmonary microvasculature, which is preventable (*7*), may be manifested by an elevated resistance. Obstruction to pulmonary venous drainage at the left atrial anastomosis may be a cause, but it too is preventable (*6*, *8*). The denervation inevitably accompanying transplantation has been thought the major cause of the high fixed vascular resistance observed in transplanted lungs (*1*, *2*, *9*). This belief was sustained by a report that division of all the connective tissue about the left pulmonary hilum, a procedure presumably denervating the left lung, prevented the vascular bed of that lung from dilating after RPA ligation (*2*). However, the electromagnetic flow-meter probes positioned about the LPA in those experiments (*2*) could have acted like the band in our experiments (group 2) and prevented LPA dilatation. The fixed vascular resistance of the left lung (*2*) also could have been produced by an indistensible LPA rather than by denervation. Our results indicate that the indistensible standard arterial anastomosis is responsible for the fixed resistance in grafted lungs and show that transplanted (denervated) lungs with normally distensible arteries and arterial anastomoses may have normal vascular resistance with the ability to vasodilate with increased flow.

Our findings regarding the regulatory mechanisms of the pulmonary vasculature (*4*, *10*) emphasize the importance of passive factors, especially the increase in cross-sectional area of the large pulmonary arteries, in the vasodilatation accompanying increased flow (*3*, *4*). Innervation is not necessary for this type of vasodilatation, and denervation of a lung does not produce a fixed high vascular resistance in that organ. Circulation in a denervated lung of an otherwise intact animal can now be studied without the necessity of measuring the flow to each lung.

Since transplanted lungs can vasodilate and carry the bulk of the pulmonary blood flow without damage and at tolerable pressures in the pulmonary artery, therapeutic lung transplantation may be considered in patients with severe pulmonary vascular disease. Total functional dependence may now be placed immediately on the grafted lung, and the efficacy of immunosuppressive programs and lung preservation techniques to be used in pulmonary transplantation can be evaluated better.

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Lactate Dehydrogenase Electrophoretic Variant in a New Guinea Highland Population

Abstract. *Six examples of a variation in the LDH-A subunit have been detected in 408 samples from three exogamous clans in the New Guinea Highlands. The New Guinea variant is similar to the Memphis-4 variant. Origin of the New Guinea variant could not be traced by genealogy but it seems likely to have persisted for several generations.*

Since Boyer and his colleagues (*1*) described the first genetically controlled variant of human lactate dehydrogenase (LDH; E.C.1.1.1.27) several further electrophoretic variants involving changes in either the A or B subunits have been reported. Vesell (*2*) has reviewed the evidence on the distribution of these variants: for 2103 whites in the United States and Europe, three A and one homozygous B; in 1585 U.S. Negroes, nine A and two B; in 223 black Africans, one A and one B; in 245 Turkish Cypriots, two A variants. In addition, an A variant has been described from Brazil. No LDH variants were found in studies of 100 Papuans, 238 Micronesians, 79 Xavante Indians in Brazil, and 284 American Indians. It appears that there is a higher chance of mutations in the A subunit being detected (16 A : 4 B) and that there is a greater frequency of LDH variants among U.S. Negroes and black Africans (0.72 percent) than among whites (0.19 percent). The number of persons studied in other ethnic groups is too small to permit useful comparison.

We have screened 408 samples from a Melanesian population in the New Guinea Highlands for LDH variants. Six persons showed identical variations in the LDH isozyme pattern after starch-gel electrophoresis of red-cell hemolyzates. For electrophoresis the bridge buffer we used was 0.2*M* phosphate–citric acid, *p*H 7.0, and gels were prepared from a 1 : 20 dilution of the same buffer