Mechanism of Epinephrine Pulmonary Edema

WITH SPECIAL REFERENCE TO RELATION OF VENOUS
RETURN TO PULMONARY CIRCULATION

TATSUYA TOMOMATSU & SUSUMU YORIFUJI

(Received for Publication, Jan. 6, 1961)

MUCH HAS been discussed in recent years on the pathogenesis of pulmonary edema. Some of the articles¹⁾⁻⁶⁾ on the subject have received good deal of attention for their interpretation of mechanism in its causation. It has been known that experimental pulmonary edema by various procedures has been attempted and the investigators have lead to accept the views that in the occurrence of pulmonary edema, a number of contributing factors seemed to participate. Because of the emphasis placed on these factors, it does appear that basic cause for pulmonary edema has become rather obscure. Since pulmonary edema is a serious complication not in frequently observed as the result in connection with open chest surgery either during or post-operative period it has aroused wide interest in recent years in this country, and extensive research has been undertaken⁷⁾⁻¹¹⁾, but the reports thus far appeared seemed far from what actually took place.

While it has been well established that pulmonary edema can be produced experimentally as well as observed in a number of clinical situations,¹²⁾ those contributing factors generally accepted were through the means of increased permeability of lung capillary besides neurogenic and cardiogenic origins. Among the three sources attributable to the edema of lung, increased permeability per se would be the most plausible reason in appreciating the mechanism, yet, there still remains a question as to what is responsible for the

abnormality of permeability.

It has been conclusively shown that the capillary permeability is dependent upon hemodynamic changes as well as deranged function of the capillary membrane. While the character of blood is another contributing factor as far as the developing edematous condition is concerned, it should be also considered that a factor which would specifically exert an effect on the pulmonary circulation must be kept in mind. And such a factor that possibly affects both the systemic and the pulmonary capillaries leaves no room for consideration. Almost probable reason yet convincing explanation for this disputed condition may be elucidated from hemodynamic change with the resultant disturbed function of the capillary membrane. Impaired capillary should be referred to the limited cases in which the damage be selectively involved in the pulmonary circuit. Such capillary malfunction is usually the result of inhaling some toxic gas or invasion by virulent microorganism and probably unrelated with the original problem of dispute.

Thus, the subject is focussed on the discussion as to how pulmonary edema be resulted from the hemodynamic changes. This implies that the investigation should be directed to the mechanism of redistribution of the blood; in another word, shifting of the blood from the blood depot in the systemic circulation to the lesser one. Authors have studied by means of inducing the pulmo-

nary edema in relation to hemodynamics with special reference to venous return which is closely related to hemodynamic regulation controlled through the buffer nerve mechanism.

METHOD

With the intravenous injection of epinephrine, pulmonary edema is induced in rats and rabbits, and progresses for a few minutes until death occurs. The minimal lethal doses are, as shown in Table I¹³⁾ and II¹⁴⁾, 0.5mg/kg in rats and 1.0mg/kg in rabbits. In order to inhibit the development of pulmonary edema induced by epinephrine, two kinds of the procedure were taken, viz., one to ligate a vessel or vessels, and the other ablation of the carotid sinus nerves. As it is difficult to produce pulmonary edema

TABLE I MINIMAL DOSE OF EPINEPHRINE REQUIRED TO PRODUCE PULMONARY EDEMA AND TO CAUSE DEATH IN RATS.

Dose of Epinephrine (mg/kg)	Number of Case	Lung-Body Index
Normal Control	25	0.78±0.12
0.1	5	0.83±0.05
0.2	5	0.86±0.03
0.3	6	0.91±0.04
0.5	20	2.54±0.67
1.0	5	2.64±0.13
2.0	6	2.65±0.57
4.0	5	3.48±0.76

TABLE III EFFECT OF PREMEDICATION OF VARIOUS KINDS OF ANESTHETICS ON EPINEPHRINE PULMONARY EDEMA.

Drugs used	Dose per kg	Route of Administration	No. of Cases	Lung-Body Index	Mortality (%)
Normal Control			25	0.78±0.12	0
Epinephrine	0.5 mg	i. v.	20	2.54±0.67	100
Urethan	10 g	i. p.			
Epinephrine	0.5 mg	i. v.	10	2.01±0.17	80
Morphine	20 mg				
Epinephrine	0.5 mg	i. v.	10	2.43±0.18	100
Phenobarbital	100 mg	s. c.			
Epinephrine	0.5 mg	i. v.	10	3.11±0.52	100
Chlorpromazine	10 mg				
Epinephrine	0.5 mg	i. v.	4	0.95±0.06	0

with epinephrine in dogs, the animals were used for observation of the hemodynamic change with epinephrine. To measure venous return, the electric conductivity method¹⁵⁾¹⁶⁾¹²⁾ was employed as reported elsewhere. For evaluating develop-

TABLE II MINIMAL DOSE EPINEPHRINE REQUIRED TO PRODUCE PULMONARY EDEMA AND TO CAUSE DEATH IN RABBITS.

Dose of Epinephrine	Number of Cases	Lung-Body Index
Control	5	0.39±0.02
0.1 mg/kg	5	0.40±0.02
0.4	5	0.47±0.09
0.5	5	0.69±0.08
0.75	5	0.37±0.15
1.0	5	0.98±0.11
1.5	5	0.94±0.09

ment or suppression of pulmonary edema it was based on the lung-body weight index as well as on the histo-pathological findings.

RESULTS AND COMMENT

With the dosage of 0.5mg/kg in rats and 1.0mg/kg in rabbits it was possible to induce epinephrine pulmonary edema without fail. The effect of anesthetics before the administration of epinephrine may have to be taken into consideration and the drugs other than chlorpromazine have indicated to give no influence as shown in the Table III¹³⁾.

The Fig. 1 shows that venous return in dogs can be enhanced with a smaller dose of epinephrine whereas with a larger dose, it will be reduced¹⁸⁾.

It was found that the epinephrine pulmo-

nary edema either in rats or in rabbits could be suppressed by ligating either jugular, portal or femoral vein and the animals survived from the pulmonary embarrassment, which signifies the fact that the lung-body weight

Fig. 1. Flow of inferior caval vein following various doses of epinephrine.

Fig. 2. Residual blood volume and pulmonary blood flow after epinephrine injection in the dogs.

Fig. 3. Simultaneous measurement of both right and left ventricular output after epinephrine in the dog.

index remained low. This was not the case, however, with the ligation of femoral artery. Because the dose of epinephrine required to produce pulmonary edema is of large one, venous return is likely to be reduced. From the results obtained in the two kinds of experiments it is to be interpreted that the reduction of venous return implies sluggish

return flow due to stagnant circulation of the right heart chamber including the central vein and the pulmonary circuit. The Fig. 2 shows¹⁹⁾ that the residual blood volume is increased in the both ventricles and the stroke volume is decreased when the large dose of epinephrine is given in dogs. The Fig. 3 indicates that in the simultaneous measure-

TABLE IV EFFECT OF LIGATION OF VESSELS ON EPINEPHRINE PULMONARY EDEMA.

Preexperimental Ligation of Vessels	Dose (mg/kg)	No. of Cases	Lung- Body Index	Mortality (%)	Effect
Control	0	25	0.78 ± 0.12	0	
	0.3	6	0.91 ± 0.04	0	
	0.5	20	2.54 ± 0.67	100	
V. Femoralis		17	1.42 ± 0.72	23	Inhibited
V. Jugularis	0.5	7	1.16 ± 0.36	26	
Portal V.		10	0.84 ± 0.23	0	
A. Mesenterica sup. & inf.		8	0.86 ± 0.15	0	
A. Femoralis	0.3	6	2.60 ± 0.56	100	Accelerated
A. & V. Femoralis	0.3	6	2.78 ± 0.29	100	
Laparotomy	0.5	8	1.94 ± 0.24	100	

TABLE V MINIMIZED EFFECT OF LIGATION OF VEIN BY TRANSECTION OR BLOCKING OF NERVES

		Number of Cases	Lung-Body Index	Pulmonary Edema	Effect
Ligation	Jugular	3	0.49	—	Suppressive
	Small Saphenous	3	0.46	—	
	Femoral Additive	3	0.43	—	
	Infusion of	3 cc/kg	3	0.49	
	Saline	15 cc/kg	3	1.03	+
Transsection or Blocking of Nerve	Femoral and Sciatic	4	0.76	+	Non Suppressive
	Lumbar cord	3	0.73	+	
	Lumbar anesthesia	3	1.13	+	
	Vagal	3	1.06	+	Accelerated ?
	Atropine	3	1.04	+	

TABLE VI EFFECT OF LIGATION OF VEINS ON EPINEPHRINE PULMONARY EDEMA IN ATROPINIZED RATS.

Procedure	Atropine 10 mg/kg	Number of Cases	Mortality (%)	Lung-Body Index.
Control	None	10	100	2.65 ± 0.28
Ligation of Vein	None	10	20	1.30 ± 0.50
or	Given	5	100	2.71 ± 0.41
Veins	Given	3	100	2.14 ± 0.27
Bilateral Femoral & Jugular	Given	5	40	1.41 ± 0.44

ment of cardiac output of both right and the left heart, cardiac output of the former apparently exceeds the one of the latter; circulation time through the lungs is retarded and the pulmonary blood volume is increased. As the animals actually survived from the fatal effect of the pulmonary edema by ligating the vein, the blood volume which is to return will be reduced, meaning the decreased blood volume would be bound to stagnate in the right heart chamber and the pulmonary vessels.

The suppressive effect of venous ligation on epinephrine pulmonary edema in rabbits, however, can be minimized as shown in the Table V²⁰⁾ by transsection of either femoral, sciatic or vagal nerve or lumbar cord, and besides by the administration of atropine. In other words, when these procedures are applied in advance it would be impossible to

prevent the epinephrine pulmonary edema by means of venous ligation, and death ensues invariably. Similarly, as the Table VI²¹⁾ shows the rats would be killed with atropine even though the ligation is applied to a femoral vein after epinephrine. It is thought, therefore, that the parasympathetic nerve is acting on the peripheral vessels as an inhibitor to venous return.

The effect of ligating any of these veins is practically identical. Not only is the reduction of inferior cava flow brought about to the almost identical level either by unilateral or by bilateral ligation of the femoral vein, but also even the superior cava flow is reduced (Fig. 4). This is well explainable on the basis of parasympathetic control of the peripheral vessel as has been referred.

The venous flow in the inferior cava vein is reducible by ligating as is shown in

Fig. 4. Change of venous flow in caval vein following ligation of a femoral vein and its release before and after transection of peripheral nerves.

Fig. 5. Change of venous flow in caval vein following ligation of a femoral artery and its release before and after transection of peripheral nerves.

the Fig. 4, but the transient reduction will be followed by marked response of increase—overshooting—when a femoral artery is ligated (Fig. 5). Nevertheless, this reactive overshooting does not appear if the sciatic nerve is cut. It does offer an explanation that the ligation of an artery serves a deferent effect from that of a vein as to epinephrine pulmonary edema. As the matter of fact, an animal subjected to the arterial ligation causes death with epinephrine even below the lethal dose.

The Fig. 6 refers to the effect of ligating a vein which varies a great deal with the time element¹³⁾. It should be noted that an effective measure would be that the ligation should be laid in a period between 20 and 60 seconds following the intravenous administration of epinephrine.

From the above two facts, it can be assumed that the development of pulmonary edema is closely associated with the buffer

Sub groups	Groups		No. of Case	No. of Death
A	1		6	2
B	2		3	1
C	2		3	0
D	3		11	2
E	3		2	0
F	3		4	0
G	4		3	3
		0 20 40 60 80 Sec.		

Fig. 6. Time element and outcome in ligation of femoral vein.

TABLE VII EFFECT OF DENERVATION OF CAROTID SINUS AND REPEATED INJECTION OF EPINEPHRINE.

	Denervation of Carotid Sinus	Repeated Injection of Epinephrine
Number of Case	14	12
Body weight (gr.)	220~110 (160.2)	210~98 (148.5)
Lung Weight (gr.)	2.50~1.00 (1.60)	2.42~0.95 (1.56)
Lung-Body Index	1.05±0.17	1.23±0.19
Mortality	13.0	8.0

Fig. 7. Blood pressure and cardiac rate after epinephrine.

Fig. 8 Cardiac rate following repeated injection of adrenalin (upper) and denervation of carotid sinus (lower).

TABLE VIII EFFECT OF CAROTID SINUS REFLEX ON EPINEPHRINE PULMONARY EDEMA.

Procedure	Number of Cases	Mortality (%)	Lung-Body Index
Control	10	90	2.27 ± 0.45
Bilateral Int. & Ext. Ligation of Common Carotid Arteries	9	78	2.30 ± 0.67
Denervation of Carotid Sinus	11	36	1.48 ± 0.28
	10	30	1.47 ± 0.26

nerve reflex. True to our thinking, the animals subjected to the ablation of carotid sinus nerves could survive, as shown in the Table VIII, even though epinephrine above the lethal dose was given, and the lung-body weight index remained low. Referring to the blood pressure and the cardiac rate, the latter begins to diminish and reaches to its minimum within thirty seconds after the lethal dose or above, and could be restored less than a minute. The restoration of the cardiac rate to the previous level was followed in less than several minutes by the second phase of bradycardia which will continue until death occurs. But in the case of sublethal dose the first phase of bradycardia gradually recovers in a few minutes to the normal level and remains. The blood pressure maintained its high level a longer period with above the lethal dose. Therefore, the effective period of venous ligation does correspond with the first phase of bradycardia.

The bradycardia of the first phase could be minimized in the rats subjected to denervation of the carotid sinus nerves or by the repeated injections of increasing dose of epinephrine; the latter procedure enables the rats to be tolerate well to above the lethal dose (Fig. 8).

The bilateral ligation of the common carotid arteries on the other hand exerts, as in the Table VIII, similar effect on epinephrine pulmonary edema to that of the ablation of carotid sinus nerves. The ligation of both internal and external carotid arteries, however, does not suppress the pulmonary edema, but may accelerate, as the ligation of the femoral artery does. Furthermore, the rats can tolerate the lethal dose of epinephrine,

Fig. 9. Blood reservoir used for maintaining the blood pressure at the constant level.

Fig. 10. Lung-Body index by application of the blood reservoir at the various stages following epinephrine.

when the blood reservoir keeping pressure 70 to 80 mmHg is connected to a femoral artery with a canula²²⁾, that is, when the canula is open death from pulmonary edema can be prevented, but the effect is lost when it is opened two minutes after epinephrine (Fig. 9, 10).

With the results observed it has been found that the carotid sinus reflex is intimately concerned in the development of pulmonary edema with epinephrine.

A question has arisen then, does the carotid sinus reflex accelerate the causation of the pulmonary edema? It has been demonstrated that the stimulation of the parasympathetic nerve ensued from the ligation of a vein acts against venous return, resulting in suppression of the pulmonary edema. Speaking of the parasympathetic stimulation, it is a yielded effect of the carotid sinus reflex. The parasympathetic discharge is working as an inhibitor in the former case and as an accelerator in the latter. Does such an inconsistency exist? If so, what can it be interpreted? Comparing the lung-body weight index after injection of

Fig. 11. Lung-Body index after injection of various doses of epinephrine when effect of carotid sinus reflex was modified by means of either bilateral ligation of common carotid arteries (left) or bilateral carotid sinus denervation (right).

TABLE IX CHANGES OF LUNG-BODY INDEX AFTER INTRAVENOUS INJECTION OF VARIOUS DOSES OF ATROPINE.

Dose of Atropine (mg/kg)	Number of Cases	Mortality (%)	Lung-Body Index
Control	25	0	0.78±0.12
0.5~ 1.0	5	0	0.84±0.09
5.0~10.0	5	0	0.78±0.12

various dose of epinephrine between the normal rats and those with the carotid sinus denervated, we have shown as in the Fig. 11. that with the dose used the lung-body weight index is increased in the normal rats, but definitely independent of the dose in the animals subjected to the ablation of the carotid sinus. As is clearly shown in the Fig. 11 the buffer nerve reflex is acting as an inhibitor, when a small dose, namely, below the lethal dose is used; but as an accelerator when a large dose, namely, the lethal or over the lethal dose. The same can be applied to the case of the bilateral ligation of the common carotid arteries in rats. Since the effect of the carotid sinus reflex is interpreted to elicit the parasympathetic discharge, the above results should be reproduced by the blockade of the vagal effect with atropine instead of carotid sinus denervation. Atropine itself dose not affect the lung-body weight index any more, as shown in the Table IX. The Fig. 12

Fig. 12. Variations of lung-body indices by atropinization of rats with administration of epinephrine.

shows that three horizontal lines give the average lung-body weight indices of 1.25, 2.27 and 2.66 obtained with epinephrine 0.3 mg/kg, 0.5mg/kg 1.0kg/mg respectively; that in the atropinized rats the lung-body weight index is elevated over the average level with a smaller dose of epinephrine, pulmonary edema being accelerated²³⁾, but is lowered by a larger dose, pulmonary edema being suppressed²⁴⁾²⁵⁾²⁶⁾²⁷⁾.

Thus, the same results have been attained. Therefore, the data being seemingly inconsistent, must be admitted to be true.

While these effects have been concerned with the parasympathetic discharge elicited by the buffer nerve reflex following the intravenous injection of epinephrine, from the hemodynamic point of view, especially venous return the authors have directed attention to the parasympathetic discharge originated following the sympathetic stimulation ensued by other measures than a direct action like epinephrine. It appears that venesection is the most suitable procedure for such purpose. As the Fig. 13¹⁷⁾ shows, venesection mobilizes blood out of the depots among which hepatic venous flow is apparently the largest,

causing reduction or loss of venous depot, which in turn acts to interfere possible occurrence of pulmonary congestion. Thus, it is readily understood that a venous depot is a source for an increment of venous return. The mobilization of blood from the depot is the result from the effect of the sympathetic stimulation presumably ensued by mediation of the carotis sinus reflex. The Table X indicates that no relation exists between the amount of blood withdrawn and the outcome²⁰⁾.

Fig. 13 Venous return in acute blood loss (upper) mobilization of blood from the depot (lower).

TABLE X EFFECT OF RAPID VENESECTION OF VARIOUS AMOUNTS OF BLOOD ON EPINEPHRINE PULMONARY EDEMA.

Volume of Blood Withdrawn %	Average	Number of Cases	Mortality (%)	Lung-Body Index
Control		10	90	2.27 ± 0.45
5	0.33	5	0	1.25 ± 0.26
10	0.65	10	0	1.01 ± 0.25
20	1.41	10	0	0.92 ± 0.16
30	1.88	5	0	0.82 ± 0.18
40	2.43	2	100	0.94 ± 0.05
50	2.89	2	50	0.78 ± 0.22
60	3.36	2	50	0.64 ± 0.08

TABLE XI EFFECT OF ATROPINIZATION ATOP OF VENESECTION ON EPINEPHRINE PULMONARY EDEMA.

	<i>Volume of Blood Withdrawn % average</i>	<i>Dose of Atropine (mg/kg)</i>	<i>Number of Cases</i>	<i>Mortality (%)</i>	<i>Lung-Body Index</i>
<i>Control</i>			10	90	2.27 ± 0.45
5	0.33	0	5	0	1.25 ± 0.26
	0.33	0.5	5	40	1.71 ± 0.59
	0.31	10.0	5	60	2.34 ± 0.67
10	0.65	0	10	0	1.01 ± 0.25
	0.63	0.5	5	0	1.23 ± 0.36
	0.70	10.0	5	20	1.40 ± 0.63
20	1.41	0	10	0	0.94 ± 0.16
	1.49	0.5	5	0	1.12 ± 0.21
	1.52	10.0	5	0	0.90 ± 0.21

CONCLUSION AND SUMMARY

If we were to assume that the sympathetic stimulation is inevitably followed by the parasympathetic discharge, just as the latter arises from the buffer nerve mechanism setting in action by the rise of the blood pressure caused with epinephrine, how can we bring the parasympathetic effect to light, which is covered by the conspicuous sympathetic conditions? The fact that the suppressive effect of venesection on the pulmonary edema is independent of the amount of blood withdrawn seems to have one believe the parasympathetic activity working behind the heightened sympathetic tone. The Table XI gives an explanation to it, that is, the suppressive effect caused by the small amount of blood loss would be minimized by atropine. In other words, one would assume that the parasympathetic tone is acting to keep retaining the rest of blood in depot. When the large amount of blood is withdrawn the parasympathetic tone would reach to the considerable high level and the large dose of atropine would be required to block the parasympathetic tone. However, such a suppressive effect was not observed even with a large dose of atropine. This evidence does not mean that the parasympathetic influence failed to enter, but it is due to presence of least reduced venous depot even under the

parasympathetic blockade, making the condition no longer possible to develop pulmonary edema.

The sympathetic stimulation induced either directly by epinephrine or indirectly by venesection, therefore, is associated with the parasympathetic effect, acting as a regulator to venous return.

At this time, the inconsistency referred previously must be explained, that is, the parasympathetic tone exerts the suppressive effect on the pulmonary edema, so long as the parasympathetic tone is at a low level, namely, when an epinephrine dose is small, it exerts a suppressive influence, but on the other hand, when the parasympathetic effect is enhanced by a larger dose, its effect is accelerative for pulmonary edema.

When such opposite results were observed, it should be natural to assume that there were two different kinds of actions functioning simultaneously. It could be concluded there are two kinds of suppressive actions; one is central, responsible for bradycardia and inhibition of the over-active cardiac work caused by epinephrine, and the other is peripheral, acting against arteriolar constriction and mobilization of venous depot. As shown in the Fig. 14, the central suppressive action exerts an accelerative influence on the pulmonary edema through which blood returning to the right heart can be retained

Fig. 14. Vagal effect elicited by sympathetic stimulation. Mild effect: peripheral suppression relatively large. Marked effect: central suppression considerably enhanced.

in the pulmonary circuit and, on the other hand, the peripheral suppressive action exerts a preventive effect on the pulmonary edema by a process to keep blood in the depots, namely, to prevent venous return from increasing. The peripheral suppressive action is dominant and more effective in a case epinephrine dose is of a small: the central suppressive action is enhanced remarkably in case of a larger dose and exceeds the former effect.

REFERENCES

- 1) Drinker, C. K., Pulmonary edema and inflammation. Cambridge, Massachusetts Harvard University Press 1950.
- 2) Altschule, M. A., Acute pulmonary edema. Grune & Stratton. New York. 1954.
- 3) Wassermann, S., Acute cardiac pulmonary edema. Charles C. Thomas. Springfield Illinois 1959.
- 4) Luisada, A. A., *Medicine* **19**: 475, 1940.
- 5) Luisada, A. A. and Cardi, F. J., *Circulation* **8**: 113, 1956.
- 6) Visschr, M. B., Haddy, F. J. and Stephens, G.: *Pharmacol. Review*, **8**: 389, 1956.
- 7) Wakisaka, J. and Kono, B., *The Lung*, **4**: 228, 1957. (in Japanese)
- 8) Hayashi, K. et al, *Ibid*, **4**: 242, 1957
- 9) Abe, K., Komatsu, H. and Kato, O., *Ibid*, **4**: 254, 1957.
- 10) Nagaishi, C., Sagawa, Y. and et al, *Ibid*, **4**: 261, 1957.
- 11) Higasa, R. et al, *Clinic All-Round*, **10**: 1750, 1960. (in Japanese)
- 12) Cameron, G. R., *Brit. M. J.* **1**: 965, 1948.
- 13) Ike, Y., *J. Jap. Soc. Int. Medicine*, **49**: 50, 1960. (in Japanese)
- 14) Odake, T., *Jap. Circulation J.*, **24**: 747, 1960.
- 15) Tomomatsu, T., Tanemoto, K., Ninomiya, H., Ito, T. and Inoue, Y., *Respiration & Circulation* **6**: 613, 1958. (in Japanese)
- 16) Tomomatsu, T. et al, *Ibid*, **8**: 109, 1960. (in English)
- 17) Ino, T., *Jap. Circulation J.* **24**: No. 11, 1960.
- 18) Cheng, K. and Janett, B. A., *Pathol. & Bacteriol.* **76**: 83, 1958.
- 19) Tanemoto, K., *Respiration & Circulation* **6**: 933, 1958. (in Japanese)
- 20) Odaka, T., *J. Jap. Soc. Int. Medicine*, **49**: 404, 1960. (in Japanese)
- 21) Morimoto, M., *Jap. Circulation J.* **25**: No. 1. 1961.
- 22) Nakamura, I., *Ibid*, **25**: No. 1. 1961.
- 23) Auer, J. and Gates, F. L., *J. exp. Med.* **24**: 201, 1917.
- 24) Campbell, G. S., Haddy, F. J., Adans, W. L. and Visscher, M. B., *Amer. J. Physiol.* **158**: 96, 1949.
- 25) Campbell, G. S. and Visscher, M. B., *Ibid*, **157**: 130, 1949.
- 26) Reichsman, F., *Am. Heart J.* **31**: 590, 1946.
- 27) Luisada, A. A. and Contro, S., *Circulation Research* **1**: 179, 1953.