Special Article

New Concept on Atherogenesis and Treatment of Atherosclerotic Diseases

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SUMMARY

"Das initiale fettfreie Ödem" has been long known by the German school as the initial stage of human atherosclerosis, and a highly similar phenomenon was found by the author and his collaborators (1960) to be regularly produced as an immediate response to a single administration of atherogenic substances like cholesterol or adrenaline in rabbits and rhesus monkeys. Moreover, the authors found that such an edematous arterial response is induced by a reduction of the active selective-permeability of endothelial cells resulting in an acute infiltration of plasma substances such as β-lipoprotein, fibrinogen and γ-globulin into the arterial wall at the same time, the repelling function of endothelial cells against platelet is reduced. As a consequence, the sticking of platelets to endothelial surfaces, as well as the reduction of adhesive platelet count and the shortening of several clotting times due to the release of platelet factors, occur and ADP-induced platelet aggregability is enhanced. When such an impairment of the function of endothelial cells was observed, these cells exhibited a contraction often accompanied by bleb formation. The contraction and blebbing of endothelial cells are proposed to be a key mechanism of atherogenesis and thrombogenesis by the author.

To prevent or treat atherosclerosis and thrombosis, a substance capable of preventing or restoring the above mentioned fundamental functions of endothelial cells, and of inhibiting the enhancement of platelet aggregability due to atherogenic stress, is essential. One such substance is pyridinolcarbamate, which has also been shown to relax endothelial cells and platelets. This compound has been tested experimentally and clinically in many countries of the world for almost 10 years, and a survey of the results of the clinical investigations has been briefly made on its efficacy as an antithrombotic and antiatherosclerotic agent.

Additional Indexing Words:
Atherogenesis Treatment of atherosclerosis Thrombogenesis
Endothelial cell-contraction Endothelial cell-relaxation Bleb
Anti-atherosclerotic agent

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ATHEROSCLEROSIS is today the major killer of human beings. Its prevention and the treatment of atherosclerotic disorders are the main concerns of modern medical science. In order to understand the present drug therapy for atherosclerosis, it is important to understand the background of the treatment.

Briefly summarized in this article are two concepts underlying the treatment of atherosclerotic disorders: the classical concept, which is widely understood and accepted; and a new concept based on recent experimental and clinical findings obtained by the worldwide application of pyridinolcarbamate.

1. The classical concept on atherogenesis and the treatment of atherosclerotic diseases

The clinical syndrome of atherosclerosis is commonly thought to be produced by inadequate irrigation of certain tissues, due to a narrowing of the arterial lumen. This condition may be chronic or may occur temporarily when an increased demand for blood exceeds supply. In the advanced stage, a dramatic syndrome is often produced by ischemic death of cells in the peripheral organs due to the thrombotic occlusion of arterial lumen, or by hypotension. This occurs when atherosclerotic plaques narrow the arterial lumen and reduce irrigation to a volume below the critical level for the peripheral tissue. It is well-known that the nerves, heart muscles and skeletal muscles lack regenerative ability, and that no drug can restore the function of these structures once it has been impaired by the death of their cells. Atherosclerotic lesions are composed of edematous fluid, atheromatous mass, regenerated smooth muscles, and scar tissue. The edematous fluid and atheromatous mass are proper targets of drug treatment and when the absorption of edematous fluid or atheromatous mass has been achieved in every part of the long arterial trees, a certain increase in the blood flow in the peripheral tissues may possibly be induced contributing sometimes, but not always, the improvement of some clinical symptoms. However, there had been no such a drug until pyridinolcarbamate. In addition, the major portion of an atheromatous lesion consists of scar or regenerated smooth muscles, especially in old and well organized lesions. Yet these are the main causes of the narrowing of the arterial lumen, which are not considered vulnerable to drug treatment. The occlusion of smaller groups of arteries by atheromatous plaque and organized thrombi is one of the common morbid conditions in advanced stages of atherosclerosis. Although fibrinolytic treatment is used in the early stage of thrombosis, the opening of such organized lesions, composed mainly of fibrous tissues, calls for surgical procedure rather than drug therapy. The above
mentioned facts suggest that drugs capable of curing edematous and atheromatous parts of the atheromatous lesions are not always effective for the relief of clinical symptoms after lesions have become established, although a certain increase in the blood flow is expected in certain peripheral tissues. Furthermore, the atheromatous lesion is known to be a progressive condition and, as such presents difficulties in the drug treatment of atherosclerosis.

In the progression of atherosclerotic lesions there are two well established mechanisms: the filtration of plasma (and especially of cholesterol-bearing lipoprotein) into the arterial wall;7)-10) and the adherence of platelets to the wall of the lesions resulting in thrombosis as well as the atherosclerosis of Rokitansky (1852)11) and Dugid (1948).12) In the treatment of such lesions the concentration of cholesterol of the plasma filtering through the arterial wall should be lowered and the sticking and aggregation of platelets should be prevented.2),3),5)

In order to reduce the filtration of cholesterol into the arterial wall, the simple lowering of the plasma concentration of cholesterol has been tried, and dietary treatment and hypocholesteremic drugs are being applied until today. Anticoagulants have also been tried in the prevention of atherosclerotic thrombosis without success. In addition, it should be emphasized here that atherosclerosis is common even among people like the Japanese, whose cholesterol level is lower than that of North Americans and Europeans. In fact, the cerebral atherosclerosis of Japanese is even severer than that of North Americans, as shown by Resch et al. (1967).13)

The limitations of the classical concept, e.i. the lipid filtration theory and thrombogenic theory as outlined above may be due to the fact that, during the period in which it became established, there was not enough knowledge on the contraction of endothelial cells and the "acute" infiltration of plasma constituents such as cholesterol-bearing plasma proteins into the arterial wall—a process now known to be induced by atherogenic stress as detailed in the next chapter.

2. A new concept on atherogenesis and a new treatment of atherosclerotic diseases

1) The acute alteration of the active selective-permeability function and repelling function against platelets of vascular endothelial cells by atherogenic stress

Even in the healthy arterial wall of rabbits, we found an acute and transient alteration of two functions of vascular endothelial cells by atherogenic stress: namely, the active selective-permeability function,10) and repelling function against platelets, leucocytes and other constituents of the blood.14) Acute infiltration of plasma constituents such as $\gamma$-globulin, $\beta$-lipoprotein and fibrinogen into the arterial wall,14) and also the sticking of platelets to vascular
endothelial surface as well as the enhancement of ADP-induced aggregability of platelets, have been clearly shown to be induced by a single administration of adrenaline (0.1–1.0 μg./Kg. i.v.) or cholesterol (1 Gm./Kg. p.o.) in rabbits as detailed below. Almost the same acute infiltration of plasma constituents such as β-lipoprotein and IgG into the arterial wall was found to be induced by the same procedures in rhesus monkeys by us as detailed below. The acute rise in catecholamines in the blood is, of course, a characteristic response of man and animals to physical and mental stress and the hypercholesteremic response to the negative mental stress has been recently reported by Shkhvatsabaya (1972). Here, it is clear that the simple hypocholesteremic procedures, used commonly today, is not enough in preventing the infiltration of cholesterol into the arterial wall, because they are ineffective in preventing the above mentioned “acute” infiltration of cholesterol into the vessel wall by atherogenic stress. Anticoagulants are also ineffective in preventing the platelet sticking and aggregation.

2) The behavior of endothelial cells and platelets under atherogenic stress

The behavior of endothelial cells and platelets under atherogenic stress is well observed by Sandison-Clark’s technique and also by direct observation of the microcirculation of the mesentery of animals, as shown by motion pictures presented at the First International Congress of Pharmacology in 1960 and the annual meeting of the American Heart Association in 1960.

In rabbits, 30 min. to 1 hour after oral administration of 1 Gm./Kg. of cholesterol or 15 to 30 min. after intravenous injection of 1 μg./Kg. of adrenaline, endothelial cells were temporarily impaired in their repelling function against the sticking of platelets and leucocytes on their endothelial surface; the margination and sticking of platelets and leucocytes to the endothelial surface were also clearly seen in some parts of endothelial surface in arterioles, venules and capillaries as well as in large arteries, and at the same time the adhesive platelet count was reduced. Concomitantly, certain hematological changes appeared, i.e. the Lee-White time, calcium clotting time and one stage prothrombin time were shortened. It was also found that either of the above mentioned challenges enhances the ADP-induced platelet aggregability.

Such responses of endothelial cells and the blood, including platelets, were repeatedly shown to accompany the acute infiltration of plasma and plasma proteins into the arterial and venous wall. In the first stage of our investigation we used hematoxylin and eosin stain and iron stain in the large arteries of rabbits and rhesus monkeys, and we demonstrated the appearance of edema in the subendothelial space and often in medial layers 30 min. after injection of adrenaline (0.1–1.0 μg./Kg. i.v.) or 1 to 2 hours after adminis-
tration of cholesterol (1 Gm./Kg. p.o.), although the edematous change was stronger in rabbits than in monkeys\textsuperscript{2,14} and such acute responses of arterial wall have been called “the edematous arterial reaction.”\textsuperscript{14} The edema shows a positive iron staining indicating the presence of acid mucopolysaccharide.\textsuperscript{2,14} Such an edematous stage in the arterial wall has been long recognized by the German school as “Das initiale fettfreie Ödem” in the

![Images](A, B, C)

Fig. 1. Immunofluorescent demonstration of the infiltration of $\beta$-lipoprotein into the arterial wall of rhesus monkey induced by atherogenic stress using the interference filter technique for FITC fluorescence by Rygaard and Olsen (1971).\textsuperscript{66}

—Transection of the thoracic aorta of rhesus monkeys—

(A) Placebo control monkey. Note: There is practically no fluorescence of $\beta$-lipoprotein.

(B) Monkey given cholesterol (1 Gm./Kg. p.o.) and sacrificed 2 hrs. thereafter. Note: The fluorescence of $\beta$-lipoprotein is shown infiltrating into the subendothelial space.

(C) Monkey given cholesterol (1 Gm./Kg. p.o.) and sacrificed 5 hrs. thereafter. Note: $\beta$-lipoprotein is still shown in the subendothelial space and also in the medial layers infiltrated
human arterial wall and thought to be an initial stage of human atherosclerosis. As early as 1958 we found that such a change can be regularly produced at will by one-shot treatment of animals with atherogenic substances such as cholesterol and adrenaline, but it took Western investigators almost 10 years to reconfirm the existence of this highly important atherogenic mechanisms. Recently we analysed further such atherogenic responses by immunofluorescent technique and found γ-globulin, IgG, fibrinogen, and β-lipoprotein as well as acid mucopolysaccharide in edematous parts of the vessel wall. Such responses were again marked in rabbits, but they also definitely appear in monkeys. This fact means that the one-shot treatment of cholesterol or adrenaline induces an acute infiltration of such plasma proteins into the vessel wall.

In another experiment it was clarified by the authors that the infiltration of large particles, e.g. proteins such as horseradish peroxidase, takes place mainly through the intercellular junctions of endothelial cells into the subendothelial space from the vessel lumen. Plasma proteins are also considered to take the same route through the intercellular junction of endothelial cells into the subendothelial space, as detailed in the following chapter. The plasma protein thus entered into the subendothelial space are considered to be transported through fenestrations of the internal elastic lamina into the medial layers. Presumably, they leave the vessel wall through lymphatic ducts to enter the extravascular space.

The relative barrier function of the internal elastic lamina was also clearly shown in this experiment. The combined administration of adrenaline and
cholesterol induced the most dramatic demonstration of the barrier function of the internal elastic lamina, damming temporarily the transport of plasma proteins into the medial layer. Plasma proteins that had infiltrated into the subendothelial space stagnated there for a while, suggesting the existence of a highly important atherogenic process that could be prevented by antiatherogenic procedure.

On the other hand, there is no such a well-developed internal elastic lamina in the vein, plasma proteins infiltrate into the venous wall diffusely and freely, and there is no stagnation in the subendothelial space. Such phenomenon may be responsible for the virtual absence of atherosclerotic changes in the vein, although the formation of thrombi is enhanced by atherogenic stress, which lowers the repelling function of endothelial cells of the vein as well as those of the artery.
3) The prevention of acute infiltration of plasma proteins into the arterial wall

The acute infiltration of plasma constituents, especially of cholesterol-bearing plasma proteins into the arterial wall by atherogenic stress is reasonably considered to be one of the essential processes. In addition, the infiltration of proteins capable of producing inflammatory breakdown products such as fibrinogen or complement may also have atherogenic significance.

This acute infiltration of plasma proteins induced by atherogenic stress comes from the alteration of the active selective-peameability function of endothelial cells, and such a phenomenon is always accompanied by the concomitant adhesion of endothelial cells to platelets and leucocytes, as mentioned in the preceding chapter. It was also found that such substances as pyridinolcarbamate and its derivatives, capable of protecting the normal selective-peameability function of the endothelial cells, can also inhibit the sticking of endothelial cells to platelets as well as the enhancement of ADP-induced platelet aggregability by atherogenic stress.

It is reasonably presumed that the prevention of such acute infiltration of plasma proteins and sticking of platelets to endothelial surface are important for the prevention of atherosclerosis and at the same time for the treatment of atherosclerotic processes. Experimentally, pyridinolcarbamate and large doses of estrogen and their derivatives, which are capable of inhibiting the acute infiltration of plasma proteins into the arterial wall and the sticking of platelets to endothelial surface as well as the enhancement of platelet aggregability induced by atherogenic stress, have been shown to inhibit significantly the atherosclerosis of cholesterol-fed rabbits and cockerels by Shimamoto and his collaborators (1966), by Wu et al. (1969) and also by Pick (1969). Pyridinolcarbamate and estrogen also have been shown by Shimamoto and his collaborators (1966) to have a curative effect, enhancing the removal of accumulated cholesterol from the atheromatous lesions, on established atherosclerosis produced in rabbits by cholesterol feeding.

4) Further fine morphological insight on atherogenesis, thrombogenesis and a new treatment of atherosclerotic diseases with pyridinolcarbamate—Contraction and bleb formation of endothelial cells induced by atherogenic stress—

According to Landis and Majno et al., it is possible to fix the living endothelial cells of capillaries in certain organs almost without changing their shape by rapid and direct application of the fixative on them. The author anesthetized rabbits and inserted surgically a relatively large catheter into the ascending aorta through left ventricle and cut abdominal aorta as an outflow and thus the ice-cold fixative (2.5% glutaraldehyde in isotonic phosphate buffer containing 0.25 M sucrose (pH 7.4) was rapidly perfused through the catheter for 10 min. in situ under the pressure of 110 mm.Hg,
Table I. Contraction of Endothelial Cells by One Shot with Cholesterol (1 Gm./Kg. p.o.)

<table>
<thead>
<tr>
<th>Animals</th>
<th>Placebo Control Group</th>
<th>Nuclear Changes</th>
<th>Cholesterol (1 Gm./Kg. p.o. 2 hrs thereafter) Group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Endothelial Cells</td>
<td>Indentations/μ.</td>
<td>Pinches</td>
</tr>
<tr>
<td>1</td>
<td>105</td>
<td>1.61±0.08</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>100</td>
<td>1.47±0.05</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>96</td>
<td>1.53±0.04</td>
<td>0</td>
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<tr>
<td>4</td>
<td>94</td>
<td>2.53±0.07</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>103</td>
<td>2.05±0.05</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>117</td>
<td>1.95±0.04</td>
<td>0</td>
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<tr>
<td>7</td>
<td>111</td>
<td>1.11±0.02</td>
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</tr>
<tr>
<td>Total</td>
<td>726</td>
<td>1.75±0.18</td>
<td>0</td>
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</table>

Cholesterol (1 Gm./Kg. p.o. 2 hrs thereafter) Group

<table>
<thead>
<tr>
<th></th>
<th>Pinches</th>
<th>Closing folds</th>
<th>Folds</th>
<th>Notches</th>
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<td>1</td>
<td>6</td>
<td>1</td>
<td>63</td>
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<td>14</td>
<td>8</td>
<td>66</td>
<td>10</td>
<td>271</td>
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<tr>
<td>Total</td>
<td>691</td>
<td>40***</td>
<td>350</td>
<td>35</td>
</tr>
</tbody>
</table>

Mean and standard error of the number of indentations per placebo control group vs cholesterol group

* P<0.05. *** P<0.001 (Wilcoxon's rank sum test)

Table II. Prevention of Contraction of Endothelial Cells by Pyridinolcarbamate

| Pyridinolcarbamate-Pretreated Group, Cholesterol (1 Gm./Kg. p.o. 2 hrs thereafter) |
|----------------------------------|-----------------|---------------|--------|--------|--------|
|                                  | Indentations/μ. | Pinches       | Closing folds | Folds | Notches |
| 15                               | 1.81±0.05       | 0             | 35     | 8      | 55     |
| 16                               | 1.70±0.04       | 0             | 21     | 0      | 79     |
| 17                               | 2.18±0.05       | 0             | 21     | 5      | 89     |
| 18                               | 1.77±0.05       | 0             | 14     | 1      | 110    |
| 19                               | 1.93±0.04       | 0             | 26     | 4      | 46     |
| Total                            | 1.88±0.08       | 0*            | 117    | 18     | 379    |

* P<0.05 cholesterol group v.s. PDC group
by which the recoil of the vessel was prevented. Thus the endothelial cells of the thoracic aorta received directly the fixative and a rapid fixation has been successfully performed. Such techniques are also available to fix the endothelial cells of arteries and veins in situ.

Tables I and II show the typical changes of nuclei of endothelial cells of thoracic aorta of rabbits due to the possible contraction of endothelial cells by one shot treatment of rabbits with cholesterol. Namely the number of the indentations of nuclei was increased (p<0.05) and the pinches of nuclei appeared (p<0.001) after oral administration of cholesterol and such changes were absent in animals received the same challenge after the pretreatment of animals with pyridinolcarbamate (10mg./Kg. p.o.). Fig. 4 shows the contraction of endothelial cells showing the appearance of pinch, deep indentations of nuclei and many filaments concentrated in definite tracts in the cytoplasm close to the strong indentations of nuclei. Such contracted cells appear spotty and the spotty distribution fits the focal distribution of subendothelial edema in the edematous arterial reaction as well as of atheroma.

The most important findings by transmission electron microscopic analysis after cholesterol or adrenaline administration were the statistically significant appearance of the contraction, and bleb formation of the endothelial cells of the aorta as reported by the author at the 14th annual meeting of international college of angiology (1972). The edematous changes of the
fine structure of protoplasma were induced by both substances and they were always accompanied by the margination and condensation of nuclear chromatin accompanied by the enlargement of perinuclear spaces. In animals received cholesterol or adrenaline, an unevenness of endothelial cells as well as of their nucleus was regularly observed by transmission electron microscope and fine and regularly running vertical lines 0.6 to 0.8 μ apart and much more fine and regularly running longitudinal lines 0.2 to 0.3 μ apart were shown on the surface of endothelial cells by scanning electron microscope, and such changes were reasonably considered to represent the contraction of contractile protein of endothelial cells which widens the intercellular junctions and results in the increase of the permeability, because such changes of endothelial cells were always accompanied by infiltration of plasma proteins in the subendothelial space. Such a contraction of endothelial cells and increase in the permeability were similarly induced by the administration of noradrenaline and angiotensin. Hypertension may also contribute to the increase of the permeability, however
Fig. 6. The luminal surface of rabbit aorta shown by scanning electron microscopy in (1) untreated rabbit; (2) rabbit treated with adrenaline (1 μg./Kg. i.v.) and sacrificed 30 min. thereafter; and (3) rabbit treated with angiotensin (1 μg./Kg. i.v.) and sacrificed 30 min. thereafter, (4) rabbit treated with cholesterol (1 Gm./Kg. p.o.) and sacrificed 2 hrs. thereafter.

Note: The endothelial surface of untreated animal shows the endothelial folds with relatively smooth surface (1), while the surface of each endothelial fold shows many fine and horizontal lines at quite regular intervals (2, 3, 4) and is marked in (2) showing the contraction of endothelial cells.
the contraction of endothelial cells of the aorta continues much longer than the hypertensive response in the experiments with the single administration of adrenaline, noradrenaline and angiotensin, so that the contraction of endothelial cells seems to contribute much more than that of hypertension to the increase of the permeability. This fact is considered to be an important new concept in atherogenesis and anti-atherogenic treatment.

The second important finding was the appearance of blebs on the endothelial cells. In rabbits, careful random sampling and statistical observation of endothelial cells revealed the appearance of blebs, especially those with a diameter over 1 μm, after the administration of cholesterol. In animals given adrenaline, the number of smaller blebs (with a diameter less than 1 μm) increased, but the larger blebs did not. Needless to say, cellular blebs due to poor fixation are familiar in electron microscopy; hence it was quite essential to consider this possibility in our preparations. However, the careful vital fixation and the controlled and statistical observation we conducted could reasonably exclude such artifacts. The formation of blebs is common in arterioles and capillaries, and it certainly disturbs the blood flow in such small blood vessels. In addition, the blebs often have platelets, adherent, and some of these produce fibrin, a fact suggesting their significance in thrombogenesis.

Such a morphological alteration of endothelial cells may represent a functional disturbance such as the impairment of their active selective permeability, resulting in the acute and abnormal infiltration of plasma constituents like plasma proteins as well as the impairment of the repelling function of endothelial cells against the sticking of platelets to endothelial surface.

According to Willms-Kretschmer and Majno et al. (1969), blebs in the endothelial cells were produced by an interruption of blood flow. We reconfirmed their evidence and also revealed the concomitant appearance of acute infiltration of plasma proteins into the vessel wall. Majno and his collaborators (1968) considered bleb formation as one of the most important causes of the no-reflow phenomenon seen in brain infarction induced by temporal interruption of the blood flow to the brain; we went further, postulating that the appearance of blebs in the vascular endothelial cells is one of the most important mechanisms in the formation of thrombosis. In the arterioles and capillaries of the heart, especially in ischemic parts of the myocardium affected by coronary sclerosis, the alteration of endothelial cells may be easily induced by certain atherogenic stress and the formation of blebs in arterioles. Such a process, in which the bleb formation has been known to take place most commonly (Willms-Kretschmer and Majno, 1969), may represent the injury of endothelial cells resulting in the adhesiveness of endothelial surface to platelets and leucocytes. Such changes may certainly slow and obstruct the regional
blood flow. Actually the slowing of the regional coronary blood flow is considered to be one of important causes of anginal attack by cineangiographical observation of Tambe et al. (1972), and it also causes stagnation of local irrigation in atherosclerotic arteries of the upper stream, and causes a thrombogenic condition in the diseased arterial segment. In addition to the stickiness of the endothelial surface, the bleb itself may enhance the formation of platelet or leucocyte thrombi in arterioles and capillaries of the myocardium.

The sticking of platelets and leucocytes induced by these mechanisms may be in itself severe enough to produce myocardial infarction in small blood vessels, and, in acute cases, to cause death without any grossly visible thrombus, as reported by Haerem (1972). Such a condition is reasonably considered to be a cause of the infarction, not only in the myocardium but also in the brain and other organs, especially when ischemic conditions are combined with atherogenic stress. Concerning the prevention of thrombosis, the above mentioned evidence suggests the importance of drugs like pyridinolcarbamate, which shows a protective effect against such alterations of endothelial cells induced by atherogenic substances such as cholesterol, adrenaline, noradrenaline and even by angiotensin as well as hypoxia.
The increase in the adhesiveness of platelets to ADP has been shown to be induced by atherogenic stresses such as injection of adrenaline or oral administration of cholesterol in animals. Needless to say, the direct effect of adrenaline and cholesterol, or some other active agent appearing after administration of cholesterol, may be responsible for such an increase in platelet adhesiveness to ADP. However, the margination and sticking of platelets to the altered membrane of endothelial cells may stimulates the transformation of platelets into a more active form. Specifically, the alteration of endothelial cells by atherogenic stress is itself considered to be one of indirect causes for the increased adhesiveness of platelets. Such evidence suggests the importance of drugs like pyridinolcarbamate, which inhibits the platelet adhesiveness directly and also preserves the repelling function of vascular endothelial cells against platelets. As in the case of the contraction of contractile proteins of endothelial cells and formation of blebs, there seems to be a similar causative relationship between the contraction of platelets and formation of their pseudopodes, which are related to the adhesiveness, because all these changes of both groups of cells were significantly prevented by pyridinolcarbamate.
Acetylsalicylate has been shown by Shimamoto and his collaborators (1966)\(^2\) to have a weak pyridinolcarbamate-like effect, inhibiting the sticking of platelets to endothelial surfaces undergoing atherogenic stress, and its direct inhibitory effect against platelet aggregation has been shown by Gast (1964),\(^9\) Evans et al. (1968),\(^3\) Zucker and Peterson (1968),\(^4\) and O'Brien (1968).\(^5\) Didisheim et al. (1971)\(^6\) demonstrated the potent inhibition effect of pyridinolcarbamate against the aggregation of human platelets induced by collagen, adrenaline and ADP and such effects of pyridinolcarbamate were reconfirmed by the author (1971).\(^7\) Pyridinolcarbamate inhibits both primary and secondary aggregation of the human platelets induced by above-mentioned substances, while acetylsalicylate is ineffective for inhibiting the primary aggregation.

Actually, the preventive effect of pyridinolcarbamate against the enhancement of platelet adhesiveness to ADP appearing after atherogenic stress in vivo is thought not to be a direct effect on platelets, but rather an indirect one presumably through its effect on the endothelial cells. Although the administration of pyridinolcarbamate (10 to 20 mg./Kg. p.o.) lowered slightly the ADP-induced platelet aggregability, this effect was not statistically significant in rabbits or in coronary patients.\(^7\) However, pyridinolcarbamate was shown by us (1970)\(^7\) to inhibit the enhancement of platelet aggregability of coronary patients induced by Master's two step test and such an inhibitory effect is undoubtedly an interesting subject to be analysed further in the prevention of human thrombosis.

3. **Clinical application of pyridinolcarbamate**

Since 1963 pyridinolcarbamate\(^5\),\(^6\) has been used in the treatment of atherosclerotic diseases and also in the prevention of cerebral apoplexy\(^3\),\(^4\) in Japan and most countries of the world. Since 1968 many controlled trials have been performed by the double blind method and its therapeutic efficacy seems to be established in various morbid conditions.

Nevertheless, this represents the first attempt in the history of medicine to use an anti-atherosclerotic drug for attacking the arterial wall and platelets directly. It was thus inevitable that the trial-and-error method was used to establish the pyridinolcarbamate treatment, and such trials are still going on in many countries.

In the first stage, optimistic as well as pessimistic opinions on the clinical effect of pyridinolcarbamate were reported, because nobody was familiar with such a treatment of atherosclerotic diseases. For instance, the reopening of arteries occluded by atherosclerosis was reported, and it is not unreasonable too, but this was considered too optimistic and presumably not always reason-
able. Occlusions are produced mainly by organized thrombi or fibrous plaque and there is little space for edematous or atheromatous parts; they are composed largely of connective tissue and smooth muscle fibres, and sometimes by calcified foci. In the majority of cases, they are obviously the target of surgical removal rather than drug therapy.

On the other hand, the dynamic aspects of atherogenic disorders have been recognized year after year, and hematological response to atherogenic stress in man\textsuperscript{21),22),45}) has been confirmed as similar to that in animals\textsuperscript{14),16),17),46}) It was also found that the hematological response is much more easily induced in patients suffering from atherosclerotic diseases than in healthy ones, as shown by the author and his collaborators (1970).\textsuperscript{46}) The same effect of pyridinolcarbamate—prevention of such hematological responses induced by similar atherogenic substances—has been confirmed in man\textsuperscript{17),19),20}) Such evidences have reasonably suggested the presence of the same alteration of the vascular endothelial function by atherogenic stress in man as is found in the case of rabbits and monkeys.

The familiar clinical symptoms and signs of atherosclerosis are due to the narrowing or occlusion of an artery, which causes an inadequacy in the blood supply to the affected organ; this results in aneurysm formation or rupture of the artery with hemorrhage into the surrounding tissues, depending on the organ or tissue affected. Such a drug as pyridinolcarbamate, which is capable of preventing the acute infiltration of plasma into the vessel wall because of its protective effect directly on the endothelial cells and platelets, seems to contribute to the prevention of the narrowing or occlusion, aneurysm formation and rupture of the artery.

In established atheromatous lesions, relaxation of contracted endothelial cells, restoration of the physiological function of endothelial cells and the enhancement of glycolytic and TCA cycle enzyme activity of vascular endothelial and smooth muscle cells by pyridinolcarbamate, shown by Mrhova et al. (1972)\textsuperscript{47}) and Numano et al. (1972),\textsuperscript{48}) may help to reduce the edematous fluid and atheromatous mass in the lesions, as shown experimentally by the author. This effect may increase blood flow through the affected organ to some extent. The prevention of the infiltration of plasma into the vessel wall due to endothelial cell-contraction and the adhesion of platelets to endothelial surfaces and to other platelets, occurring after stress, may enhance microcirculation including collateral circulation in the affected organ. Thus, symptoms accompanying physical stress such as an exercise,\textsuperscript{22),49}) which calls for an increase in the blood supply, may be relieved by such a drug as pyridinolcarbamate.
Fig. 9. Disappearance of ulcer in patients with atherosclerosis obliterans by pyridinolcarbamate treatment in the author's institute. This figure shows the duration of ulcers and their period required for complete healing during the course of pre-treatment with other drugs including vasodilators (shown on the left) as well as pyridinolcarbamate (shown on the right) treatment. Ulcers were completely cured in 25 cases out of a total of 31 cases, although ulcers did not heal in 6 cases. Lumbar sympathectomy was performed in 2 cases of the uncured cases. The ulcer was cured in one of the 2 cases after sympathectomy. In the uncured cases, amputation of the patients' feet was required. (Atsumi et al.: Jap. Heart J. 12: 335-346, 1971)

4. Pyridinolcarbamate treatment
(a) Peripheral atherosclerotic disorders

The morbid conditions known as arteriosclerosis obliterans and thromboangiitis obliterans have been subjected to pyridinolcarbamate treatment since 1963 in Japan and abroad, and many controlled clinical trials have been successfully conducted under the double blind technique in Japan, England, Belgium, Germany, Italy, and the USSR. Improvement was noted mainly in the crest time of toe-plethysmography, ischemic signs such as cyanosis and ulcers of affected fingers and toes, and intermittent claudication. In the treatment of ischemic ulcer with this compound alone, almost all investigators have experienced a relatively high rate of cure, which averages about 75% in 150.

In an open study, Cotton (1972) treated 13 cases of arteriosclerosis obliterans with pyridinolcarbamate (1.5 Gm. daily) for many weeks and observed clinical improvement while measuring the change of platelet aggregability by ADP using his filtration pressure method. He observed that the pyridinolcarbamate treatment normalized slowly but steadily the elevated ADP-induced platelet aggregability of patients in the course of 5 to 6 weeks, and was followed by the improvement of clinical signs.
In many reports, the dosage of pyridinolcarbamate used was 1.0 to 2.0 Gm. or 20 mg. per Kg. of body weight, and this seems to be an adequate daily dose in the treatment of patients suffering from peripheral atherosclerosis. The favorable response started at the end of the first week in some cases and the intolerable pain of arteriosclerotic ulcer subsided within 1 to 3 weeks. Pale ischemic ulcers with poor granulation turned pink after 1 or 2 weeks and the growth of healthy granulation started relatively soon. Definite improvement of some other symptoms—notably a distinct reduction in the crest time of toe-plethysmography—started in the course of 4 to 6 weeks of the treatment.
in quite a number of cases.

Pyridinolcarbamate treatment seems to increase the blood flow not only in the skin of the ulcer, but in the muscles of the affected extremities as well. Atsumi et al. (1971) actually measured the blood flow in anterior tibial muscles of affected legs in patients suffering from atherosclerosis obliterans by the \textsuperscript{133}Xe-injection method and, even using such an insensitive method, a definite increase of blood flow at the height of reactive hyperemia was serially demonstrated in the course of pyridinolcarbamate treatment in some of their patients and the increase in blood flow was accompanied by clinical improvement.

(b) Coronary atherosclerosis\textsuperscript{2)}

This condition\textsuperscript{2)} is also called arteriosclerotic or atherosclerotic heart disease or coronary heart disease. The major clinical manifestations are angina pectoris, acute myocardial infarction, sudden death, disturbances in rhythm and electrical activity of the heart, and congestive heart failure.

Angina pectoris is believed to be induced by ischemia of the heart muscle due to the narrowing of the coronary artery. This symptom usually occurs only intermittently from physical exertion or emotional stress, which temporarily increases the need of the heart muscle for blood beyond the available supply delivered by the affected coronary artery. As a rule such pain subsides promptly with rest or drug therapy. The usual treatment of angina pectoris\textsuperscript{1)}

![Fig. 11. Changes of ADP-induced platelet aggregation after Master's two step test. Enhancement of platelet aggregation was observed 1 min. after Master's test on coronary patients with statistical significance under $10^{-6}$, $3 \times 10^{-6}$ and molar of ADP. The enhancement was not observed in the same patients when they were pretreated with pyridinolcarbamate. (Yamazaki et al.\textsuperscript{31})](image-url)
is designed to relieve chest pain either by avoidance of activities which produce the discomfort, or the use of nitroglycerin.

Using the double blind cross-over technique with pyridinolcarbamate and placebo in one group of 40 patients suffering from angina pectoris, and in another such group of 13 patients, the author found that anginal pain, ECG changes and the above mentioned hematological changes such as a transient reduction of adhesive platelet count, a shortening of clotting times and the enhancement of ADP-induced aggregability of platelet were induced by Master's two step exercise test, and that such changes are inhibited to a statistically significant degree after pretreatment of patients with pyridinolcarbamate. Russek (1972) also reported that in some of his coronary patients the exercise test changed from positive to negative in the course of pyridinolcarbamate treatment.

Controlled clinical trials in the treatment of coronary atherosclerosis with angina pectoris have been tried under the double blind technique and they have shown its favorable effect. In the author's experience, pyridinolcarbamate treatment of the intermediate form, which is known to induce acute myocardial infarction often, seemed to prevent such infarction significantly. Such clinical evidence may suggest the effect of pyridinolcarbamate on the affected coronary arteries and especially on microcirculation including that of the collaterals in the myocardium.

As shown by Haerem (1972), sudden death is often induced by a hampering of the intramyocardial circulation by platelet aggregation without major acute lesion in the epicardial arteries. Even in thrombosis of epicardial arteries, which is the common cause of acute myocardial infarction, the above mentioned hampering of local intramyocardial circulation due to platelet aggregation may contribute to slow and to stasis of the local upper stream in the atherosclerotic epicardial artery and result in thrombosis. In such a condition, it is tempting to use pyridinolcarbamate for the prevention of sudden death or acute myocardial infarction, which is also under way. This new type of anti-thrombotic treatment was first suggested by the author in 1962.

(c) Cerebral atherosclerosis

Atherosclerosis of a given cerebral vessel causes no symptoms if the area of the brain supplied by the diseased artery receives adequate compensatory blood supply from other arteries or through collateral circulation. In the treatment of cerebral atherosclerosis, the prevention of acute infiltration of plasma into the cerebral arteries by stress is also important as a precaution against further narrowing and the progress of atherosclerosis. At the same time, the microcirculation of the brain, especially collateral circulation, should
be protected from the excessive sticking of platelets by stress. For such a purpose, pyridinolcarbamate treatment has been extensively applied. There are already many reports on its administration under the open or double blind trials, and its therapeutic efficacy has been reported.\(^{66)-77)}\)

Patients who had survived a first stroke (mainly from cerebral thrombosis) were subjected to controlled clinical trials by Murase et al. (1969)\(^ {44)}\) for 2 years, and the statistically significant effect of pyridinolcarbamate in preventing relapse was observed.

Side effects of pyridinolcarbamate: Gastrointestinal symptoms such as gastric distress, anorexia, constipation or diarrhea were encountered in about 5% of patients receiving pyridinolcarbamate, but it was tolerated by the great majority. In Japanese, jaundice was reported on rare occasions. Careful observations have recently been made on the side effect in the United States of America and European countries during the past over 4 years, and the results were collected and published for the international congress held in Tokyo on May 19–21, 1972, but no such case was reported except minor side effects. More than 30 patients who had received over 1,005 Gm. to 2,483 Gm. of pyridinolcarbamate for 5 to 7 years in the author's hospital were subjected to various organ function tests, and analysis disclosed no abnormality that could be attributed to the drug.

**DISCUSSION ON THE CLINICAL APPLICATION OF PYRIDINOLCARBAMATE**

It seems to be an essential effect of pyridinolcarbamate to prevent or diminish the contraction of endothelial cells and at the same time to prevent or diminish the infiltration of plasma into the arterial wall as well as to inhibit or reduce platelet aggregation and adhesion to endothelial surfaces induced by atherogenic stresses, and such preventive effects have been shown in monkeys and other animals. The worldwide clinical applications and our own experience with double blind trials and open studies suggest the appearance of the same phenomena in man. A definite improvement of clinical signs used to appear slowly in the course of 2 to 10 weeks of pyridinolcarbamate treatment, even in the improvement of the enhancement ADP-induced platelet aggregability of arteriosclerotic patients as shown by Cotton (1972).\(^ {29)}\) Among such improvement, the most impressive result of the administration of pyridinolcarbamate was the relatively rapidly appearing gain of pink color, accompanied by the growth of healthy granulation tissue, in the majority of cases of the pale and desperate ischemic ulcer of patients suffering from arteriosclerosis obliterans. Such ulcer had previously showed almost no improvement, and had often remained pale without the growth of healthy granulation
for over several months. For such patients, amputation of the affected limb would have been indicated in the past. For this reason, the need is felt for a drug capable of protecting the active selective-permeability and repelling functions of endothelial cells as well as reducing platelet aggregability. Pyridinolcarbamate, which has shown this capability in the experiments of thrombosis and atherosclerosis.

The author mainly introduced the cytological aspect on atherogenesis in this article, however the biochemical approach is needless to say highly important. Pyridinolcarbamate was shown by Mrhova et al. (1972),47 and by Numano et al. (1972)48 to accelerate the glycolytic enzyme and TCA-cycle enzyme activity of the human and animal arterial wall. ATP is required in quantities sufficient for the production of enzymes and proteins needed for the prevention or repair of the injury and atheromatous lesions, and therefore the accelerating effect of pyridinolcarbamate on such enzyme activity is obviously important. However, it is not yet clear whether such an effect is actually essential to the above mentioned effect of this drug.

Diabetes mellitus is known to be an accelerating factor in the progress of atherosclerosis in man, and diabetic angiopathy itself is an important cause of death today and pyridinolcarbamate was stated to be effective in the treatment of retinal angiopathy by Takaku et al.76 Using a large daily dose of pyridinolcarbamate, Camerini et al. (1972)77 succeeded in preventing diabetic nephropathy due to diabetic angiopathy in K.K. mice. This finding is important, also, in the analysis, although it is not clear whether such a preventive function is induced by the inhibitory effect of pyridinolcarbamate against the acute infiltration of plasma into the vessel wall or by its effect on some enzyme activity of the vessel wall. The acute infiltration of plasma proteins into arterial wall by atherogenic stress found by the author and his collaborators, may induce a profound altering of the metabolism of the arterial wall, which has to be analysed in its relationship to atherogenesis and is now under way.

Needless to say, patients with hypercholesteremia should have their abnormal lipid metabolism corrected. However, they need also to be protected from the contraction of endothelial cells resulting into the acute infiltration of cholesterol-bearing plasma proteins into the arterial lesion, and from the thrombotic tendency induced by atherogenic stress. The prevention of contraction of endothelial cells as well as the relaxation of contracted endothelial cells seem also essential in the treatment of established atheromatous lesions. Such a protective procedure is required for the common treatment of atherosclerotic patients with or without hypercholesteremia.
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