Experimental Studies on the Pathogenesis of Arteriosclerosis with Special Reference to Blood Coagulation

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It is now generally accepted that lipemia has a close relationship to the pathogenesis of arteriosclerosis, and blood coagulation has been reported to be accelerated by alimentary lipemia. Recently the atherogenic factor of lipemia has been discussed with reference to this hypercoagulability induced by lipemia. This paper presented the experimental investigations on the influence of hypercoagulability in the pathogenesis of the arteriosclerosis. Authors found that blood coagulability was markedly accelerated in vivo after the injection of celite, which was demonstrated in the decrease of whole blood clotting time, the increase of serum thromboplastic factor and the decrease of some blood clotting factors which were assumed to be due to the increased consumption of them in vivo.

The atherosclerotic changes in aorta and pulmonary arteries produced with cholesterol feeding were accelerated by this experimentally induced hypercoagulability.

From these results authors considered that hypercoagulability of blood probably plays the primary role in the pathogenesis of arteriosclerosis.

In 1913 Anitschkow showed that atherosclerosis was produced experimentally in rabbits by cholesterol-feeding. Thereafter the cholesterol hypothesis in the pathogenesis of arteriosclerosis has been markedly prevailed, and it has been thought that hypercholesteremia is the basic factor for arteriosclerosis.

Lately, however, some authors have reported that the correlation between the level of serum cholesterol and the grade of arteriosclerosis can not always be found, and that hypercholesteremic patients do not necessarily show arteriosclerosis.

Thus, as to the disturbances of lipid metabolism in arteriosclerosis, other factors than cholesterol, such as phospholipid, C/P, lipoprotein, or fatty acid components in serum, have been studied.

On the other hand, experimental arteriosclerosis have been produced by the methods which have no direct relation to lipid metabolism. Therefrom, the metabolic disturbances of blood vessel wall have been studied especially of the beginning pictures of arteriosclerosis.

In thromboembolic patients and alimentary lipemia hypercoagulability has been reported to be observed frequently. Thus recently it has been thought that the role of fat in accelerating the occurrences of thromboembolism or arteriosclerosis should be attributable to the effect on the blood coagulation factors. Blood vessel wall has been thought to be affected by such an abnormal acceleration of blood coagulation.

In this paper authors reported the effect of experimentally accelerated blood coagulation on the occurrences of arteriosclerosis.

Methods and Materials

Twenty-four male rabbits were divided into the following five groups. Body weight of these
rabbits was approximately 1.5 kg. As the method of accelerating the blood coagulability in vivo, celite suspension, which was reported to activate Hageman factor, was intravenously injected very slowly. 5 mg of celite (Filter-cel) suspended in 5 cc of normal saline and sterilized in bold water bath, was injected 3 times a week for about 40 days. Rapid injection of celite suspension caused the sudden death of experimental animals because of the thrombus formation in right auricle and ventricle, as shown in plate 1.

Plate 1 The intracardial thrombus by the rapid intravenous injection of celite. The upper, right auricle and ventricle in which the total blood was clotted, the lower, left ventricle in which fibrin deposition was observed.

Experimental groups.
Group A: Injection of celite suspension was performed to 5 normal rabbits.
Group B: Celite suspension was injected to 5 rabbits which were fed with cholesterol (0.1 g/day).
Group C: Injection of celite suspension was performed to 5 rabbits which were received anticoagulant, phenylindandione (Indion), about 1 week before this treatment and their prothrombin level were decreased below 5 per cent of normal. Anticoagulant was successively administered in the course of this treatment.
Group D: Five rabbits were injected 4000 units of streptokinase immediately after the injection of celite suspension in order to enhance the fibrinolytic activity in vivo.
Group E: Four rabbits were fed with cholesterol (0.1g/day) and this group were received no other treatment.

Serum cholesterol levels in group B and E were controlled not to be elevated more than 200 mg/dl.

Rabbits of each group were sacrificed after about 40 days of upper described treatment and their aorta and lungs were investigated histologically.

Blood coagulability after the injection of 5 mg of celite was investigated on other rabbits, corresponded to group A, C, D, respectively and they were described as group a, c, d. The methods of blood coagulation studies were as follows.

1. Whole blood clotting time was estimated by Lee-White's method in siliconized test tube. Blood was collected from the prepared femoral arteries with paraffin coated syringe.

2. Platelet count was performed by Rees-Ecker's direct method.

3. Plasma fibrinogen level was estimated by Quick's tyrosine method modified by Matsuoka.

4. Thromboplastin generation test was performed as described by Biggs and Douglass modified by Matsuoka, but in this experiments BaSO4-adsorbed plasma was diluted 1 in 10, and serum 1 in 20. Maximum activity of formed blood thromboplastin was expressed in per cent.

5. Prothrombin was estimated by the one-stage method described by Matsuoka, but in these experiments diluted plasma 1 in 4 was used.

6. Fibrinolysis was estimated before and after the injection of 4000 units of streptokinase by the method of euglobulin lysis time.

7. Serum cholesterol level was estimated by the method described by Zak, Dickenman, Burnett and Cherney modified by Shibata.

RESULTS

(1) Results of coagulation studies.

a) Whole blood clotting time. In cases of group which were injected celite only the whole blood clotting time was decreased markedly as shown in fig. 1. In group c which were received anticoagulant the whole blood clotting time was very long, and it was longer than that of normal rabbits even after the injection of celite suspension, although it caused slight decrease of clotting time.

b) Platelet count. As shown in fig. 2 platelet count in group a was decreased in average from $23.6 \times 10^4$ to $16.9 \times 10^4$ 10min. and to $16.1 \times 10^4$ 30min. after celite injection. This decrease was less in group c

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whose blood coagulability was reduced by anticoagulant treatment previously, i.e. the average of group c was \(20.8 \times 10^4\) before, \(18.1 \times 10^4\) 10 min. after and \(17.9 \times 10^4\) 30 min. after the injection of celite suspension.

c) Plasma fibrinogen level. As shown in fig. 3 before the injection of celite the average level of plasma fibrinogen was 246.6 mg/dl. It was decreased to 197.5 mg/dl 10 min. and to 207.5 mg/dl 30 min. after celite injection respectively. In group c which were received anticoagulant previously no remarkable changes of plasma fibrinogen level were observed, i.e. 243.0 mg/dl before, 231.7 mg/dl 10 min. after, and 240.1 mg/dl 30 min. after injection of celite suspension.

d) Blood thromboplatin generation test. As shown in fig. 4 sera obtained after injection of celite suspension showed markedly increased activity in the thromboplatin generation test in 3 out of 5 cases, but in 1 case this activity was slightly decreased. The average of the serum thromboplastic activity was 112.8 per cent 10 min. and 111.4 per cent 30 min. after celite injection. On the contrary, sera of group c which were received anticoagulant did not show any change in their activity. They were almost about 2 per cent before and after celite injection.

Plasma thromboplastic activity of group a as shown in fig. 5 was decreased after celite injection. The decrease of this factor was inhibited by the anticoagulant therapy as can be seen in group c.

e) One-stage prothrombin activity. As shown in fig. 6 the decrease of plasma prothrombin activity was induced by the celite injection in group a. In group c, the reduced prothrombin activity which was induced by anticoagulant treatment did not show any change after celite injection. They were left below 3 per cent of normal.

From these results it was thought that blood coagulability was markedly accelerated...
in vivo by the injection of the celite suspension. The decrease of some clotting factors, such as platelet count, plasma fibrinogen, prothrombin, plasma thromboplastic activity, was thought to be attributable to the consumption in vivo by these acceleration of coagulability. This assumption was sustained by the fact that the changes of various coagulation factors after celite injection were inhibited, when blood coagulability was reduced in advance by anticoagulant therapy.

f) Fibrinolytic activity after the injection of streptokinase. As shown in table 1, the fibrinolytic activity estimated by euglobulin lysis time was markedly enhanced after the injection of 4000 units of streptokinase.

g) Serum cholesterol level in group B and E. As shown in fig. 7 the total serum cholesterol level was gradually elevated after cholesterol feeding. On the 38-th the maximal elevation was 294 and the minimal 194 mg/dl.

(2) Histological investigation.

a) Aorta. In one case of group A whose blood coagulability was markedly accelerated by the celite injection, thoracic aorta showed macroscopically gray white, slightly elevated patch of rice size, resembling to atheromatous plaque. In the microscopic findings, as shown in plate 2 and 3, nodular hyperplasia of subintimal and medial layer was observed. Splitting or irregular arrangement of elastic fibre was observed. Fat stain was negative. In 2 cases out of 5 rabbits in group B which were fed with cholesterol and received celite injection as shown in plate 10, 11, 12, and

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Figure 3: The change of fibrinogen level by the injection of celite in group a and c.

Figure 4: The changes of serum thromboplastic activity after the injection of celite in group a and c.

Table 1: Euglobulin lysis time before and after the injection of 4000 units of streptokinase immediately after the injection of celite (in minute).

<table>
<thead>
<tr>
<th>Case</th>
<th>Before</th>
<th>10 min.</th>
<th>30 min.</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>840</td>
<td>10</td>
<td>22</td>
</tr>
<tr>
<td>2</td>
<td>1180</td>
<td>32</td>
<td>30</td>
</tr>
<tr>
<td>3</td>
<td>1180</td>
<td>16</td>
<td>25</td>
</tr>
<tr>
<td>4</td>
<td>1320</td>
<td>45</td>
<td>35</td>
</tr>
<tr>
<td>5</td>
<td>1200</td>
<td>28</td>
<td>23</td>
</tr>
<tr>
<td>Average</td>
<td>1144</td>
<td>26.2</td>
<td>27.0</td>
</tr>
</tbody>
</table>

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13, thickening of intima and lipid accumulation in the intima and media was obviously observed and their elastic fibre was irregularly formed. Those histological changes of aorta were observed neither in cases of group C which were received anticoagulant before the celite injection nor group D whose fibrinolytic activity was enhanced by streptokinase, as shown in plate 20, 21, 25 and 26.

Atherosclerotic change was not found in any rabbits of group E which were fed with cholesterol. (plate 30, 31, 32, and 33)

b) Pulmonary arteries. Middle sized pulmonary arteries of group A showed diffuse or localized thickening in the intima, suggesting thrombus deposition, and their internal elastic membrane at those sites were fragmented and elastic fibres of the media were also irregularly arranged. (plate 4, 5, 6) Some of the small pulmonary arteries were occluded obviously by thrombi. These occluded arteries were again recanalized and at this site intima and media was united by the fragmentation

Plate 10
Plate 13
Plate 11
Plate 14
Plate 12
Plate 15

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of internal elastic membrane. (plate 7, 8, and 9) Those changes of pulmonary arteries were also found in group B. (plate 14, 15, 16, and 19) In this cases, however, lipid deposition into the thickened intima was observed in one case. (plate 17, and 18) In group C and D such changes of intima and media were only very slight and no thrombus was found. (plate 22, 23, 24, 27, 28, and 29)

DISCUSSION

As to the pathogenesis of arterio-and atherosclerosis, it has been studied from various points of view. Especially the disturbances of serum lipid metabolism have been thought to be the most basic factor in the production of atherosclerosis since Anitschkow's experiments.

But recently this atherogenic properties of lipid have been discussed with special reference to its effect on blood coagulation. Ailmentary hyperlipemia induced by animal fat has been reported to cause the acceleration of blood coagulability\textsuperscript{9,10,11} and the inhibition of spontaneous fibrinolytic activity in vivo\textsuperscript{12}. Authors shared this view and also reported that vegetable fat intake was not resulted in such an acceleration of blood coagulation and inhibition of fibrinolysis. Furthermore, unsaturated fatty acid component which was thought to have inhibitory effect on experimental atherosclerosis, inhibited the hyper-coagulability by alimentary hyperlipemia\textsuperscript{13}. Some saturated fatty acid which have long chain of carbon were found to have a high activity in the formation of experimental thrombus in vivo. This activity is thought by Connor\textsuperscript{14} to be due to its activating mechanism of Hageman factor. Activated Hageman factor promotes further stages of blood coagulation, and thus thrombus formation or fibrin deposition will be caused.

On the other hand in patients with throm-
bosis, closely related to atherosclerosis, the thromboplastin generation was reported to be accelerated\textsuperscript{(30)}. Atherosclerotic subjects were found to have a mean platelet survival time significantly shorter than that in normal control subjects.

It is not clearly studied whether this shortening of platelet survival time is due to the increase of sequestration in a specific organ or an increasing tendency of its deposition in the endothelial surfaces of blood vessels.

Normal endothelium was thought to have a deposition of fibrin which was called as the endo-endothelial fibrin film by Copley\textsuperscript{(30)}. He assumed that this fibrin film would be regulated in vivo by the dynamic balance between latent coagulation and fibrinolytic activity. This concept was verified by Roos\textsuperscript{(7)} who has investigated blood vessels with electron-microscopic method. He found that fibrin like fibriles were observed in normal subjects but scarcely or almost absent in the state of hypocoagulability.

Normal intima and the internal layer of media is thought to be nourished by the diffusion of intravascular streaming blood. Therefore the overdeposition of such fibriles would distrube the physiological metabolism of intima which would accelerate further stages of histological changes of blood vessel wall.

This paper reported that blood coagulablity was markedly accelerated in vivo by celite injection. The decrease of some clotting factors in this experiment would be caused by their consumption in vivo.

Thrombus observed in the pulmonary arteries suggested apparently the deposition of fibrin which resulted in the decrease of platelet count or plasma fibrinogen level. Anticoagulant therapy inhibited these changes of blood coagulation factros. Histological investigations showed that the occurrences of atherosclerotic change of aorta by cholesterol-feeding was accelerated by the injection of celite, com-
pared with that by cholesterol-feeding only.

It will be difficult, however, to be con-
cluded without delay whether this acceleration
of atherosclerosis production is only due to the
enhancement of blood coagulability with celite
or to the other moments such as the distur-
bance of non-wettable surface properties of
blood vessel wall with celite.

However it would be possible that en-
hanced blood coagulability would have an
influence on the normal metabolism of blood
vessels wall, as the histological changes in
the arteries were found only slightly in rabbits
which were received anticoagulant simultane-
ously with celite injection. These histological
changes, as reported in the experiments, were
the most markedly observed in the small pul-
monary arteries. The same changes would
possibly be produced in the vasa vasorum of
aorta, although it could not be demonstrated
in this paper.

The damage of outer layer of aorta has
been reported also to cause the disturbances
of intima\(^{50}\). The disturbed intima was
thought to activate strongly the PTC activity
in plasma and this activated PTC promotes
the thrombus formation in the site\(^{20}\).

In this experiment, plaque, macroscopi-
cally and histologically observed in the aorta
of group A would be the basic changes which
could accumulate lipid if hypercholesterol-
emia would be present.

Recently Friedman et al\(^{30}\) reported that
a cellular plaque was led from a thrombus
experimentally produced in the aorta of rab-
bbits, and in the presence of hypercholesteremia
the same histological findings observed in
human atherosclerosis were produced.

Thrombotic theory of atherosclerosis was
set forth by Duguid \(1946\)^{30}.

He reported in his studies on the coro-
nary thrombosis that thrombi became orga-
nized and formed fibrous thickening which
was indistinguishable from atherosclerosis and
the same changes were also observed in the
aorta. This Duguid's thrombotic theory was
strongly opposed by Stewart et al\(^{20}\), as they
found the occurrence of atherosclerosis in a
73 years old hemophilic patient. To this
opposition, however, Astrup\(^{20}\) stated that by
adding tissue thromboplastin hemophilic blood
could be clotted namely as that of normal and
furthermore that intima had a strong thrombo-
plastic but a very weak fibrinolytic activity.
So the hemophilic blood would have the pos-
sibility to form fibrin thrombi.

Perlick\(^{30}\) also reported that the activity
of inhibitor or inactivator of the tissue thrombo-
plastin was higher in the intima than in other
layer of arteries.

Marchand\(^{30}\) reported that fibrinoid de-
generation or homogenization of the inter-
cellular substance in arteries was the same
phenomena as that of exudated fibrin. This
fibrin exudation in the arterial wall was
thought to be closely related to the intravas-
cular coagulation. Klinge\(^{30}\) also showed that
the fibrinoid degeneration of the connective
tissue has a characteristic aminoacid, oxyprolin,
which is not found in the human fibrinogen
and that fibrinoid showed histochemically
marked tryptophan- and tyrosine reaction
which are demonstrated only slightly in col-
lagen.

In lipemia chylomirca were found rich
in the wall side of blood stream and form a
thin film on the wall of blood vessel which
was thought to disturb the nourishing of
internal layer of blood vessel. Lipemia clearing
factor activity has been reported to decreased
in senile or atherosclerotic patients\(^{30}\). The
fibrinolytic activity closely related to lipemia
clearing factor has been found to be inhibited
by antifibrinolysin which is contained in \(\beta-
lipoprotein\)^{20}. This \(\beta\)-lipoprotein is increased
by hyperlipemia, alimentary or by hyper-
cholesteremia. This decrease of fibrinolytic
activity by lipemia or the decrease of lipemia
clearing factor by senile or atherosclerotic
patients would be thought to have the same
role on the pathogenesis of such a sclerotic
disease.

Clinically it has been found that the
occurrence of atherosclerotic change of aorta
is rare or slight in patients with liver cirrho-
sis\(^{30}\). This fact has been thought to be attribu-
table to the acceleration of fibrinolytic
activity in this disease.

The authors found that the thickening of intima or fragmentation of elastic fibre in the pulmonary arteries or aorta by celite injection were inhibited by the enhancement of fibrinolytic activity in vivo with streptokinase.

Fibrinogen was reported to have a high turnover rate in the circulating blood, i.e. a half-life of about 6 day, which indicated that it was rapidly metabolized. This fact was thought by Astrup to have some significance for the problem of the pathogenesis of atherosclerosis.

As mentioned above, blood coagulability would be thought to play some role on the occurrences of atherosclerosis, especially in hyperlipemia.

However these atherosclerotic changes have been reported to be found quite differently in various organs in spite of the fact that blood chemical properties or blood coagulability would be universally the same at least in arterial blood. This would be one of the most significant problem to be studied in the pathogenesis of atherosclerosis in the future.

**Summary**

1. Hypercoagulability was produced by the intravenous injection of celite which was resulted in the consumption of some blood clotting factors in vivo as platelet count, fibrinogen, plasma thromboplastic factor, prothrombin. These phenomena were inhibited by the administration of anticoagulant. Streptokinase injection was also resulted in the marked enhancement of fibrinolytic activity.

2. In pulmonary arteries, thrombus formation and the thickening or hyperplasia of intima and media which seemed to be produced by thrombus organization were observed in the state of this hypercoagulability. Some pulmonary arteries of rabbits which had simultaneous injection of celite with cholesterol feeding showed positive fat stain.

3. Atherosclerotic change in aorta was produced in relative short period of cholesterol feeding when celite suspension was injected simultaneously.

4. These histological changes of arteries by celite injection were inhibited by the administration of anticoagulant or streptokinase.

5. From these results it would possibly be considered that blood coagulation has a close relationship and plays a primary role in the pathogenesis of arteriosclerosis.

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**REFERENCES**

29. Greig, H. B. W., and Runde, I. A., Lancet. II:
31) Astrup, T. Connective Tissue, Thrombosis, and