143. **Method of Finding the “Arteriosclerogenic Substance” and Producing Intimal Arteriosclerosis on Various Species of Animals**

By Takio Shimamoto, Hiroh Yamazaki, Tsutomu Fujita, Toshiaki Sunaga, Tadao Ishioka, Shigeo Iwahara, and Gonpachi Yajima


The intimal arteriosclerosis may be divided into two types. One is the non-fatty type, to which belongs the intimal fibrous or elastofibrous thickening with or without the accumulation of fibrinous substance in the subendothelial layer and the other type is the atherosclerosis characterized by the accumulation of lipids in the intimal lesion and both types have been found in man and animals.

The non-fatty type of intimal arteriosclerosis was produced in the large arteries of the dog by Hueper (1941, 1942) using high molecular weight substances like polyvinyl alcohol, methylcellulose, pectin, and acacia. Also Rinehart and Greenberg (1949) found a non-fatty type of intimal arteriosclerosis in the monkey with experimental pyridoxine deficiency and Vitale and their collaborators in the dog with experimental magnesium deficiency. Wexler and Miller (1958) reported on a destructive intimal and medial arteriosclerosis in female rats produced by ACTH, but, however, the researchers of UCLA failed to reproduce this experiment.

The authors (1957-1959) succeeded in producing the non-fatty type of arteriosclerosis in rabbits, using serotonin, angiotensin, and several high molecular weight substances capable of eliciting the host response of Landy and Shear like bacterial polysaccharides, tissue polysaccharides, glycogen, dextran, and kaolin not only in rabbits but also in the chick, rat, and guinea pig, and the frequent appearance of the visceral complications like myocardial infarction or cerebral apoplexy was emphasized in the authors' arteriosclerosis. Moreover, the authors found that the non-fatty type of intimal arteriosclerosis thus produced changes in the atherosclerosis in their later stage. Such substances, used by the authors, were found to possess a common property capable of eliciting the injury of endothelium, swelling, and abolishing its “silicone-like” property forming the platelet-thrombi or platelet-leucocyte thrombi on some injured parts of endothelium of various species of the animal and were nominated “arteriosclerogenic substances”. These substances have been considered as the “germ” of the intimal arteriosclerosis by the authors.

In this paper the method of finding a new arteriosclerogenic substance as well as of determining the dosage to produce intimal arteriosclerosis in individual animals of various species is described in detail.
(1) How to find the authors’ "arteriosclerogenic substances".

This picture and Photos 2–6 illustrate the injury of the endothelium by the arteriosclerogenic substance. The endothelium shows swelling and on some parts of the injured endothelial surface the platelets stick forming platelet-thrombi or platelet-leucocyte thrombi.

The authors nominated the substances capable of injuring the endothelial cell and destroying its "silicone-like" property on its some surface forming platelet- or platelet-leucocyte thrombi "arteriosclerogenic substances" when the substance does not exhibit other toxic effects, killing animals before the appearance of arteriosclerosis, because the authors succeeded in producing an intimal arteriosclerosis using various substances possessing such properties, like serotonin, angiotensin, glycogen, bacterial substances, tissue polysaccharides, dextran, and...
kaolin, in rabbits, chicks, guinea pigs, and rats.\textsuperscript{7}

The method of detecting the arteriosclerogenic property of the test substance is very simple, when the substance acts acutely. One, two, or four hours after the administration of test material the animal was sacrificed for ordinary histochemical demonstration of the artery (Photos 1–6). The use of electron-microscopic exploration of the endothelium of the artery is also useful.

<table>
<thead>
<tr>
<th>Substances (Effective dose for rabbits)</th>
<th>Fatty substance</th>
<th>Non-fatty arteriosclerogenic substances</th>
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<tbody>
<tr>
<td>* Lanolin 3–5 gm/kg</td>
<td>Serotonin 100 (\mu g/\text{kg})</td>
<td></td>
</tr>
<tr>
<td>* Cholesterol 1–5 gm/kg</td>
<td>Bacterial polysaccharide 50–100 (\mu g/\text{kg})</td>
<td></td>
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<tr>
<td></td>
<td>Glycogen 30 mg/\text{kg}</td>
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<td></td>
<td>Dextran 30 mg/\text{kg}</td>
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</tr>
<tr>
<td></td>
<td>Kaolin 10 (\mu g/\text{kg})</td>
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</tr>
<tr>
<td></td>
<td>* Killed Escherichia coli 100 (\mu g/\text{kg})</td>
<td></td>
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<tr>
<td></td>
<td>Angiotensin 100 (\mu g/\text{kg})</td>
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1 hour

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<tr>
<th>Duration</th>
<th>Intimal swelling + Decrease of number of circulating platelets +</th>
<th>Intimal swelling + Decrease of number of circulating platelets +</th>
<th>Intimal swelling + Decrease of number of circulating platelets +</th>
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<tbody>
<tr>
<td>1 hour</td>
<td>Hypercholesteremia + &quot;Lipomatous type of atheroma&quot; ±</td>
<td>Hypercholesteremia – Intimal fibrous and elastofibrous thickening +</td>
<td>Hypercholesteremia – Intimal fibrous and elastofibrous thickening +</td>
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<tr>
<td>1–2 months</td>
<td>&quot;Fibrous type of atheroma&quot; +</td>
<td>&quot;Fibrous type of atheroma&quot; +</td>
<td>&quot;Fibrous type of atheroma&quot; +</td>
</tr>
<tr>
<td></td>
<td>Damages of visceral organs +</td>
<td>Damages of visceral organs +</td>
<td>Damages of visceral organs +</td>
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3–4 months

<table>
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<tr>
<th>Duration</th>
<th>Atherosclerosis</th>
<th>Atherosclerosis</th>
<th>Under experiment</th>
</tr>
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</table>

* Oral administration.

This table shows the summary of the authors' experiment on arteriosclerosis induced by "arteriosclerogenic substances". The substance was
administered to rabbits intravenously 3 times a week except cholesterol, lanolin, and killed Escherichia coli, which were administered orally 3 times a week using a stomach tube.

The most noticeable finding in the authors' experiment is the appearance of non-fatty type of intimal arteriosclerosis in the initial stage followed by the fatty type of intimal arteriosclerosis, i.e. the atherosclerosis in the later stage by the use of the authors' non-fatty arteriosclerogenic substances under a low fat diet.

(2) How to determine the proper dosage. The determination of proper dosage of arteriosclerogenic substance is very important in producing an intimal arteriosclerosis. The authors' principle is to measure the change in the number of circulating blood platelets using Olef's counting method. Five minutes, 30 minutes, 1 hour, 2 hours, 3 hours, and 4 hours after the administration of test material the number of circulating platelets was counted by Olef's method. The arteriosclerogenic substances induce abrupt swelling of the endothelium resulting in injury of its silicone-like property of endothelial surface in some places, so that the circulating platelets adhere on to the injured surface (Figs. 2–4). Thus the number of circulating platelets may show an abrupt decrease.

Here is one precaution, because there might exist some substance which causes injury to the endothelium and at the same time causes mobilization of platelets from the bone marrow etc. like adrenaline and acetylcholine, of which the arteriosclerogenic effect was found by us and is now under experiments. In such cases the counting of circulating platelets might not be useful and only the morphological change of the endothelium might be the indication.

Except such cases, the dosage which decreases the number of circulating platelets by half, at least once within the period of 5 minutes to 4 hours after the administration of the test material, has been found effective in producing an intimal arteriosclerosis when the administration of such a dose 3 times a week for over 4 weeks might be performed on the same test animal. According to the authors' experience one-half of such a dose has also been shown to be effective to produce intimal arteriosclerosis when it was administered 3 times a week for over 4 weeks. However, one-third of the dose has been shown to be ineffective in producing arteriosclerosis in the 4–5 weeks of such repetitious administration.

Discussion. For the purpose of producing atherosclerosis the cholesterol feeding technique is commonly used and among the various species of animals there exists a species-difference in the dosage and duration of cholesterol administration. The rabbit and chick are well known to be the most susceptible animals and the guinea pig is the next susceptible, the rat being resistant to cholesterol feeding. In the authors' experiment the arteriosclerogenic effect of bacterial polysaccharide and glycogen showed the same relationship as to the species-difference in their arteriosclerogenic property.
Not only in the case of the administration of cholesterol but also of lanolin the abrupt swelling of endothelium, appearance of platelet-thrombi, or platelet-leucocyte thrombi and the decrease in the number of circulating platelets have been confirmed by the authors when the effective, though large, dose of these substances was administered orally at once to animals and the authors confirmed that this method is also useful in the oral administration of cholesterol or lanolin using a stomach tube to find the proper dosage of these materials.

Recently Cohn, Pick, and Katz found that the oral administration of cholesterol to chick exhibits a striking difference in the production of atherosclerosis according to the mode of administration. The group receiving cholesterol twice a day exhibited strikingly severe atherosclerosis as compared with the group nibbling the cholesterol all day long although the former group received a smaller amount of cholesterol as compared with the latter group. Also More and Haust examined 150 aortas and 100 coronary arteries of man and found that the atherosclerotic lesions did not start from the deposit of grossly or microscopically visible lipid. Many lesions were found to be initiated and many progressed by means of inflammation of the intima and/or thrombosis. The thrombosis has been proposed as the mechanism involved in the formation of atherosclerosis by Rokitansky and Duguid.

The principle of the authors' method of producing intimal arteriosclerosis may correspond with the findings of these investigators on atherosclerosis of man and animals.

McGill et al. (1959) stated that the values for coronary fibrous plaques strongly correlated with the percentage of natural deaths due to ischemic heart disease in the various necropsy groups, while values for fatty streaks do not. The authors' intimal arteriosclerosis is also characterized by intimal fibrosis or elastofibrosis as well as by the high incidence of myocardial infarction, and on the other hand the atherosclerosis produced by the classic method with the cholesterol feeding is characterized by the extremely low or no incidence of myocardial infarction despite the severe accumulation of lipids in the coronary lesion.

The appearance of cerebral apoplexy in the authors' intimal arteriosclerosis is also characterized by the venous hemorrhage in the thalamostriatal region which is a characteristic feature in the hypertensive or arteriosclerotic patients described by Scheinker (1945) and such a cerebral apoplexy was produced for the first time by the authors' method.

Before the discovery of Koch's mycobacterium the cause of tuberculosis had been discussed on its correlation with various factors, for instance the nutritious or hereditary factor. On the cause of atherosclerosis hundreds of such environmental and hereditary factors have been also discussed until the present time. The finding of the "germ" was the most important fact concerning the solution of the old troublesome problem of tuberculosis. Also in the problem of arteriosclerosis the finding of the "germ" seems to be the most important fact as compared the finding of some facilitating or aggravating
factors. What is the "germ" of the intimal arteriosclerosis and of atherosclerosis? We believe that the authors' method may be able to give an answer to such a question.

**Conclusion.** The authors emphasized the importance to find out the "arteriosclerogenic substance", i.e. the "germ" causing the arteriosclerosis, in the problem of aging.

Various substances, capable of eliciting the injury of endothelium, swelling and abolishing its silicone-like property forming the platelet- or platelet-leucocyte thrombi on some parts of the endothelium, have been found by the authors and considered as the "germ" causing the intimal arteriosclerosis including atherosclerosis. The process involves some inflammatory, thrombogenic as well as infiltrative mechanism.

The method to find out the arteriosclerogenic substance as well as its potency was detailed in this paper.

The authors' method of using cholesterol or other fatty arteriosclerogenic substances, especially with the combination of the other non-fatty arteriosclerogenic substance, may be a suitable technique to produce atherosclerosis which appears among people with hypercholesteremia and the authors' method of using a non-fatty substance may be a technique to produce a condition simulating the non-fatty intimal arteriosclerosis or atherosclerosis of man and animals not only under the high fat diet but also under the low fat diet.

The combination of several arteriosclerogenic substances with various endogenous substances or with different environmental conditions may produce various experimental types of arteriosclerosis with different visceral complications and such experimental intimal arteriosclerosis may present new information regarding the various morbid conditions of man, the causes of which remain obscure yet.

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**References**

3) Vitale, J. J.: Personal communication.
6) Personal communication.