Experimental Studies on Coronary Atherosclerosis

By

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(Received for Publication, July 16, 1960)

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Summary

Introduction

Experimental cholesterol atherosclerosis is now widely utilized for the study of human atherosclerosis, and in some points is considered as a model of the human disease. But from another point of view, there are certain differences found between the experimental and human diseases. The following facts are well recognized as the main differences:

1. In experimental atherosclerosis, the arterial lesions mostly consist of foam cell plaques containing enormously large amounts of lipids, and are combined with hyperlipemia and general organ and tissue lipid deposition. In the human disease, however, no such constant and marked organ lipid deposition or hyperlipemia are seen and the arterial lesion contains more fibrotic or hyalinized or calcified areas.
2. In the experimental disease, no necrosis, hemorrhage, ulcer or thrombus-formation of the focus can be induced and thus such organ injury as myocardial infarction or encephalomalacia can seldom be induced.

From the clinical point of view, the most important process of the pathology is the occlusion of the vessel lumen and the following ischemic injury of the organs, especially that of the heart and the brain. However most of the experimental studies on atherogenesis are focused upon the aortic atherogenesis, and there are but few reports on experimental coronary atherosclerosis and its following heart diseases.

Therefore, the author has taken up the experimental production of coronary sclerosis and resultant coronary heart diseases in the rabbit, and carried out comparative studies of these rabbit-materials with those of human lesions. In the latter part of the paper, the action of some anti-atherogenic substances and the experimental methods for the screening of these drugs are discussed.

I. Experimental Production of Coronary Heart Disease in the Rabbit

Experimental methods (Table I)

Albino-rabbits, weighing 2.5 kg in average, were maintained on the following experimental diet— anhydrous lanolin was dissolved in half its volume of cottonseed oil and 7 gm of this mixture, added
TABLE I  METHOD OF EXPERIMENTAL PRODUCTION OFATHEROSCLEROSIS.

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet</th>
<th>Combined Procedures</th>
<th>Sex</th>
<th>No. of Animals</th>
<th>Period of Feeding</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Okara + Lanolin</td>
<td>Long Period of Feeding</td>
<td>M</td>
<td>35</td>
<td>13-45 W</td>
</tr>
<tr>
<td>II</td>
<td>Okara + Lanolin</td>
<td>NaCl 5-7 g per day per os</td>
<td>M</td>
<td>5</td>
<td>23-30 W</td>
</tr>
<tr>
<td></td>
<td>Okara only</td>
<td></td>
<td>M</td>
<td>3</td>
<td>26 W</td>
</tr>
<tr>
<td>III</td>
<td>Okara + Lanolin</td>
<td>Allylamine 70-80 mg</td>
<td>M</td>
<td>10</td>
<td>26-28 W</td>
</tr>
<tr>
<td></td>
<td>Okara only</td>
<td>2 times per week (i. v.)</td>
<td>M</td>
<td>3</td>
<td>26 W</td>
</tr>
<tr>
<td>IV</td>
<td>Okara + Lanolin</td>
<td>NaCl (p. o.) + Allylamine (i. v.)</td>
<td>M</td>
<td>5</td>
<td>26 W</td>
</tr>
<tr>
<td></td>
<td>Okara only</td>
<td>10% NH₄Cl 2cc per day (i. v.)</td>
<td>M</td>
<td>5</td>
<td>20 W</td>
</tr>
<tr>
<td>V</td>
<td>Okara + Lanolin</td>
<td>Nicotine 4 mg per day (i. m.)</td>
<td>M</td>
<td>5</td>
<td>20 W</td>
</tr>
<tr>
<td>VI</td>
<td>Okara + Lanolin</td>
<td>Castrated</td>
<td>M</td>
<td>5</td>
<td>20 W</td>
</tr>
<tr>
<td></td>
<td>Okara only</td>
<td></td>
<td>M</td>
<td>5</td>
<td>20 W</td>
</tr>
<tr>
<td>VII</td>
<td>Okara + Lanolin</td>
<td>Not Castrated</td>
<td>M</td>
<td>5</td>
<td>20 W</td>
</tr>
<tr>
<td></td>
<td>Okara only</td>
<td></td>
<td>M</td>
<td>5</td>
<td>20 W</td>
</tr>
<tr>
<td>VIII</td>
<td>Okara + Lanolin</td>
<td>India Ink daily (i. v.)</td>
<td>M</td>
<td>10</td>
<td>17 W</td>
</tr>
<tr>
<td></td>
<td>Okara only</td>
<td></td>
<td>M</td>
<td>10</td>
<td>17 W</td>
</tr>
<tr>
<td>IX</td>
<td>Okara + Lanolin</td>
<td>Trypan blue (i. v.) Large Doses</td>
<td>M</td>
<td>10</td>
<td>14 W</td>
</tr>
<tr>
<td></td>
<td>Okara only</td>
<td>Trypan blue (i. v.) Small Doses</td>
<td>M</td>
<td>10</td>
<td>14 W</td>
</tr>
<tr>
<td></td>
<td>Okara only</td>
<td></td>
<td>M</td>
<td>10</td>
<td>14 W</td>
</tr>
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</table>

To the basic diet “Okara” (the refuse left in making soy bean curd) was the daily ration for the experimental rabbits. As possible methods for the production of coronary sclerosis, the following procedures were added to the experimental feeding; prolongation of the plain experimental feeding (group I), oral use of NaCl (group II), intravenous use of allylamine (group III), combination of NaCl and allylamine (group IV), NH₄Cl acidosis (group V), intravenous use of nicotine (group VI), castration in both sexes (group VII), intravenous use of India ink (group VIII) or trypan blue (group IX) for reticuloendothelial blocking (group X). Eight rabbits in group I were treated with Neo-Minophagen AT (Minophagen Co.) injections in order to increase the calcification of the focus. This drug contains histidine, lysine and arginine, and is known to have a calcifying action of tuberculous lesions.

**Experimental results (Table II)**

**General condition of the rabbits:** Most of the rabbits maintained on prolonged lanolin feeding showed emaciation in the late stadium of the experiment. Fat deposition on the sciera was seen in almost all the animals. Many of the rabbits showed depilation of the body and limbs, which was most frequent in the rabbits treated with allylamine. Some showed jaundice (4 in 35 rabbits of prolonged plain lanolin feeding, 3 in 8 rabbits treated with allylamine, 1 in 4 treated with NaCl) and soon died after its onset.

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![Fig. 1  E.C.G. of the rabbit (No. 162) fed plain lanolin. Depression of ST1 aVL in the 41st week, deeply inverted. T1 aVL in the 42nd week, and abnormality of QRS with inverted T, just before death.](image-url)
**Electrocardiograms:** Electrocardiograms of rabbits show a good deal of spontaneous variability so it is not always easy to detect truly significant changes. Only 4 of 35 rabbits of group I developed a definite abnormality of the coronary type, consisting of a deeply inverted T or depression of RS-T segment, (Fig. 1) and these rabbits exhibited a well marked myocardial lesion at autopsy.

**Serum cholesterol level:** The serum total cholesterol level before the experimental lanolin feeding ranged between 50–100 mg/dl, reached its highest point in a certain period of the experiment and decreased thereafter, though the levels showed marked individual variations. The combined use of NaCl

<table>
<thead>
<tr>
<th>Table II Production of Atherosclerosis in Lanolin Fed Rabbits.</th>
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<tbody>
<tr>
<td>Feeding Periods</td>
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<tr>
<td>-----------------</td>
</tr>
<tr>
<td>13/14 W</td>
</tr>
<tr>
<td>20 W</td>
</tr>
<tr>
<td>28 W</td>
</tr>
<tr>
<td>30 W</td>
</tr>
<tr>
<td>35/37 W</td>
</tr>
<tr>
<td>40/45 W</td>
</tr>
</tbody>
</table>

* Treated with Neominophagen AT.

seemed to have an increasing effect on the serum cholesterol elevation.

**Autopsy findings:** As shown in table II (group I), gross visible aortic atherosclerosis was first constantly recognized in the 13–14th week of the experimental feeding, and later the degree of lesions increased in proportion to the term of lanolin feeding. Along with this aortic atherogenesis, coronary arterial or arteriolar atherosclerosis appeared in some animals and the lesion was almost constantly produced in the animals fed for 26 weeks or more. In the early stage of the experiment, the atheromatous lesions consisted simply of the foam cell-plaque, but in the later stage, 30–45th week, marked fibrotic, hyalinizing and occasionally even calcifying tendencies were observed and the lumen of the coronary artery was narrowed or almost occluded (Fig. 2). The calcified lesions (Fig. 3) occurred rather frequently in the animals treated with Neo-Minophagen AT (3 cases in 8 animals). Myocardial lesions resulting from the marked coronary arterial narrowing or occlusion were almost constantly found in the animals fed for 30 weeks or more, and the commonest finding of the myocardium was the destruction of myocardial parenchyma as a scattered.

![Fig. 2](image_url) Huge atheromatous plaque of left coronary artery which was markedly fibrotic. The vessel is almost completely occluded. (No.36)
or localized fibrosis or scar (Fig. 4). These lesions were most constantly located in the left ventricular wall, especially the subendocardial myocardium and its papillary muscles. Thus from many points, the lesions were quite parallel with those of human coronary heart disease.

As for arteriosclerosis in other organs, pulmonary arteriosclerosis was produced most frequently, with splenic and renal lesions occasionally and in a few cases gross renal infarction was recognized.

The influence of combined procedures: The combined use of sodium chloride and allylamine, and reticulo-endothelial blockage (by India ink or a large dose of trypan blue) intensified the aortic as well as the coronary arterial atherogenesis. It is of much interest that one rabbit treated with sodium chloride died in the 23rd week of experimental feeding and showed a fresh myocardial infarct with marked hemorrhage (Fig. 5) at autopsy. The picture was quite similar to that of human recent infarction.

![Fig. 3 Calcified atheromatous plaque of the coronary artery which is completely occluded.](image)

![Fig. 4 Extensive tear in the left ventricular wall. (No. 35)](image)

![Fig. 5 Fresh myocardial infarction with hemorrhage. The animal (No. 10) kept on lanolin+NaCl died in the 23rd week of feeding.](image)

The injection of a small dose of trypan blue, though its accelerating effects on the reticulo-endothelial function was reported by some authors, showed in our experiment no remarkable influences on atherogenesis. The combination of nicotin injections, ammonium chloride acidosis or castration in the male and female did not produce significantly different results from those obtained with lanolin alone.
II. Studies on the Pathogenesis of Atherosclerosis

From the etiological standpoint, it is a very important problem to determine whether lipid materials are the primary cause of the vascular pathology, or merely secondary deposits in the injured arterial wall, possibly induced by some other cause. More recently, some authors found rearrangement of mucopolysaccharides of the arterial wall preceding the lipid deposition, and took up this fact as data for opposing the lipid theory of atherogenesis[2]-[12]. From this point of view, the formerly reviewed rabbit arterial materials, human aortas of various ages, and arteries of rats injured by agents other than cholesterol, were compared in every stage of development, and the mechanism of the lipid deposition and infusion of some material into the vessel wall were also observed.

Comparative observation of the development of human and experimental atherosclerosis

(Table III)

In the human aortas of a fetus and female child 8 years old, the toluidin blue-metachromasia positive substances at pH 4.6 were universally diffused in the medial layer, this being a normal and a non-pathologic feature. In the second decade, toluidine blue-metachromasia at pH 2.5 was first visible in the media, and in the 3rd decade the metachromasia positive substances at pH 4.6 started its pathological irregular rearrangement and increase in the media as well as in the intima. In the 5th decade the metachromasia positive substances at pH 2.5 also appeared in the intima. These acid mucopolysaccharide changes were intensified in proportion to age, but in the extreme later stage these changes in the media located just under the serious intimal atheroma or calcified lesion were inversely retarded and decreased in degree. Thus it can be seen that the acid mucopolysaccharide changes preceded the lipid deposition as some authors have stated.

In the rabbit, normal aortic medial layer showed diffuse homogenous distribution of the metachromasia positive substances at pH 4.6 similarly as in the human fetal or child cases. At the end of the first experimental lanolin feeding, the above mentioned rearrangement of the mucopolysaccharides first appeared and gradually were intensified towards the 14th week of experimental feeding. The cholesterol was first visible in the 3rd to 7th week and the intimal thickening in the 12th week, when the changes of the acid mucopolysaccharides appeared in the thickened intima which also progressed gradually thereafter. In the extreme late stage, the mucopolysaccharide changes in the media just adjacent to the intimal atheroma was found to be weakened as seen in the human elderly cases, though the changes in the intima were yet in progress.

Lipase activity after Gomori's method was also tested in both the human and rabbit material. In the human material it was positive in the early slight atheromatous lesion, such as seen in the cases of the 4 to 6th decade, and the location of the positive reaction was situated in the so-called mono-
nuclear cells appearing in the intimal lesions. In the younger cases or too senile cases with marked findings, the reactions were both negative. In the rabbit material, it was markedly positive from the end of the first week to the 4th week of experimental feeding, and was negative in the untreated or in the later stage of the experiment, just like as in the human child or senile cases.

The calcification of the experimental atheroma was mostly found in the deep layer of the thickened intima just adjacent to the media, similarly as in the human cases. And most of the deposited calc seemed to consist of calcium phosphate, because the phosphate reaction was constantly positive in the area strictly equal to the microscopically observed area of calcification in both materials.

Thus from all points of view as above reviewed, human and experimental atherosclerosis are quite similar. The early mucopolysaccharide changes of the vessel wall preceding the lipid deposition do not support the opponent opinion to the lipid theory of atherogenesis, because these changes are just equally found in the experimental lesions caused by lipid alone. Also such changes are found in our other experiment, in the arteries of the rats fed NaCl or treated with repeated allylamine injections, and therefore must be considered as unspecific signs of early vascular injury.

The progress of the organ lipid deposition in the experimental lanolin feeding

At the end of the 2nd, 5th and 10th week of experimental lanolin feeding, 5 rabbits each were sacrificed to estimate the cholesterol and phospholipid content of the aorta, liver and blood plasma (Fig. 6). In the liver, the cholesterol content reached its maximal level as early as in the end of the 2nd week and no further elevation seen, but the phospholipid content showed no such marked elevation at any stage; the highest being only 1.5 times of normal value in the 5th week. The plasma cholesterol level reached its peak in the 5th week and decreased thereafter, and the plasma phospholipid level took a paralleled course with the cholesterol level.

The cholesterol content of the aorta, however, showed very little elevation in the 2nd or 5th week though sharp marked elevation occurred in the 10th week, and phospholipid of the aorta reached its peak — only twofold of the normal level — in the 5th week and decreased in the 10th week, in spite of the marked cholesterol elevation.

This early cholesterol elevation in the plasma or liver and phosholipid elevation in the aorta, which precedes the aortic cholesterol elevation seems to suggest the existence of some general precursing state for atherogenesis or some atherosclerosis inhibiting reaction. The stadium of these phenomena corresponds to that of mucopolysaccharide changes in the vessel wall, and once the lipids are infused into the vessel wall they may be phagocytized by mononuclear cells to form foam cell plaques, which may later be substituted by fibrotic tissues.

Experimental studies on ultravital lipid deposition

In order to examine the ultravital lipid deposition, the excised rabbit aorta was ligated at one end, filled with lipemic rabbit serum and connected with a syringe by a plastic tube. This aorta was placed in phosphate buffer of 37°C and a pulsating pressure equal to blood pressure was applied to the inner vessel wall for 3 hours. After this procedure the aortic material was sectioned and histochemically studied for lipid deposition. By this experimental procedure, there was no lipid infusion detectable in the normal aorta, but a diffuse lipid infusion was recognized in the aortic specimen which had been injured by repeated injections of allylamine before sacrifice. This may show the ready affinity of the injured vessel wall to fat infiltration,
Infusion of some substances other than lipid into the vessel wall

Daily intravenous injection of carbowax solution (20 cc of 5% solution per day per animal) for 10 months did not yield any atheromatous lesions. Trypan blue, Congo red, Arizarin, Sudan III, Orange G and Fuchsin are not so toxic and the rabbits were able to tolerate to repeated intravenous injection of the dyes for 1-3 months. Among these dyes, Trypan blue and Congo red stained the arterial inner wall markedly, though the staining of the wall was diffuse and no plaque formation detectable. In paper electrophoretic studies, these dyes showed little tendency to conjugation with serum protein, though many other dyes readily conjugated with serum albumin when incubated with human serum in 37°C for 30 minutes. The author was unable to find any agent other than cholesterol that caused atheroma-like vascular lesions.

Thus in the range of our above experiments, there is no data that opposes the lipid theory of atherogenesis, although perhaps not enough to support the theory. And the experimental cholesterol atherosclerosis was recognized as valuable for the study of the human disease.

III. ON ANTI-ATHEROGENIC DRUGS

There are many hypcholesterolizing antiatherogenic drugs introduced and some of which are appearing on the market. The author studied the effects of some these drugs upon the experimental atherogenesis of rabbits.

The drugs were administered to the animals all through the experimental period up to sacrificing, and the data was read by blood plasma cholesterol level and gross aortic findings. The effects of the drugs on the coronary atherogenesis seemed to be represented by its effects on the aortic lesions, the previous experimental data showed the parallelism of the both. The period of experimental feeding was about 4 months, which seemed to be optimal for comparison of the data. The number of animals in each group was 10, because the variation of individual data was so marked that to reduce the number of animals would make statistical consideration impossible.

1. Chondroitin sulfate

Effects in experimental feeding: Chondroitin sulfate was used, 0.5 gm oral or 100 mg intravenous, along with the lanolin feeding. Because of the marked individual variation of the data, the experimental feeding was repeated for three times. In the first experiment, cholesterol level on sacrificing showed an average of 385±229 mg/dl in the group orally dosed, and 869±451 mg/dl in the control group, while the aortic atherogenesis seemed to be inhibited in the former group. In the second experiment, the serum cholesterol level in the 5th week was about half that of the control group in both oral and intravenous chondroitin sulfate group, but in the 14th (final) week the level was rather higher in the oral group than in the control, and aortic findings were rather inhibited in the oral group and rather increased in the intravenous group. In the third experiment, the intravenous as well as the oral use was both effective on serum cholesterol level and aortic atherogenesis. (Fig. 7)

![Graph showing effects of chondroitin sulfate on plasma cholesterol levels of lanolin fed rabbits.](image)

Fig. 7 Effects of chondroitin sulfate on plasma cholesterol levels of lanolin fed rabbits.

Considering the above 3 experiments, it seemed that the drug was effective as a serum cholesterol depressant and antiatherogenic.

On the mode of action of chondroitin sulfate: The drug can not be a cholesterol absorption inhibitor in the intestine because it was effective by intravenous use.

Because its chemical structure is a sort of sulfated polysaccharide like that of heparin, the action mode of both substances was comparatively observed. The serum of the rabbit which was dosed with chondroitin sulfate, when added to lipemic dog serum did not show a lipemia clearing activity like serum of the heparin dosed rabbit, and the lipoprotein ratio ($\beta$-lipoprotein: $\alpha$-lipoprotein) of the dog serum did not show an decrease in the ratio by the addition of chondroitin sulfate treated rabbit
serum. As an "in vivo" experiment, lipemic rabbit serum was intravenously injected in the condroitin sulfate treated rabbits, and serum turbidity decrease in the latter was estimated periodically. No acceleration was observed in the condroitin sulfate treated animals but in the heparin treated rabbits an acceleration was observed, as many authors have reported. In this experiment, however, the total cholesterol of the condroitin sulfate treated rabbit that was injected with the lipemic serum, was more rapidly removed than that of the untreated rabbit, and the decrease of the lipoprotein ratio was also accelerated compared to the control rabbit. In this "in vivo" experiment, in place of the lipemic serum, a cholesterol suspension was injected, with similar effects.

2. Phenylethyl acetic acid derivatives\(^{16,18}\)

Effects in experimental feeding: The following 6 derivatives of phenyl-butyrate were tested by orally administrating 0.5 gm daily in the lanolin feed.

**Chemical Structure of Derivatives of Phenyl-ethyl-acetic Acid**

1) \(\text{CHCOONa} \quad \text{C}_2\text{H}_5\) 
   Sodium-\(\alpha\)-phenyl-N-butyrate
   P.B.-Na

2) \(\text{CHCO-NHCH}_2\text{CH}_2\text{CH}_2\text{CH}_2\text{CHNHCO-CH} \quad \text{C}_2\text{H}_5\)
   N-N-sis dl-\(\alpha\)-phenyl butyryl-l-lysine salt
   D.P.B.-Lysine

3) \(\text{CHCONH}_2 \quad \text{C}_2\text{H}_5\)
   \(\alpha\)-phenyl butyryl amide
   P.B.-amide

4) \(\text{CHCONHCH}_2\text{SO}_3\text{Na} \quad \text{C}_2\text{H}_5\)
   \(\alpha\)-phenyl-N-butyryl taurine salt
   P.B.-Taurine

5) \(\text{CH} \quad \text{CONH-CH} \quad \text{C}_2\text{H}_5\) 
   \(\text{OH-CH} \quad \text{O} \quad \text{H-CHOH} \quad \text{HO-CH} \quad \text{HO-CH}_2\)
   \(\alpha\)-phenyl-N-butyryl-\(\alpha\)-glucosamine
   P.B.-Glucosamine

6) \(\text{CHCONH} \quad \text{HOOC-CH-CH}_2\text{CH}_2\text{SCH}_3 \quad \text{C}_2\text{H}_5\)
   \(\alpha\)-phenyl-N-butyryl-methionine
   P.B.-Methionine

P.B.-Na had no effect on the cholesterol level nor aortic atherogenesis.

P.B.-Methionine, P.B.-Taurine, and P.B.-Glucosamine were all effective in lowering the cholesterol level, and P.B.-G and P.B.-T were especially effective; the cholesterol of the group treated with either of the drugs showed levels 1/5–1/7 of the control group in the 5th and 10th week of feeding, though P.B.-G was used only for the first 13 weeks and afterward this group was fed the plain lanolin mixed diet as the control group to the end of the 17th week, when all animals were sacrificed (Fig. 8).

![Graph](https://example.com/graph.png)

**Fig. 8** Effects of P.B.-T., P.B.-G., & P.B.-M. on plasma cholesterol levels in rabbits fed lanolin.

The aortic atherogenesis was mostly inhibited in the P.B.-T. treated group, followed by the P.B.-G. group and only slightly in the P.B.-M. group. The P.B.-amide and D.P.B.-lysine were also tested in the lanolin fed rabbit, with the former showing a slight serum cholesterol-lowering effect only in the 4–6th week and the latter rather elevating effect. In autopsy findings both showed no effects.

**On the action modes of the derivatives:** After administrating these derivatives into rabbits, a cholesterol suspension was intravenously injected, and the rate of removal from the blood was compared with a control case. None of the derivatives however, were able to accelerate the removal.

Experimental oral administration of P.B.-T., which showed the most definite effect in the first experimental feeding, was again attempted under different conditions. In the second experiment, administration of P.B.-T was started two weeks after the lanolin feeding has begun, but no effect of the drug was observed on the plasma cholesterol level or atherogenesis. In the third experiment, P.B.-T with lanolin was started 1 month after the cessation of a preliminary 2 weeks lanolin feeding,
when the plasma cholesterol level had returned to normal. Here again, however, the drug inhibition of the lanolin induced hypercholesterolemia was less than in the first experiment. These findings suggest a decrease in the effectiveness of P.B.-T. after the onset of some organ dysfunction caused by lanolin feeding.

In this experiment, the cholesterol content of the liver was estimated in the rabbits sacrificed at the 2nd, 5th and 10th week of the lanolin feeding. The liver cholesterol level equally increased in the P.B.-T. treated, as well as in the control group, until the 5th week of feeding. But thereafter the liver cholesterol in the P.B.-T. treated group increased still more towards the 10th week, while that in the control group showed no further elevation. This 10th week, coincides with the time when the hypocholesterolemic action of P.B.-T. is no longer recognized. Thus some close relationship between the liver function and the hypocholesterolizing effect of the drug is suggested.

3. Sunflower oil

Two experimental groups in which 25 or 50% of sunflower oil was added to the 4gm of daily lanolin per rabbit were used, and the animals kept on these oil contained lanolin diets for 6 weeks. The plasma cholesterol levels in both groups on the oil supplemented diet were lower than in the control group fed lanolin alone for the first 2 weeks, but later a lowering of cholesterol level was only recognized in the group fed the 50% oil supplemented diet and not in the other group (Fig. 9). The cholesterol content of the aorta or the liver showed no significant differences among these groups.

4. Female sex hormone

Daily intramuscular injection of 1 mg per kg of estradiol benzoate from the 5th week to the 10th week of lanolin feeding in rabbits, reduced the plasma cholesterol elevation but not the phospholipid elevation, thereby causing the plasma C/P ratio to descend more significantly (Fig. 10). In autopsy findings, however, inhibitory effects of the hormone were never significant on aortic as well as coronary atheromatosis, although in this case the administration of the hormone was continued only for 5 weeks.

IV. STUDIES ON THE METHODS FOR THE EXPERIMENTAL FEEDING OF THE RABBIT AS A SCREENING METHOD FOR ANTI-ATHEROGENIC DRUGS

The question whether drugs found effective by this method will have any significant clinical value, will not be taken up, and the author will comment only on methodological problems which yet remain unanswered.

(1) There are marked individual variations of the serum cholesterol elevation as well as aterogenesis observed from animal to animal even in the same experimental group, and the severity of atherogenesis is not always parallel to the plasma cholesterol elevation.

(2) The severity of the atheromatous lesions is usually graded by the point system or by a simple (+) or (--). These methods leave much to be desired in as much as it is difficult to avoid subjectiveness in the evaluation. Therefore, a more suitable quantitative appraisal method is desirable for pure objective estimation and statistical treat-
ment of the data.

(3) It is also desirable to reduce the period of the experimental feeding when adopting this method for screening, whereas, at present, production of grossy visible atherosclerotic lesions are usually not seen before the 12–20th week of feeding. The following experiments were carried out from the above mentioned point of view.

1. The relationship between the lipid concentration of plasma and some organs and the severity of atheromatosis

The plasma cholesterol and phospholipid levels were determined every 3rd or 4th week in 18 rabbits fed lanolin following the method previously described, and the animals were all sacrificed in the 17th week to determine the cholesterol content of the aorta and liver to compare them with serum lipid values.

A fair parallelism was obtained between the severity of the aortic atheromatosis (presented by + or − signs) and the cholesterol levels in the 6th or 9th week of feeding, but there was no significant correlation with the levels in the later or terminal stage. The cholesterol content of the liver was not related to the degree of atheromatosis. But the total cholesterol extracted from the aorta itself correlated to some extent to atheromatosis of the aorta (Fig. 11). This relationship between the aortic lesion and its cholesterol content is naturally expected, so long as the lesion is not too advanced to contain much fibrosis. Therefore the aortic atheromatosis can be said to be represented by its cholesterol content. The correlation coefficients between the cholesterol content of the aorta as the indicator of atherogenesis, and the total cholesterol level of each experimental stage showed the highest peak at the 6th and the 9th week (r = +0.558 and +0.566 respectively) and then declined towards the terminal week (Fig. 12).

Fig. 12 Correlation between the cholesterol contents of aorta and plasma in each stage of lanolin feeding.

The correlation between the plasma C/P ratio and atherogenesis (aortic cholesterol level) also showed a similar tendency to that of plasma cholesterol and atherogenesis. The integral of the plasma cholesterol level in time represented by the area between the cholesterol curve and the base line, was also considerably correlated to the aortic cholesterol content. Among these several factors, the correlation of the plasma cholesterol in the 6–9th week to the aortic cholesterol content was most significant, so the severity of atheromatosis of the rabbit fed lanolin may be predicted by the estimation of plasma cholesterol in the 6–9th week of experimental feeding. Thus we can appraise the effect of the hypocholesterologizing antiatherogenic drugs by the plasma cholesterol levels of the 6th and/or 9th week of lanolin feeding, and this will reduce the period of experimental feeding, as the foregoing relation between plasma and aortic cholesterol was applicable in the investigation of the P.B. derivatives and chondroitin sulfate as shown in our past experiment.

Besides, aortic cholesterol estimation as an indicator of its atherogenesis offers an advantage in that statistical treatment can be made of the data.

2. On the individual variation of the experimental data
As formerly reviewed, the individual variation of the data in plasma cholesterol elevation, as well as in atherogenesis, was marked from animal to animal. The author made some observations concerning these problems in order to predict the possible individual variation of such data in future, by which the number of animals in each experimental group might be decreased.

The plasma cholesterol elevation in the rabbits after a 2 week period of lanolin feeding is, according to our study, fairly correlated with the future atherogenesis though not so highly as that of 6 or 9 weeks feeding. As a preliminary trial, a number of rabbits were fed lanolin for a 2 week period and they were devided into 2 groups; one which consisted of animals with little plasma cholesterol elevation and the other with high plasma cholesterol elevation, such as 500 mg/dl or more. After the cholesterol estimation, the animals were kept on a plain "Okara" diet until the cholesterol level was restored to the normal value and the following examinations were performed.

1. Hepato-sulphalein test
2. Congo red test for the function of the reticulo-endothelial system
3. Determination of the reduction rate of the intravenously infused cholesterol suspension

But there was no distinct tendency seen in the results from each group.

After these examinations, the animals were kept on the plain "Okara" diet for 3 months and again a 2 week cholesterol feeding was carried out, and plasma cholesterol levels estimated. These, however, were not quite parallel to the former two week cholesterol values. This data shows the plasma cholesterol elevation after a two week lanolin feeding has no constant reproducibility in such experimental conditions.

Therefore in order to eliminate individual variation possibly caused by an ununiformity of intestinal cholesterol absorption, the parenteral (intravenous) use of a cholesterol emulsion instead of oral lanolin was tried, though the following prescribed emulsions were all too toxic for prolonged use.

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<td>Tween 80</td>
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<td></td>
<td>cholesterol</td>
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<td></td>
<td>olive oil</td>
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Thus at the present stage of our experiment, the problem of individual variation is unsolved and the use of a great number of animals for each group is unavoidable.

**SUMMARY**

1. Coronary atherosclerosis in the rabbit along with aortic disease induced by a long term lanolin feeding in the animal, and the resultant stenosis or occlusion of the vessels caused the coronary heart disease, were similar to such lesions as acute myocardial infarction or chronic myocardial fibrosis in humans.
2. The combined use of vascular injury by allylamine or sodium chloride treatment, or of reticuloendothelial disturbances through India ink or trypan blue administration along with the lanolin feeding, precipitated the coronary and aortic atherogenesis.
3. In experimental cholesterol atherosclerosis, the fibrotic, hyalinizing or calcifying tendencies of the lesion and the occlusion of the vessel lumen, may be produced as the pathologic progress just like as in human atherosclerosis. And further as a resultant of these changes, organ parenchymal lesions may likely be produced.
4. In the experimental cholesterol atherosclerosis, the mucopolysaccharide changes in the vessels ground substance were seen to precede the intimal fat deposition, equally like as in the human pathology. The changes of mucopolysaccharides, however, may represent a sort of vascular injury which occurs in many vascular disease other than atheromatosis, and the fact can not support any opposing opinion to the lipid theory of atherogenesis.
5. Chondroitin sulfate, α-phenyl-N-butryltaurine salt, α-phenyl-N butyryl-d-glucosamine or estradiol benzoate seemed to be effective on lowering the plasma cholesterol level and inhibiting atherogenesis.
6. As a screening method for anti-atherogenic drugs through a plasma cholesterol lowering action, the administration of the drug can be terminated in 6 to 9 weeks of experimental feeding and the final plasma cholesterol value can be evaluated as
an indicator of their effects.

(7) As a method for comparing the degree of aortic atherosclerosis, the estimation of the aortic cholesterol content is valuable, so far as the lesions are not in a too advanced stage, because the method offers the statistical treatment of the data.

I wish to offer the thanks to Prof. Imasato Donomae for his continuous guidance. Thanks are also due to my co-workers, Dr. T. Kokubu, Dr. M. Sakai, Dr. R. Koide, Dr. N. Hosokawa, Dr. R. Kobayashi and Dr. T. Hashimoto for their cooperation in preparing this report.

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