
ALTSCHUL • SELECTED STUDIES ON A SCLERO MAS

Selected Studies

on

ARTERIOSCLEROSIS

By

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TO THE MEMORY
OF MY SISTER WILMA KATZ AND OF HER 12 YEAR OLD BOY TOMY
AND TO THE MEMORY
OF MY BROTHER FRANTIŠEK ALTSCHUL

PREFACE

The title of the present book was chosen intentionally to indicate that this is a collection of various studies on arteriosclerosis. It should be added that it deals both with human and with experimental arteriosclerosis and that the various chapters are only loosely associated. The book therefore makes no pretense of being either a review or a final and complete report on arteriosclerosis. It makes no claim of being of assistance to the clinician or even to the hospital pathologist.

In it are observations which may be new and others which are not. With some hesitancy certain results are correlated and others are dissociated. Also considered are lesions of tissues and organs other than the vascular system. They are included because they result from the same factors that elicited experimental arteriosclerosis.

Looking over the book after it was written and recognizing its incompleteness and the paucity of its conclusions, I was tempted to label it: "Volume I," thus stressing that it is an unfinished work and that at a later date additional reports and conclusions will be published. However I was afraid to anticipate such results and to make promises which may never be realized. Therefore I do not call this book "Volume I," though I do hope to extend the work and later to record the findings thus obtained in another volume.

R. A.

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CONTENTS

I

Relationship between human and experimental arteriosclerosis

SOME AUTHORITIES believe that the riddle of arteriosclerosis will eventually be solved by the biochemist rather than by the pathologist. But even if it be ultimately proven that chemical changes in the blood are the principal pathogenic factors, the active help and knowledge of the morphologist will be required to find out whether the alleged chemical changes are responsible for the various vascular alterations which constitute the arteriosclerotic syndrome. Therefore the histopathologist should be well prepared to meet such a request, aside from the possibility of establishing the pathogenesis of arteriosclerosis primarily by means of morphological studies.

In the present volume we shall deal with some aspects of human and of experimental arteriosclerosis. Therefore it may be worthwhile to give a few introductory lines on experimental arteriosclerosis for those pathologists who are not acquainted with this aspect. However, for a thorough study of the subject, the symposium by Cowdry and the reviews by Duff and by Hueper should be consulted.

In the past, various substances have been used in the attempt to produce arteriosclerosis in animals, the most important being epinephrine, vitamin D, nicotine, bacterial toxins, cholesterol and cholesterol derivatives. Hemodynamic and traumatizing agents have also been tried as well as combinations of some of these methods, for example, vitamin D and cholesterol, cholesterol and trauma.

Concomitant with attempts to produce arteriosclerosis experimentally, arose the problem whether or not results obtained by

such experimental methods are identical with or essentially similar to spontaneous arteriosclerosis. At present, this problem is not yet solved.

One of the most frequently used means for producing arteriosclerosis has been and still is the feeding either of pure cholesterol or of diets rich in cholesterol. The results thus obtained have not been accepted by many authorities as identical with human arteriosclerosis nor even as closely related. There are several reasons for this attitude: feeding of cholesterol in excess causes distinct vascular changes only in rabbits, less distinct changes in guinea pigs, and either no changes in carnivores and omnivores or alterations which are not accepted as experimental arteriosclerosis on the grounds that they may be spontaneous lesions. Another reason to reject identity or essential similarity of human and experimental cholesterol arteriosclerosis is the fact that the former is not generally accompanied by cholesterolemia (Hirsch and Weinhouse) though an increase in blood cholesterol was recorded by some authors (Morrison et al.) in the majority of patients under 60 years of age who had coronary occlusion.

One objection against accepting the identity of common spontaneous arteriosclerosis and experimental cholesterol arteriosclerosis is the nature of the alterations: in experimental cholesterol arteriosclerosis, lipoid cushions and foam cell accumulations are the main pathognomonic features; they are relatively scarce in spontaneous arteriosclerosis, except perhaps for the diabetic type (Sobotka) and for the vascular changes in lipoidoses. Vice versa, mucoid degeneration is rare in experimental arteriosclerosis. As will be shown later, the prevalence of gross lipoid accumulations and of innumerable foam cells in experimental cholesterol arteriosclerosis may be the reason why moderate alterations, which resemble much more the spontaneous type of arteriosclerosis, were and still are being overlooked.

One other reason for not accepting experimental arteriosclerosis as an equivalent of the human form was the fact that changes of cerebral vessels could not be elicited in animals (Duff's review; Pollak, 1945), though they are very frequent in man. This reason is no longer valid: by feeding dried and heated egg yolk to rabbits I succeeded (1946) in producing, in a number of animals, foam

cell formation in the lining of cerebral vessels and around their wall and that not only in the choroid plexus where it had been observed previously (Versé), but also in the region around the third ventricle, in the meninges, in the pineal gland and recently also in the posterior lobe of the pituitary. Only once was a vascular lesion found in the basilar artery. Admittedly, frequency and intensity of changes in the cerebral vessels are much slighter than in man, also the morphological picture is not identical.

It may be well to defer making any decision regarding the similarity or dissimilarity of spontaneous and experimental arteriosclerosis, the more so as we do not know or do not agree as to what exactly arteriosclerosis is. We may however continue to use the experimental approach not merely for studying arteriosclerosis per se, but also for studying any changes which can be elicited in the vessel wall, whether or not they should be regarded as arteriosclerotic.

For the sake of accurate studies it is desirable to produce both exaggerated and moderate alterations, affecting the endothelium, the ground-substance, the elastic tissue, and the media of vessels. The combination of such single alterations may supply pathological syndromes similar to or identical with the kaleidoscopic aspect of human arteriosclerosis. However, the morphological and especially the cytological study of arteriosclerosis has almost been abandoned, because for a number of years the biochemical approach and the study of pathogenesis have been considered more promising. Further, the overall picture of human arteriosclerosis makes a microscopical diagnosis easy and produces a feeling of knowledge, which, as I shall try later to show, we do not possess.

Species and individual differences in experimental arteriosclerosis and, still more, differences between experimental and spontaneous arteriosclerosis in animals deserve much attention because they may help to explain the causes of differences in the human disease. This aspect is of such importance that it will be discussed extensively in a later chapter.

II

Experimental cholesterol arteriosclerosis

TERMINOLOGY-TECHNIQUE

THE TERMINOLOGY used in experimental arteriosclerosis meets with difficulties. First of all, it is not clear whether the experimental vascular alterations are to be called arteriosclerosis. Also the name *experimental atherosclerosis* has been used, though this is hardly an improvement, since proliferative sclerosis may readily show atheromatous decay in human, and, more rarely, also in experimental arteriosclerosis. To distinguish at least one type more clearly, Duff used the term experimental cholesterol arteriosclerosis. In view of the fact that, in addition to blood vessels, other organs and systems become primarily affected in experiments with cholesterol, Schönheimer introduced the term "cholesterol disease of rabbits" (*Cholesterinkrankheit der Kaninchen*), thus avoiding the narrower term of cholesterol arteriosclerosis. However, in my opinion even Schönheimer's proposal is not appropriate, because "disease" implies signs and symptoms during life which in these cases are lacking, not detectable or neglected. Thus it may be best to use the term "lipoidosis," though it has already been given special significance in naming such diseases as Gaucher's, Niemann-Pick's, Hand-Schüller-Christian's, etc. On the other hand, cholesterolemia may elicit changes other than lipoidosis, e.g. proliferation of endothelium, and hyalinization with secondary calcification of muscle. Therefore the pathological changes produced by continuous cholesterolemia could perhaps be covered by the name of cholesterolosis.

In the present report, I shall use the term *experimental cholesterol arteriosclerosis* when the vascular damage is to be characterized; the terms *lipoidosis* when the foam cells and lipoid cushions are meant,

whether they developed in the vessel wall or elsewhere; and *cholesterolosis* or cholesterol damage if the changes include lipoidosis as well as necrosis with or without calcification.

Various experimental techniques have been used on different species. Feeding cholesterol, or a diet rich in cholesterol, is mainly used on rabbits but results are also obtained with guinea pigs, golden hamsters, and chickens. Monkeys, dogs, cats, rats, and mice have been tried, but unless the cholesterol technique was combined with some other procedure and thus the establishing of arteriosclerosis facilitated, the results were either negative or not accepted as being caused by cholesterol feeding.

In our investigations of experimental arteriosclerosis we used mainly rabbits, but also guinea pigs, golden hamsters (*Cricetus auratus*), prairie gophers (*Citellus Richardsonii, Sabine*) and white rats. The animals were fed capsules containing either cholesterol or cholesterol derivatives, with cottonseed oil added either directly to the powder, or in separate capsules, or spread on oats. Some of the animals received milk, the fat of which seemed to promote the cholesterol absorption. Other animals were given a diet consisting of milk with yolk powder and yolk cake. The latter was prepared by mixing dried yolk powder with flour in the proportion of 4:1, adding yeast, some salt and enough water to make a dough. After rising, the latter was baked in an oven at 300°–500° F. This cake was given *ad libitum*. Twice a week, hay and occasionally some bread were added to the food. In several cases the cholesterol capsules were alternated or combined with the yolk diet.

Most of the rabbits, hamsters, some guinea pigs, all the gophers and, as could be expected, all the rats took readily to the milk-yolk diet. Many rabbits however refused it before the end of the second month and cholesterol capsules together with milk were then substituted for the yolk diet. There were greater difficulties in feeding the milk-yolk diet to guinea pigs. Many refused it, others died after approximately one week, sometimes with paraplegia. In such cases, the microscopical examination of the spinal cord and of the brain was negative. An avitaminosis is tentatively assumed to have been the cause of death. Ten hamsters and seven gophers took the diet very well; the latter especially thrived and became fat.

Some of the animals were killed intentionally at a certain period,

but several rabbits and guinea pigs died spontaneously at various periods as a sequel to the experiment.

Previously, cholesterol dissolved in oil, or fresh yolk, had been fed by means of a stomach tube, or the cholesterol had been administered in capsules. Our mixing of the yolk powder with milk and baking it into a cake proved very expedient but implied a deviation from the technique of others in that the cholesterol compounds of yolk were probably altered by the drying and heating. This is a possible explanation of the somewhat different results obtained in our experiments, in contrast to those of other experimenters. Accordingly, we started to alter cholesterol intentionally.

In view of the fact that previous experiments on rats had been carried out only with fresh yolk we included a number of rats in our experiments with dried and heated yolk. However, even this modification gave no results. Finally, to bypass the alimentary tract, we painted heated cholesterol, dissolved in benzol and some vegetable oil, on the skin in the interscapular region. The following list gives our technical procedures:

(1) Milk-yolk diet was fed to rabbits, guinea pigs, hamsters, gophers and rats;

(2) Rabbits and guinea pigs received pure cholesterol in daily doses from 0.06 gm. (guinea pigs) and 0.3 gm. (rabbits) to 0.48 gm. (guinea pigs) and 2.4 gm. (rabbits);

(3) Some rabbits received pure cholesterol heated for 35 minutes at 300° C, following the routine of Roffo who by this procedure elicited stomach cancer in rats;

(4) Vessels were ligated in rabbits and rats, some of which had previously been given cholesterol or the milk-yolk diet;

(5) Plain catgut sutures were inserted subcutaneously and intramuscularly in animals which had received a cholesterol treatment for various times, to study the granulation tissue in animals with cholesterolemia;

(6) Skeletal muscles of one leg were denervated in rabbits prior to feeding the milk-yolk diet, the denervated muscles being compared after death with those of the opposite side;

(7) Pure cholesterol and also dried yolk powder were irradiated extensively with ultraviolet light, a procedure used by Roffo for oxidizing cholesterol at low temperature;

(8) Rabbits and rats were given cholesterol capsules or fed the milk and yolk diet and were daily exercised for two hours in a rotating drum (a technique previously used by Pfleiderer on rats together with feeding them minimal doses of cholesterol and either vitamin D or epinephrine);

(9) Vitamin D was given to rabbits in amounts of 10,000–50,000 units daily or sporadically for 5–67 days with the intention of studying possible damage to skeletal muscle;

(10) Liver extract was given to rabbits along with the milk-yolk diet, to find out whether this treatment prevents or attenuates the damage attributed to cholesterol;

(11) Pure or heated cholesterol, cholesterol derivatives or stigmasterol were dissolved in benzol and oil and painted on the skin of rabbits, guinea pigs, and rats;

(12) Rabbits, guinea pigs, and rats were given the milk-yolk diet or cholesterol capsules and were in addition exposed to chloroform or benzol vapors with a view to studying the influence of chloroform and benzol as fat solvents and also as noxious agents to the liver, bone marrow, etc.;

(13) Cholesterol derivatives (as 7-ketocholesterol, cholestenone, cholesterilene), and stigmasterol, were fed; and also

(14) Cholesterol which had been finely pulverized and then treated with strong hydrogen peroxide.

Little attention was paid to sex, age or body-weight of the animals and no exact record was kept of their milk-yolk consumption. This was deemed useless, because it had been shown by previous investigators that sex, age, and the amount of cholesterol given have no direct influence on the type and intensity of experimental arteriosclerosis (Duff, Hueper). Contrariwise, Pollak (1947) stressed the importance of standardizing the cholesterol technique by considering weight and age of the animals used. However, the same author had stated previously (1945) that the threshold for cholesterol damage differs from animal to animal.

Our own results did not support Pollak's view for it appears that individual differences and differences in animal strains influence the course of the development of lesions much more than do weight, age or sex (if these have an appreciable influence at all). It seems for instance that albino rabbits are more susceptible than

others. However, even animals from the same litter, kept in the same cage, and fed milk with yolk and yolk cake, developed vascular and organ changes of different intensity, in spite of the fact that the food was supplied in excess so that the stronger animal should not deprive the weaker one of its share. Attempts to standardize the technique of cholesterol arteriosclerosis will perhaps prove successful if they are carried out on inbred animals and attention is given to seasonal changes in the endocrine system, to gestation, exercise, etc. A major factor may be the state of nutrition of the animals at the onset and during the experiments. The amount of available adipose tissue may play a role in the establishment of cholesterol arteriosclerosis (see also page 152). It has been reported that rabbits, given amorphous cholesterol without added fat, mobilize their own fat reserves (Popjak). However the animals deplete their fat reserves even if they receive the milk and yolk diet, which contains much fat. The question arose during the present series of experiments whether any standardization is necessary or desirable. The standardization may be indicated if the investigations are carried out for studying measures of prevention and cure. But for studying the pathological pattern of experimental arteriosclerosis, which was our aim, a very wide range of possible alterations should be attempted. Therefore we did not try to suppress differing reactions of single animals, of animal strains, and of species.

Human material was used in the investigation for studying the cellular changes in the intima and media of sclerotic arteries. For this purpose, mainly vessels of the extremities and cerebral vessels of persons over 50 years of age were examined.

In the experiments with oral or percutaneous administration of cholesterol, cholesterol derivatives, or stigmasterol, and with feeding of cholesterol-rich diets, 111 rabbits, 20 guinea pigs, seven prairie gophers and ten golden hamsters were used, besides a considerable number of rabbits, guinea pigs, rats, golden hamsters, prairie gophers and chickens which served for controls.

There is little to say about the preparation of the material: the tissues were fixed in formaldehyde solution or in Susa fixative or the latter was applied after preliminary formaldehyde fixation. Methods used were: hematoxylin-eosin, iron hematoxylin, Mallory's connective tissue stain or its modifications (Azan or Masson's