value$<$0.001. They also indicate that penicillin is effective in preventing wound infections due to "penicillin-resistant" staphylococci not previously exposed to penicillin (18/18 as compared to 4/17) provided the inoculum size is small and the penicillin dosage is adequate ($P<0.001$).

As originally observed by Kirby(1) the resistance of staphylococci to penicillin depends upon ability of penicillinase, synthesized by the bacteria, to inactivate penicillin before the bacterial cell itself is irreversibly injured. The individual cell in such a penicillin-resistant population is relatively susceptible to penicillin. The inoculum must be large enough to provide and maintain sufficient penicillinase in the environment to inactivate a given concentration of penicillin. This is the probable explanation for the difficulty in demonstrating penicillinase production when working with low density populations of staphylococci.

To demonstrate *in vitro,* a difference in penicillin-susceptibility of populations used, the large populations which were cultivated in broth for only 2 hours, with and without penicillin, were studied by tube-dilution technic. No *in vitro* evidence of modification of penicillin-resistance was observed. This seemed surprising in view of the partially substrate-adaptive nature of penicillinase synthesis by staphylococci(4,5). However, it has been observed(6) that synthesis of penicillinase by staphylococci is significantly influenced by the composition of the nutritional environment. Therefore, increased penicillinase production stimulated by previous exposure to the substrate, penicillin, could have occurred in the more complex nutritional environment *in vivo* in spite of the fact that it was not manifested *in vitro.*

In the continuing effort to control hospital infections due to "penicillin-resistant" staphylococci, it appears that control of penicillin in the environment may be as important as the distribution and genetic character of the staphylococci. This appears to be an instance in which the phenotype, as well as the genotype, of a pathogen is important.

Summary. Experimental wound infections with small inocula of staphylococci were regularly produced in rabbits. Prophylactic penicillin was effective in preventing such experimental wound infections due to "penicillin-resistant" staphylococci when size of inoculum was small and when the infecting organisms were derived from environment containing *no* penicillin. Under similar conditions penicillin was not effective in preventing wound infection due to small inocula of "penicillin-resistant" staphylococci which were derived from an environment *containing penicillin.*

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Intimal Lesions in Arteries of Vit. E Deficient Rats.* (25429)

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During an investigation on effects of Vit. E deficiency on nerve cells in the rat, it was incidentally observed that in the few instances in which carotid arteries were removed and processed simultaneously with the superior cervical ganglia, the intimal lining of these vessels was characterized by proliferative changes. Since the rat is generally con-

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TABLE I. Lesions in Arteries of Control and Experimental Groups of Rats.

Group No.	No. of rats	Diet	Supplement	No. of animals with arterial lesions	No. of days required to produce lesions
I	76	E-def.		70	280–450
II	20	"	fat	20	150–450
III	"	stock		0	
IV	10	E-def.	vit. E	0	
V	"	"	cholesterol	9	250–450
VI	"	stock	"	0	

sidered to be highly resistant to development of atheromatous arterial lesions, it was considered worth while to investigate this phenomenon in greater detail. Previous attempts to produce atherosclerosis in the rat have met with variable results. According to Okey(1), Page and Brown(2), and Malinow, Hojman and Pellegrino(3), administration of cholesterol does not lead to atherosclerosis. Page and Brown reported that feeding cholic acid and cholesterol to hypothyroid rats elicited severe hypercholesteremia with accompanying fatty liver and lipid infiltration of kidney, heart and aorta, but no evidence of atherosclerotic changes. Malinow, *et al.*, on the other hand, observed "moderately advanced lesions" in arteries of rats which were fed cholesterol together with methylthiouracil. "Moderately advanced lesions" were defined as lesions which showed endothelial or subendothelial proliferation. They also observed similar or more advanced lesions in rats made perinephritic and given methylthiouracil, methylthiouracil and oil, or cholesterol and methylthiouracil. Bragdoon and Mickelsen (4) observed atheromata-like lesions as masses of foam cells. These most frequently occurred on aortic and mitral valves and in endocardium of left ventricle, following intravenous or intraperitoneal injections of lipoproteins obtained from serum of cholesterol fed rabbits. Such changes were never observed in the aorta. These investigators concluded that to date, no one has succeeded in producing atheromatous lesions in aorta of the rat. They explained the lesions described by Hartroft, *et al.* in choline-deficient rats(5) as representing primarily medial degeneration.

Methods. The animals included 126 rats of Wistar (MW-2) strain, weighing 50 g, 150 g and 300 g respectively. Most animals (*Group I,* Table I) were maintained on a tocopherol-deficient test diet which had the following composition: 20% vitamin-free casein GBI, 56% dextrose, 10% lard, 4% salt mixture(6), 10% Brewer's yeast powder together with the following fat-soluble vitamins/100 lb: 523 g cod liver oil, or 68 g carotene in oil, GBI type 3a, and 0.001 g calciferol. Other rats (*Group II*) were placed on above diet to which was added 15 to 20% Vit. E-free fat. This was a hydrogenated cottonseed oil which had been dissolved in saturated ether solution of ferric chloride to oxidize any tocopherol which might be present in the fat. The ether was then removed by a rotating evaporator. *Group III* was placed on stock laboratory diet of Purina Rat Chow, and *Group IV* on tocopherol-deficient diet to which was added pure α-tocopherol. The effects of adding 2% cholesterol to Vit. E-deficient diet (Group V), or to stock diet (Group VI), were also studied. In later studies, 4 rats from 5 different strains (C. F. Nelson, HLA, Long-Evans, Wistar and Sherman) were placed on tocopherol-deficient diet while 2 animals from each strain were maintained on Rat Chow diet. Animals were kept on these diets for various intervals of time from 100 to 480 days before being sacrificed. The tissues were rapidly removed following pneumothorax, fixed in 10% formalin and processed in routine manner. Our study deals with carotid arteries and aorta. These arteries were removed *in toto* and cut into small pieces so that representative sections could be studied from all parts of the arteries. One-half of each piece of artery was set aside for frozen sections and for cholesterol and lipid studies. Blood cholesterol studies were made on 3 control animals and 4 animals that were on the tocopherol-free diet. In these instances blood was drawn directly from the heart following its exposure while the animal was still alive.

Results. The number of rats exhibiting lesions in the aorta and carotid arteries after being subjected to various experimental procedures is shown in Table I. When 15 to 20% tocopherol-free fat was added to tocopherol-deficient diet, arterial lesions occurred much earlier than in animals maintained on the same diet without added fat, the intimal lesions becoming noticeable in a little more than one half the time. Addition of cholesterol to tocopherol-deficient diet, on the other hand, did not alter length of time required to produce the lesions.

In some instances, intimal lesions were quite extensive and completely surrounded the circumference of vessel wall. Such lesions ordinarily extended longitudinally through large segments of the artery and could be observed in most sections of the particular vessel. On the other hand, the majority of lesions were quite localized and appeared in only a few sections of any given segment of artery. Since the lesions in most animals were localized, it was necessary to study large numbers of sections from all of the segments of an artery to determine whether or not arterial alterations existed in an individual animal.

Lesions in the intima varied in size and in nature in different rats as well as in different arterial segments of the same rat. Curiously, there appeared to be no correlation between size of lesions and length of time during which the animal had been on Vit. E-deficient diet.

In several animals, intimal lesions were observed in both carotid arteries and aorta, whereas in others the lesions were limited to the aorta, or to carotid arteries.

Lesions varied from a simple thickening of the endothelium in a localized region comprising only a few sections (Fig. 1) to large, well formed plaques, with fibrotic subendothelial tissues, which protrude into the lumina of the vessels (Fig. 2). In some instances, small lesions extended completely around the circumference of the artery. These could be observed almost throughout the entire length of the respective artery (Fig. 3). The initial thickening of endothelium is due to an increase in height of the normally flattened endothelial cells. This is followed by proliferation of elongated cells resulting in small localized endothelial plaques. In sections in which the endothelium is undergoing active proliferation, this is followed by invasion of connective tissue cells and fibers which arise either

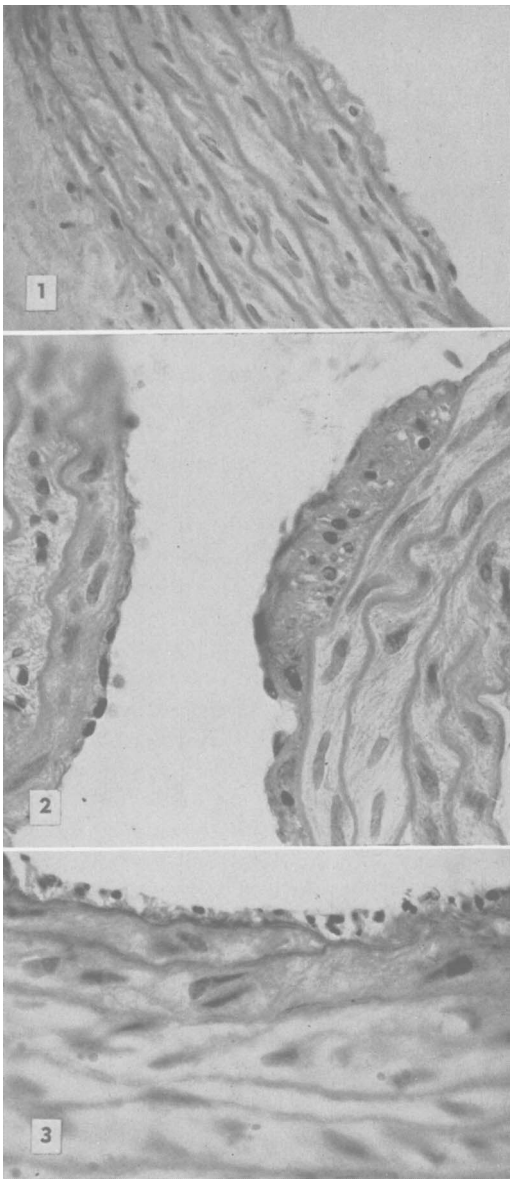

FIG. 1. Photomicrograph of section of aorta from vit. E-deficient rat, showing a small intimal plaque.

FIG. 2. Photomicrograph of section of carotid artery from vit. E-deficient rat showing typical large atherosclerotic plaque.

FIG. 3. Photomicrograph of section of aorta from vit. E-deficient rat showing intimal proliferation which completely surrounds circumference of the vessel.

from subendothelial elements or from the media. This leads to formation of highly organized intimal plaques, some of which are of considerable extent.

Occasionally there is a desquamation of thickened endothelium into the lumen which, in some instances, appears to be still attached to the intima of the artery at one or more points. Moreover, a colorless material is in certain instances deposited below the endothelial cells with subsequent formation of a subendothelium. This is a phenomenon which does not normally occur in arteries of the rat.

Formation of foam cells, which occurs in experimental atherosclerosis in the rabbit coincident with hypercholesteremia, has not been observed in the rat. Moreover, sections of arteries from Vit. E-deficient rats, including sections with intimal lesions, when subjected to Schultz(7) modification of the Liebermann-Burchardt reaction, did not reveal presence of cholesterol. This was true even in animals whose diet had been supplemented with cholesterol. In arteries of rats fed cholesterol in addition to Vit. E-deficient diet, the lesions were identical in both nature and occurrence to lesions of animals fed Vit. E-deficient diet without supplementation. Blood cholesterol levels of 4 rats on high cholesterol Vit. E-deficient diet were essentially the same as those of rats fed the diet without cholesterol.

Study of frozen sections of arteries from rats on various experimental and control diets stained with Sudan black revealed no remarkable differences in sudanophilic lipid material. In most cases, this material was not demonstrable either in the lining of vessels or in the intimal plaques. In only a few instances a small number of sudanophilic granules were present in these areas.

There was no indication that age differences of animals resulted in differences in nature, time of onset, or occurrence of lesions.

Since all observations were made on one particular strain of rats, the possibility presented itself that the results obtained might be due to genetic factors in this strain. For this reason, 4 animals from each of 5 additional strains of rats were subjected to the Vit. E-deficient diet, whereas 2 animals from each strain were placed on the stock diet. No arterial alterations were present in these animals. The group fed the Vit. E-deficient diet exhibited arterial lesions comparable in both nature and extent to those found previously in the larger series of animals, which suggests that the lesions observed were not the result of genetic factors of a particular strain.

Discussion. There is some question concerning the designation of the intimal lesions described here as atheromatous lesions. Although they are characterized by a thickening and proliferation of the intima with eventual formation of intimal plaques there was no marked deposition of lipids, a phenomenon usually considered characteristic of atherosclerosis. For this reason the lesions have been referred to as "intimal arterial lesions."

The effects of Vit. E, or of its deficiency on the vascular system, have been subjected to numerous investigations, the results of which are not always in agreement.

According to some investigators(8,9), increased amounts of tocopherol result in increase in occurrence or severity of atheromatosis and other lesions. Others(10,11,12) state that Vit. E failed to influence atherogenesis or other vascular lesions, while still others(13, 14,15) reported that Vit. E prevents the formation of such lesions or causes their regression. Loewi(16) observed that rabbits and guinea pigs maintained for 7 months on a semi-synthetic Vit. E-deficient diet, develop lesions which resemble atheromata, whereas rats do not.

Although numerous investigators have studied the effect of Vit. E-deficiency in the rat, occurrence of arterial lesions has not been previously observed. There is the possibility that since the majority of lesions are highly localized, their occurrence might not have been noted unless large numbers of sections were studied from any given segment of an artery.

It is of interest that addition of fat (but not cholesterol) to the Vit. E-deficient diet accelerated formation of arterial lesions. Since both saturated and unsaturated lipids were present in fat added, as well as in the diet, these data give no indications concerning the effect of unsaturation on production of the

lesions. Furthermore it should be noted that addition of 15 to 20% fat diluted the diet so that protein content was reduced. This reduction in protein may be related to the acceleration of the lesions.

Lesions differed from those described in cholesterol-fed rabbits(17) or in rats made hypercholesteremic by a variety of procedures (3) or following injections of lipoprotein from serum of cholesterol-fed rabbits(4). In all these instances foam cells, cholesterol and large amounts of sudanophilic lipids were present, with the exception of some described by Malinow, *et al.*(3). The lesions here described were characterized by absence of foam cells and cholesterol, and by presence of only small amounts of lipids.

Summary. 1) The aorta and carotid arteries of rats maintained on a Vit. E-deficient diet for 280 to 450 days were characterized by presence of intimal changes. Addition of 15-20% Vit. E-free hydrogenated fat accelerated formation of lesions. A daily supplement of pure alpha-tocopherol to Vit. E-deficient diet inhibited formation of intimal changes. Addition of cholesterol to the diet had no effect on incidence or severity of the lesions. 2) Intimal alterations varied from a simple thickening of endothelium to large well formed plaques with fibrotic subendothelial tissue.

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Heparin-Latex Test in Rheumatoid and Non-Rheumatoid Serums.* (25430)

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At the first conference on serological reactions of rheumatoid arthritis, in Jan., 1957, sponsored by the Arthritis and Rheumatism Fn., "rheumatoid factor" was defined as "the substance in blood of patients with rheumatoid arthritis and some related diseases responsible for agglutination of sensitized sheep erythrocytes and latex particles in the presence of F II, etc., under appropriate conditions." This definition implies the obligatory presence of an "outside" α-globulin in serological reactions in which the rheumatoid factor participates. It was, therefore, of interest when Gofton *et al.*(1) reported that some polysaccharides, in this case heparin, chondroitin sulphate and hyaluronic acid, may be used in place of α-globulin to sensitize polystyrene latex particles in the latex fixation test. Using a slightly modified procedure, we

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