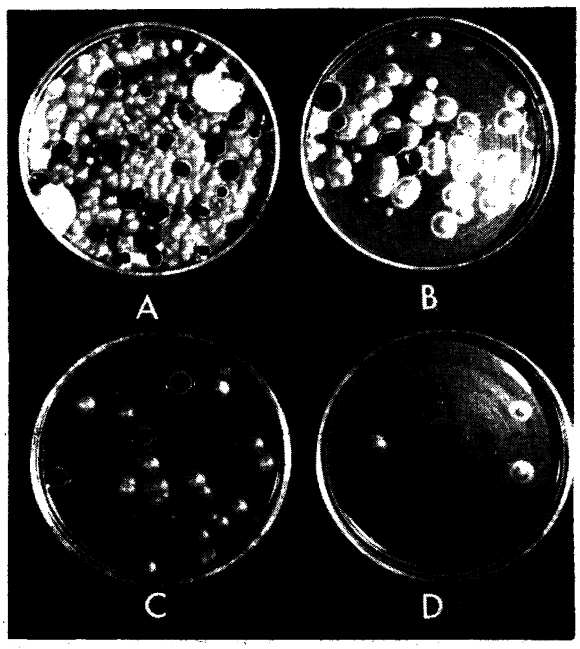

FIG. 1. Isolation of *C. immitis* from a mixture of saprophytic spores. A mixed spore suspension was placed on plate A, which contained 0.5 mg actidione/ml basal medium, and serially spread on plates B, C, and D, which contained the same medium. A few saprophytic colonies can be seen on plates A, B, and C; but the grey, slightly moist, dome-shaped colonies of *C. immitis* can be seen on all the plates, and appear in pure culture on plate D.

taining 0.1 mg actidione/ml, *C. immitis* colonies were recognizable but were overgrown with saprophytes, whereas on the remaining plates of the series saprophytic growth was negligible and many isolated colonies of the pathogen appeared. On a series containing 0.5 mg actidione/ml, the growth of the saprophytes was further restricted, and, even after 12 days, the plates contained *C. immitis* in almost pure culture (Fig. 1).

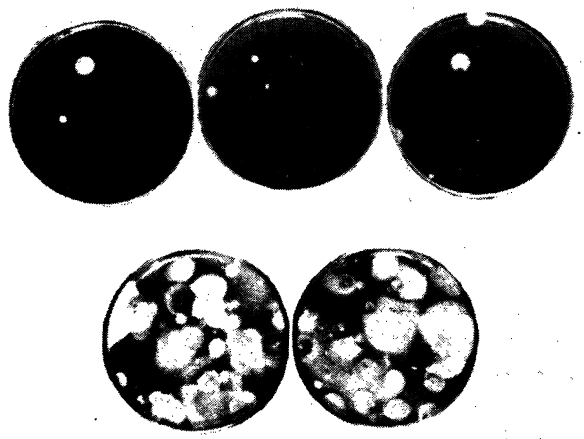

FIG. 2. Exposure of actidione media to the air for 4 hr. Plates in top row contain 0.1, 0.5, and 1.0 mg actidione/ml. The two plates below contain no actidione. After incubation, a few saprophytic fungus colonies appeared on the actidione plates, but the control plates were covered with the growth of saprophytic fungi.

The effectiveness of the actidione media in suppressing the growth of airborne saprophytic fungi was tested by exposure of plates out of doors for periods of 1–6 hr. The plates contained 0, 0.1, 0.5, and 1.0 mg of this antibiotic per ml. After exposure, the plates were covered and incubated at 25° C. After 6 days only a very few restricted colonies of saprophytic fungi had developed on the plates containing actidione, whereas the controls were completely covered with saprophytes (Fig. 2).

These preliminary tests suggest that 0.1 mg actidione/ml of the basal agar may be of value in the isolation of *C. immitis* from the air, whereas the higher concentrations, 0.5–1.0 mg/ml, might be required for isolation from more heavily contaminated materials.

Field tests of these media are in progress.

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The Experimental Production of Lipid Deposition in Excised Arteries

Sigmund L. Wilens

Departments of Pathology, Bellevue Hospital, and *New York University-College of Medicine, New York*

Anitschkow (*1*), after many years of investigation, was of the opinion that there is normally a constant passage of fluid through the walls of arteries in the direction from the lumen to the adventitia. He believed that atherosclerosis resulted from disturbances in this fluid transport. Little effort has been made to substantiate or disprove this simple hypothesis. A series of experiments was therefore undertaken to study the filtration properties of excised human arteries.

During the course of these experiments, it was observed that visible lipid would deposit in the tissues of normal arteries if normal human blood serum was filtered through its walls at normal arterial pressures for 24 hr or longer. The present communication is a preliminary report of this observation.

Common and external iliac arteries were removed at necropsy[1] within 24 hr of death from individuals 19–26 years of age who had died suddenly following trauma. They were rinsed in 0.9% saline and the loose adventitial adipose and areolar tissue removed. One end of the vessel was made watertight by inserting a short glass rod with a bulbous tip and ligating it in position with coarse soft thread. The other end was fastened with a similar ligature to a glass cannula. This was then attached to a manometer system, and the internal air pressure raised slowly to 300 mm Hg. The distended vessel was then submerged in saline to

[1] The arteries were obtained through the courtesy of Milton Helpern, Robert Fisher, and Henry Weinberg, of the Medical Examiner's Office of the City of New York.

TABLE 1

CHEMICAL COMPOSITION OF POOLED HUMAN SERUM FILTERED THROUGH EXCISED EXTERNAL ILIAC ARTERIES

Pressure (mm Hg)	Serum	Total cholesterol (mg/%)	Total protein (mg/%)	Albumin globulin ratio (mg/%)	Calcium (mg/%)	Non-protein nitrogen (mg/%)	Chlorides (mg/%)
45	Before filtration	323	7.2	4.5/2.7	11.6	30	130.4
	Retained in artery after filtration	452	11.5	6.4/5.1	—	—	—
	Filtrate	None	2.5	1.4/1.1	8.4.	30	113.6
145	Before filtration	323	7.6	4.5/3.1	10.4	28	126.4
	Retained in artery after filtration	552	14.1	7.8/6.3	12.8	—	112.0
	Filtrate	25	2.65	1.5/1.15	9.2	32	108.0
245	Before filtration	215	7.5	5.7/1.8	10.25	—	—
	Retained in artery after filtration	566	11.5	6.5/5.0	—	—	—
	Filtrate	None	3.4	2.8/0.8	5.76	—	—
320	Before filtration	323	7.2	4.5/2.7	11.6	30	—
	Retained in artery after filtration	663	15.2	7.8/7.4	—	30	—
	Filtrate	43	3.8	1.2/2.6	8.4	33	—

test for leaks and minute nutrient vessels. The latter are present in about one of every five iliac arteries and are somewhat more frequent in the common than in the external iliac artery. Points of leakage were easily determined by escaping air bubbles. Only vessels that were leakproof at 300 mm Hg were used.

Pooled serum of human blood donors and of patients drawn within 24 hr of the test was obtained from the Serology Laboratory at Bellevue Hospital. This was aspirated into a graduated 5-ml pipette, and the latter was attached in a vertical position to a manometer. The cannula at one end of the arterial segment was attached by rubber tubing to the lower end of the pipette, all air bubbles dislodged, and the pressure adjusted to the desired level with due allowance for the length of the column of serum above the vessel. The approximate inner surface area of the distended vessel was calculated from its length and external diameter. The rate of filtration was determined by measuring the amount of serum lost from the pipette during the first 4 hr of filtration at room temperature and expressed for convenience as ml of filtrate/hr/100 cm^2 of intimal surface. The distended artery was placed in a test tube and the filtrate collected.

Total cholesterol (Bloor), total protein, albumin/globulin ratios, calcium, nonprotein nitrogen, and chloride determinations were done on the original serum, on the fluid left in the vessel after 24 hr of filtration and on the filtrate obtained. From these determinations and from the volumes of filtrate and serum retained with the vessel lumen, the amounts of cholesterol and protein that had been retained in the vessel wall were computed.

The filtrate differed so strikingly from the original serum in appearance and chemical composition that it is obvious that it had diffused through the tissue of the arterial wall and had not escaped through leaks or minute intramural vasa vasora. The fluid was watery, very pale yellow, and had lost its opalescence. It contained only minute amounts of cholesterol or none at all and relatively little protein. In some instances the globulin was completely removed by filtration. The albumin content was reduced. The filtrate contained 25–50% less calcium than the original serum, indicating that much of the protein-bound fraction had been removed. Inorganic substances such as chlorides and nonprotein nitrogen were present in approximately the same concentration in the filtrate, in the retained fluid within the vessel lumen, and in the original serum.

The serum retained within the lumen of the vessel was viscid and dark yellow, especially after prolonged filtration. The cholesterol content of the retained fluid was markedly increased. Values of 600 mg% were not uncommon. The protein content, especially the globulin fraction, was also greatly increased and in some instances the albumin/globulin ratio was reversed. The calcium content was moderately elevated. Sample protocols are shown in Table 1.

It is obvious, therefore, that large molecular sub-

TABLE 2

FILTRATION RATE OF SERUM THROUGH ARTERIAL WALLS AT VARYING LEVELS OF PRESSURE

Pressure (mm Hg)	Rate of filtration (ml/100 cm^2 surface/hr)	
	Common iliac artery	External iliac artery
20	0.0	0.0
30	.29	.40
45	0.59	0.92
70	1.04	1.12
120	1.56	1.22
170	1.36	2.29
245	3.64	2.26
320	4.91	2.71

TABLE 3

CALCULATED AMOUNT OF CHOLESTEROL RETAINED IN WALLS OF EXCISED ILIAC ARTERIES AFTER FILTRATION OF POOLED HUMAN SERUM

Filtration pressure (mm Hg)	Observation	Serum retained in lumen and filtered	Serum retained in lumen	Filtrate	Cholesterol retained in artery wall (calc)	
					mg	%
45	Volume (ml)	5.3	2.5	2.8	—	—
	Cholesterol mg%	323 (initial)	452	None	—	—
	Cholesterol mg (calc)	17.12	11.30	"	5.82	34.0
95	Volume (ml)	9.4	4.0	5.4	—	—
	Cholesterol mg%	270 (initial)	522	None	—	—
	Cholesterol mg (calc)	25.38	20.88	"	4.50	17.7
120	Volume (ml)	5.8	3.4	2.4	—	—
	Cholesterol mg%	270 (initial)	279	None	—	—
	Cholesterol mg (calc)	15.66	9.49	"	6.17	38.1
145	Volume (ml)	8.9	3.0	5.9	—	—
	Cholesterol mg%	303 (initial)	519	85	—	—
	Cholesterol mg (calc)	26.97	15.57	5.02	6.38	23.7
245	Volume (ml)	6.0	2.0	4.0	—	—
	Cholesterol mg%	215 (initial)	566	None	—	—
	Cholesterol mg (calc)	12.90	11.32	"	1.58	12.3
270	Volume (ml)	10.7	4.2	6.5	—	—
	Cholesterol mg%	190 (initial)	380	60	—	—
	Cholesterol mg (calc)	20.33	15.96	3.90	0.47	2.3
320	Volume (ml)	6.2	2.7	3.5	—	—
	Cholesterol mg%	323 (initial)	496	None	—	—
	Cholesterol mg (calc)	20.03	13.49	"	6.54	32.6

stances do not readily enter the arterial intima within the range of filtration pressures tested (20–320 mm Hg). At 20 mm Hg pressure no fluid filtration occurred. At 30 mm Hg a very slow rate of filtration was noted. At increasing pressures the rate of filtration was correspondingly accelerated (Table 2), but the character of the filtrate was essentially unchanged.

By calculation (Table 3), it was found that 20–50% of the cholesterol present in the original volume of serum filtered could not be accounted for by concentration within the lumen of the vessel. It was concluded, therefore, that some of the cholesterol had entered the arterial wall and had been retained within it. It was estimated that 2–38% of the cholesterol in the filtration system was deposited intramurally. Similar computations revealed that a small proportion of protein was consistently lost during filtration and was presumably deposited intramurally. The relative amounts of cholesterol and protein retained in the vessel wall did not depend upon the pressure level of filtration. These did increase with the length of time of filtration, but there was considerable unexplained variation in individual experiments.

The presence of lipid in the arteries was confirmed by Sudan IV staining of frozen sections (Figs. 1, 2). After 18 hr of filtration at high pressures a light diffuse deposit of lipid was usually observed in the intima of the artery. This was most pronounced at the proximal end of the arterial segments. If filtration was continued for 36–48 hr at 200–300 mm Hg, sufficient lipid had accumulated in the entire exposed intimal surface to give a reddish color when the artery was stained *in toto* with Sudan IV. The amount of lipid deposited depended chiefly upon the amount of fluid that had filtered through the vessels per unit of surface area. It also depended to some extent upon the original thickness of the fibrous intima and for this reason was generally more pronounced in the thicker common iliac than in the external iliac artery. At physiological blood pressures (75–125 mm Hg) scanty lipid deposits were first noted after 24 hr of filtration. The lipid material appeared to spread uniformly throughout the entire thickness of the intima in some instances; in others, it had concentrated at the internal elastic lamella. The latter often acted as an effective barrier against the further penetration of lipid material. There was no tendency to lipid droplet formation, and the lipid in the impregnated intimal tissue was not doubly refractive.

At points where ligatures had been applied and the intimal tissue crushed, lipid often had penetrated through the media but was arrested at its junction with the adventitia. In several experiments where high filtration pressures were used, stainable lipid material in appreciable amounts had diffused through the media and had concentrated at its external margin. After prolonged filtration at high pressures, the media were impregnated with lipid throughout. There is little doubt, however, that the intact internal elastic

lamella serves as a resistant barrier to serum cholesterol, whereas the intimal surface is semipermeable to this material as constituted in normal human blood serum.

The artificial conditions of experiments on excised nonviable arteries are such that it is impossible to state that filtration of blood serum occurs during life through the walls of arteries in an identical manner. There are several circumstances that suggest that the mechanism of filtration observed does reflect in some measure what may occur physiologically. The filtration properties of excised arteries are fairly constant and are not altered appreciably by post-mortem changes. If two vessels are obtained from a person within a few hours of death and one is tested immediately and the other after 48 hr in the icebox, no significant change in rate or character of filtration is noted. Second, the semipermeability of the arterial wall to serum constituents is similar to that which exists in capillary walls *in vivo*. Third, the pressure threshold of filtration at 20 mm Hg is very close to that at which capillary filtration is believed to occur. Furthermore, the arterial wall retains much of its physical characteristics after it has lost its viability; for example, its elastic properties are not greatly altered. It is noteworthy that arterial grafts obtained up to 28 hr after death have been successfully transplanted (*2*).

It is possible, therefore, that the filtration of serum through excised arteries may not be totally dissimilar to what obtains during life. The filtration properties of the vessel wall may depend more upon the size and character of interstices in the framework of the vessel than upon the permeability of living cell membranes. The character of intimal lipid deposit produced artificially so closely resembles that occurring spontaneously in the very stages of arterial lipid deposition that it is likely that a somewhat similar mechanism is involved in both processes. There is no reason to believe that an inert membrane such as the internal elastic lamella should change very greatly within a few hours of death. It is suggested, therefore, that the filtration of serum through artery walls under the conditions of the experiments provides direct and substantial evidence in favor of the filtration theory of lipid deposition in atherosclerosis.

The failure of cholesterol to pass freely through capillary walls is generally attributed to its linkage with large protein molecules. It is likely that the major fraction of serum cholesterol fails to enter the arterial intima for the same reason. The fraction that does enter the intima is probably united to relatively small protein molecules or entirely dissociated from protein. Moreton (*3*) and Gofman *et al.* (*4*) have claimed that serum lipids may exist in aggregates of varying particulate size. It is possible, therefore, that the smaller particles of lipid in the serum are

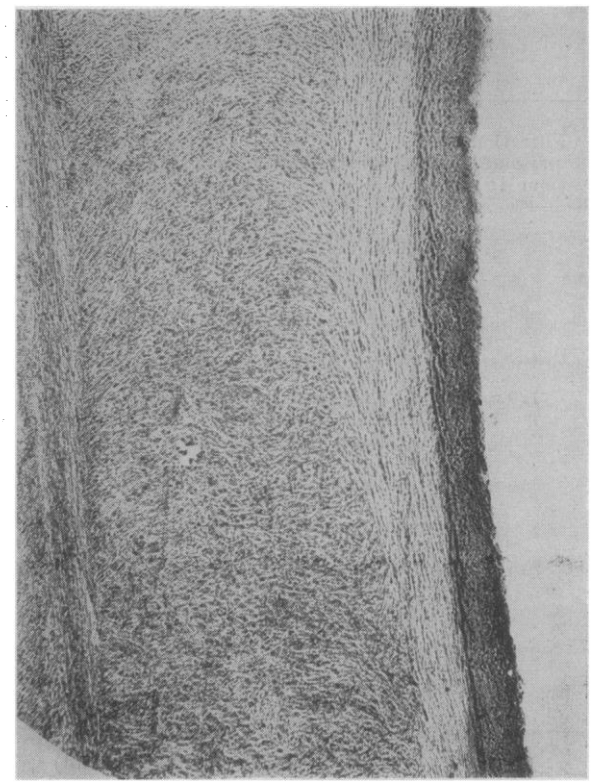

FIG. 2. Photomicrograph of Sudan IV-stained frozen section of iliac artery distended with serum for 48 hr at 100 mm Hg. The intima is uniformly impregnated with darkly stained lipid.

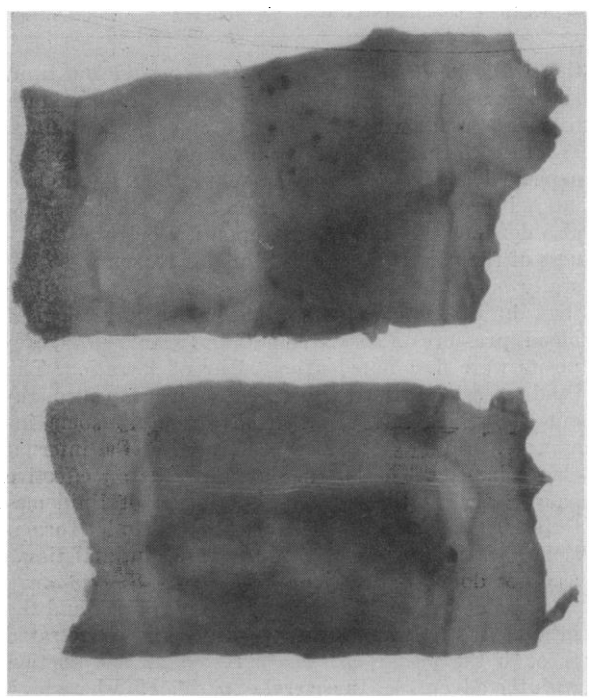

FIG. 1. Photomicrograph of intimal surfaces of common iliac arteries stained with Sudan IV after being distended with serum at 300 mm Hg for 48 hr. The pink-stained lipid-containing areas appear as broad dark grey bands that are sharply outlined from the paler borders of intimal surface that lay outside the zones of distension. The dark punctate areas in the upper specimen are points at which the internal elastica was ruptured and lipid penetrated into the media.

able to enter the intima, whereas the larger ones are rejected.

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Mango Grafting in Eight Weeks

L. B. Singh

Fruit Research Station, Saharanpur, U. P., India

Mango is commercially propagated by inarching. Age of the seedlings to be inarched varies from 1½ to 2½ years, and the grafts are separated from the parent tree in about 3 months. Thus, it takes 2–3 years before a mango graft is ready for transplanting in the field. During this period, the nurseryman must take very good care of the seedlings; besides, copious watering of grafts, essential for good union, makes the method cumbersome and expensive. Further, such grafts, being on 2-year-old seedlings, have a relatively poor root system. They also do not transport well.

Inarching of mangoes on 4-week-old seedlings was, therefore, tried by the author in order to overcome the serious disadvantages mentioned. Mango stones planted in the the first week of July started germinating by the end of the month. About 30 days after germination, the seedlings attained a height of approximately 1 ft and a girth of ⅛ in.–¼ in. One hundred such seedlings were lifted from the seedbed along with stones and sprouting roots, and the soil clinging to the stones was removed. The stones were then covered with wet sphagnum moss about ½ in. in thickness, held in position by a thin string. The seedlings were taken to the parent tree and inarched with new shoots of equal thickness in early September (Fig. 1).

FIG. 1.

Complete union took place in about a month, and the grafts were detached from the mother plant by the end of September and potted. Eighty per cent success was obtained. Watering was completely withheld since the entire operation was completed in the rainy season, when the rain water absorbed by the moss furnished the required moisture. This method also obviated the necessity of lifting of stock with a ball of soil for food material, as this was supplied by the stones.

Temperature-dependent Characteristics of an Adenylpyrophosphatase Preparation from Potatoes[1]

Kwan-Hua Lee and John J. Eiler

College of Pharmacy,
University of California Medical Center, San Francisco

A need for a means of selectively hydrolyzing the acid-labile phosphate groups in ATP arose in our studies (*1*) on the turnover of labeled phosphate in the ATP present in preparations from animal tissues. Although crystalline myosin (*2*) and purified myokinase (*3*) proved useful, the time and effort involved in the preparation of these enzymes, together with the lack of stability of myosin, prompted a study of other preparations (*4*) that might be both stable and easily available. We report here on a preparation from potatoes which, in suitable dilution, possesses the desirable property that at temperatures above 7° C it catalyzes the hydrolysis of the 2 acid-labile phosphates in ATP, and at 7° or below it catalyzes the hydrolysis of only the terminal group. The preparation is quite stable and may be prepared in a period of 24 hr. One sample, saturated with toluene, maintained its activity over a period of a year. Between periods when aliquots were withdrawn for use in the analysis of ATP, the solution was stored at 2°–5°. The usefulness of our preparation in the large-scale conversion of ATP to ADP is being studied.

Kalckar (*5*) and, later, Krishnan (*6*) reported on an enzyme preparation from potatoes catalyzing the hydrolysis of the acid-labile phosphates in ATP. Kalckar (*5*) suggested that a single enzyme was involved. Meyerhof (*7*) proposed that the name apyrase be reserved for the dephosphorylating enzymes that do not distinguish between ATP and ADP. Our preparation differs sufficiently from those reported by Kalckar and Krishnan to suppose that we are dealing with a different enzyme or a mixture of enzymes.

[1] This investigation was supported by a research grant from the National Advisory Heart Council, National Institutes of Health, USPHS.