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Tissue Cytochrome *c* and Prevention of Experimental Atherosclerosis

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It has been shown that the protective action of iodides on the experimental atherosclerosis induced in rabbits by cholesterol-rich diets is exerted through the thyroid, since iodides are not effective in the absence of the gland (*1, 2*) or when given simultaneously with thiourea (*2*).

Drabkin (*3*) has pointed out that there is a clear positive correlation between the thyroid activity and the content of cytochrome *c* in the tissues. Consequently it seemed important to investigate whether changes will occur in the cytochrome *c* content of tissues of rabbits on a cholesterol-rich diet with potassium iodide, in the presence and in the absence of thyroid gland.

Rabbits on a cholesterol-rich diet (nearly 0.5 g cholesterol, in the form of cattle spinal cord, daily) and with constriction of the upper abdominal aorta inducing hypertension, which acts synergistically with diet in producing atherosclerosis (*4*), were divided into 4 groups: one control, another with thyroidectomy, and two on protective doses of potassium iodide (0.3 g orally every other day), one of them thyroidectomized. The animals were sacrificed 120 days after starting the diet. The development of atherosclerosis was judged macroscopically and evaluated on a scale of 0 to 10 (*4*). The cytochrome *c* of liver and kidney was extracted by the method of Potter and Du Bois (*5*) and determined spectrophotometrically according to Rosenthal and Drabkin (*6*).

The results on liver cytochrome *c* are given in Fig. 1. The changes in kidney cytochrome *c* were similar to those in the liver.

The correlation coefficient between liver cytochrome *c* and development of atherosclerosis is $r = -0.533$, with $t = 2.88$, a value regarded as statistically significant.

These findings confirm, in the rabbit, Drabkin's results in the rat (*3*) of the influence of the thyroid

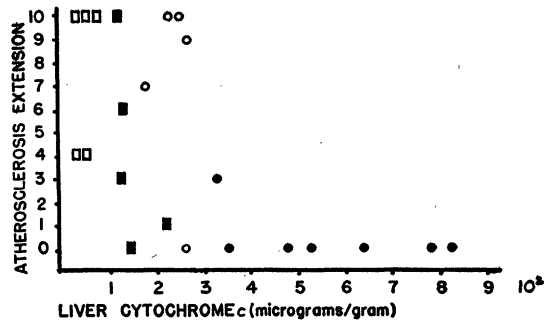

FIG. 1. Relationship between liver cytochrome *c* and degree of atherosclerosis in rabbits on cholesterol-rich diet, sacrificed at 120 days. Squares, thyroidectomized; circles, normal; open squares and circles, animals without treatment; solid squares and circles, animals given potassium iodide.

gland and thyroxine upon the level and content of cytochrome *c* in tissues. It is deduced from the data that the action of iodide may be one of stimulation of the thyroid gland, since the concentration of cellular cytochrome *c* is increased when the drug is administered to animals with thyroid. The results furthermore suggest that the augmentation of cellular cytochrome *c* must be considered as a factor in the prevention of the experimental atherosclerosis by means of potassium iodide.

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A Selective Medium for the Isolation of *Coccidioides immitis*

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Cultural procedures commonly used in attempting isolation of *Coccidioides immitis* from clinical specimens or from the physical environment frequently fail because of overgrowth of the pathogen by saprophytic fungi and bacteria. Laboratory tests indicate that a newly developed medium shows great promise in overcoming this difficulty.

Sabouraud dextrose agar fortified with penicillin (20 units/ml) and streptomycin (40 units/ml) was selected as a basal medium. This combination is inhibitory to most bacteria but does not prevent the growth of fungi, except the actinomycetes.

On the basis of the findings of Leach, Ford, and Whiffen (*1*), Whiffen (*2*), and Phillips and Hanel (*3*), which demonstrated the selective antifungal ac-

TABLE 1

No. days of incubation	Sabouraud dextrose agar + penicillin and streptomycin*			Sabouraud dextrose agar + penicillin and streptomycin* + Actidione I (0.1 mg/ml)			Actidione II (0.5 mg/ml)			Actidione III (1.0 mg/ml)		
	2	5	8	2	5	8	2	5	8	2	5	8
Alternaria	1+	4+	4+	0	2+	3+	0	2+	3+	0	1+	2+
Aspergillus (Strain 1)	4+	4+	4+	0	3+	3+	0	0	0	0	0	0
Aspergillus (Strain 2)	4+	4+	4+	0	1+	2+	0	0	0	0	0	0
Fusarium	2+	4+	4+	0	0	0	0	0	0	0	0	0
Helminthosporium	0	3+	4+	0	0	0	0	0	0	0	0	0
Hormodendrum (Strain 1)	1+	4+	4+	0	1+	2+	0	1+	1+	0	1+	1+
Hormodendrum (Strain 2)	3+	4+	4+	1+	4+	4+	1+	3+	4+	0	2+	3+
Mucor	3+	4+	4+	0	1+	2+	0	0	0	0	0	0
Oospora	1+	4+	4+	0	0	0	0	0	0	0	0	0
Paecilomyces	3+	4+	4+	0	0	0	0	0	0	0	0	0
Penicillium (Strain 1)	1+	4+	4+	0	3+	3+	0	1+	2+	0	1+	2+
Penicillium (Strain 2)	3+	4+	4+	0	2+	2+	0	0	0	0	0	0
Rhizopus	4+	4+	4+	0	0	0	0	0	0	0	0	0
Scopulariopsis	2+	4+	4+	0	3+	3+	0	1+	2+	0	0	1+
Trichoderma	2+	4+	4+	0	1+	2+	0	0	0	0	0	0
Coccidioides immitis (3 strains)	1+	3+	4+	1+	3+	4+	0	2+	3+	0	2+	3+

* Penicillin, 20 units/ml, and streptomycin 40 units/ml.

tivity of actidione,[1] this antibiotic was added to the basal medium. These workers showed that actidione was active against a variety of saprophytic fungi as well as a few of the human pathogens, but concentrations as high as 1.0 mg/ml of medium were found not to inhibit *C. immitis*.

Actidione is soluble in water in amounts up to 2%, and solutions are stable for several weeks at pH 3–5. For this investigation, stock solutions of actidione were prepared in aqueous potassium di-hydrogen phosphate (*M*/15), sterilized by filtration and stored at 5° C. Measured quantities were added to sterile Sabouraud broth or to sterilized, melted, and partially cooled Sabouraud agar in combination with penicillin and streptomycin.

Three strains of *C. immitis* recently isolated from active cases of coccidioidomycosis were used in these studies, as well as 15 strains of common saprophytic fungi comprising 12 different genera.

The degree of resistance of *C. immitis* to actidione was tested by adding 0.1 ml of a dilute suspension of washed spores (approx 100 spores/ml) of this organism to a series of tubes of Sabouraud dextrose broth containing graded amounts of the antibiotic, and incubating at 25° C for 1 week. At the end of this period no inhibition of growth of *C. immitis* was observed in tubes containing as much as 1.0 mg of actidione per ml. Very slight inhibition was seen in tubes containing 2.0–4.0 mg actidione/ml.

Washed spores of a series of common saprophytic fungi, as well as of the 3 recently isolated strains of *C. immitis*, were then inoculated on slants of the basal medium containing varying concentrations of actidione and on controls without actidione. The tubes were incubated at 25° C, and growth was recorded as 1+ to 4+ after 2, 5, and 8 days. No inhibition of growth of the 3 strains of *C. immitis* was observed on media containing 0.1 mg actidione/ml. Colonies first appeared on the second day and could be identified by the fifth day. Although colonies were slower to appear on media containing 0.5–1.0 mg/ml, they also were readily identifiable by the fifth day. Detailed results of this experiment are recorded in Table 1.

Attempts were made to isolate *C. immitis* in culture from a mixture of its spores with those of the 15 saprophytes. Spore suspensions of equal densities were prepared from washed packed spores of each of the saprophytic species. These were combined and a suspension of washed *C. immitis* spores (approx 2,000 spores) was added. Calculating on the basis of the volumes of packed spores used, there were approximately 2,000 times as many saprophytic spores as *C. immitis* spores in the mixture. A series of agar plates with and without actidione was inoculated by placing 0.5 ml of a saline suspension of the spore mixture on the surface of one plate and streaking it serially by means of a bent glass rod over 3 additional plates. After 3 days of incubation the plates containing no actidione were covered with a heavy growth of the saprophytic fungi and no colonies of *C. immitis* were detectable. On the first of a series of plates con-

[1] The Upjohn Company, Kalamazoo, Mich.

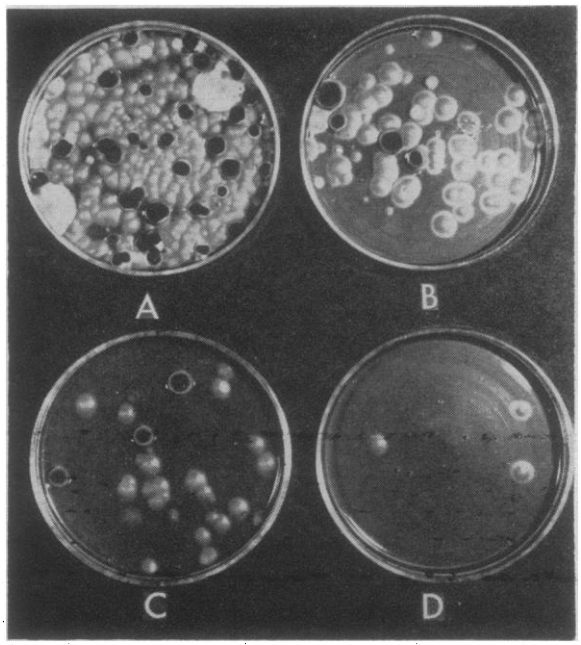

FIG. 1. Isolation of *C. immitis* from a mixture of saprophytic spores. A mixed spore suspension was placed on plate A, which contained 0.5 mg actidione/ml basal medium, and serially spread on plates B, C, and D, which contained the same medium. A few saprophytic colonies can be seen on plates A, B, 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).

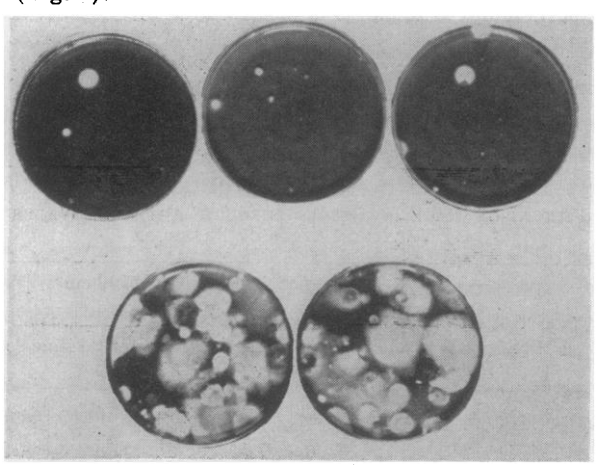

FIG. 2. Exposure of actidione media to the air for 4 hr. Plates in top row contain 0.1, 0.5, and 1.0 mg actidione/ml. The two plates below contain no actidione. After incubation, a few saprophytic fungus colonies appeared on the actidione plates, but the control plates were covered with the growth of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

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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.