obtained from both non-growth and growth techniques with rapid-growing mycobacteria species such as *M. smegmatis* and *M. fortuitum* which have had extensive previous carbohydrate investigation by growth techniques(12).

Some mycobacteria will surely be found to yield reactions intermediate to those of well-defined species. Also, some strains definitely belonging to a particular species may show individual reactions. Such "aberrant" or "peculiar" reactions should not occasion confusion; these reactions should lead to further study towards distinguishing organisms and towards correlating metabolism with various characteristics. Thus, special carbohydrate reaction differences between strains of the same species may correlate to differences in morphology, pathogenicity, and serology.

Summary. 1. A massive inoculum non-growth carbohydrate testing procedure provides results not obtained previously with slow-growing mycobacteria. 2. The results obtained indicate usefulness of carbohydrate typing for aiding differentiation of species and strains of acid-fast organisms. 3. The trehalose and xylose reactions may correlate with pathogenicity for certain types of mycobacteria.

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Modification of Solubility of Cholesterol During Incubation with Growing Cells of *Aerobacter aerogenes.** (27031)

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During the course of experiments involving the catabolism of C^{14}—labeled cholesterol by growing cells of *Aerobacter aerogenes*, a significant transfer of label into the acid fraction was noted. Subsequent studies indicated that this phenomenon was due, in large part, to production during cell growth of some factor which modified the solubility of cholesterol, rather than to a conversion of the sterol itself to an acidic product.

Methods. A strain of *A. aerogenes* described earlier(1) was grown on a sterile medium containing 1% agar, 1.5% Difco Nutrient Agar, 0.5% yeast extract, and 0.01% colloidal cholesterol(1) labelled with C^{14}, at 35°C for 2 to 4 weeks, either in screw cap bottles containing vials of strong alkali as CO_2 traps, or in cotton plugged flasks. Both randomly labelled cholesterol prepared by incubating

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1-C^{14}-acetate with rat liver slices(2) and purified via the dibromide(3), and 4-C^{14}-cholesterol (Nuclear-Chicago Corp.) were used. The purity of the latter compound was confirmed by paper chromatography using Bush's system A(4). At the end of the growth period, the mixture was hydrolyzed with 2N HCl by refluxing for one hour under N_2. Acid was used for hydrolysis, since the metabolized cholesterol was suspected of being linked to a carbohydrate by a glycosidic bond. The hydrolysate was made alkaline with $NaHCO_3$ and extracted 3 times with ether (separatory funnel). The aqueous phase was acidified with HCl in the cold, and again extracted in the same manner. The latter ether extract, termed acid fraction (AF), was then chromatographed on Whatman No. 1 filter paper. Either collidin/H_2O/NH_4OH (conc.) — 100:30:5 (v/v/v) (5, with slight modifications), or Sjövall's system (mobile phase: isopropylether /heptane — 60:40 (v/v)) (6) were used as developing solvents. Following the development of the chromatogram, the paper was dried in an oven at 95-100°C, and then treated with either a saturated solution of $SbCl_3$ in $CHCl_3$, or a 2% solution of 2,4-dinitrophenylhydrazine in 2N HCl, for location of products.

Portions of the same chromatogram, untreated with color reagents, were cut into strips which were eluted with methanol. The eluates were placed on planchets, and after drying, their radioactivity was measured at infinite thinness using a gas flow counter.

Results. In the presence of growing cells of *A. aerogenes*, 6-10% of the total radioactivity of the cholesterol applied appeared in the AF (Table 1). The AF of identically treated, uninoculated controls contained no significant quantities of C^{14}. Nor did the AF prepared by a somewhat different procedure from resting cell samples, incubated with radioactive cholesterol for 7.5 hours, show detectable amounts of C^{14}(7).

TABLE I. Transfer of Radioactivity from C^{14}-Cholesterol to the Acid Fraction during Growth of *Aerobacter aerogenes*.

Exp.	Sample	Incubation time	Total radioactivity applied	Total radioactivity rec. in AF	
		weeks	cpm.	cpm.	%
1	Inoculated	2	23,500*	1,500	6.4
	"	3	12,400*	740	6.0
	Uninoc. control	3	12,500*	130	1.0
2	Inoculated	4	500,000*	47,100	9.4
	"	4	900,000	88,200	9.8
	Uninoc. control	5	500,000	6,000	1.2

* Avg of duplicate samples.

Paper chromatography indicated that 2 radioactive substances were present in the AF

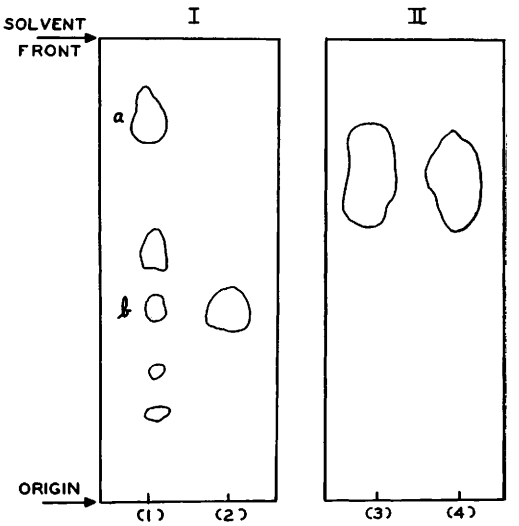

FIG. 1. I. Paper chromatogram of AF (1), and of pure cholic acid (2), developed with collidine/H_2O/NH_4OH. Only spots *a* and *b* contained detectable amounts of radioactivity. II. Paper chromatogram of radioactive compound eluted from spot *a* and purified as described in text (3), and of pure cholesterol (4), using Bush's system A. Staining with $SbCl_3$.

(Fig. 1, I). Spot *a* which contained about 98% of the radioactivity recovered from the AF, was identified as cholesterol by means of elution and rechromatography along with the known compound, using Bush's system A(4), and also by elution followed by digitonin precipitation, splitting of the complex(8), and rechromatography, using again Bush's system A (Fig. 1, II). The second radioactive spot, *b*, which contained about 2% of the counts, ran at a rate similar to that of cholic acid in collidin/H_2O/NH_4OH, but when rechromatographed in Sjövall's system(6), it traveled somewhat faster than the bile acid.

Discussion and Conclusion. It is seen that growth of *A. aerogenes* in a cholesterol-containing nutrient medium resulted in an alteration of the solubility behavior of the sterol: Significant amounts of the latter were found in the aqueous phase, apparently as the result of

an artifact. Some substance, as yet unidentified, which was produced during cell growth, appears to be the responsible agent. The absence of significant quantities of C^{14} in the AF from both sterile incubated controls and incubated resting cell suspensions seems to preclude improper extraction, interfering substances in the medium used, or autooxidation during incubation and extraction as sources of this effect.

Increased water solubility of cholesterol in biological systems such as, for example, bile and serum(9, 10) has long been known. In addition, it is possible to modify the water solubility of this sterol *in vitro* by use of proteins, phospholipids(11), bile salts(12), or detergents(13).

It seems important, therefore, to use caution in interpretation of results of biological experiments dealing with cholesterol catabolism, in particular, where relatively small changes detectable only by means of tracers are involved. Simple classical extraction procedures, without further fractionation and a more detailed characterization of the products, were shown to be both inadequate and misleading.

Summary. C^{14}-labeled cholesterol was incubated with growing cells of *A. aerogenes*. Following hydrolysis of the incubation medium, a significant portion of the sterol was recovered in the saponifiable fraction. Apparently, the solubility of the sterol was modified by a substance produced during the growth of the *Aerobacter*.

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The Effect of Continuous Antibiotic Feeding on X-Irradiation Mortality in Mice. (27032)

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The occurrence of bacteremia in animals after exposure to lethal or sublethal total body X-irradiation has been reported(1-4). The bacteremia was caused primarily by microorganisms normally present in the lower intestinal tract; however, due to apparent increase in intestinal wall permeability these organisms were dislocated into the peritoneal cavity(1). Since the generalized infection develops during the period of greatest mortality, it may be concluded that infection plays an important role as one of the factors in death following irradiation. Various workers(5-6) have found that clinical doses of chlortetracycline when administered orally in the feed or by injection following irradiation prolonged the lives of rats, dogs and mice. The following experiments were undertaken to determine the effect of low level chlortetracycline feeding administered prior to and after exposure to total body X-irradiation on a lifetime basis. During the early weeks of the first experiment, a *Bartonella muris* infection was detected in the experimental animals. A second experiment was included to determine possible interactions of irradiation, disease, time and chlortetracycline feeding.