Effect of Streptomycin on the Metabolism of Certain Mycobacteria

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A nonpathogenic strain of tubercle bacillus, No. 607 of the American Type Culture Collection, rapidly oxidizes benzoic acid. The hydroxybenzoic acids, however, are not oxidized. This confirms Lehmann's observation (*2*) that the oxygen uptake of nonpathogenic strains is not affected by hydroxybenzoic acids, whereas that of pathogenic strains, as previously shown (*1*), is definitely increased by o-hydroxybenzoic acid as well as benzoic acid. The evidence indicates, however, that although the pathogenic strains of tubercle bacillus have an increased oxygen uptake in the presence of benzoic and o-hydroxybenzoic acids, these compounds are not oxidized in the process. Because of the apparent importance of benzoic acids in the metabolism of these bacteria, it was of interest to study the effect of streptomycin on it.

Resistance to streptomycin was induced in *Myco. tuberculosis* No. 607 by successive passage in Long's medium containing increasingly higher concentrations of the drug. Suspensions of the resistant strain and the normal parent strain were prepared from 3-day cultures on Long's medium by the method previously described (*1*). The bacteria were resuspended in M/20 phosphate buffer pH 6.7 so that 1.0 cc. of buffer contained 0.1 cc. of the packed centrifuged organisms,

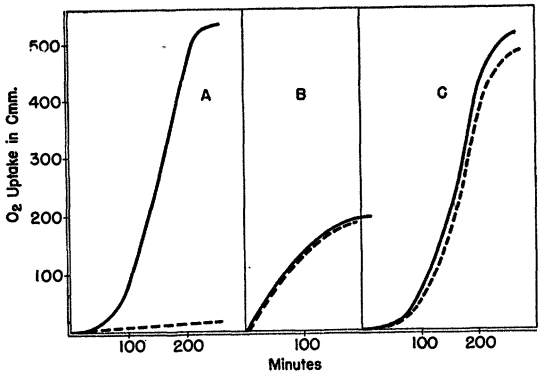

FIG. 1. A—Effect of 10 γ streptomycin on the oxidation of 1.0 mg. sodium benzoate by the normal 607 strain; B—Effect of 100 γ streptomycin on the oxidation of 2.0 mg. sodium pyruvate by the normal 607 strain; C—Effect of 100 γ streptomycin on the oxidation of 1.0 mg. sodium benzoate by the streptomycin-resistant 607 strain. The control uptakes have been subtracted. The dotted lines represent the addition of streptomycin.

and 0.5 cc. of this suspension was used in each Warburg vessel, which contained a final volume of 2.0 cc.

Ten γ of streptomycin (Merck) completely inhibits the oxidation of 1.0 mg. of benzoic acid by the normal strain, whereas 100 γ is without effect on the oxidation by the resistant strain. The oxidation of pyruvic acid by the normal strain as well as its oxygen uptake without added substrate is not affected by 100 γ of streptomycin. This indicates that the inhibition of the benzoic acid oxidation by streptomycin may be fairly specific. The results are shown in Fig. 1. However, the increased oxygen uptake of the virulent H37 strain in the presence of benzoic acid is not affected by 300 γ of streptomycin. Other mechanisms must, therefore, be inhibited in this pathogenic strain.

References

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Suppression of Axillary Growth in Decapitated Tobacco Plants by Chemicals

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In the commercial production of tobacco the plant is topped or decapitated at the flowering stage and later "suckered," *i.e.* at intervals all subsequent axillary growth is removed. An inexpensive substitute for the operation of suckering without detriment to quality or yield would be of practical importance to growers.

It was with this final objective in mind that greenhouse trials were begun on suppression of axillary growth of topped plants. The chemical compounds employed included certain synthetic growth-regulating substances, some of which have been reported to possess the ability of retarding axillary growth. Powdered compounds were applied to the stem wound with a spatula after topping, and liquid compounds with a dropper. Decapitated plants retained 7 leaves at the time of treatment. The plants were harvested 35 days after treatment

TABLE 1

Treatment	Green moist weight				
	Leaf		Stem	Suckers	Total (grams)
	(grams)	(%)	(grams)		
Control 1, suckered.....	145.6	100.0	55.8	46.2	247.6
Control 1, unsuckered.....	114.7	78.6	52.7	132.3	299.7
Control 2, suckered.....	146.3	100.0	45.3	63.9	255.5
Control 2, unsuckered.....	140.1	96.0	53.7	145.9	339.7
γ-(Indole-3)-n-butyric acid..	162.4	111.2	56.9	111.8	326.1
4-Chlorophenoxyacetic acid*.	168.0	115.1	55.3	180.3	403.6
α-2-Chlorophenoxypropionic acid*.....	174.7	119.7	59.5	156.6	390.8
α-Naphthylacetic acid methyl ester*.....	165.8	113.6	68.2	0	234.0
2,4-Dichlorophenoxyacetic acid methyl ester*.....	175.2	120.0	78.0	72.0	325.2

*Courtesy of the Dow Chemical Company.

A few of the responses are given in Table 1. The figures, which are average values for three plants in grams of fresh weight, clearly indicate the possibility of controlling the relative development of the various parts of the tobacco plant.

The responses fall into several categories. In one, chemical suppression of axillary growth is accomplished in a degree equal to manual suckering, with as great or greater increase in yield of leaf. In another category, the yields of leaf, stem, and suckers are all increased considerably above those of the

controls—apparently a stimulation response. Fuller details of the work will appear elsewhere.

These data indicate the possibility that it may be economically feasible to employ chemical suppression of suckers in the production of tobacco. However, experimental field work with these compounds under the actual conditions employed in the production of the different types of tobacco must be undertaken before their use can be recommended for this purpose. Such work is now under way with Maryland tobacco and with Rustica tobacco grown for nicotine.

Crystalline Kitol[1]

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Kitol has the interesting property of decomposing at elevated temperatures into vitamin A (*1*). This, and the possibility that it acts physiologically in inverse fashion to detoxify excessively high stores of vitamin A, has stimulated research to determine its structure. As a preliminary step we have prepared kitol in crystalline form and also its di-p-phenylazobenzoate ester.

The concentration procedure was different at certain stages from that previously described (*1*) and appeared to give a concentrate of slightly higher potency. Whale-liver oil (0.8 per cent kitol, 21,000 units of vitamin A/gram) was saponified, and the unsaponifiable matter was freed of sterols and other solids as far as possible by low-temperature crystallization from acetone. The resulting oil was mixed with constant-yield oil and residue oil (*2*) and distilled in a molecular still. A fraction rich in kitol was collected at 190–260°. This was stripped at 160°, after the addition of constant-yield oil and residue oil, to give a distilland rich in kitol and largely free of vitamin A. The unsaponifiable matter from the distilland was esterified with succinic anhydride; the acid succinate was neutralized in 83 per cent ethanol; and nonhydroxylic materials were separated by extraction with petroleum ether. By saponification a kitol concentrate [$E^{1\%}_{1\,cm.}$ (290 mμ) = 586] was obtained which was sufficiently pure so that a crystalline di-p-phenylazobenzoate ester could be prepared. We were unsuccessful in preparing a crystalline 3,5-dinitrobenzoate, as described by Embree and Shantz, probably because of the nature of the impurities present.

Crystallization of the unsaponifiable matter from the phenylazobenzoate from methyl alcohol gave kitol in the form of colorless, elongated prisms. The yield was approximately 10 per cent, based on the amount present in the original whale-liver oil. Direct crystallization of the kitol concentrate, even after its extinction coefficient had been raised to 650 by chromatography, gave only a poor yield of crystals. This behavior may indicate the presence of geometric isomers.

Kitol melted at 88–90° (capillary tube method—CT; 95–97° in the Fisher-Johns apparatus—FJ), and $E^{1\%}_{1\,cm.}$ (290 mμ) = 707. The analysis agreed with the formula $C_{40}H_{58}(OH)_2$, twice that of vitamin A. The crystals exhibited considerable stability toward atmospheric oxidation, for their ultraviolet absorption curve was unchanged after 53 hours exposure at room temperature in the dark. Kitol is more stable in air than vitamin A. In diffused daylight, however, only 44 per cent of the initial extinction coefficient was recovered in 46 hours. A solution of kitol in ethanol was relatively stable, losing only 7 per cent of its initial extinction coefficient after storage for 10 days in the dark at room temperature.

By distillation of kitol palmitate [$E^{1\%}_{1\,cm.}$ (290 mμ) = 379] in a cyclic molecular still (from peanut oil residue; temperature, 240–270°; pressure, 3μ), values of 0.67 and 0.75 were found for the moles of vitamin A palmitate collected in the distillate per mole of kitol palmitate destroyed. Similar results were obtained by heating refined cottonseed-oil solutions of kitol and its acetate in sealed tubes, but less reliance could be placed on these data, since the vitamin A formed during decomposition was not removed from the heating zone and a correction of uncertain accuracy had to be made for this source of error. The data suggest that the breakdown of kitol by heat is not a clean-cut process in which one molecule of vitamin A and another fragment of unknown composition is formed. It appears more probable that a number of degradative changes occur, with vitamin A constituting one of the major products formed.

Kitol diphenylazobenzoate was found to exist in two forms. One melted at 125–126° (CT) and was obtained by chromatographing the crude ester on silicic acid, followed by crystallization from methyl acetate. A higher melting form [m.p., 149–150° (CT), 153–155° (FJ)] was isolated by chromatographing the crude ester on zinc carbonate, followed by crystallization from acetone. Refluxing the higher melting ester in acetone, chromatographing it on sodium aluminum silicate, or treating it with iodine in benzene, gave the lower-melting ester, but the conversion was not usually quantitative. The high- and low-melting forms gave colorless hydrazo compounds which appeared to be the same. They had nearly the same melting point (124–126° and 127–129°, respectively), and a mixed melting point determination showed no depression [127–129° (CT)]. It seems probable that the two azoates are geometric isomers of the carbon-carbon type, although the data on the hydrazo compound does not exclude nitrogen-nitrogen stereoisomerism.

A crystalline anthraquinone carboxylate ester [m.p., 195–197° (CT)] was prepared, but this proved to be less suitable for concentrating kitol. It also appeared to exist in a lower-melting form which was not obtained pure. The palmitate and dinitrophthalate esters were prepared but could not be crystallized.

Whale-liver oil is not unique in containing kitol. The substance was found also in dogfish-liver oil (0.06 per cent) and in shark-liver oil (0.8 per cent). The oils were conveniently assayed by chromatographing the unsaponifiable matter on sodium aluminum silicate. Kitol is adsorbed more strongly than vitamin A alcohol and a relatively sharp separation could be effected by inspection of the column with ultraviolet light. The purity of the kitol fraction was then estimated by its extinction coefficient at 290 mμ, using the value of 700 for the pure material. This procedure is recommended for the assay of kitol in fish-liver oils.

References

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[1] Communication No. 111.