# Fat Content of Nocardia Corallina[1]

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It has been reported (Clark and Aldridge, 1960) that the stainable fat material in *Nocardia corallina* disappears from the cells just prior to the stage of fragmentation and reforms in older, coccoidal cells. The staining technique used to demonstrate the presence of the fat inclusions was the standard procedure using Sudan black B. However, in order to improve photographic contrast in the illustrations used in the publication, the preparations were counterstained with a thin film of nigrosin. This procedure aids in decolorizing the cytoplasm and gives a dark background for better cell contrast. Adams and McClung (1962) found in their application of this method that many lipid granules were completely or partially decolorized as a result of the action of nigrosin. The decolorizing action of nigrosin can be partially controlled by variation in the thickness of the film applied to the slide. A thin, rapidly drying film normally yields a minimum of decolorization. Our experience with this technique showed only minor, partial decolorizing of the lipid inclusions.

The original stain work did not reveal whether the total fat content (as indicated by extractable fat) of the cells was reduced at the time of fragmentation, or whether the fat content remained constant and only the amount of stainable fat was reduced. To clear up this point, and to elucidate the possible action of the nigrosin counterstain leading to false results, the following procedure was followed.

Cultures of *Nocardia corallina* ATCC 4273 were grown on nutrient agar containing 1 per cent fructose. These were harvested at 18 hours to obtain cells containing no stainable fat, and at 48 hours to obtain cells which contained stainable fat inclusions. Fat stains were run on each batch of cells to make certain of the presence or absence of stainable material. Each batch of cells was then dried, weighed, and the fat extracted in a micro-Soxhlet using the system reported by Prince (1960). The solvents, in order of use, were petroleum ether, absolute ethanol, chloroform, acetone, and acidified ethanol. After extraction, each solvent was evaporated and the residue dried to constant weight. No attempt was made to characterize the various fractions.

As shown in Table 1, the cells containing no stainable fat had less extractable fat than the cells which contained demonstrable fat bodies. On the basis of dry weight, cells with no stainable fat contained 8.3 per cent extractable fat compared to 15 per cent extractable fat for cells containing fat inclusion bodies. It can thus be concluded that there is a correlation between the presence of stainable fat inclusion bodies and the amount of extractable fat in the cells of *N. corallina*. Therefore, there is also a correlation between the fat content of the cells and the stage of growth in the life cycle.

[1]This work was supported in part by grants from the National Institute of Allergy and Infectious Diseases, U.S. Public Health Service, under contracts E-1916 and E-3923, and the Faculty Research Committee of the University of Oklahoma.

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*Experimental Procedure*

Standard manometric techniques (Umbreit, et al., 1957) were used. Forty-seven carbon compounds consisting of alcohols, carbohydrates, creosols, and phenol, were used as carbon sources. These sources were in concentrations of 0.01 M and 0.005 M. The average dry weight of cells used was 0.019 mg. per ml. The organisms were *Nocardia corallina*, ATCC 4273, and *Nocardia asteroides*, ATCC 3308. These microorganisms were suspended in phosphate buffers of pH 7.2 and 8.0. Experiments were allowed to run until oxygen uptake was essentially completed. Temperatures of the manometric experiments were 29C in all cases. Paraffin utilization was determined by the method of McClung, 1958. In this method, the ability of the organism to grow with paraffin as the sole carbon source is the criterion of utilization.

*Results*

Those compounds in which the rate of oxygen uptake was significantly greater than the endogenous rate of oxygen utilization for *Nocardia corallina* are listed in Table 1 as being capable of serving as the sole carbon source for the organism. If the oxygen utilization was equal to or less than the endogenous rate, the compounds are listed as being negative. The results obtained from *Nocardia asteroides* are given in Table 2.

*Discussion*

The fact that *Nocardia asteroides* is capable of utilizing a significantly wider variety of carbon compounds than *Nocardia corallina* can be used as a justification for speculation as to the occurrence of these organisms in nature. *Nocardia corallina* has been isolated from soil in England, Australia, and New Zealand. To date, some 74 isolates have been reported. *Nocardia asteroides* has been isolated from soil in many places throughout the world. McClung (1960) was able to isolate *Nocardia asteroides* from a majority of the soils that he tested. It must be considered, however, that more work has been done on the isolation of *Nocardia asteroides* than of *Nocardia corallina* because of the medical significance of the *Nocardia asteroides*. As a result, the reports and the literature may be quite misleading.

On the basis of carbon utilization studies, it would be expected that some strains of *Nocardia asteroides* would be capable of growing under a much wider variety of conditions than would be expected of *Nocardia corallina*. The work reported here includes only one strain of each organism. It is entirely possible that other strains of *Nocardia asteroides* would give a different pattern of utilization from that found on this one strain, since *Nocardia asteroides* is a taxonomic designation that covers organisms of many different descriptions. It should also be pointed out that McClung's and Webb's work on *Nocardia corallina* were on the same ATCC strain as reported here. The results obtained by these various workers were different, showing perhaps the difference in technique leading to different results, or perhaps indicating that the organism itself may have changed over a period of years. We have found the metabolic patterns of *Nocardia corallina* ATCC to have changed slightly over a period of several years study. Certainly more work of a similar nature must be done before the true ecological significance of carbon utilization can be elucidated.

TABLE 1. CARBON SOURCE UTILIZATION BY *Nocardia corallina*

| Utilized | Not Utilized | | |
|---|---|---|---|
| CREOSOL USP | ADONITOL | GALACTOSE | β-NAPTHALAMINE |
| M-CREOSOL | ARABINIC ACID | GLYCEROL | HYDROCHLORIDE |
| O-CREOSOL** | d-ARABINOSE | INOSITOL | RAFFINOSE |
| FRUCTOSE | l-ARABINOSE* | INULIN | RHAMNOSE |
| GLUCOSE | ARABITOL | LACTOSE | d-RIBOSE* |
| β-GLUCOSE | CELLOBIOSE | β-LACTOSE | SALICIN |
| MANNITOL | CELLOBIOSE OCTA | d-LYXOSE | l-SORBOSE |
| NAPHTHALENE | ACETATE | MALTOSE | TURANOSE |
| PARAFFIN | DULCITOL | d-MANNOSE* | XYLAN |
| PHENOL | i-ERYTHRITOL | MELIBIOSE | d-XYLOSE |
| SORBITOL | ESCULIN | MELIZITOSE | |
| SUCROSE | l-FUCOSE | α-NAPHTHALAMINE | |
| TREHALOSE | | | |

* — Slight utilization
** — Purity of compound doubtful

TABLE 2. CARBON SOURCE UTILIZATION BY *Nocardia asteroides*

| Utilized | | | Not Utilized |
|---|---|---|---|
| ADONITOL | GALACTOSE | PARAFFIN | ARABINIC ACID |
| d-ARABINOSE | β-GLUCOSE | PHENOL | CREOSOL USP |
| l-ARABINOSE | GLYCEROL | RAFFINOSE | i-ERYTHRITOL |
| ARABITOL* | INOSITOL | RHAMNOSE | GLUCOSE |
| CELLOBIOSE | d-LYXOSE* | SALICIN | INULIN |
| CELLOBIOSE OCTA | MALTOSE | SORBITOL | LACTOSE |
| ACETATE | MANNITOL | TREHALOSE | β-LACTOSE |
| M-CREOSOL | d-MANNOSE* | d-XYLOSE | MANNOSE |
| O-CREOSOL** | MELIBIOSE* | l-XYLOSE | d-RIBOSE |
| DULCITOL | MELIZITOSE | | l-SORBOSE |
| ESCULIN* | α-NAPHTHALAMINE | | SUCROSE |
| FRUCTOSE | β-NAPHTHALAMINE-HCl* | | TURANOSE |
| FUCOSE* | NAPHTHALENE* | | XYLAN |

* — Slight utilization
** — Purity of compound doubtful

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