Oxidative Dissimilation of Nonnitrogenous Compounds in *Acetobacter suboxydans*[1]

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Since the isolation of *Acetobacter suboxydans* by Kluyver and De Leeuw in 1924 (*1*), this organism has been used extensively in several fermentations. It is characterized by restricted oxidative capacity; thus, yields of sorbitol from sorbose are high (70% or over), and nearly quantitative conversion of glycerol to dihydroxyacetone (DHA) has been obtained (*2, 3*). Similarly, more than a score of other polyhydroxy compounds are oxidized to the corresponding monoketones (*1–6*).

Work in this laboratory has focused upon *A. suboxydans* metabolism because of the organism's preferential use of conjugated forms of pantothenic acid (PAC [*7*]; coenzyme A [*8, 9*]) over the free vitamin. Since this vitamin (or one of its conjugates) is essential to the growth of *A. suboxydans,* the present study has attempted to locate in the organism certain processes that are known to be coenzyme A-dependent. Failure to observe these, together with the discovery that resting *A. suboxydans* cells can cause extensive dissimilation of sorbitol or glycerol beyond sorbose or DHA, have emphasized the need for further study of the oxidative patterns that exist for this organism. The preliminary results are summarized in the following paragraphs.

A. suboxydans cells[3] were grown in a purified medium essentially the same as previously described (*10*). The cells were harvested by centrifuging, washed at 4° C, and immediately dried *in vacuo* from the frozen state. The dried cells were then used as test material. All oxidative studies were performed by standard manometric techniques in a Warburg apparatus.

Coenzyme A (CoA) participation in oxidative metabolism has been indicated at the point of entry of a C_2 unit (at the oxidation level of acetate) into the Krebs cycle (*11, 12*). Although hot water extracts of the dried cells contained liberal amounts of CoA,[4] all attempts to oxidize acetate or any member of the cycle by *A. suboxydans* failed completely, even in the presence of glycerol as a potential "sparker." The inert character of acetate, as well as the absence of other CoA-dependent processes, was further indicated by the failure of the organism to form citrate or to acetylate sulfanilamide,[5] even in the presence of an external source of CoA and adenosine triphosphate (ATP). Under such conditions both activities were exhibited by liver systems, with or without extra CoA and ATP. Barring a complete lack of permeability of the cells toward all these substances, it would appear that the citric acid cycle, if it operates at all, does not play a major role in the oxidative metabolism of *A. suboxydans*; also, the function of CoA in this organism still remains to be established.

The oxidation of various nonnitrogenous substrates was then studied. Lactic and pyruvic acids were dissimilated in nearly quantitative fashion to give equimolar quantities of acetic acid and CO_2. The R. Q. of the former oxidation was about 1, whereas with pyruvic acid it approached 2. Ethanol oxidation involved the uptake of 2 oxygen atoms, with no CO_2 evolution, as reported by other workers (*2, 16*). However, the oxidation of glycerol by resting cells in phosphate buffer required about 4 atoms oxygen per mole of glycerol. As shown in Table 1, dihydroxyacetone (DHA) was also further oxidized in the Warburg flasks; approximately 3 atoms of oxygen were consumed per mole, which is consistent with the idea that glycerol is oxidized by a pathway which includes DHA as an intermediate. On the other hand, when the cells had been thoroughly washed with distilled water and separated by centrifuging at room temperature for 90 min, oxidation of glycerol stopped at 1 atom/mole, and activity toward DHA was reduced practically to zero. These activities were restored by the addition of diphosphopyridine nucleotide (DPN) up to a concentration of 10^{-4} *M*. Under the conditions used, DPN did not appear to be required either in the oxidation of glycerol to DHA or ethanol to acetic acid.

TABLE 1

OXIDATION OF GLYCEROL AND DHA BY RESTING *A. suboxydans* CELLS*

Substrate	Time of oxidation (min)	O_2 uptake (μatoms)	CO_2 produced (μmoles)
40 μmoles glycerol	300	150	47
40 " DHA	330	116	55

* The system contained 0.05 *M* phosphate, 0.01 *M* $MgCl_2$ and 10 mg (dry wt) of cold-washed cells per Warburg flask. Total volume = 2.8 ml; pH = 6.0; temp. = 29° C; air in gas phase. All values corrected for endogenous blanks.

The cold-washed cells showed retarded oxidation of glycerol in the later steps when no external phosphate was present, but this phenomenon was not observed in the case of ethanol oxidation or in the initial step of glycerol oxidation. The unusual nonparticipation of phosphate suggested that 2,4-dinitrophenol (DNP),

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[3] ATCC strain Nos. 621 and 9322 were employed, as well as a strain of No. 621 given to us by L. A. Underkofler. The latter strain had been maintained in his laboratory since 1935.

[4] By acetylation (*13*) and by bound pantothenic acid assay (*14, 15*).

[5] Acetylation and citrate formation were performed in systems similar to those used by Kaplan and Lipmann (*13*) and Stern and Ochoa (*11*). The resting cell was employed as the enzyme.

TABLE 2

EFFECT OF DNP ON THE OXIDATION OF VARIOUS SUBSTRATES BY RESTING *A. suboxydans* CELLS*

Amt substrate (μmoles)	DNP concentration	Time of oxidation (min)	O_2 uptake (μatoms)
40 Glycerol	—	400	147
40 "	10^{-4} *M*	400	43
20 DHA	—	320	61
20 "	10^{-4} *M*	320	7
90 Ethanol	—	120	170
90 "	10^{-4} *M*	120	179
40 Sorbitol	—	450	158
40 "	10^{-4} *M*	450	53

* The system contained 0.05 *M* phosphate, 0.01 *M* $MgCl_2$, 10^{-4} *M* DPN and 10 mg (dry wt) of room temperature-washed cells per Warburg flask. Total volume = 2.8 ml; pH = 6.0; temp. = 29° C; air in gas phase. The substrate was tipped into the main compartment containing DNP after 5 min preincubation. All values corrected for endogenous blanks.

which is generally regarded as a phosphate "uncoupling" agent (*17, 18*), might affect only the reactions beyond DHA from glycerol. Representative data, summarized in Table 2, show this to be true. The first atom of oxygen was rapidly consumed in glycerol oxidation either in the presence or absence of DNP. However, after 1 atom of oxygen had been used, the reaction virtually ceased in the flasks containing 10^{-4} *M* DNP. With DHA, almost no oxidation occurred in the presence of DNP. Likewise, in the oxidation of sorbitol, DNP affected only the oxidation steps beyond sorbose, so that 1 atom of oxygen was utilized per mole. Ethanol oxidation, on the other hand, was completely unaffected by DNP.

The growth of *A. suboxydans,* either in glycerol or sorbitol media, was unaffected by DNP even at concentrations of the inhibitor up to 4×10^{-4} *M*. The results of some preliminary experiments showed that the filtrate of the fermentation liquor from a medium containing 5% sorbitol and 0.5% yeast extract had higher reducing power in the presence of DNP than in its absence, indicating an increased yield of sorbose. The above observations raise the question whether some of the energy storage mechanisms in this organism may differ from those in higher animals or other bacteria, in which coenzyme-linked energy-rich phosphate is regularly generated. Further details of these studies will be reported later.

References

1. KLUYVER, A. J., and DE LEEUW, F. J. G. *Tijdschr. Vergelijk. Geneesk.*, **10**, 170 (1924); KLUYVER, A. J. *The Chemical Activity of Microorganisms.* London: University of London Press (1931).
2. WIELAND, H., and BERTHO, A. *Ann. (Chem.)*, **467**, 95 (1928).
3. FULMER, E. I., and UNDERKOFLER, L. A. *Iowa State Coll. J. Sci.*, **21**, 251 (1947).
4. BUTLIN, K. R. *Dept. Sci. Ind. Research (Chem., Brit.), Special Rept. No. 2*, 1936.
5. VISSER'T HOOFT, F. Diss., Delft (1925).
6. HANN, R. M., TILDEN, E. B., and HUDSON, C. S. *J. Am. Chem. Soc.*, **60**, 1201 (1938).
7. KING, T. E., FELS, I. G., and CHELDELIN, V. H. *Ibid.*, **71**, 131 (1949).
8. NISHI, H., KING, T. E., and CHELDELIN, V. H. *J. Nutrition*, **41**, 279 (1950).
9. NOVELLI, G. D., FLYNN, R. M., and LIPMANN, F. *J. Biol. Chem.*, **177**, 493 (1949).
10. KING, T. E., and CHELDELIN, V. H. *Ibid.*, **174**, 273 (1948).
11. STERN, J. R., and OCHOA, S. *Ibid.*, **179**, 491 (1949).
12. NOVELLI, G. D., and LIPMANN, F. *Ibid.*, **182**, 213 (1950).
13. KAPLAN, N. O., and LIPMANN, F. *Ibid.*, **174**, 37 (1948).
14. NIELANDS, J. B., and STRONG, F. M. *Arch. Biochem.*, **19**, 287 (1948).
15. HOAG, E. H., SARETT, H. P., and CHELDELIN, V. H. *Ind. Eng. Chem., Anal. Ed.*, **17**, 60 (1945).
16. BUTLIN, K. R. *Biochem. J.*, **30**, 1870 (1936).
17. LOOMIS, W. F., and LIPMANN, F. *J. Biol.. Chem.*, **173**, 807 (1948).
18. TEPLY, L. J. *Arch. Biochem.*, **24**, 383 (1949).

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The Absorption of Human Skin between 430 and 1,010 mμ for Black-Body Radiation at Various Color Temperatures

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It is an accepted premise that if dark and light skins are irradiated by the same source radiating in the visible and near infrared part of the spectrum the dark skin will absorb more than will the light skin. As far as the author can ascertain nothing has been published concerning the quantitative relations for the absorptance of skins of various colors. This paper is written to show the expected relative absorption of tanned and untanned white and Negro skin for the radiation between 430 and 1,010 mμ emanating from sources radiating at various color temperatures.

It is known that for black-body radiation at color temperatures less than 8×10^{3}° K there is no attenuation due to the transport of radiation through the atmosphere. As the color temperature of a source increases, the transmission coefficient for air decreases until, at 25,000° K, the transmission coefficient is approximately 0.4. Since the temperature of the ball of fire (which is considered to be a good approximation to a black-body radiator) resulting from the explosion of a nominal atomic bomb falls to 8×10^{3}° K in approximately 20 μsec when the radius of the ball of fire is thought to be about 8 yards (*1*) (a distance at which thermal radiation has not yet begun to play an important part), attention is not given to atmospheric attenuation. Neither is attention given to the fact that the flux density of the radiation varies with distance, nor that the type of day (clear or hazy) has an effect on the amount of radiation reaching a given point. The concern here is with the comparison of the maximum expected absorption of tanned and untanned skin of the white and Negro races. Although the ordinate of the accompanying graph is expressed in units of energy, it should be kept in mind that this energy is for the maximum expected absorption at the surface of the radiator and that no attempt has been made to calculate the energy arriving at a particular distance, as some authors have done.

The average absorptance values were calculated from average reflectance values obtained from indi-