

These observations seem to indicate that the blood of athreptic infants may show the presence of reaction bodies to cow's milk both by the presence of a precipitine to cow's milk protein and by the fact that the blood of the infant can passively sensitize guinea pigs to cow's milk.

Whether the presence of these reaction bodies bears a causative relation to the clinical condition we do not feel in a position to discuss with assurance at the present time. That this may be true seems quite possible and is in accord with many clinical observations. On the other hand, the presence in the blood of athreptic infants of reaction bodies to cow's milk protein may be due merely to the fact that the intestinal tract of such infants has permitted the passage of cow's milk protein into the blood stream, thereby causing antibody formation, and may not be of etiological importance. Observations are being conducted at present which may throw some light on this question.

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Carbon assimilation and respiration of autotrophic bacteria¹

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The autotrophic bacteria, or those bacteria which are capable of assimilating carbon dioxide chemosynthetically utilizing for that purpose the energy obtained from the oxidation of simple inorganic substances, range from obligate to facultative forms. In other words they range from those which are capable of obtaining their energy only from the oxidation of specific inorganic substances but from no other source to those that can exist autotrophically and heterotrophically; the latter can obtain their energy both from inorganic sources and, lacking these, also from the oxidation of organic compounds. The nitrite, nitrate and certain sulfur bacteria are the only strict autotrophic forms, while all the others, including various sulfur bacteria,

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the methane, hydrogen and particularly iron bacteria belong to the second group.

Respiration in the case of autotrophic bacteria is different from that of the heterotrophic forms; it consists in taking in carbon dioxide and giving out oxygen, at the same time consuming large quantities of oxygen for purposes of oxidation which yields the energy necessary for the activities of the organisms. Meyerhof was the first to demonstrate conclusively that the nitrite and nitrate forming bacteria obtain their carbon only from the carbon dioxide of the atmosphere or in the form of bicarbonate in solution. To demonstrate the carbon assimilation by the sulfur-oxidizing organism, *Sulfomonas thiooxidans*, the following procedure was followed: A medium consisting of 0.2 gm. $(\text{NH}_4)_2\text{SO}_4$, 3 gm. KH_2PO_4 , 0.5 gm. MgSO_4 , 0.25 gm. CaCl_2 , 0.01 gm. FeSO_4 and 10 gm. of elementary sulfur per 1000 c.c. of distilled water, placed in 100 c.c. portions in 250 c.c. Erlenmeyer flasks was sterilized and inoculated in the usual manner. To some of the flasks 1 per cent. of dextrose was added, or 0.1 per cent. of NaHCO_3 , or 0.1 per cent. NaHCO_3 and sufficient $\frac{\text{N}}{1} \text{H}_3\text{PO}_4$ to neutralize the excess alkalinity. The

flasks were divided into two series; (1) placed in the incubator kept at constant temperature and (2) placed under bell jars, with a carbon-dioxide free atmosphere. The air passing through the jars was drawn through a soda-lime tube and bottles containing 50 per cent. solution of KOH. The jars were sealed on to a bench in the same incubator (at 28°C) with the first series of flasks. At the end of 7 days, the hydrogen-ion concentration and titratable acidity (for 10 c.c.) of the cultures were determined. These serve as an index of the amount of sulfur oxidized.

Treatment	Atmosphere			
	Ordinary		CO ₂ free	
	Final P _H	Titre	P _H	Titre
Regular medium—Control	4.2	2.20	4.2	2.20
Regular medium—Inoculated	1.2	12.15	3.8	2.25
1 per cent. Dextrose—Control	3.0	2.20	3.0	2.2
1 per cent. Dextrose—Inoculated	1.2-	13.15	2.8	2.33
0.1 per cent. NaHCO ₃ —Control	6.6	1.3	6.6	1.3
0.1 per cent. NaHCO ₃ —Inoculated	5.4	2.0	6.0	1.75
0.1 per cent. NaHCO ₂ + H ₃ PO ₄ —Control	6.2	2.2	6.2	2.2
0.1 per cent. NaHCO ₂ + H ₃ PO ₄ —Inoculated	1.5	9.3	5.3	2.5

No growth took place in the carbon-dioxide free atmosphere both in the absence and presence of dextrose. The slight increase in acidity of the inoculated culture is due to the inoculum introduced (3 drops of culture in 100 c.c. of medium). However, in the presence of the bicarbonate, a slight amount of growth took place even in the CO₂-free atmosphere. The relatively small growth made in the presence of the bicarbonate is due to the change in reaction of the medium, the organism having its optimum at P_H 2.0 to 5.0 (1.0-5.6).

For studying the respiration of bacteria, the methods of Osterhout² and Meyerhof³ are available. The former is not very suitable for the study of the autotrophic bacteria, since the organisms consume carbon dioxide and produce acids which rapidly change the reaction of the medium. The method used by Meyerhof was found more suitable. By this method we measure the amount of change in the concentration of substratum produced in a definite period of time, as a result of the energy utilization by the organism. We can thus differentiate between the growth and respiration processes.

Sulf. thiooxidans was grown in the above medium till the maximum rate of growth was attained, as determined from the

² Osterhout, W. J. V., *J. Gen. Physiol.*, 1918, i, 171.

³ Meyerhof, O., *Arch. Ges. Physiol.*, 1916, clxiv, 353; clxv, 229; clxvi, 240.

autocatalytic curve of growth. This took place at the end of the fourth to the fifth day of incubation. The culture was then filtered through filter paper to get it free from sulfur. 50 c.c. portions of the uniform culture were then placed in small flasks (100 c.c. Erlenmeyer), to each of which 1 gram of elementary sulfur was added. The stimulating or repressive agents were then added and the flasks placed in the incubator and kept at 30°C for about 20 hours. The change in hydrogen-ion concentration, titration or sulfate content during that period indicates the amount of sulfur oxidized and can serve as an index of oxygen consumption or carbon dioxide assimilation. One is particularly justified in doing that, since the ratio between the sulfur oxidized and carbon dioxide assimilated is constant under the conditions of the experiment, as pointed elsewhere. The change in titration, using phenolphthalein as an indicator, is the simplest index of the respiration of the culture, since at the very high acid concentration at which the organism is active, particularly in well buffered media, the change in hydrogen-ion concentration is not a sensitive index.

If the normal rate of respiration is taken as a 100, the respiration of the organism as influenced by various treatments is as follows:

INFLUENCE OF GASES, NITRATES AND ORGANIC SUBSTANCES
UPON THE RESPIRATION OF SULF. THIOOXIDANS

Control			100
(NH ₄) ₂ SO ₄	0.025	molar	108
(NH ₄) ₂ SO ₄	0.050	molar	112
(NH ₄) ₂ SO ₄	0.100	molar	112
(NH ₄) ₂ SO ₄	0.250	molar	108
NaNO ₃	0.025	molar	29.2
NaNO ₃	0.050	molar	12.5
NaNO ₃	0.100	molar	0
NaNO ₃	0.250	molar	0
Ca(NO ₃) ₂	0.025	molar	27.3
Ca(NO ₃) ₂	0.050	molar	8.5
Ca(NO ₃) ₂	0.100	molar	10.0
Ca(NO ₃) ₂	0.250	molar	0
NaNO ₃ +(NH ₄) ₂ SO ₄	0.0125	molar each	62.5
NaNO ₃ +(NH ₄) ₂ SO ₄	0.025	molar each	25.0
NaNO ₃ +(NH ₄) ₂ SO ₄	0.050	molar each	16.5
NaNO ₃ +(NH ₄) ₂ SO ₄	0.125	molar each	0
KNO ₃	0.005	molar	71
KNO ₃	0.010	molar	57
KNO ₃	0.020	molar	43
KNO ₃	0.050	molar	21
Mg(NO ₃) ₂	0.050	molar	16
NaCN	0.0004	molar	0

Peptone	0.2 per cent.	50
Peptone	0.4 per cent.	4.2
Peptone	1.0 per cent.	0
Peptone	2.0 per cent.	0
Dextrose	0.25 per cent.	133
Dextrose	1.00 per cent.	120
Tyrosin	0.1 per cent.	64
Glycocoll	0.1 per cent.	100
Glycocoll	0.5 per cent.	45
Urea	0.1 per cent.	100
Urea	0.5 per cent.	57
Ethyl Alcohol	0.1 per cent.	54
Ethyl Alcohol	0.3 per cent.	46
Amyl Alcohol	0.1 per cent.	43
Amyl Alcohol	0.3 per cent.	28
Normal atmospheric pressure		100
CO ₂ -free atmosphere		60
Increased CO ₂ pressure		300
Hydrogen atmosphere		50
Increased atmospheric pressure (5-10 per cent.)		130
Reduced atmospheric pressure (10 per cent.)		100

It is seen from the above data that the nitrates have a depressing effect upon the respiration of the organism while the sulfate does not. 0.025 molar concentration of the nitrate is sufficient to reduce respiration to 27-29 per cent.; 0.05 molar only to about 10-20 per cent., while still higher concentrations practically depress respiration. The action of nitrates is not antagonized by the presence of ammonium sulfate, since 0.025 molar nitrate depresses respiration in the presence of an equimolar concentration of ammonium sulfate to the same extent as in its absence.

Peptone proves to be another substance injuring respiration even in comparatively small concentrations. The injurious action of nitrates and peptone can also be readily demonstrated by adding these substances to the culture media upon which the organism is grown. The amino acids and acid amides have some depressing effect, but not to such an extent as peptone.

Cyanides repress respiration completely, even in concentrations of 0.0004 molar. Dextrose has no depressing effect on respiration, even a 1 per cent. concentration tends to favor it.

The influence of the gas pressure is of particular interest, particularly that of carbon dioxide. When the flasks, in which respiration was taking place, were covered with air tight bell jars, it was found that a CO₂-free atmosphere reduces respiration to 60 per cent., while an atmosphere rich in CO₂ (which was allowed to bubble from a gas tank into the bell jar) in-

creased respiration 300 per cent. An hydrogen atmosphere (no precautions were taken to free the gas from traces of oxygen and CO₂) reduced respiration to 50 per cent. Reducing the atmospheric pressure 5-10 per cent. has no appreciable effect on respiration, while increasing the pressure 10 per cent. has a stimulating effect.

The ratio between the sulfur oxidized and carbon assimilated by the culture is about 32, this ratio varying greatly by changing the conditions of growth and by adding various depressive substances. The presence of nitrates, for example, greatly increases the ratio. When thiosulfate is used as a source of energy the ratio is about 65. Of the total amount of energy made available only about 6.5 per cent. is utilized by the organism. The amount of energy utilized by the nitrite and nitrate bacteria is about 5 per cent. These quantities in comparison with the low utilization of energy by higher plants point to the greater efficiency of the autotrophic bacteria.

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A study of light waves in relation to their protective action in rickets

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In a communication presented a year ago we showed that rickets can be prevented in rats by daily exposures to direct sunlight for about fifteen minutes¹. A similar result was reported at the same time by others². When rats were placed in a box having flint glass windows it was found that the sun's rays, in traversing the glass, had lost their protective power. In a later communication it was shown that the pigment of the skin also hinders the action of the effective rays, that black rats require more radiation than do white rats³.

¹ Hess, A. F., Unger, L. J., Pappenheimer, A. M., PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 8.

² Shipley, P. G., Park, E. A., Powers, G. F., McCollum, E. V., PROC. SOC. EXP. BIOL. AND MED., 1921, xix, 43.

³ Hess, A. F., Unger, L. J., Pappenheimer, A. M., PROC. SOC. EXP. BIOL. AND MED., 1922, xix, 238.