tracted from blood or plasma with isoamyl acetate at *p*H 7.0, and then extracted from the solvent with isonicotinic acid hydrazide and sodium hydroxide. A yellow color develops with an intensity which is proportional to the concentration of chloramphenicol at 430 mμ. The results compared well with microbiological assays.

We have used this procedure (*7*) to evaluate concentrations of chloramphenicol in blood and plasma of patients receiving multiple antibiotics and have found no interference during studies in vitro or in vivo when sulfadiazine (400 μg/ml), procaine, streptomycin (200 μg/ml), penicillin (30 units/ml), erythromycin (50 μg/ml), and kanamycin (50 μg/ml) were added. The intact chloramphenicol succinate and palmitate esters (25 μg/ml) gave zero readings on the spectrophotometer, indicating that only the free form of chloramphenicol was measured. Since pure chloramphenicol glucuronide was not available, this compound could not be tested, but in previous experiments Kakemi *et al.* (*6*) demonstrated that it was not extracted by isoamyl acetate. D-Threo-1-*p*-nitrophenyl-2-amino-1,3-propanediol, a diol hydrolysis product of chloramphenicol, produced no absorption at 430 mμ in concentrations of 50 μg/ml.

Concentrations of chloramphenicol in the plasma of two patients 1 to 2 hours after they received the succinate ester were determined by the methods of Kakemi *et al.* (*6*) and Levine and Fischbach (*5*) (without extraction of hydrolyzed ester with sodium bicarbonate). The results are shown in Table 1. The method of Levine and Fischbach gave higher results, as expected, since the unhydrolyzed ester of chloramphenicol at the peak plasma concentration accounts for about 30 to 40 percent of the unconjugated chloramphenicol measured by this or similar methods (*8*). On the other hand, the results obtained by the method of Kakemi *et al.* have previously indicated a close correlation with those of microbiological assay (*6*).

The test is simple, requires few reagents, and can be performed relatively rapidly (*7*). The absorbance at 430 mμ is linear up to concentrations of at least 50 μg/ml of chloramphenicol. Agreement between duplicate sample readings has been of the order of 5 to 10 percent.

We wish to emphasize the usefulness of the method and to stress its value in cases where multiple antibiotics are being administered, an important feature not mentioned by other workers. By this method unduly high concentrations of chloramphenicol during therapy can be detected and measures can be taken to avert the possible toxic effects on bone marrow.

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7. The method is as follows. Fresh, heparinized blood or serum may be used. Whole blood or packed red cells are lysed by repeated freezing and thawing, or by adding 0.5 ml 1 percent saponin per milliliter of sample. The reagents required are: isoamyl acetate (Fisher Scientific Co.); isonicotinic acid hydrazide, 3 percent solution (Eastman Kodak Co.); sodium hydroxide, 1.5*N* or 6 percent solution; and 0.1*M* phosphate buffer, *p*H 7.0, prepared by dissolving 2.21 g of $NaH_2PO_4 \cdot H_2O$ and 7.61 g of $Na_2HPO_4 \cdot 7H_2O$ in 1 liter of distilled water. Pure chloramphenicol is used as standard. Stock solution 500 μg/ml (50 mg to 100 ml of distilled water). Duplicate standards are prepared, equivalent to 10, 20, and 30 μg for the assay. Procedure: 2.0 ml of phosphate buffer is added to all tubes; 0.5 to 1.0 ml of plasma, blood, or a standard solution is added, distilled water being put in the blank reagent tube. Isoamyl acetate, 3.0 ml, is pipetted into each tube, after which all tubes are stoppered, shaken well for 10 minutes, and then centrifuged. To each tube, containing 2 ml of the supernatant solvent, 1.0 ml each of 1.5*N* NaOH and 3 percent isonicotinic acid hydrazide are added. The tubes are then stoppered and incubated in a water bath at approximately 30°C for 45 minutes, the tubes being shaken well at intervals to ensure good admixture. At the end of the incubation period the yellow-colored underlayer is aspirated and measured in a spectrophotometer at 430 mμ against the reagent blank. A line best fit for the standard solutions is then plotted on arithmetic graph paper so that the values for the test solutions can be interpolated. The procedure may be modified by using double quantities of all solutions to give larger volumes.
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9. This research was supported in part by grants from the John A. Hartford Foundation, Inc., and the National Heart Institute (HTS 5255).

4 March 1964

Anaerobic Formate Oxidation: A Ferredoxin-Dependent Reaction

Abstract. *The formate oxidizing system of* Methanobacillus omelianskii *is specific for nicotinamide-adenine dinucleotide and requires ferredoxin, a biological carrier with the lowest known potential. Ferredoxin from other obligate anaerobes is active in the reaction studied.*

In this report we present evidence for another ferredoxin-dependent reaction, the oxidation of formate to carbon dioxide by *Methanobacillus omelianskii*.

$$\underset{E'_0\ (-425\ \text{mv})}{HCOOH} + Fd_{ox} \longrightarrow \underset{E'_0\ (-417\ \text{mv})}{CO_2} + Fd_{red}$$

$$Fd_{red} + NAD \xrightarrow[E'_0\ (-319\ \text{mv})]{} Fd_{ox} + NADH_2$$

Ferredoxin in the oxidized form is represented by Fd_{ox} and in the reduced form by Fd_{red}. The oxidation-reduction potential at *p*H 7 is shown by E'_0; NAD and $NADH_2$ represent the oxidized and reduced forms of nicotinamide-adenine dinucleotide, respectively.

Since the discovery of ferredoxin in extracts of *Clostridium pasteurianum* (*1*), a variety of electron transport reactions in obligate anaerobes (*2*, *3*), blue-green algae (*4*), and plants (*5*) have been found to require ferredoxin. Ferredoxin has also been found in photosynthetic bacteria (*5*). The biological mediation of electrons by ferredoxin occurs in the presence of a suitable substrate and its oxidizing enzyme. The electrons from ferredoxin

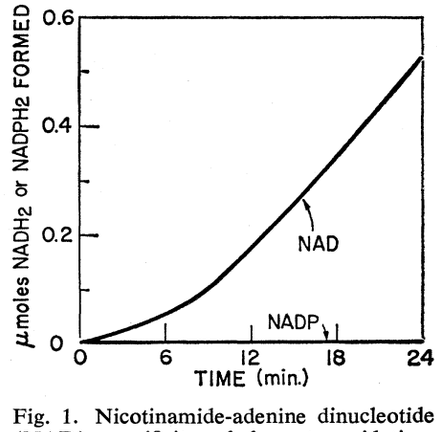

Fig. 1. Nicotinamide-adenine dinucleotide (NAD) specificity of formate oxidation. Each cuvette contained NADP or NAD, 1 μmole; sodium formate, 100 μmole; phosphate buffer, *p*H 7.5, 100 μmole; and Sephadex-treated extract, 4.8 mg of protein.

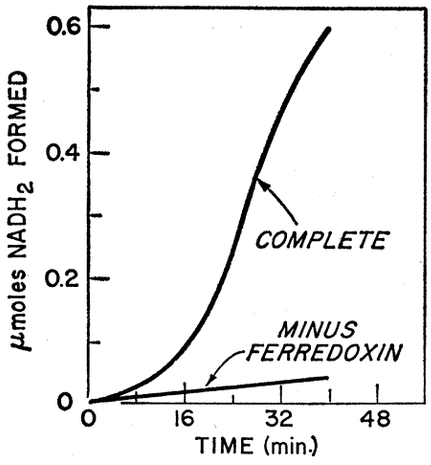

Fig. 2. Ferredoxin dependency of formate oxidation. Each cuvette contained NAD, 1 µmole; sodium formate, 100 µmole; phosphate buffer at *p*H 7.5, 100 µmole; and treated extract (Sephadex and diethylaminoethyl-cellulose), 11 mg of protein. Partially purified ferredoxin (0.07 mg of protein) from *M. omelianskii* was used.

may be transferred enzymically to such substrates as pyridine nucleotides (*6, 7*), nitrite (*8*), hydroxylamine (*9*), and urate (*2*), or may accept protons to form hydrogen (*1*). Examples of electron donors are light-activated chlorophyll (*5*), α-ketoglutarate (*10*), pyruvate (*1*), hydrogen (*6*), and hypoxanthine (*2*).

Cell-free extracts of *Methanobacillus omelianskii* were prepared as described by Wolin *et al.* (*11*). The endogenous pyridine nucleotides and low molecular weight substrates were removed by adding 10 ml of the crude extracts (40 mg/ml) to a Sephadex G-25 column (24 × 2.75 cm) at 4°C and then eluting with demineralized water. Pyridine nucleotide reduction was assayed in an anaerobic cuvette which had been evacuated and flushed with argon several times.

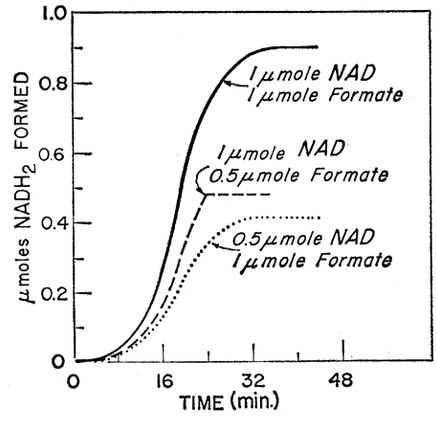

Fig. 3. Stoichiometry of formate oxidation. Each cuvette contained NAD and formate as indicated; potassium phosphate buffer, 100 µmole, *p*H 7.5; and Sephadex-treated extract, 7.0 mg of protein.

The main compartment of the cuvette contained, in a total volume of 3 ml, 1 µmole of nicotinamide-adenine dinucleotide (NAD) or nicotinamide-adenine dinucleotide phosphate (NADP), 100 µmole of potassium phosphate buffer at *p*H 7.5, and a suitable amount of cell-free extract. Protein was assayed by the biuret method (*12*). Sodium formate (100 µmole) was tipped from the side arm to initiate the reaction which was followed in a Cary model 14 recording spectrophotometer by measuring absorbancy at 340 mµ. As shown in Fig. 1, the formic dehydrogenase is NAD specific.

The ferredoxin dependency of formate oxidation is shown in Fig. 2. When ferredoxin was removed from the crude extract by passage through a small diethylaminoethyl-cellulose (phosphate) column (1 × 4 cm), as described previously (*1*), the ferredoxin-free extract did not appreciably reduce NAD from the formate, but when ferredoxin was added back to the system, $NADH_2$ was formed. Partially purified ferredoxins from *C. pasteurianum, M. omelianskii,* and *Clostridium acidi-urici* were interchangeable in this reaction. We have obtained no evidence to support the possibility that hydrogen is a product of formate cleavage in *M. omelianskii.* Certain aged, frozen extracts showed negligible hydrogenase activity; yet NAD reduction by formate occurred rapidly, showing that hydrogen is not an intermediate in the reduction.

Evidence to show that both formate and NAD are quantitatively utilized is presented in Fig. 3. The reaction also was studied using Warburg vessels, showing the dependency of carbon dioxide evolution on ferredoxin and NAD.

The formic dehydrogenase of *C. acidi-urici* has been studied (*13*), but was not found to reduce pyridine nucleotides. Using the techniques presented here, we have shown this organism to have an NAD-specific, ferredoxin-dependent formate oxidation system similar to that of *M. omelianskii.* The mechanism by which *M. omelianskii* is able to reduce carbon dioxide to methane is still an unsolved problem. Perhaps formate or an active formyl complex is a direct intermediate in the reduction of carbon dioxide.

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22 November 1963

Cytological Evidence for Crossing-Over in vitro in Human Lymphoid Cells

Abstract. *In human blood cells dividing in vitro two chromosomes are occasionally found intimately associated in a quadriradial configuration (Qr). A Qr by traditional interpretation is the result of chromatid interchange. Since the configurations in blood cells often are equal and symmetrical and are composed of homologous chromosomes, they are considered cytological evidence that somatic crossing-over may occur in mammalian cells.*

In meiosis, in addition to chromosomal segregation during reduction division, crossing-over normally contributes additional genetic diversity to gametes. As reported in his classical paper of 1936, Stern (*1*) demonstrated by genetical methods that crossing-over