feature of the crystalline substance, and both effects show a direct correlation with the amounts added to the bath—that is, the higher the dose the stronger the initial contraction and the subsequent depression.

From its chromatographic behavior with Sephadex G-25 it appeared that the active principle had a molecular weight in the range 1000 to 2000. It was therefore of interest to compare the crystalline crown gall substance with tomatine, a known constituent of tomato plant of similar molecular weight (1035).

The crystalline crown gall product and tomatine (*7*) were spotted on Whatman No. 1 paper and chromatographed according to the method of Tukalo (*8*). The chromatograms were dried at room temperature in a draft of air. Spots were developed with ninhydrin and phosphomolybdate reagents (*9*), the latter to locate steroid-containing component(s). The results indicated that similar, if not identical, steroid-containing components were present in both samples. It was therefore of interest to study the biological effects of tomatine.

Figure 2 shows that the effect of crystalline tomatine on histamine-induced contractions, when administered in concentrations of 3×10^{-6} and 4×10^{-6} to the bath, is very similar to the effect of crystalline crown gall substance. To reduce the time required for each assay, it was necessary to double the original dose of histamine so as to obtain responses similar to those of the standard controls. When tested against contraction induced by bradykinin, serotonin, and acetylcholine, the efficacy of both tomatine and the crystalline crown gall substance was the same as against histamine.

The antihistaminic activity of crown-gall tumor extracts up to the stage of Sephadex fractionation has been firmly established in vivo. However, we have not yet accumulated a sufficient quantity of the crystalline product for testing in vivo. Three batches of crystalline commercial tomatine have been tested by the histamine aerosol technique, on 40 guinea pigs. When given intraperitoneally, in a dose of 10 mg/kg, only one batch showed definite protection against the lethal effect of 0.2 percent histamine aerosol.

From these findings it appears that the principle(s) found in crown gall tumors is unique in its properties since it inhibits equally four major smooth-muscle contracting agents for a long period of time. This prolonged effect is also apparent in vivo, as evaluated against histamine aerosol. Although crystalline, there may be more than one principle, and it seems quite probable that one of them is tomatine or something similar. We are aware of the implications of a naturally occurring antihistaminic with a steroid nucleus.

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16 March 1964

Chloramphenicol in Blood: Simple Chemical Estimations in Patients Receiving Multiple Antibiotics

Abstract. *A simple method is described which obviates the difficulties of estimating the concentration of chloramphenicol in the blood in the presence of other antibiotics. This is of importance in avoiding hematologic depression from chloramphenicol toxicity.*

Chloramphenicol has proved itself an extremely valuable antibiotic, but its use has been occasionally associated with blood dyscrasias. The commonest form of hemopoietic toxicity is transient erythropoietic depression, which appears to be closely related to the concentration of the drug in the plasma (*1*), and measurements of such concentrations in the blood may therefore be of value in predicting its harmful effects.

After the administration of chloramphenicol, the free or biologically active form is converted into two major products (*2*), the glucuronide and aryl amines, both of which are biologically inert. When chloramphenicol succinate (*3*) or palmitate (*4*) is employed, the esters are first hydrolyzed, liberating free chloramphenicol. The free form of the drug assumes importance in evaluating both its therapeutic and toxic effects.

In the past, there have been disadvantages with both the chemical and biological methods of assaying chloramphenicol when the patients have been receiving multiple antibiotics, as in cases of meningitis. The biological assay, though measuring free chloramphenicol, requires the use of aseptic techniques and is of limited value when the patient is receiving multiple antibiotics. Chemical procedures (*3*, *5*) dependent on extraction with ethyl acetate, followed by evaporation, reduction of the nitro compound, and diazotization and coupling, are more sensitive, but may be time-consuming and are rendered useless when sulfonamides and procaine penicillin are also being administered, owing to gross interference by aryl amine compounds (*3*). In addition, when chloramphenicol succinate is used, the ethyl acetate solution has to be washed with sodium bicarbonate in order to extract the unhydrolyzed ester (*3*).

Kakemi *et al.* (*6*) recently described a chemical method for assaying chloramphenicol in which the drug is ex-

Table 1. Concentrations of chloramphenicol in the plasma of two patients, estimated by the methods of Kakemi *et al.* (*6*) and Levine and Fischbach (*5*).

Patient	Unconjugated chloramphenicol (μg/ml)	
	Kakemi *et al.*	Levine and Fischbach
A	14	22
B	9.5	15

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

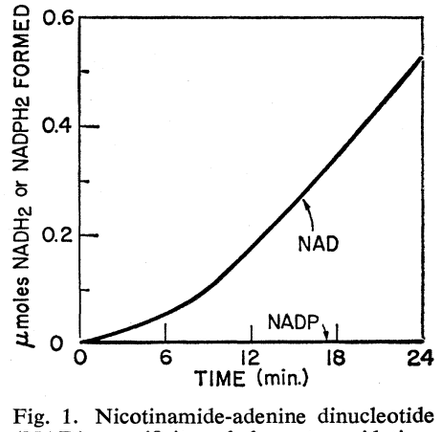

Fig. 1. Nicotinamide-adenine dinucleotide (NAD) specificity of formate oxidation. Each cuvette contained NADP or NAD, 1 μmole; sodium formate, 100 μmole; phosphate buffer, *p*H 7.5, 100 μmole; and Sephadex-treated extract, 4.8 mg of protein.