on the intrathoracic airways, primarily reflecting changes in tidal volume; (*b*) the pretreatment with morphine antagonizes the action of the antibiotic; (*c*) the dicloxacillin activity is unaffected by the pretreatment with atropine, dibenamine, INPEA, methysergide, chlorpheniramine, cyproheptadine, and hexamethonium; and (*d*) the action of the antibiotic persists after cutting of the vagi.

Consequently, only *in vitro* is it possible to note the myolytic activity of the antibiotic on the bronchial musculature, while *in vivo* it is more likely that dicloxacillin, as ampicillin, induces at least in part an action on the medullary respiratory center.

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COMMUNICATIONS

Spectrographic Determination of Traces of Cobalt in Vitamin B_{12} Preparations

Keyphrases □ Cobalt determination—vitamin B_{12} dosage forms □ Emission spectrograph, direct—analysis

Sir:

The assay methods available for determination of vitamin B_{12} fall into four groups: biological, microbiological, chemical, and radioactive (1–3). Most of these methods are time consuming or difficult to perform, and often large samples must be taken for analysis.

A quick and simple analytical assay was needed for evaluation of active ingredients in pharmaceutical preparations containing vitamin B_{12} or its analogs, using only a few centigrams of the test substance.

We have developed a direct emission spectrographic method for rapid analysis of vitamin B_{12} in biological materials, where the amount of nonvitamin B_{12} cobalt is negligible. The method is based upon the cobalt content of cyanocobalamin ($C_{63}H_{88}CoN_{14}O_{14}P$, 4.35% Co in dry form). Mineralization, fusion, or ashing of organic substances is superfluous in this determination; separation or extraction of traces of Co is similarly unnecessary.

One milliliter of a liquor containing 25–125 mcg. vitamin B_{12}/ml. (corresponding to 1.1–5.4 mcg. Co/ml.) is mixed with 0.1 g. spectroscopically pure graphite powder and evaporated to dryness at 105°. Or, approximately 0.05 g. powdered solid sample is thoroughly mixed with 0.1 g. graphite powder. Then 20 mg. of this mixture or the residue is vaporized in a cupped carbon electrode, using a 14-amp. d.c. arc, and the spectrographic plate is measured as usual (4).

The entire procedure takes about 30 min. for a single determination. In batch analyses the time required is considerably reduced.

The results of this determination are expressed in terms of anhydrous cyanocobalamin. When less stable but more naturally occurring forms of vitamin B_{12}, like hydroxocobalamin and metabolically active coenzyme B_{12}, are also present in the test solution, total vitamin B_{12} activity is directly obtained in this method (calculated as cyanocobalamin equivalents).

Fraudulent addition of inorganic cobalt interferes with this assay.

The most sensitive cobalt line in the UV region, at 3435.5 Å, is chosen as the analytical line. Its intensity is suitable for the range 1–5 mcg. Co/ml. which is usually encountered in pharmaceutical preparations.

There are few ions that interfere with this determination, and it may be termed as specific. However, the intensity of the cobalt line is increased several times in the presence of organic matter existing usually in vitamin preparations. A similar increase of line intensity is observed in flame photometry when using solutions made with combustible organic solvents like methanol or methyl isobutyl ketone (5). For this reason, standard powders are made according to the "addition method," in which increasing amounts of Co are added to the same solution that is being analyzed (4). This "standard addition method" is equivalent, by its nature, to a recov-

ery test. Therefore, experiments for the complete recovery of added vitamin B_{12} are not necessary in this assay.

The cobalt line is measured against the background of the plate, using a densitometer. To prepare standard synthetic powders, 0.1, 0.2, 0.3, and 0.4 ml. of a standard cobalt solution containing 10 mcg. Co/ml. [49.38 mg. cobalt nitrate, $Co(NO_3)_2 \cdot 6H_2O$, dissolved in 1000 ml. distilled water] are added to four 1-ml. portions of the vitamin solution being analyzed. Each sample is treated as mentioned previously, after mixing with 0.1 g. graphite powder.

An atomic absorption spectrophotometer may be used for this determination. While freedom of interference is better in emission spectrography, because of the higher resolving power of the large spectrographs used, the sensitivities of detection are of the same order of magnitude. Although the atomic absorption method may be superior in rapidity and probably precision, we can mention the following points in favor of emission spectrography:

1. Solid samples can be analyzed directly after drying and grinding with graphite powder.

2. Solvent extraction is not needed for the range of cobalt concentration usually encountered in biological materials.

3. The spectrographic plate is a valuable and permanent record of analytical data, enabling a complete qualitative and quantitative check at any time. This fact is particularly important in legal cases.

In the authors' opinion, emission spectrography and atomic absorption must be used simultaneously and concurrently for the exact determination of traces of metals in biological materials. Their respective results for every metal should doublecheck each other.

In the spectrographic procedure, a "National" carbon cupped electrode, type L 3900, is used as the lower electrode, and a "National" L 3957 is used as the counter electrode. The charge weighed into the cavity is 20 mg. The distance between electrodes, or analytical gap, is 3 mm., which is further increased to 6 mm. during arcing. A d.c. arc, 14-amp., generated from N.S.L. Spec Power,[1] is used as the excitation source. The time of exposure is set to 13 sec., during which up to 3 mcg. of Co in 20 mg. dry residue is completely volatilized. Prearcing is not necessary. A Bausch & Lomb Large Littrow spectrograph is used for this assay, with a fixed slit of 20-μ width and 3-mm. height. The spectrographic plate is Kodak No. 1, size 4 × 10 in., which is developed and dried using a N.S.L. processor. It is measured with a N.S.L. reader (densitometer).

The characteristic curve of the plate is obtained by plotting a preliminary curve, using an iron arc spectrum taken with a two-step filter. This method of plate calibration seems to have a satisfactory precision, because random scatter of experimental points is compensated for in drawing the preliminary curve. The characteristic curve is practically unchanged for a period of laboratory work. Concentrations are plotted against intensities on ordinary graph paper.

[1] National Spectrographic Laboratories Inc., Cleveland, Ohio.

By taking three spectra from each powder, a precision of ±10% was attained.

A large number of vitamin B_{12}-containing preparations such as liver extract and liver injection were analyzed by this method. It was also used to follow separation or purification steps in manufacturing processes (fermentation, extraction, *etc.*). To indicate the precision obtained, we are giving here the results of a routine determination. The analysis of a vitamin B_{12} preparation claimed to contain 40 mcg. vitamin/ml. gave the following results: 1.7, 1.8, 2.0, 2.0, 1.6, and 2.0 mcg. Co/ml. in six different plates. The mean value, 1.85, multiplied by 100/4.35 = 23 gives 42 mcg. cyanocobalamin/ml.

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Synthesis of *O*-Alkyldihydroxyacetone and Derivatives

Keyphrases □ *O*-Alkyldihydroxyacetone, derivatives—synthesis □ IR spectrophotometry—structure □ GLC—identity

Sir:

Recently, Snyder and coworkers (1–4) proposed a biochemical pathway and identified intermediates that demonstrated the biosynthesis of *O*-alkyl ether bonds in glycerolipids from fatty alcohols and dihydroxyacetone-P in cell-free systems. This new metabolic scheme included *O*-alkyldihydroxyacetone and *O*-alkyldihydroxyacetone-P as intermediates. We have now further substantiated the previously reported biochemical reaction sequence (3) by the chemical synthesis of one of the intermediates, namely *O*-alkyldihydroxyacetone (I) (R = octadecyl or hexadecyl) (Scheme I). This preliminary communication describes the synthesis of I which can exist as a keto-enol tautomer; the properties of I are identical with those of the ^{14}C-*O*-alkyldihydroxy-