the accuracy of the foregoing and other procedures tested, however, we have made many analyses of silk, chosen because of its relative simplicity and the detail with which it has been studied by classical methods of analysis. The data in Table 1 illustrate the kind of accuracy furnished by these chromatographic procedures.

The results of columns 2 and 3 are single readings of representative chromatograms. For column 2 the acids were extracted from the paper after treatment with ninhydrin and measured colorimetrically; for column 3 the procedure was that described above as the spot-dilution technique. A comparison between columns 4 and 5 indicates the kind of agreement to be expected between duplicate runs. Previous analyses are reproduced in columns 6 and 7.

There are several significant differences between our results and the earlier ones. We agree with analysis (a) and disagree with analysis (b) in finding a high serine content and little, if any, phenylalanine. According to chromatographic analysis, there is less tyrosine and more alanine than was found by earlier methods. Valine was not previously identified; on the other hand, there has been no chromatographic evidence for methionine. The solvents used in this work were not appropriate for determinations of the other two acids, histidine and threonine, that have been reported.

The methods used and results obtained in this study will be described in detail elsewhere.

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Illuminator for Melting-Point Blocks

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Berl and Kullman (*2*) and Bergstrom (*1*) have described copper melting-point blocks which give more accurate results than the open beaker type of apparatus. However, the copper block is only as accurate as the amount of illumination supplied to its interior. Most investigators have used quartz rods to conduct the light to the interior. Although this gives a good source of light, the rods are expensive and subject to breakage. To overcome these disadvantages, the illuminator described below was constructed.

A piece of aluminum alloy tubing, $\frac{7}{16}$ inch in diameter, was bent to form a circle 10 inches in diameter. A piece of sheet aluminum alloy $\frac{1}{16}$ x $1\frac{1}{4}$ x 10 inches was bolted to this circle and trimmed so that the outside edge was 9 inches long and its center was $2\frac{7}{8}$ inches from the center of the block. Two cones were constructed from sheet copper. One end had a diameter of $\frac{15}{16}$ inch, and the other tapered to $\frac{5}{16}$ inch. Two brass light fixtures, each holding a 7-watt lamp, were mounted in these cones and then bolted to the aluminum ring. Wires from the lamps passed through the ring and out at the rear, where they were attached to a female plug (Fig. 1).

The illuminator was connected to the block by glass rods, $\frac{5}{16}$ inch in diameter, cut to fit the block used. The entire apparatus usually is mounted by clamping it to a ring stand. The addition of a magnifying glass or telescopic sight mounted

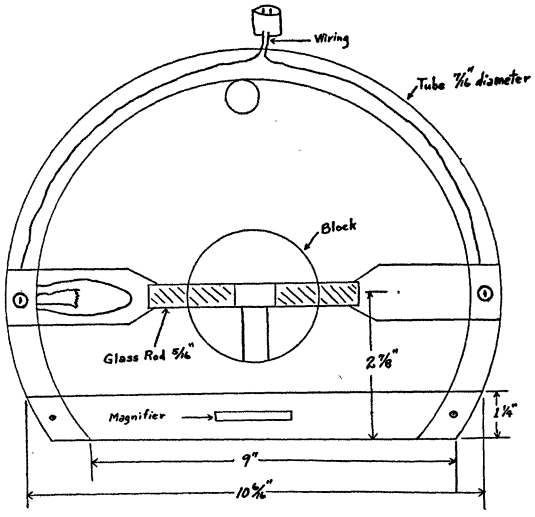

FIG. 1

in the center of the cross brace and sighted on the observation hole in the block completes the apparatus.

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Interference by ATP in the Estimation of Coenzyme I

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Chemical and physicochemical methods cannot be relied upon for the estimation of coenzyme I in tissue extracts; biochemical methods involving the use of enzyme systems are the most advantageous. Among these, the most widely used has been the fermentation method of von Euler and Myrbäck (*4*), where apozymase is used as the source of enzymes and the coenzyme is estimated by the volume of carbon dioxide liberated when excess sugar is fermented in the presence of inorganic phosphate and hexose diphosphate. In the method due to Warburg (*6*), partially purified enzymes, protein A and protein B, obtained from Lebedev's juice, are used, the other components of the system being coenzyme I, adenine nucleotide, Robison ester, acetaldehyde, phosphates and magnesium, manganese and ammonium ions. Jandorf, Klemperer, and Hastings (*2*) described a new method based on the catalysis by coenzyme I of the breakdown of hexose diphosphate in the presence of arsenate, a dialysed aqueous extract of cat muscle being used as the source of glycolytic enzymes. Haas (*1*) has suggested

[1] Government of India scholar. The author is grateful to J. B. Sumner for his interest in this investigation and to the Rockefeller Foundation for financial support.

that coenzyme I may be estimated spectrophotometrically by the reduction of 2,6-dichlorophenolindophenol by dihydrocoenzyme formed in the presence of suitable substrate and apodehydrogenase. Very recently (*3*, *5*) two other methods have been reported from this laboratory.[2] In one, use is made of lactic

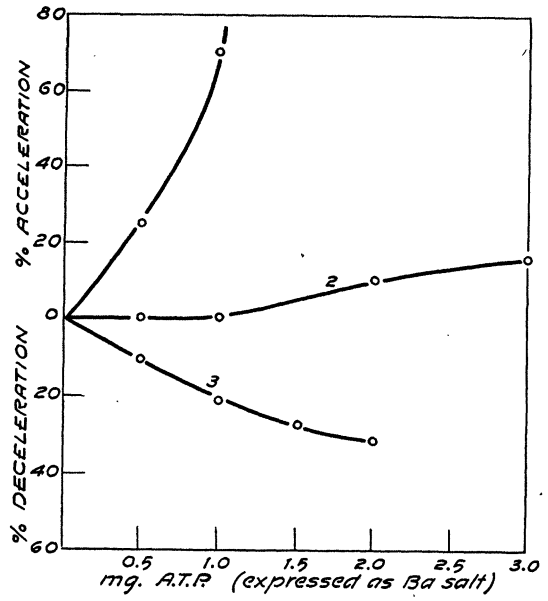

FIG. 1. Method of von Euler and Myrbäck, 1; method of Krishnan, 2; method of Jandorf, *et al.*, 3.

apodehydrogenase and diaphorase in the test system, and in the other, aetiozymase is used as the source of aldolase and triose phosphate dehydrogenase. While all these methods give satisfactory results with comparatively pure solutions of coenzyme I, no unequivocal proof has yet been furnished as to their applicability to tissue extracts, where interfering substances might be present.

The present report deals with one such possible interfering substance, namely, ATP, which is present in certain tissues in concentrations several fold that of coenzyme I. We have worked with three different test systems: the fermentation method of von Euler and Myrbäck, the manometric method of Jandorf, *et al.*, and the oxygen-absorption method of Krishnan (*3*). The concentrations of ATP, expressed in terms of the original dibarium salt, ranged from 0.1 to 3 mg./reaction flask, and that of coenzyme I, from 8 to 16 γ. The results obtained are represented in Fig. 1.

It is apparent that very small amounts of ATP (of the order of 0.1 mg.) have practically no effect on results given by the methods mentioned above. This is in agreement with the statement by Jandorf, *et al.*, who found that ATP is without influence on the course of the reaction developed by them. However, when the concentration is increased, it exerts a pronounced effect on the rate of the reaction. In the fermentation method of von Euler and Myrbäck (where readings were taken of the maximum rate of fermentation in a 10-minute interval, as recommended by Axelrod and Elvehjem) and in the oxygen-absorption method of Krishnan, the reaction rate is accelerated, whereas in the method of Jandorf, *et al.* the effect is of an inhibitory nature. It may also be noted that the interfering effect of ATP is least in the oxygen-absorption method. This is to be expected, since ATP is not known to influence the stages of glycolysis used in the test system. ATP exerts its maximum effect on the fermentation method utilizing apozymase[3]—an effect different from that of hexose diphosphate, which is believed to function merely by decreasing the induction period.

The conclusion has to be drawn, therefore, that in such tissue extracts and test systems, where there is a preponderance of ATP over coenzyme I, appreciable errors will be introduced in the estimation of the latter by the conventional biochemical methods.

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[2] Details of these methods will be published elsewhere.

[3] Apozymase was prepared by drying brewer's bottom yeast (obtained from Haberle Congress Brewing Company, Syracuse) for 24 hours at room temperature and washing 5 times with large volumes of distilled water. The specimen of ATP used in the above experiments was supplied by I. C. Gunsalus, to whom our grateful thanks are due.