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The Micro Determination of Citric Acid by the Thunberg Methylene Blue Method.

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This method[1] is based on the capacity of an enzyme found in cucumber seed to liberate hydrogen from citric acid for the reduction of methylene blue to the leuco compound. The speed of decolorization varies with the amount of citrate up to a certain concentration. Beyond this "critical" concentration there is no further decrease in decolorization time.

The least amount of citrate necessary for maximum speed of decolorization is influenced by the nature of the enzyme extract; this depends on the source of the cucumber seed, the amount of grinding and centrifuging in its preparation, the H ion concentration (during extraction and the reaction proper) and on the time the extract stands before use. Enzyme extracts less than 40 minutes old rapidly decolorize Me-blue even in the absence of citrate; with increasing age of the extract the decolorization time is greatly prolonged unless citrate is present. In practice enzyme extracts about 2½ hours old have proved most satisfactory in revealing the "critical" citrate concentration. Of 20 determinations made on standard freshly prepared citrate solutions 15 were within 6% of the average. The other 5 varied more widely perhaps because of slight irregularities in the preparation of the enzyme extract. Adams and Boothby[2] suggested extraction of the seed with water instead of with 0.87% K_2HPO_4 at pH 8.2 because water extracts contain less extraneous substances which have an accelerating effect like that of citrate. The advantage gained by this procedure is somewhat offset by the higher "critical" concentration of citrate necessary, and by the absence of buffer, other than that supplied by the seed.

The sensitivity of the method is directly proportional to the amount of methylene blue present in the reaction tube. Large amounts of methylene blue (0.3 cc. of 1:30,000 solution) permit accurate reading; small amounts (1:120,000) are difficult to read but allow the estimation of as small amounts of citrate as 0.2 mg. per 100 cc.

The modification of the method for the determination of citric

[1] Thunberg, T., *Biochem. Z.*, 1929, **206**, 109.

[2] Adams and Boothby, as reported at Montreal meeting, Am. Soc. Biol. Chem.

acid in the blood suggested by Benni, Schersten, and Östberg[3] gives low and unreliable results because it compares a tube containing a high concentration of Na and Ca with one in which no salts are present and without reference to the "critical" point. The presence of Na, Ca, or hexose di-phosphate alters the time required for decolorization but does not change the least amount of citrate necessary for maximum speed of decolorization. By increasing the sensitivity of the method through use of a smaller concentration of methylene blue, the effect of these substances is avoided.

Oxalic acid in concentrations 3 times that required to prevent coagulation increases the amount of citrate necessary for maximum speed of decolorization. Oxalates therefore can not be used as anticoagulants if plasma citrate is to be determined by this method.

Preliminary experiments on rabbits showed that the citric acid content of the serum dropped very markedly during a 3-day fast, and that the administration of $NaHCO_3$ to fasting rabbits increased the serum citrate.

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The Psychopathological Effect of Sodium Amytal.

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Isoamylethylbarbiturate was introduced into psychiatry by Lorenz and Bleckwenn in 1929. It was recommended to produce profound sleep which in certain neuropsychiatric patients was followed by peculiar changes in behavior. Lucid intervals with good contact and almost complete insight were produced in certain cases of catatonic dementia precox. Depressed or excited patients had periods of calmness and contentment. In some cases, improvement, in others a rapid recovery followed the repeated induction of sodium amytal narcosis.

We conducted experiments guided by the assumption that the striking changes following sodium amytal administration cannot be sufficiently explained on the basis of the narcosis produced. We studied the drug action, therefore, in very small doses not leading to any narcosis or sleep at all. In examining patients' responses as compared with the observations by the authors mentioned above we found that after the injection of less than half of the dose used by

[3] Benni, Schersten, and Östberg, *Biochem. Z.*, 1930, **223**, 443.