Fig. 1. Paper chromatogram of dimethyl leucine (1), dimethyl isoleucine (2), dimethyl phenylalanine (3), and a mixture of these acids (4). For details see text.

subsequent treatment with photographic developer reveals the dimethylamino acids, the basic and sulphur containing amino acids as black spots. The identification is simplified by the fact that a dimethylamino acid in butanol-acetic acid-water-systems travels in front of and not widely separated from the corresponding unsubstituted amino acid.

Finally we wish to point out the possibility of using the proposed method for detection of all kinds of substances capable of reacting with methyl iodide with formation of iodide ions (amines, organic sulphides *etc.*). The method has also been used by us to detect ninhydrin-negative impurities in commercial amino acid samples and contaminants obtained in eluates from ion exchange resins.

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The Use of ^{110}Ag in Quantitative Paper Chromatography of Sugars

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In connection with other investigations it was necessary to determine quantitatively very small amounts of paper-chromatographically separated reducing sugars. The method of Trevelyan *et al.* [1] was used, involving the precipitation in the spots of metallic Ag from $AgNO_3$-acetone solution. The present communication deals with some preliminary studies on the conditions under which reducing sugars can be determined quantitatively with the aid of ^{110}Ag incorporated in $AgNO_3$.

Methods: The sugars were separated in descending chromatograms on sheets of Whatman No. 4 or 54 filter paper, size 30 × 50 cm. The temperature was 20° C and the duration of the chromatography was 24 hours. Two solvents were used: (a) *n*-butanol, pyridine, and water in the ratio 3:2:1.5, and (b) ethyl acetate, glacial acetic acid, and water in the ratio 60:17:17.5[2].

Ag of high purity grade was activated in the Norwegian uranium reactor to a specific activity of about 1.5 mC ^{110}Ag/g Ag. The activated Ag was dissolved in pure nitric acid and the nitrate was prepared. It was then found suitable to dilute this nitrate ten times with inactive $AgNO_3$. The experiments included two aldoses: glucose and galactose, two ketoses: fructose and sorbose, and two disaccharides: maltose and lactose. These sugars were determined singly or in different combinations. It should be mentioned that glucose and fructose were only separated with solvent (b) and that glucose and galactose were not separated from the same solution with these solvents. Small volumes (3—10 μg in one ml) of the sugars to be tested were applied to the papers with an Agla micrometer syringe. In some cases larger quantities, up to 40 μg were used, the spots being allowed to dry between successive 1 μl loadings of the paper. The position of the separated spots were identified with the aid of standard solutions placed on narrow edge strips of the chromatographic paper. R_F values could not be used as the solvent front leaves the paper in about 9 hours and the R_F values in such a case are not sufficiently reproducible. Around the spots,

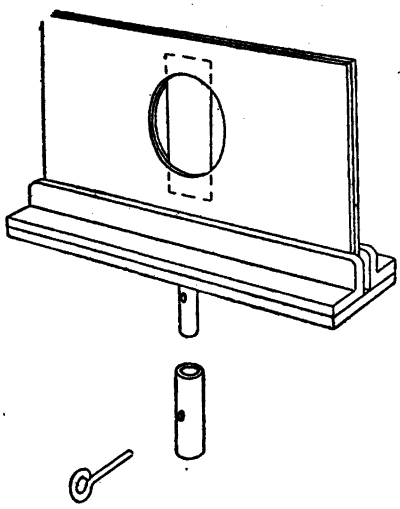

Fig. 1. Frame for developing the spots.

strips of identical sizes, 4 × 10 cm, were cut out. These strips were dipped in the $AgNO_3$-acetone solution and dried at 20° C. They were then sprayed with a 0.5 *N* solution of sodium hydroxide in aqueous ethanol. In order to attain reproducible conditions a special, simple turnable frame (Fig. 1) was designed. From the sprayer, the position of which was fixed, a well definied volume of NaOH solution could be uniformly distributed on the paper strip. At a given time ("development-time") following the spraying, the strip was immersed into 6 *N* NH_3. After washing in running water and air drying, the spots were cut out into squares of equal sizes, the radioactivity of which was determined. When the spots were large compared with the standard size chosen, they were measured in two parts. The background, which is due to the reduction ability of the paper and probably also to the starting hydrolysis of the cellulose, was determined from a square of equal size cut from the vicinity of the spot. The most reproducible values were obtained when the total β- and γ-radiations were determined, *i. e.* without shielding. A thin windowed (2 mg mica/cm²) Geiger counter-tube was used.

Results: The reduction values, expressed as counts per minute, are found to be rectilinear functions of the amount of sugar applied (Fig. 2a), whence it is possible to determine quantitatively the sugars studied in amounts down to about 3 μg and up to about 40 μg (the higher concentrations not included in the figure). Since there is no simple stoichiometric relationship between the amount of sugar applied and the reduction value (*cf.* the different and lower values for the two ketoses as compared to the aldoses), the determination has to be performed relative

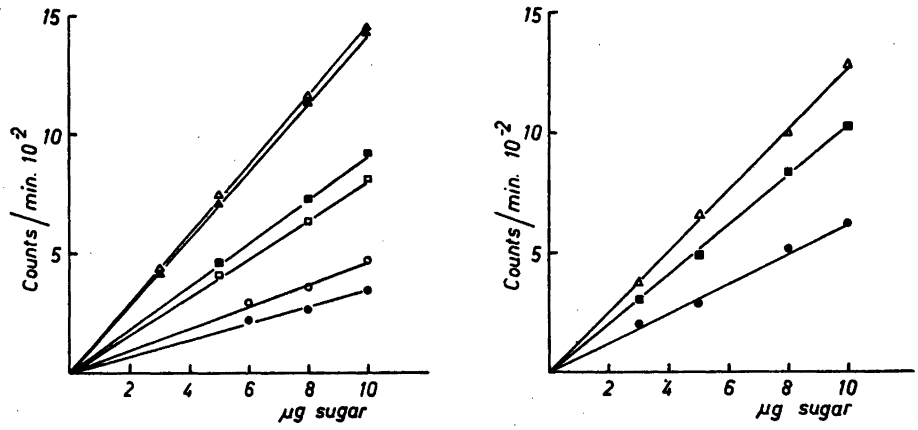

Fig. 2a and b. Reduction values (counts/min.) as a function of the amount of different sugars. △ *glucose,* ▲ *galactose,* ■ *fructose,* □ *sorbose,* ○ *lactose,* ● *maltose. 10 secs. in $AgNO_3$-acetone. The dots represent a mean value of at least six different determinations. Washed with 6 N NH_3. a. Development-time 5 minutes. Subtracted background 50 counts/min. b. Development-time 12 minutes. Subtracted background 200 counts/min.*

to an identically treated standard. It should be mentioned that the ketoses require a longer development-time than the aldoses and the disaccharides an even longer time. Experiments with a varied development-time has disclosed that a maximum reduction value is reached after 5 minutes in the case of the monoaldoses, after 10 minutes in the case of the ketoses, and after 10—12 minutes in the case of the disaccharides. However, even at the maximum value, the difference between aldoses and ketoses is retained. This fact is in agreement with the results of experiments using the method of Wallenfels *et al.*[3], where fructose and sorbose required treatment in steam for 1.5 minutes whereas a greater amount of aldoses could be developed in 10—20 seconds. The longer development-time required in the case of the ketoses can be explained on the basis of the chemical structure and mode of reaction. It is more difficult to explain why the disaccharides require such a long time for complete development. It could be expected that the mode of reaction of glucose and maltose is identical and that the reduction value per weight of the disaccharide is half that of the monosaccharide. This relation is obtained after 12 minutes development (Fig. 2b). With these extended development-times the background becomes correspondingly higher and the values therefore less reliable. — When the method of Wallenfels *et al.*[3] is applied, the spots of maltose and glucose are developed at equal rates.

A complicating factor is the experience that, in washing with 6 *N* NH_3, a part of the Ag is extracted. This is particularly evident in the case of tri- and oligosaccharides, where the washing produced a total decoloration of the spots. Since this extraction does not occur in 2 *N* NH_3 it seems most likely that the extraction is due not to the presence of Ag_2O but to the oxidation and dissolution of the finely dispersed Ag.

Conclusion: ^{110}Ag can be used for chromatographic determinations of reducing sugars. The isotope is useful for investigations of reaction conditions in chromatographic methods involving development with Ag. The fact that di- and monoaldoses give reduction values proportional to the number of reducing groups (Fig. 2b), indicate the possibility of estimating oligosaccharides even in cases where standards can be obtained only of the mono- and disaccharide of the series.

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Ruminant Bloat and Foaming Activity of Clover

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Many explanations have been proposed for ruminant bloat, in which thousands of cows die each year. As the bloating can be stopped, and the animal cured with anti-foaming agents as oil if given in good time, it is most likely that bloat is caused by the intense formation of foam in the rumen. Solid particles from the contents of the rumen gather into this foam, and the passage for gas escape becomes accordingly blocked. The great amounts of gas which are normally formed by fermentations in the rumen thus cause the bloat.

In order to find out if a great amount of substances with strong foaming activity are found in red clover which in Finland is the most dangerous cause of bloat during the latter part of summer, we made some determinations on the foam producing qualities of clover and timothy. The method used was the following.

Measurement of foam produced by shaking of crushed samples of plants in water. Parallel experiments with different amounts of water showed that a 5 % suspension of crushed fresh clover in water was the most satisfactory concentration for examination