

SCIENTIFIC PROCEEDINGS.

NEW YORK MEETING

College of the City of New York, April 15, 1925.

174 (2697)

Preventing glucolysis in blood samples.

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When blood samples are set aside at room temperature with no preservative, the sugar content as measured by the reduction of picric acid diminishes markedly. This is most pronounced on the first day, and continues, until in the course of two to four days the glucose may have entirely disappeared. Commercial glucose added to the sample is similarly destroyed. If the samples are not chilled or treated chemically, sugar determinations are of little value unless the analyses are made immediately. It is not always practicable to make determinations promptly, nor can the samples always be kept on ice in the interim. An inquiry was therefore made to find practical means of checking, or if possible, completely preventing glucolysis.

Blood preservatives have been recommended in various scientific journals¹ but these were found to be unsatisfactory, and a systematic investigation was therefore undertaken.

The work has been done on the blood of herbivorous animals with a large number of substances, and is now being continued

¹ Major, R. H., *J. Am. Med. Assn.*, 1923, lxxxix, 1952; Denis, W., and Beven, J. L., *J. Lab. Clin. Med.*, 1924, 9x, 674; Sander, F. V., *J. Biol. Chem.*, 1923, lviii, 1; Denis, W., and Aldrich, M., *J. Biol. Chem.*, 1924, xlv, 203.

on human specimens. The fluorides recommended by other workers¹ were first tested. These inhibit glucolysis, and in most human specimens keep the blood sugar nearly constant for several days, but can not be relied on for all specimens over the 10 days required as a minimum by the Prudential Laboratory. In addition to the fluorides a large number of substances varying widely in their properties have been tried. Eventually the work has narrowed down to halogen derivatives of hydro-carbons. It was found that trichlor-ethelene mixed with NaF is fully as effective as thymol and NaF. Neither $\text{CCl}_2=\text{CHCl}$ nor NaF alone are of any value. Trichlor-methane and tetra-chlor-methane were soon discarded as worthless for our purposes. Chlor-benzol and brom-benzol keep the sugar content of blood samples near its initial value for days, and in combination with NaF have so far proven the best.

Increasing the halogen on the benzene ring diminished its anti-glucolytic properties. Between chlor and brom derivatives of benzol no difference could be noted, but iodo-derivatives proved less useful as preservatives.

Introducing aliphatic side chains into the ring gave less effective preservation.

In case of the sheep's blood which has been so far mostly used in these studies, no combination has yet been found which will prevent a fluctuation of 0.01 per cent glucose in the samples during the first three days. These fluctuations are either a drop the first day with a rise on the second day, or, less frequently, a rise followed by a decrease in sugar. After the fourth day there is very little change when a halogen benzene compound is present in the blood sample, but the non-halogen preservatives allow the amount of sugar to drop very abruptly on the third day, becoming a mere trace on the fourth or fifth day. In a large number of cases where the halogen-derivative fluoride combination has been used, the glucose at the end of 15 to 20 days is within 0.015 per cent of the initial value. Two samples were kept for 72 days with a final discrepancy of less than 0.015 per cent, whereas the same blood treated with NaF and thymol lost half its glucose in three days, and all of it in six days.