Contribution of the Major Constituents to the Refractive Index of Milk

RECENT investigations[1,2] have shown the possibility of measuring the refraction in milk and the variations of the value under routine conditions of management.

A comparative examination of the refractive indices of more than 70 bi-weekly samples of milk and its rennet serum, prepared with one drop of Hansen's liquid rennet in 25 c.c. of milk, gave the following limits of variation of the constant. (In this proportion rennet caused no detectable addition to the refractive index of milk, as judged immediately after addition of rennet, and before coagulation set in.)

REFRACTIVE INDEX OF MILK AND MILK SERUM

Sample	Cow (R.I. 20° C.)	Buffalo (R.I. 20° C.)
Milk	1·3474–1·3506	1·3484–1·3534
Milk serum	433–447	436–453

These figures show not only that the elimination of casein reduces considerably the refractive index (fat having been shown to contribute nothing to this value[1]), but also that the variability of the constant of the serum is less than that of milk. Analysis of the entire data also showed that the refractive index of the serum does not always follow that of milk. This fact, and the lack of an exact linear relation between the refractive index and the solids-not-fat of milk[2], is perhaps caused by the differences in mutual proportions of the several constituents of milk. In other words, although two samples of milk (or of milk serum) may have the same solids-not-fat, the difference in the ratio of protein : lactose : minor constituents in the samples is responsible for the difference in refractive index.

Refractive index being a cumulative property, the presence of each solute adds to the refractive index of the water in milk. It has not been possible, so far, to determine the share of the several constituents in the total refraction, since there was no method of estimating the refractive index of milk. The method[1] recently devised enables such an estimation to be made.

A process of successive elimination of the major constituents and determination of the refractive index of the resulting fluids was used in determining the share of each. Casein (together with fat) was first eliminated with a drop of rennet. The lactalbumin and globulin in the rennet serum[3,4] was then removed by ultrafiltration with 'Cellophane' under a pressure of 20 kgm./cm.^{2}. The refractive index and composition (including lactose[5]) of the milk sample and filtrates were also estimated. The excess of the value of the refractive index of the ultrafiltrate over that of a solution containing the per cent lactose in the sample determined the share of the minor constituents (mineral salts, non-protein nitrogen, citric acid, etc.). From a number of samples of cow milk so examined the following conclusions have been drawn.

In the refractive index of milk, proteins contribute, next to water, the largest fraction, lactose ranks next and minor constituents the least. But *in proportion to their percentages*, water, proteins, minor constituents and sugar contribute in descending order. Among the proteins, considering their proportion, the lactalbumin and globulin contribute much more than casein. These experiments consistently gave a refraction of 0·0021 gm. casein in 100 c.c. of milk. This is slightly higher than the values given by Robertson[6] (0·00152) and Brereton and Sharp[7] (0·00181) for a 1 per cent solution of casein in 0·1 *N* sodium hydroxide. The higher value in the present series of experiments is perhaps to be attributed to difference in dispersion and composition in milk and in the sodium hydroxide solution.

The data also show that milk sugar contributes the most constant value to the refractive index, and confirms the inference from the table above that the wide changes in the refractive index of milk are accounted for largely by the variations in the protein content of milk.

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Determination of Benzene Hexachloride in the Blood of Cattle

DE MEILLON[1] reported that various arthropods are killed when fed upon rabbits which had received 'Gammexane' by ingestion. Similarly, Dr. S. G. Wilson, of the Veterinary Research Laboratories, Entebbe, Uganda, has found that the blood of cattle which had received benzene hexachloride by oral ingestion was toxic to tsetse flies for a considerable time after treatment[2]. Although biological tests thus showed that the insecticide was circulating in the blood, it seemed useful to apply the normal methods for the analysis of benzene hexachloride to estimation of the concentration in the blood of treated animals.

The simplest estimation of this insecticide depends upon the removal of three atoms of chlorine from each molecule of any of the benzene hexachloride isomers by refluxing with ethanolic potassium hydroxide and afterwards titrating the chloride ions present in the solution.

Outline of the method : 10 ml. of fresh oxalated blood were ground with anhydrous sodium sulphate and extracted with benzene. After evaporation of the benzene, chloride formed by dehydrohalogenation of the residue was determined by one of two methods. Method 1 is the Volhard procedure as used by Neal *et al.*[3]. This was found to be too insensitive for the quantities normally recovered from blood, and Method 2 was substituted, in which the silver chloride was filtered off before titration with potassium thiocyanate solution. Recoveries of 'Gammexane' (the gamma isomer of benzene hexachloride), added to blood from untreated animals, were satisfactory, and the error does not appear to be greater than 2–3 per cent in the range of concentrations which have so far been found in cattle bloods. Details of the method will be published later.

Some results are given in Table 1.

One experiment has been performed in which a calf was sprayed with the insecticide in oil solution (an extract of crude benzene hexachloride with a mixture of equal parts by volume of power kerosene and cotton seed oil).