A NOTE ON PAPER ELECTROPHORESIS OF MILK PROTEINS

B. C. Ray Sarkar, L. A. Ramanathan and C. Rajsekar

INTRODUCTION

The various limitations of the paper electrophoresis technique in the fractionation of serum proteins have been studied [1, 2, 3]. Usually not more than four to five bands could be apparent, whereas these conventional bands have been separated into twentytwo zones by starch gel electrophoresis [4]. As the paper technique is much simpler, it is of interest to see how this works in the case of other biological materials. The study reported in this paper relates to fractionation of milk proteins. Instead of attempting at this stage to isolate each fraction for quantitative estimation, only a gross picture as examined so far will be presented.

EXPERIMENTAL

Electrophoresis was carried out according to the hanging strip technique as standardized in this laboratory. The period of run was 17 hours under 100 to 110 volts. Five centimetre wide strips of Whatman filter paper No. 1 were used and the quantity of fluid milk, either whole or separated, delivered to a strip was 0·008 ml. to 0·016 ml. The method of taining was the same as followed in the case of serum and the dye used was bromophenol blue.

RESULTS

Electrophoretic patterns of milk using veronal and veronal plus Tris as buffers are presented in Figs. 1 and 2 respectively. The phosphate buffer (PH 7·8) proved relatively much less satisfactory and hence was discarded.

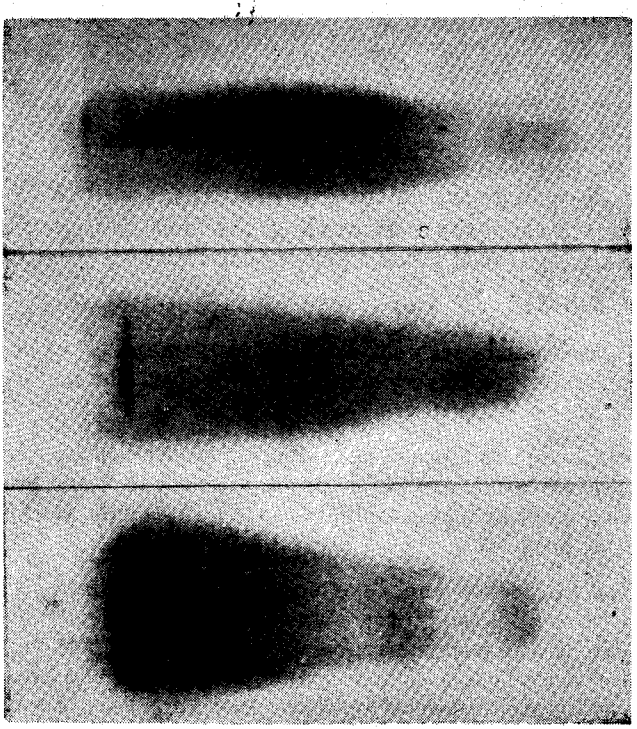

Fig. 1. Electrophoretic patterns of milk using veronal buffer.

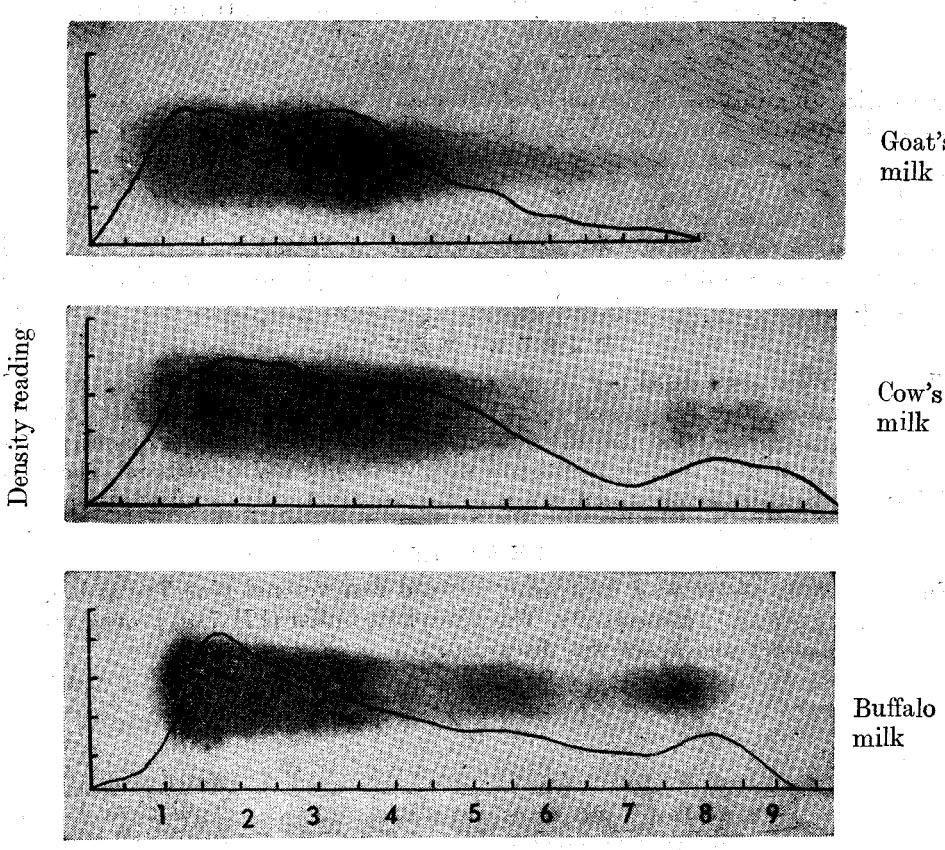

Fig. 2. Electrophoretic patterns and the corresponding densitometric curves for milk using 0·05M veronal plus 0·05M Tris.

1. *Using Veronal (0·025 M—0·075M, pH 8·6)*

Buffalo milk—Generally three bands are very apparent. In some cases, a fourth one faster than the rest appears on staining as a very small and faint spot. The major portion of the proteins is in the first one.

Cow's milk—In most cases, separation into bands is not apparent, rather it represents a continuous picture. However, two bands are visible in some cases, one being broad and elongated and the other small but showing higher mobility. As in buffalo milk, the first band contains major portion of the proteins.

Mixed milk—For examination, four different compositions were obtained by mixing buffalo milk with cow's milk in the following proportions—9 : 1, 3 : 1, 1 : 1, and 1 : 3. Two bands are generally apparent, the faster one appears to correspond with the fourth one in buffalo milk. The slower one, which is the major zone, is elongated and broad. This represents presumably an overlapping of several proteins. In other words, a continuous band in the case of any milk sample may mean an overlapping either due to trailing or that each fraction moves very close to the other in front of it leaving no distinct gap.

2. Using a mixed buffer (0·05 M veronal and 0·05 M Tris-trishydroxymethyl aminomethane)

Buffalo milk—Four distinct zones are apparent with no faint spot, corresponding to the fastest one, as perceived earlier using veronal buffer only. The gaps appear to be not absolutely free from staining. If these gaps are then presumed to represent some proteins there would in all be seven zones.

Cow's milk—Two distinct zones are apparent with more or less a clear but small gap between them. The slower band is an elongated one as usual.

Goat's milk—The band is continuous without any separation into zones, but an intense spot slightly apart from the line of application is obvious. This picture is apparently different from what has been obtained in the case of either cow or buffalo milk.

DISCUSSION

The addition of Tris helps more rapid migration of the proteins with less lateral diffusion and the separation into zones becomes more distinct. The limited observations made so far point to an apparent species difference in milk protein patterns. There are indications that cow-milk proteins have been separated into seven zones by paper electrophoresis using barbital buffer of *pH* 7·9 [5] and into eight fractions by moving boundary method [6] which are α—casein (45-63 per cent, relative mobility—6·7), β—case in (19-28 per cent, relative mobility—3·1), γ—casein (3—7 per cent, relative mobility—2·0), β—lactoglobulin (7—12 per cent, relative mobility—5·1), α—lactalbumin (2—5 per cent, relative mobility—4·2), "blood" serum albumin (0·7—1·3 per cent, relative mobility—6·7), euglobulin (0·8—1·7 per cent, relative mobility—1·8) and pseudoglobulin (0·6—1·4 per cent, relative mobility—2·0 to —2·2). However, in these studies the procedures of treating the samples before electrophoresis were different and elaborate. The continuous band observed after using fluid milk in the present work represents therefore incomplete fractionation. Though the limitations of the technique as followed here are evident, subsequent study to improve its resolution power for the purpose of identification, together with the quantitative assay of the various fractions may reveal more interesting features.

Acknowledgement—The authors are thankful to the Director of Defence Science Laboratory for his kind permission to publish this work.

REFERENCES

[1] Ray Sarkar, B.C. and Murthy, M.S., *Ind. J. Physiol & Allied Sci.*, **12**, 126 (1958).

[2] Murthy, M. S. and Ray Sarkar, B.C., *Ind. J. Physiol and Allied Sci.* **13**, 11 (1959).

[3] Ray Sarkar, B. C., *Sci & Industr. Res.*, **20C**, 239 (1961).

[4] Poulik, M. D. and Smithies, O., *Biochem. J.*, **68**, 636 (1958).

[5] Schulte, K.E. and Muller, E.F., *Milchwissensch*, **10**, 90 (1955).

[6] Jenness, R., Larson, B.L., McMeekin, T.L., Swanson, A.M., Whitnah, C.H., and Whitney, R. Mcl., *J. Dairy Sci.*, **39**, 536 (1956).