

CONTRIBUTIONS TO THE STUDY OF BLOOD CONSTITUENTS IN DOMESTIC ANIMALS IN SOUTH AFRICA.
6. NORMAL VALUES FOR SERUM PROTEIN FRACTIONS IN SHEEP AS OBTAINED BY ELECTROPHORESIS ON CELLULOSE ACETATE STRIPS

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Studies on the chemical pathology of various diseases of sheep in this department have made it essential that a set of normal figures for various determinations be compiled for comparative and diagnostic purposes.

The results of a statistical analysis of data relating to the serum protein fractions, as determined by electrophoresis on cellulose acetate strips, are presented in this paper.

MATERIALS AND METHODS

A total of 396 normal Merino sheep was used in these studies. They were divided into groups as follows: 82 rams and wethers 2 to 5 $\frac{3}{4}$ years of age, 125 ewes 2 to 7 years old, 141 lambs of 6 to 13 months and 48 lambs 4 to 6 months of age.

Since statistical differences have been shown to exist between certain blood or plasma constituents of sheep born and raised in the Karoo and those of sheep raised elsewhere (Wagner, 1964; Wagner & Brown, 1966 a, b.) it is of interest to note the place of origin of the animals used in this work.

Eighty-one of the ewes emanated from the Eastern Cape Midlands, twenty-one from Griqualand West, ten from the Karoo, one from the Eastern Cape Province, eleven from the Transvaal and one from the Orange Free State. Forty-one of the rams and wethers emanated from the Eastern Cape Midlands, twenty from the Karoo and twenty-one from various parts of the Transvaal.

The 6 to 13 month old lambs were taken mainly from the Eastern Cape Midlands (117) and 24 were born and raised in the Transvaal. This whole group of lambs consisted of 68 ewes and 73 rams and wethers. For the purpose of this study the

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results from both sexes were combined. A separate group of lambs aged 4 to 6 months were all born at Onderstepoort. Values for serum protein fractions obtained from their blood were used for comparative purposes only.

The numbers involved in this study are insufficient for a statistical survey of any differences which might exist between these various groups of animals. They have therefore been combined into the various age groups mentioned above. The statistical results presented in this paper are therefore representative of sheep in general in South Africa and not of any particular population group. Such data may be used for clinical laboratory studies on patients drawn from the general run of sheep flocks encountered in practice since the precise place of origin of many of these animals is often not known as a result of the very extensive traffic of sheep in this country.

The animals received a daily ration of $\frac{1}{2}$ to 1 lb of a concentrate mixture consisting of: 71 lb yellow maize meal, 20 lb lucerne meal, 5 lb blood meal, 2 lb bone meal, 1 lb salt and 1 lb urea. In addition each sheep received approximately 1 lb teff hay each day.

The blood was collected after jugular venupuncture directly into centrifuge tubes, allowed to stand for twenty minutes and then centrifuged. All analyses were done on serum thus obtained as soon as possible after bleeding.

The total serum protein figure for each sample was determined by the biuret method of Weichselbaum (1946).

The different serum protein fractions were determined electrophoretically using the Beckman Microzone apparatus and cellulose acetate membranes according to procedures laid down by the manufacturer. Subsequent scanning of the stained membrane was done on the Beckman model RB Analytrol Densitometer (Beckman instruction manuals, 1965).

RESULTS

The data were compiled and processed as described previously (Wagner, 1964; Sion, 1966).

The cumulative relative frequency curves and histograms constructed from the results obtained are presented at the end of this paper. The conclusions drawn are shown in Table 1.

TABLE 1.—Serum protein ranges

Fraction	Figures shown by median (50 per cent) (gm per cent)						Ranges shown per group (gm per cent)					
	80 per cent		10 per cent Lower		10 per cent Upper		80 per cent		10 per cent Lower		10 per cent Upper	
	R & W	E	L	R & W	E	L	R & W	E	L	R & W	E	L
n =	82	125	141	82	125	141	82	125	141	82	125	141
Total Protein.....	7.45	7.60	7.25	6.87-8.15	6.85-8.23	6.57-7.90	6.51-6.86	6.51-6.84	6.0-6.56	8.16-9.00	8.24-9.00	7.91-9.00
Albumin.....	4.45	4.05	4.28	4.0-4.91	3.66-4.51	3.74-4.80	3.0-4.75	3.1-4.33	3.10-3.73	4.92-5.70	4.52-5.30	4.81-5.50
α_1 -Globulin.....	0.26	0.34	0.28	0.16-0.35	0.21-0.51	0.15-0.42	0.1-0.15	0.1-0.20	0.10-0.14	0.36-0.60	0.52-0.70	0.43-0.55
α_2 -Globulin.....	0.64	0.72	0.74	0.52-0.80	0.58-0.87	0.59-0.90	0.41-0.51	0.51-0.57	0.51-0.58	0.81-1.10	0.88-1.30	0.91-1.10
β -Globulin.....	0.41	0.46	0.45	0.27-0.57	0.36-0.61	0.28-0.67	0.21-0.26	0.21-0.35	0.21-0.27	0.58-0.70	0.62-0.80	0.68-1.10
Main γ -Fraction.....	1.40	1.52	1.16	1.02-1.82	1.04-1.96	0.78-1.71	0.71-1.01	0.71-1.03	0.51-0.77	1.83-2.50	1.97-2.90	1.72-2.30
Trailing γ -Fraction.....	0.16	0.29	0.13	0.05-0.52	0.11-0.56	0.05-0.34	0.01-0.04	0.01-0.10	0.01-0.04	0.53-0.90	0.57-1.00	0.35-0.70
A/G Ratio.....	1.30	1.04	1.30	1.02-1.76	0.82-1.36	0.94-1.86	0.71-1.01	0.71-1.35	0.81-0.93	1.77-2.50	1.37-2.30	1.87-2.60

R & W = Rams and Wethers

E = Ewes

L = Lambs

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PLATE 1.—Comparison of ovine (top) and human electrophoretograms

A set of typical electrophoretograms of ovine serum proteins together with a similar set of preparations from human sera are shown in Plate 1. Differences in the rate of migration and density of the β -globulin bands are apparent between the two sets of electrophoretograms. The greatest differences are to be observed in the γ -globulin fractions. Sheep sera yield a broad dense band on the leading edge of this fraction. The corresponding band in human sera is much narrower and far less distinct. This has been designated the "main γ -fraction" for the purposes of this study. This is followed in both sets of sera by a diffuse trailing band which on very close inspection appears to contain one or sometimes two ill-defined protein bands. In many sheep sera this fraction was found to contain a dense band located at the end of the ill-defined area. We have, for the purposes of this discussion, designated this diffuse trailing band the " γ -trailing fraction". Both main and trailing fractions have been grouped together for the purpose of calculating the albumin-globulin ratio.

DISCUSSION

The histograms (Fig. 1a to 8c) show reasonably normal to moderately skewed types of distribution curves except in the cases of the γ -trailing fraction and A/G ratio where the distributions are markedly skew. In view of these facts all the data were analysed by means of the methods described earlier (Wagner, 1964; Sion, 1966).

It is apparent from Table 1 and the cumulative relative frequency curves (Fig. 1 to 8) that sex differences exist between the groups of animals, as can be judged from the figures for the median values. In the case of the globulins these figures are higher for ewes than for rams and wethers and in the case of albumin the reverse is apparent. As can therefore be expected from these data there is a sex difference in the A/G ratio.

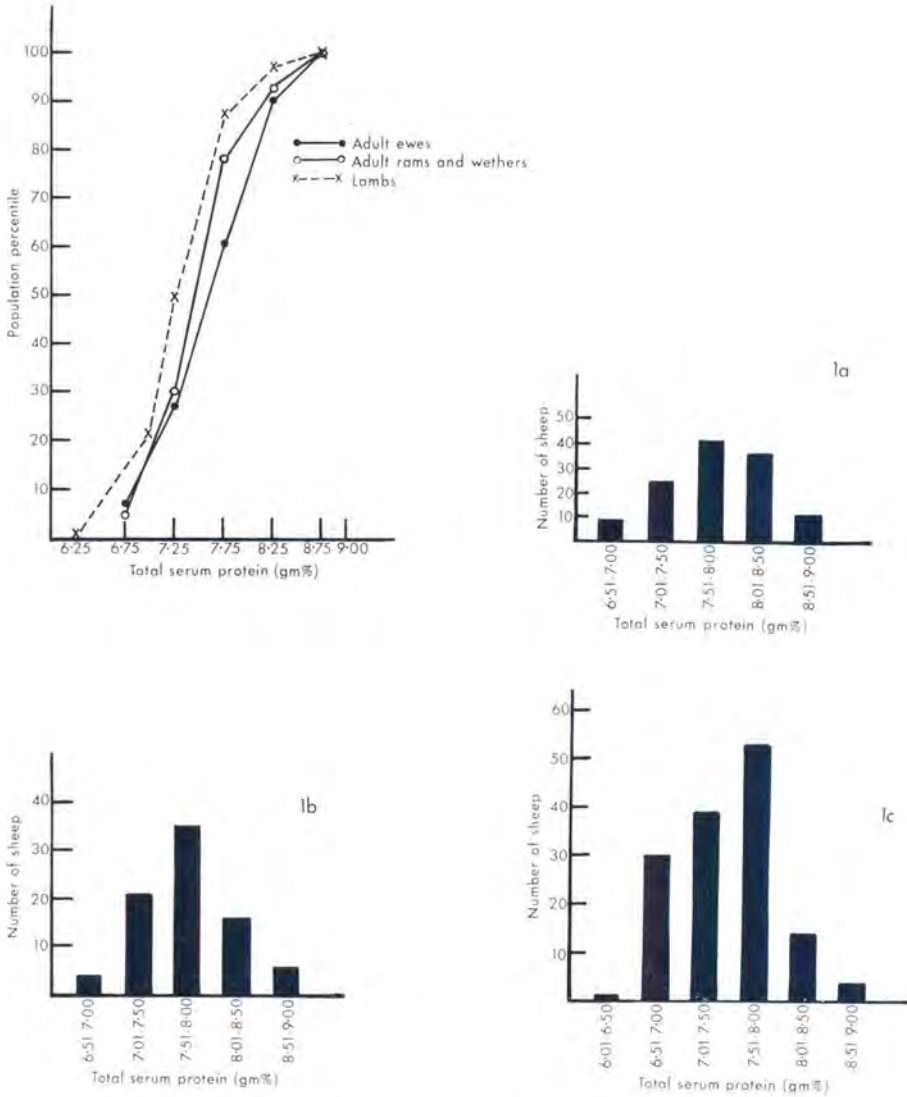


FIG. 1.—Total serum protein. 1 (a) Total serum protein in ewes. 1 (b) Total serum protein in rams and wethers. 1 (c) Total serum protein in lambs

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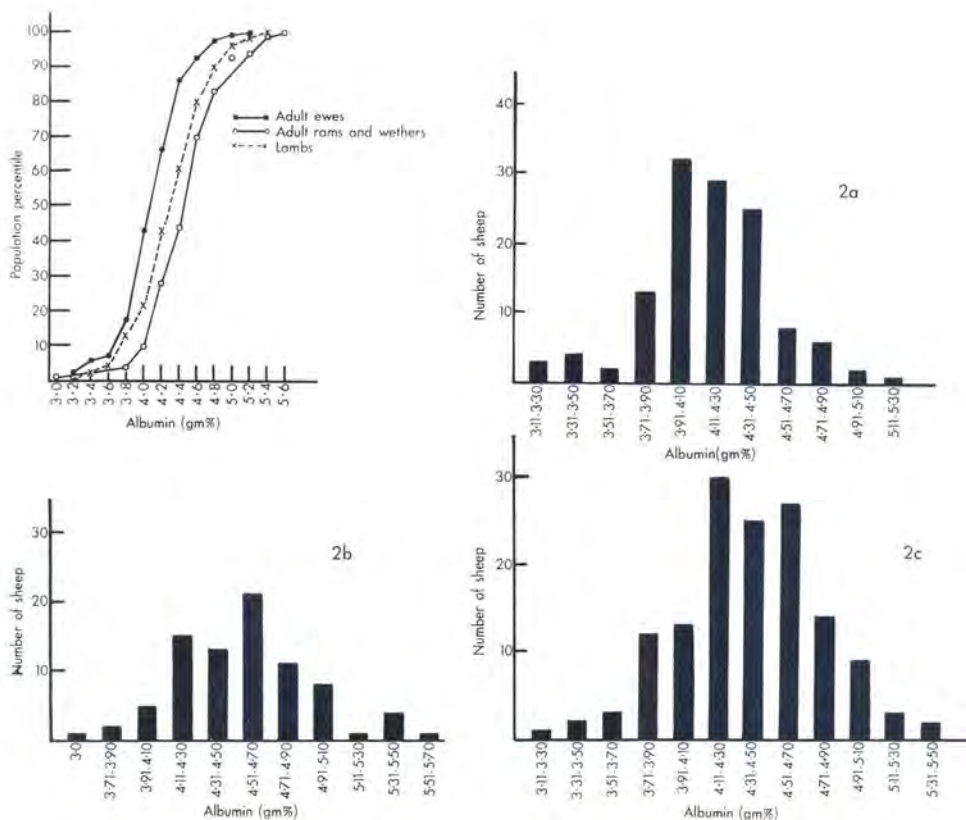


FIG. 2.—Serum albumin fraction. 2 (a) Serum albumin fraction in ewes. 2 (b) Serum albumin fraction in rams and wethers. 2 (c) Serum albumin fraction in lambs

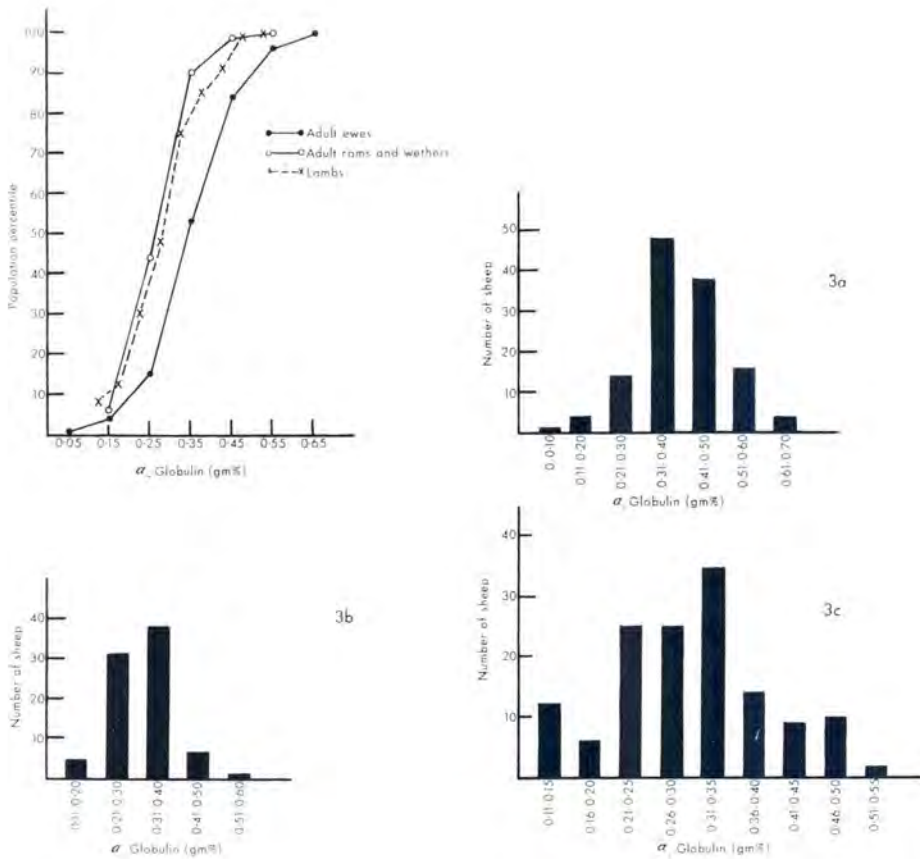


FIG. 3.—Serum α_1 -globulin fraction. 3 (a) Serum α_1 -globulin fraction in ewes. 3 (b) Serum α_1 -globulin fraction in rams and wethers. 3 (c) Serum α_1 -globulin fraction in lambs

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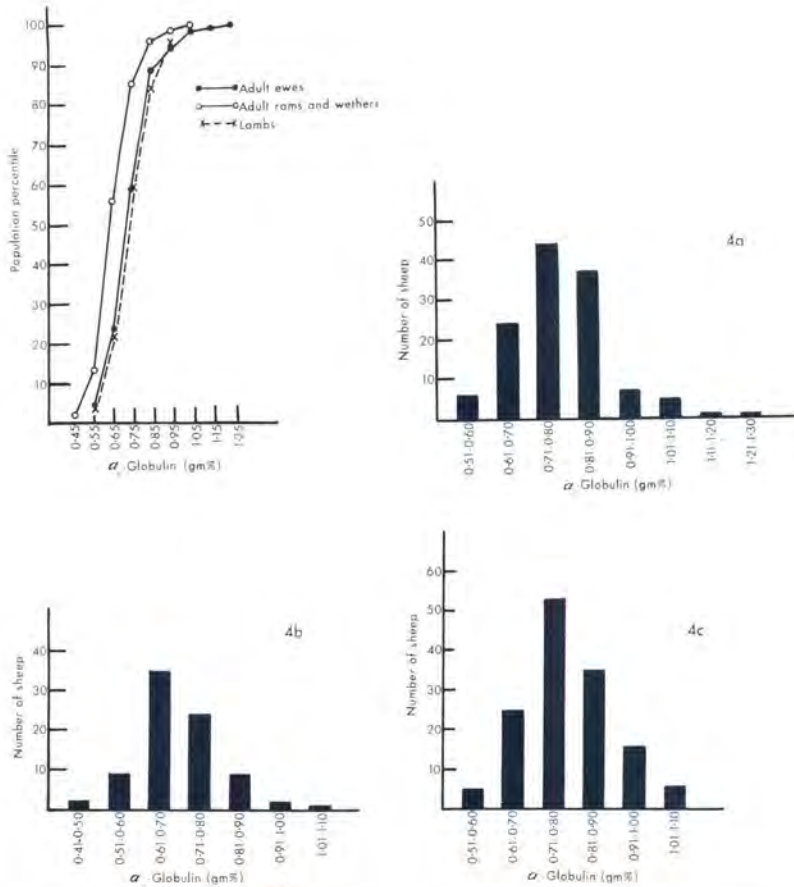


FIG. 4.—Serum α_2 -globulin fraction. 4 (a) Serum α_2 -globulin fraction in ewes. 4 (b) Serum α_2 -globulin fraction in rams and wethers. 4 (c) Serum α_2 -globulin fraction in lambs

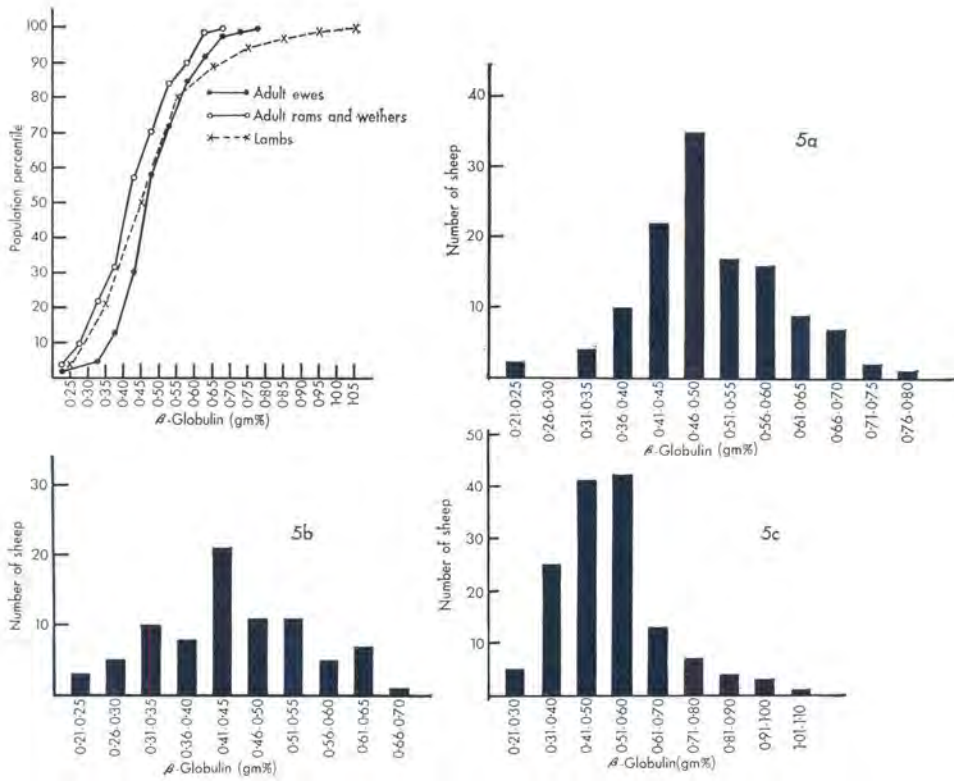


FIG. 5.—Serum β -globulin fraction. 5 (a) Serum β -globulin fraction in ewes. 5 (b) Serum β -globulin fraction in rams and wethers. 5 (c) Serum β -globulin fraction in lambs

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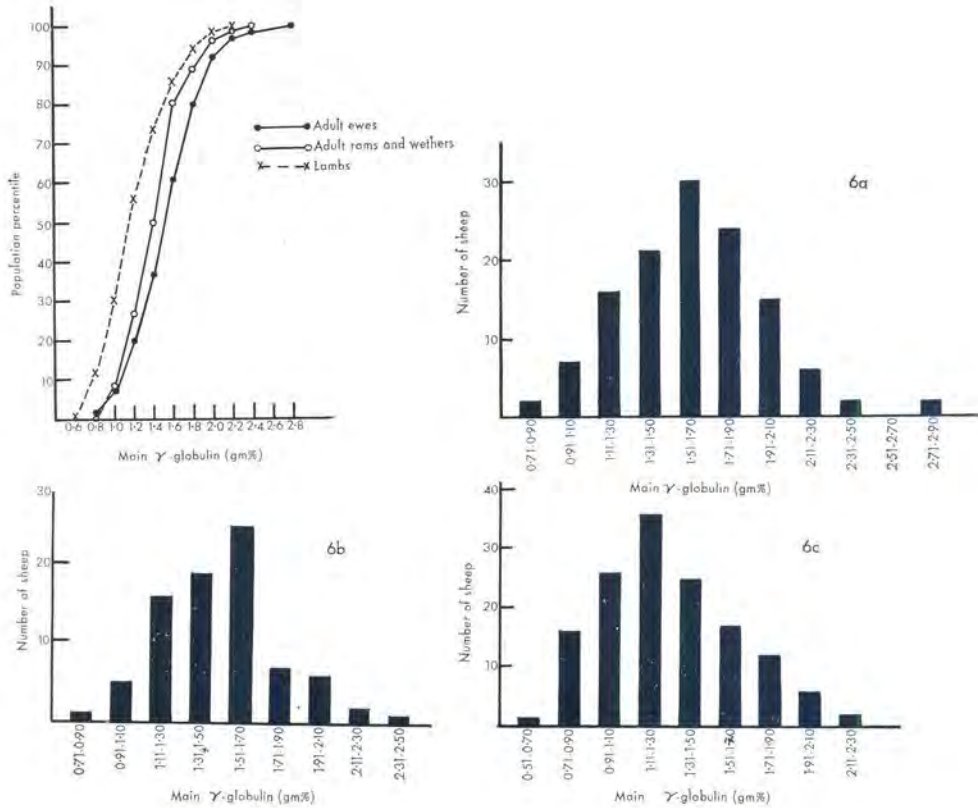


FIG. 6.—Main serum γ -globulin fraction. 6 (a) Main γ -globulin fraction in serum of ewes. 6 (b) Main γ -globulin fraction in serum of rams and wethers. 6 (c) Main γ -globulin fraction in serum of lambs

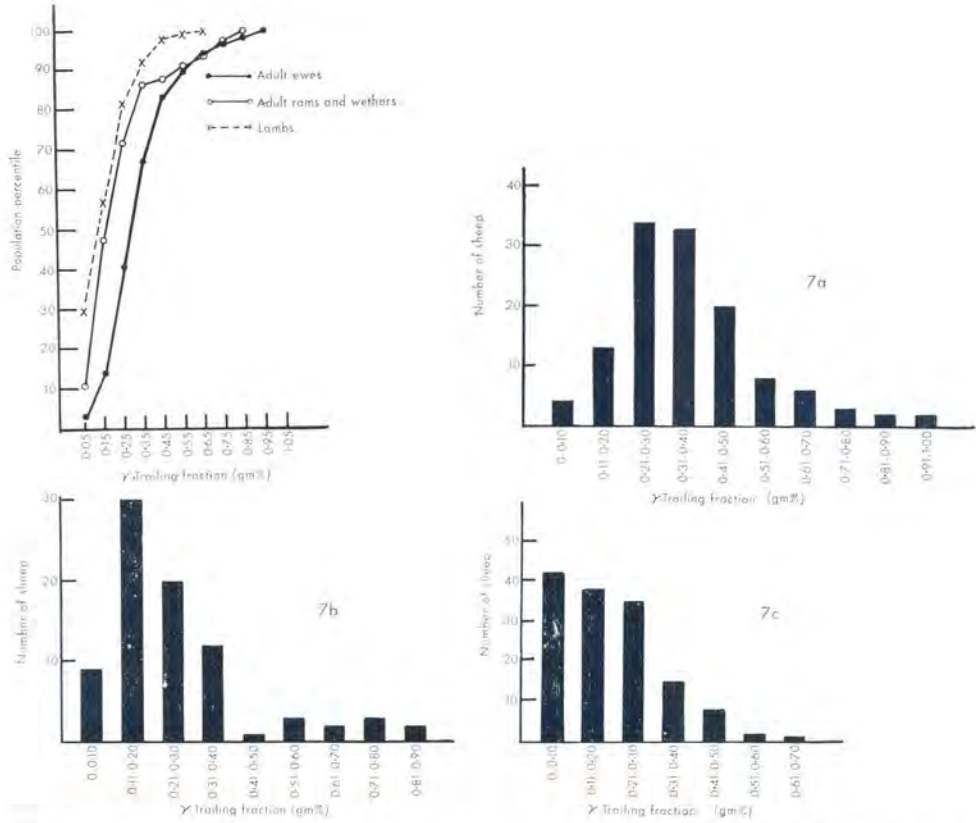


FIG. 7.—Serum γ -trailing fraction. 7 (a) Serum γ -trailing fraction in ewes. 7 (b) Serum γ -trailing fraction in rams and wethers. 7 (c) Serum γ -trailing fraction in lambs

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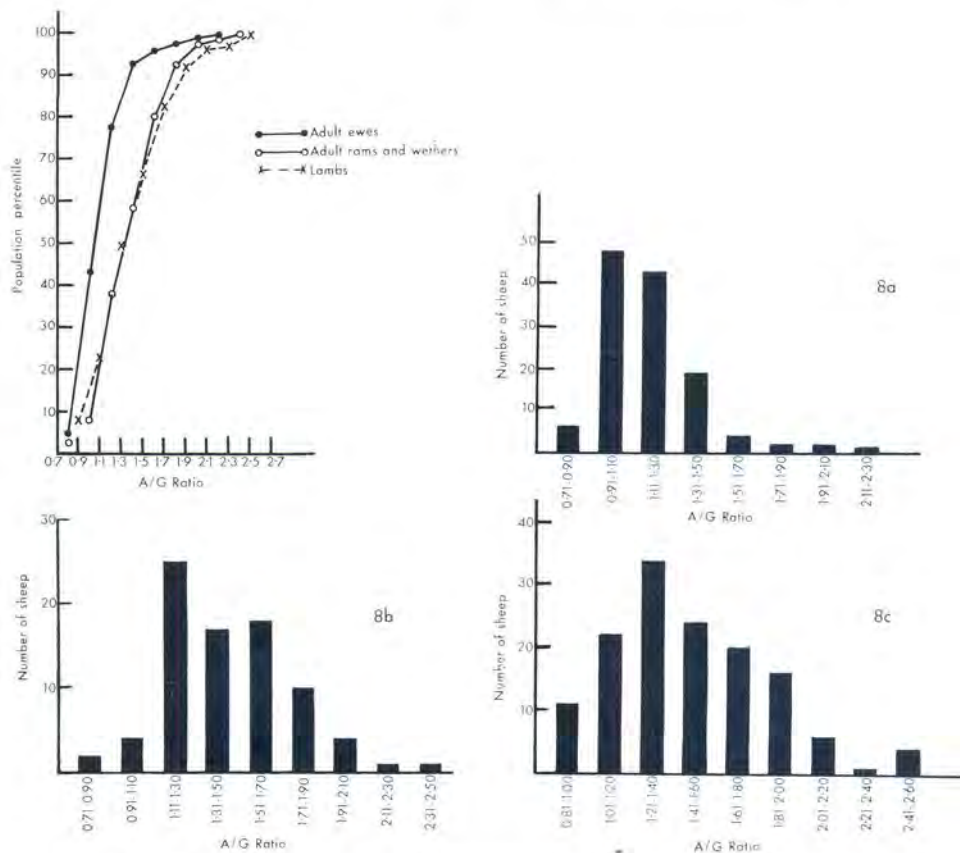


FIG. 8.—Serum A/G ratio. 8 (a) Serum A/G ratio in ewes. 8 (b) Serum A/G ratio in rams and wethers. 8 (c) Serum A/G ratio in lambs.

The 6 to 13 month old lambs are interesting in that the medians for the various frequency curves generally occupy a position between those of the two sexes of adult animals or may be higher or lower than the other figures. This is no doubt due to the mixed nature of the lamb population.

For practical purposes the 80 per cent limits show little difference between the three groups of animals. In general this holds true for the upper- and lower 10 per cent limits as well.

Owing to the small number of 4 to 6 month old lambs they have not been included in this statistical survey (48 as opposed to 141 in the older group). In general, however, the following differences are apparent from the limited number of figures available: lower total serum protein (median 6.92), lower albumin (median 4.02) and slightly higher main γ -fraction (median 1.25) than the older group of animals.

Electrophoresis on cellulose acetate membranes as performed by the "microzone" technique yields very sharp bands for the various protein fractions and in addition resolves the globulin fractions far better than filter-paper strip or cellulose acetate strip electrophoresis as done in the conventional type of bath (Van Zyl, 1966). This has enabled us to present a more precise calculation of the albumin: globulin ratio in the serum of apparently normal sheep than has been possible before. (See for instance the work of Horak & Clark, 1963, where some of the difficulties inherent in the older methods are mentioned and where the A: G ratio is given as 0.76 ± 0.1 for typical South African Merinos using such methods.)

SUMMARY

Normal values for the various serum protein fractions of Merino sheep in South Africa have been established. Use was made of cellulose acetate membrane electrophoresis by the microzone technique and after processing the data these were plotted on cumulative relative frequency curves. Some sex differences in the values for albumin and globulins are apparent. The albumin fraction was found to be higher in rams and wethers than in ewes. Age differences are also apparent particularly with respect to the values for total serum protein and albumin which are lower in lambs and young animals than in adult sheep. The precision of the microzone technique has permitted a re-appraisal of the albumin: globulin ratio in ovine blood.

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REFERENCES

- BECKMAN INSTRUCTION MANUAL RIM-5, Nov. 1957. Model RB Analytrol Densitometer.
- BECKMAN INSTRUCTION MANUAL RM-IM-3, Aug. 1965. Model R-101 Microzone electrophoresis cell.
- HORAK, I. G. & CLARK, R., 1963. Studies on paramphistomiasis. V. The pathological physiology of the acute disease in sheep. *Onderstepoort J. vet. Res.*, 30, 145-160.
- SION, J. J. G., 1966. Contributions to the study of blood constituents in domestic animals in South Africa. 5. The dog: normal values for red cell counts, haemoglobin, blood sugar and blood urea nitrogen with a practical statistical method for evaluation of such data. *Onderstepoort J. vet. Res.*, 33, 353-362.
- VAN ZYL, L. C., 1966. Electrophoresis of ovine plasma proteins on cellulose acetate: technique as adapted to standard equipment for use with filter paper strips. *Jl S. Afr. vet. med. Ass.*, 37, 460-464.

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- WAGNER, A. M., 1964. Contributions to the study of blood constituents in domestic animals in South Africa. 1. Normal values for methaemoglobin reductase, erythrocyte fragility, blood glutathione, plasma catalase and the total erythrocyte pyridine nucleotides in sheep. *Onderstepoort J. vet. Res.*, 31, 77-90.
- WAGNER, A. M. & BROWN, J. M. M., 1966a. Contributions to the study of blood constituents in domestic animals in South Africa. 2. Normal values for the activity of some enzymes of the Embden-Meyerhof pathway and tricarboxylic acid cycle in the plasma of sheep. *Onderstepoort J. vet. Res.*, 33, 325-336.
- WAGNER, A. M. & BROWN, J. M. M., 1966b. Contributions to the study of blood constituents in domestic animals in South Africa. 3. Normal values for the activity of glyceraldehyde-phosphate dehydrogenase and glucose-6-phosphate dehydrogenase in the erythrocytes of sheep. *Onderstepoort J. vet. Res.*, 33, 337-341.
- WEICHSELBAUM, T. E., 1946. An accurate and rapid method for the determination of proteins in small amounts of blood serum and plasma. *Am. J. clin. Path.*, 16, 40.