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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 OCCURRING IN THE PLASMA OF SHEEP

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Introduction

The recent studies of Brown and his co-workers on the pathogenesis of geeldikkop (tribulosis ovis) and enzootic icterus in sheep in South Africa have indicated the existence of certain fundamental biochemical lesions in affected animals. Both syndromes include severe icterus, intravascular haemolysis and varying degrees of photosensitivity. The icterus of geeldikkop has been classified as an intrahepatic cholestasis. Apart from severe bile pigmentation and variable fatty infiltration the liver cells are usually free from any histological evidence of cell destruction, when examined under the ordinary light microscope. One of the main biochemical lesions appears to be a decreased permeability of the hepatic cell wall towards compounds such as bilirubin glucuronides, porphyrins, bromsulphalein, bile salts and copper ions. Profound disturbances in carbohydrate metabolism and in the function of enzymes such as succinic dehydrogenase and glyceraldehyde-phosphate dehydrogenase have been observed in affected animals at the height of the clinical disease. These various features of the disease have been connected with a low grade chronic subclinical selenium intoxication and the action of severe nonspecific stressors. The most important of the latter is now thought to be an inapparent or subclinical infection with a relatively mild virus. (Brown, 1962, 1963, 1964; Brown, Le Roux & Tustin, 1960; Brown & De Wet, 1962; Brown, Wagner & Brink, 1966).

A prerequisite for further progress in the biochemical studies on these icterus and photosensitivity syndromes was the establishment of " normal values " for some of the tests used on sheep and for the blood levels of various enzymes and co-factors under consideration. This work has been complicated by the fact that marked differences have been found to exist in the levels of certain blood constituents between apparently healthy sheep raised in areas where these diseases do not occur and those from areas in which geeldikkop and enzootic icterus are prevalent (Wagner,

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1964). Previous studies have indicated the existence of profound biochemical disturbances and varying degrees of liver pathology in apparently normal animals from the latter areas (Brown, 1959, 1962, 1963; Brown, Le Roux & Tustin, 1960; Brown & De Wet, 1962).

Materials and Methods

The animals used for this study, their nutrition and management were the same as reported in a previous paper of this series (Wagner, 1964), viz.

(*a*) *Group* 1.—Fully grown Merino sheep drawn from the pool of animals available for research work at this Institute. Approximately 75 per cent of these animals were purchased in areas where geeldikkop and enzootic icterus are enzootic i.e. the Karoo and notably the Laingsburg and Beaufort West districts. The remainder were obtained from farms on the Transvaal Highveld.

(*b*) *Group* 2.—Fully grown Merinos and Dorpers bred and raised at the experimental farm of the University of Pretoria, situated in a grass pasture area in which geeldikkop and enzootic icterus have never been observed.

The method of statistical evaluation of the data collected was as described previously (Wagner, 1964).

The following enzyme assays were done on fresh ovine plasma or serum by the methods indicated in parentheses immediately following the procedure mentioned: Lactic dehydrogenase (Wroblewski & La Due, 1955), isocitric dehydrogenase (Taylor & Friedmann, 1960), glutamic-oxalacetic transaminase and glutamic-pyruvic transaminase (King, 1958), aldolase (Sibley & Lehninger, 1949, as simplified in the Sigma Technical Bulletin No. 750, Sigma Chem. Co. St. Louis, Mo., 1961) and phospho-hexose isomerase (Bodansky, 1954).

The abbreviations which will be used in the text which follows for the names of these enzymes are as follows:—

lactic dehydrogenase, LDH;
isocitric dehydrogenase, ICD;
glutamic-oxalacetic transaminase, GOT;
glutamic-pyruvic transaminase, GPT;
aldolase, Ald;
phospho-hexose isomerase, PHI.

In all cases, except in that of LDH, the unit of enzyme activity used was as defined or stated in the original procedure employed. One Wroblewski & La Due (1955) unit of LDH activity is defined as a decrease in optical density of the reaction medium of 0·001 per minute per ml of serum used, optical density readings being taken over a 3 to 5 minute period. During the course of this work we found that reaction rates are not linear for high values of LDH activity in the plasma of apparently normal sheep and in known abnormal cases. This is illustrated by three examples shown in Fig. 1. The curves shown represent three cases, 1, 2 and 3, which presented plasma LDH values of 393, 920 and 2030 units respectively in terms of the original definition.

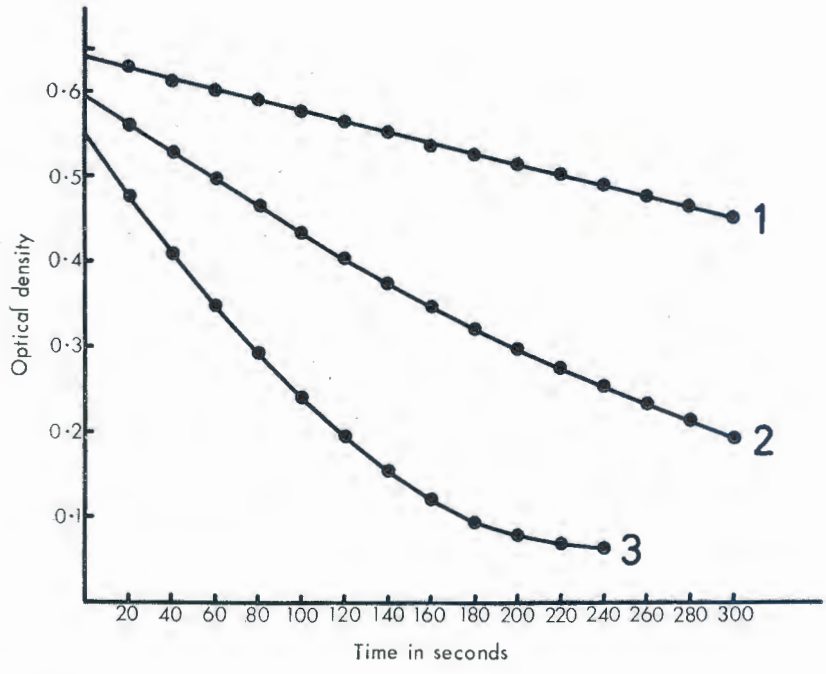

FIG. 1.—Studies on the linearity of the method for LDH in normal and abnormal sheep

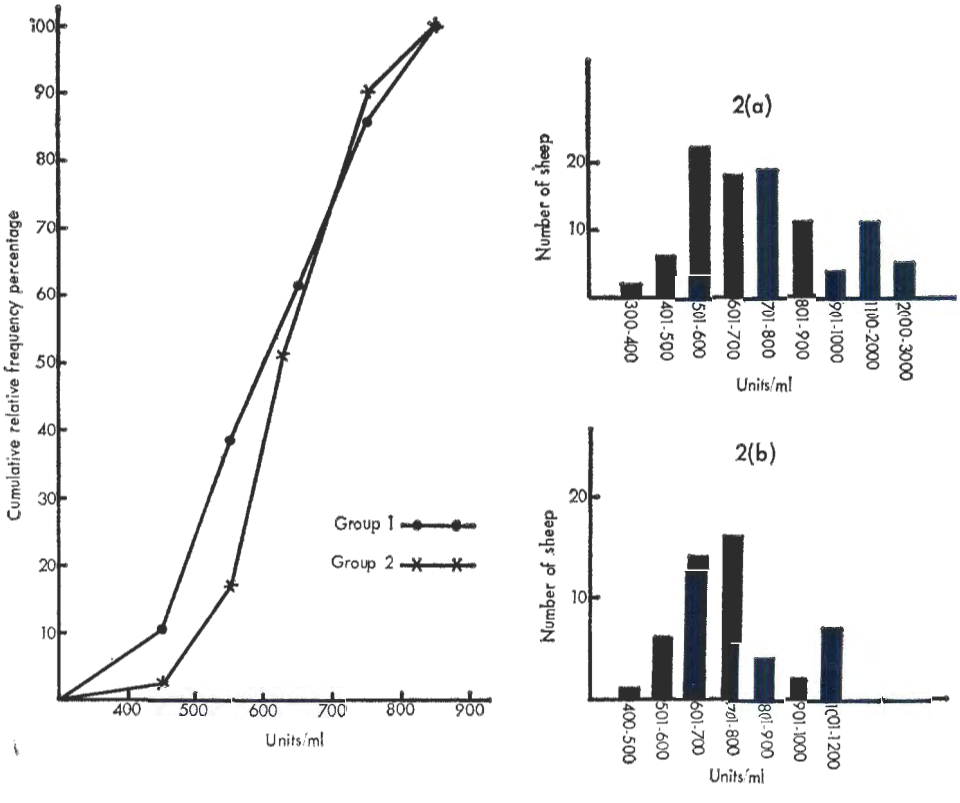

FIG. 2.—Lactic dehydrogenase. 2(a) Lactic dehydrogenase, Group 1. 2(b) Lactic dehydrogenase, Group 2.

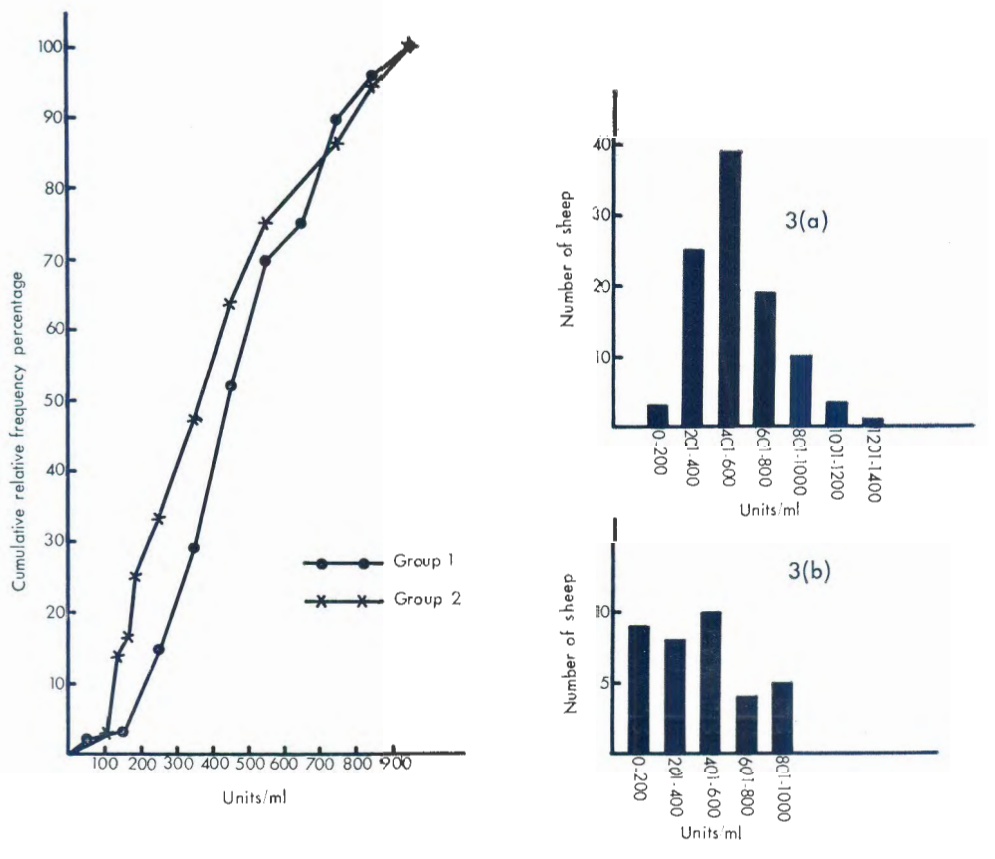

FIG. 3.—Isocitric dehydrogenase. 3(a) Isocitric dehydrogenase, Group 1. 3(b) Isocitric dehydrogenase, Group 2.

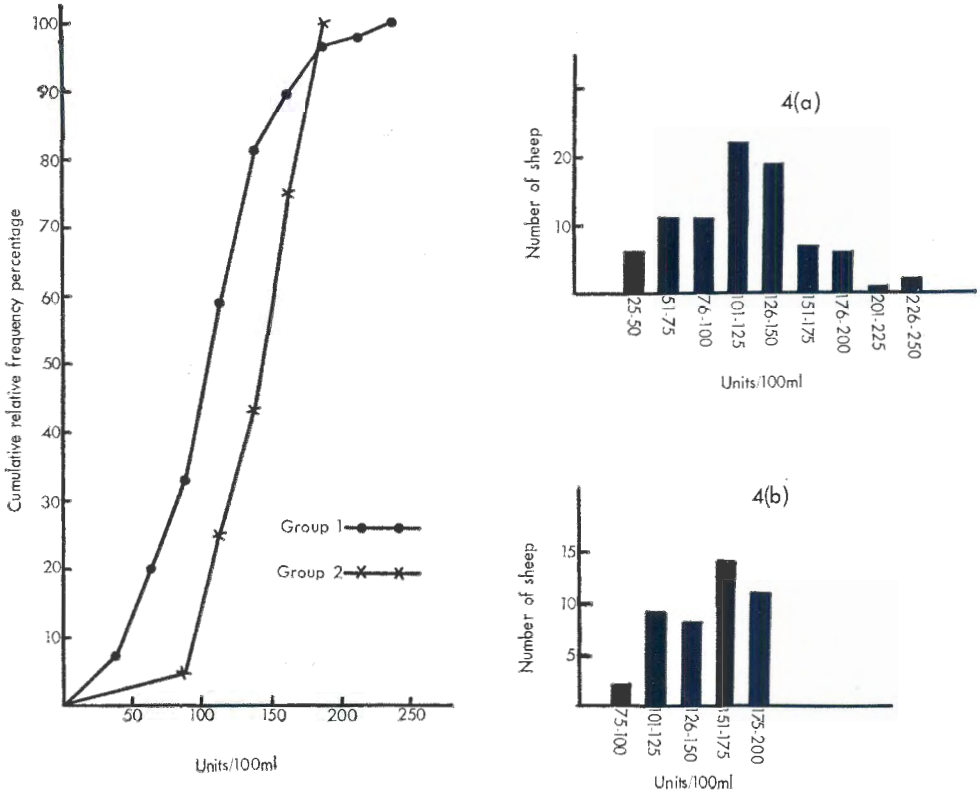

FIG. 4.—Glutamic-oxalacetic transaminase. 4(a) Glutamic-oxalacetic transaminase, Group 1. 4(b) Glutamic-oxalacetic transaminase, Group 2.

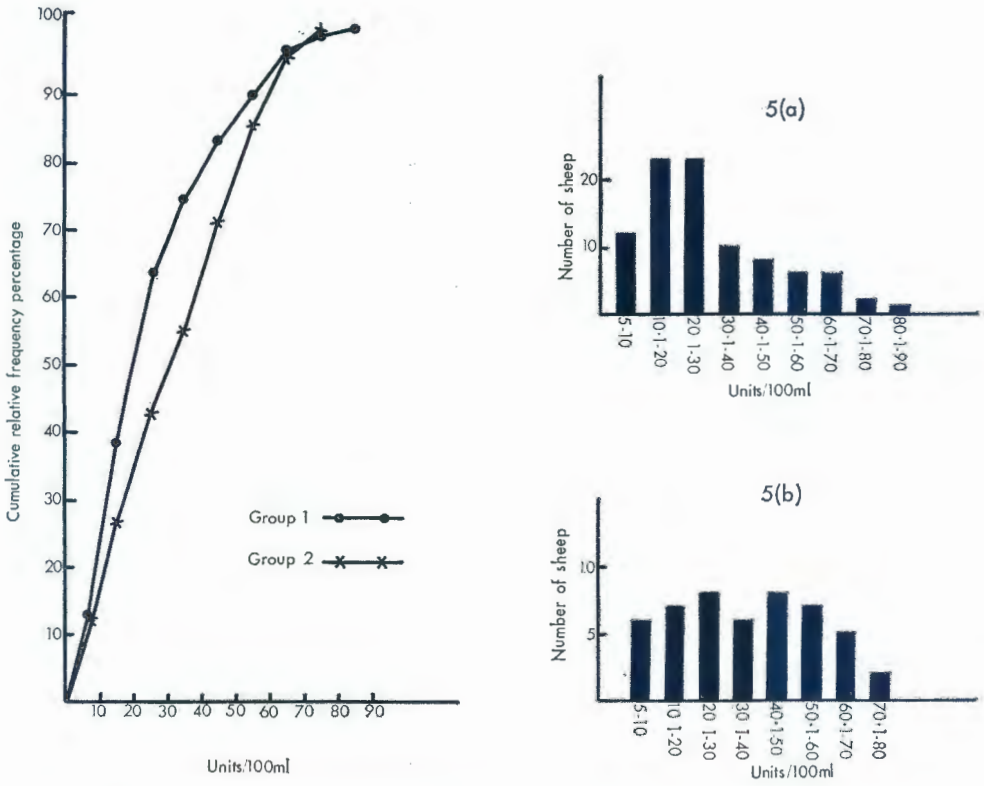

FIG. 5.—Glutamic-pyruvic transaminase. 5(a) Glutamic-pyruvic transaminase, Group 1 5(b) Glutamic-pyruvic transaminase, Group 2.

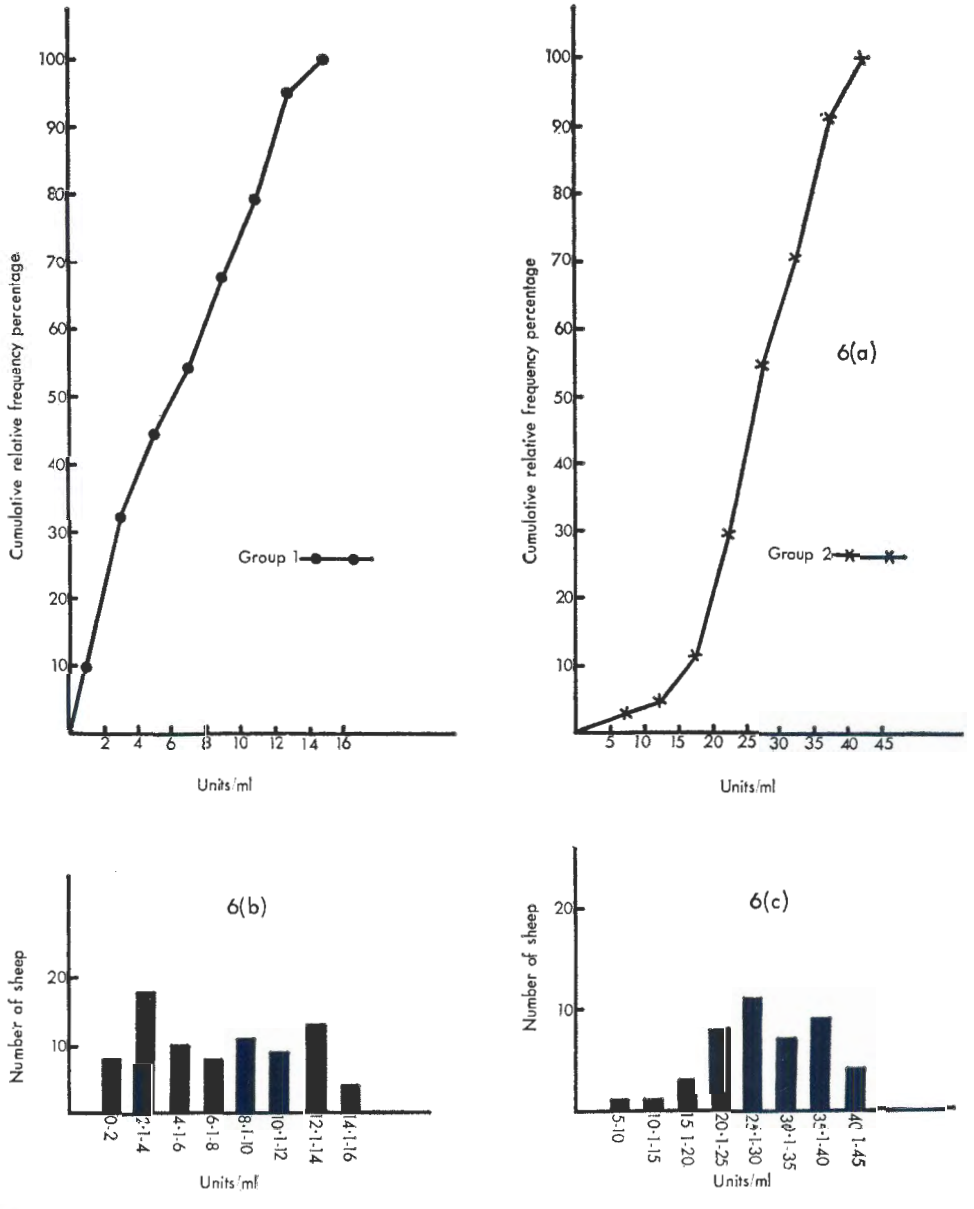

FIG. 6.—Aldolase, Group 1. 6(a) Aldolase, Group 2. 6(b) Aldolase, Group 1. 6(c) Aldolase, Group 2.

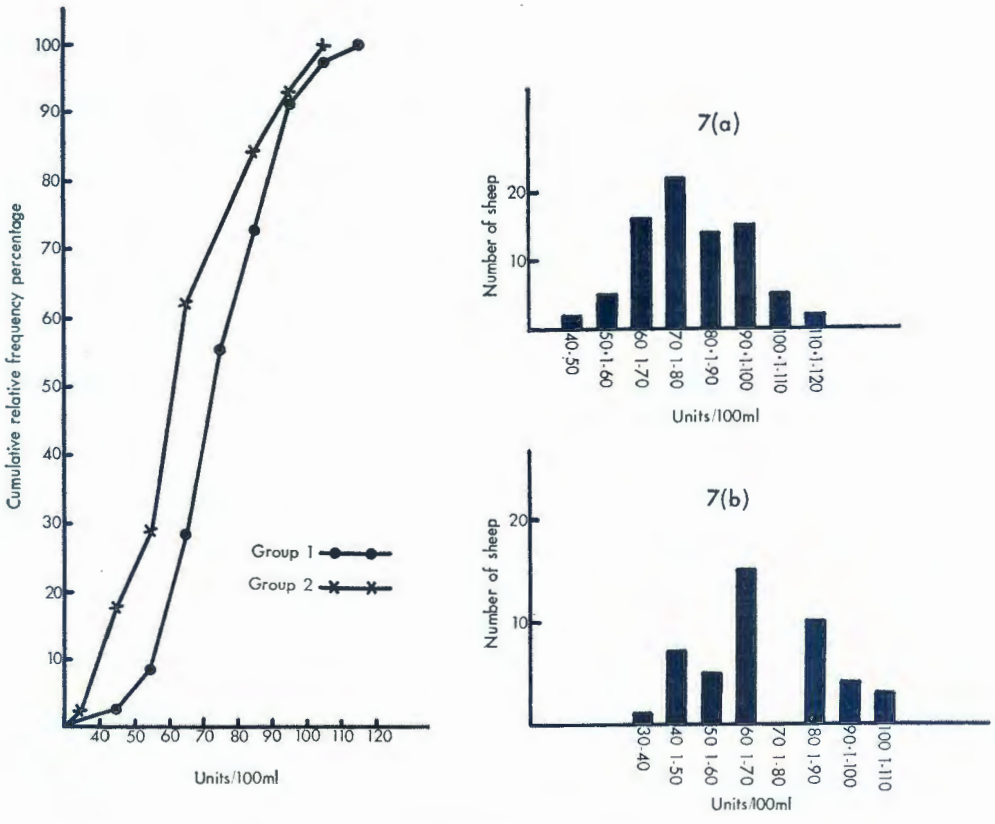

FIG. 7.—Phosphohexose isomerase. 7(a) Phosphohexose isomerase, Group 1. 7(b) Phosphohexose Group 2.

From this figure it can be seen that in cases 2 and 3 (high values) the reaction rates tend to slow down after about two minutes, if the change in optical density is greater than 0·07 per minute. Amador, Dorfman & Wacker (1963) also found similar nonlinear decreases with this method. They suggested employing the " forward " reaction, viz. lactate→pyruvate, in which there is an increase in optical density as a result of reduction of nicotine-adenine-dinucleotide (NAD). This increase was found to be linear in all instances where the change in optical density was less than 0·100 per minute. In spite of the several advantages inherent in the Amador, Dorfman & Wacker procedure, we have continued to use the Wroblewski & La Due method with the following proviso: optical density readings are taken every twenty seconds over the first three minutes of the reaction period. These are then averaged for the purposes of the final calculation, except in instances such as case 3 above which are obviously highly abnormal and in which a one minute reaction time is preferable.

Heparin was used throughout as the anticoagulant for the collection of blood samples; a Unicam SP500 spectrophotometer was used in all the assays; all chemicals used were *Analytical Reagent* grade and all the substrates and coenzymes used for the various procedures were obtained from the Sigma Chemical Co., St. Louis, Mo. The methods were standardized against pure enzyme preparations obtained from the same firm.

Results

The cumulative relative frequency curves and histograms constructed from the results obtained from the various assays performed are presented for each group of sheep at the end of this paper. The conclusions drawn are shown in Table 1.

Discussion

As was found in previous work of this nature (Wagner, 1964), the distribution curves were with a few exceptions of the " skew " type. The method of expressing the results as a standard deviation about a mean cannot, therefore, be employed.

For the reasons given in the previous publication (Wagner, 1964) the upper and lower 1 per cent limits for each set of values have been omitted from Table 1. These limits are readily determined by inspection of the cumulative relative frequency curves.

The homogeneity of the populations of Groups 1 and 2 have been discussed in the previous paper (Wagner, 1964) and no further comment need be added.

From the results presented here it is once more clear that for all the assays performed differences are observed in the " normal values " obtained from the two groups of sheep. These differences are particularly striking in the values for ICD, GOT, Ald and PHI. These findings serve as further substantiation of the contention of Brown and his co-workers (loc. cit.) that marked differences exist between apparently healthy sheep raised in areas where geeldikkop and enzootic icterus normally occur and those raised outside of these areas. Furthermore, as pointed out earlier (Wagner, 1964) these data emphasize the differences which may be present between different geographical population groups of a single species and the fallacy of accepting so-called " normal values " for a species regardless of environmental influences.

Summary

Normal values have been established for the levels of activity of lactic dehydrogenase, isocitric dehydrogenase, glutamic-oxalacetic transaminase, glutamic-pyruvic transaminase, aldolase and phospho-hexose isomerase in the plasma of sheep emanating from areas in which geeldikkop and enzootic icterus are prevalent and those raised in areas where the diseases do not normally occur. Some marked differences between the two groups are apparent which support recent contentions regarding the aetiology of the two syndromes mentioned.

TABLE 1.—*Ranges found for the various enzyme assays performed*

Determinations	Figure shown by median (50%)		Ranges per group					
			80%		10% Lower		10% Upper	
	Group I	Group II	Group I	Group II	Group I	Group II	Group I	Group II
Lactic dehydrogenase	600·0 (n = 78)	625·0 (n = 41)	445–780	500–750	393–444	470–499	781–896	751–860
Isocitric dehydrogenase	440·0 (n = 96)	370·0 (n = 36)	210–760	130–640	65–209	105–129	761–950	741–990
Glutamic-oxalacetic transaminase	105·0 (n = 85)	142·6 (n = 44)	45·0–165	95·0–177	41–44·9	77–94·9	165·1–212	177·1–197·5
Glutamic-pyruvic transaminase	20·0 (n = 91)	31·0 (n = 49)	7·5–55	6·0–59·0	7·0–7·5	5·0–5·9	55·1–87·5	59·1–68·0
Aldolase	6·0 (n = 81)	27·0 (n = 44)	1·0–11·0	16·5–37·0	0–0·9	6·5–16·4	11·1–14·7	37·1–45·0
Phosphohexose isomerase	73·0 (n = 81)	61·5 (n = 45)	56·0–94·0	40·0–91·0	47·0–55·9	37·0–39·9	94·0–117	91·1–107·0

NOTE.—Units are as given in the original procedures used or as stated in the text of this paper

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