ELECTROLYTE AND HAEMATOCRIT CHANGES IN THE BLOOD OF SHEEP FROM FOETAL TO POSTNATAL LIFE

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Summary

Foetal and postnatal packed cell volume percentages and whole blood, plasma, and erythrocyte potassium and sodium concentrations have been studied in the sheep.

A significant increase in packed cell volume and whole blood potassium was found between 70 and 140 days postconception.

A significant decrease in erythrocyte potassium concentration occurred over the same period. The equation

$$Y = 64 \cdot 23 + (1 \cdot 017x - 0 \cdot 0059x^2) \text{ m-equiv/l},$$

where x = foetal age in days, best fits the data.

Postnatal data fits well with prenatal data and a decrease in packed cell volume, whole blood potassium, and erythrocyte potassium associated with an increase in whole blood and erythrocyte sodium was recorded over the first 105 days of postnatal life. The red blood cell potassium concentration fell from 91·4 to 15·5 m-equiv/l over this period.

The results are discussed in relation to previous work and the possibility of a change over from one type of cell to another over the period in which the blood factors were studied.

I. Introduction

Differences in the concentration of potassium between foetal and adult erythrocytes have been shown for several species. Green and Macaskill (1928) reported that whole blood from calves had higher concentrations of potassium than blood from adult cows. Hallman and Karvonen (1949) noted similar differences in the whole blood of sheep, and demonstrated that these differences were related to differences in the concentrations of potassium in the erythrocyte. A fall in the concentration of potassium in the calf erythrocytes was shown to occur during the first 90 days of postnatal life by Wise *et al.* (1947) and in the lamb by Wright *et al.* (1958). A decrease with age in the concentration of potassium in the erythrocytes of foetal lambs has been shown by Widdas (1954), the relationship being represented by the equation $K = (115 - 0 \cdot 196d)$ m-equiv/l, where d is the foetal age in days. The reverse situation in man and pig has been reported by McCance and Widdowson (1956) who showed that concentrations of potassium in the erythrocytes of these species are lower in the foetus than the adult.

The concentration of potassium in the erythrocytes of adult man and pig is of the order of 100 m-equiv/l, and no evidence of marked variation from this value in normal animals has been reported. Adult sheep can be divided, however, into two

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groups according to the concentrations of sodium and potassium in their erythrocytes (Kerr 1937; Hallman and Karvonen 1949; Evans 1954). Any individual has red blood cells which are either of the low potassium/high sodium type or of the high potassium/low sodium type. A similar bimodality was found to exist in the erythrocytes of the goat, but cattle were found to be uniformly of the low potassium/high sodium type by Evans and Phillipson (1957). Recently evidence for high potassium erythrocytes in cattle has been found (Evans, unpublished data). These two types in the sheep have been designated *LK* and *HK* respectively (Evans 1954). This difference in the electrolyte composition of erythrocytes has been shown to be a permanent characteristic (Evans 1957), and to be genetically determined in a simple Mendelian manner, *HK* being recessive (Evans and King 1955; Evans *et al.* 1956). Most breeds are polymorphic for these characters (Evans and Mounib 1957; Evans, Harris, and Warren 1958).

Widdas (1954), who was using foetuses from *HK* and *LK* Welsh mountain ewes, fitted a single linear regression to his data. This implies that the difference between the genotypes is only expressed after birth and that the postnatal fall in the concentration of potassium in the erythrocyte of *HK* and *LK* lambs must be at different rates.

It is difficult to imagine that the expression of the genotype controlling the concentration of potassium in the red blood cells of sheep is absolutely dependent upon extra-uterine life particularly when it is known that adult haemoglobin is initially synthesized no later than the last third of pregnancy and that at birth 20–50% of the circulating haemoglobin is of the adult type (Karvonen 1949; Barron 1951). It would be most interesting, however, if the gene did express itself only after pulmonary respiration had commenced.

If the blood of the foetuses which are potentially *LK* animals was to be examined over the last 70 days of pregnancy any tendency for a greater rate of decrease in the concentration of potassium in the red blood cells over the last 4 weeks should be demonstrable and it should be possible to relate it to the fall which is known to occur in *LK* lambs from birth to 4 months of age (Wright *et al.* 1958).

The experiment reported here was an attempt to study these relationships.

Unfortunately, *HK* animals are uncommon in most of the breeds of sheep which are readily available in Australia (gene frequency for *HK* in Merino = approx. 0·07 (Evans 1960)) and it was not possible therefore to compare the rate of decrease of potassium in the red blood cells of potential *HK* with that in potential *LK* animals. This will be done when suitable flocks have been established.

II. Materials and Methods

(*a*) *Materials*

(i) *Prenatal.*—Foetal blood was collected from the progeny of an *HK* Southdown ram and *LK* Romney ewes which had been slaughtered at 10-day intervals from the 70th–140th day of gestation. Altogether 26 foetuses from 24 ewes were examined. Blood was expressed from the umbilical vessels and collected into 30-ml heparinized screw-cap bottles which were both sodium- and potassium-free.

(ii) *Postnatal*.—Six Merino lambs, the progeny of *LK* Merino ewes and an *HK* Merino ram, were used. Blood samples were taken within 24 hr of birth and again at 3-weekly intervals for a period of 15 weeks. 10 ml of blood was withdrawn from the jugular vein on each occasion into a sodium- and potassium-free heparinized syringe.

(*b*) *Methods*

Plasma samples were obtained by centrifugation at 1750 *g* for 30 min. Both plasma and whole blood samples were diluted 1:100 with deionized water and potassium and sodium estimations carried out on an "EEL" flame photometer by the method of King and Wootton (1956). Red blood cell values were calculated from these data and the haematocrit. This method has been shown to give values equivalent to those obtained by a direct analysis of red blood cells (Barker 1958).

Table 1

MEAN POTASSIUM AND SODIUM CONCENTRATIONS IN WHOLE BLOOD AND ERYTHROCYTES AND PACKED CELL VOLUME IN BLOOD OF LAMBS FROM 70–140 DAYS POSTCONCEPTION

Days of Pregnancy	No. of Animals	Packed Cell Volume (%)	Whole Blood Potassium (m-equiv/l)	Erythrocyte Potassium (m-equiv/l)	Whole Blood Sodium (m-equiv/l)	Erythrocyte Sodium (m-equiv/l)
70	2	17·7±2·1	22·5±0·4	110·0±10·3	—	—
80	3	25·7±3·0	30·0±5·6	104·9±10·9	—	—
90	3	31·7±3·7	36·4±4·8	106·0± 4·7	—	—
100	2	40·0±5·2	47·5±2·7	111·4± 6·5	87·8*	17·3*
110	7	43·0±6·8	48·2±8·9	104·8± 6·7	73·8±11·9†	34·7±11·3†
120	3	48·6±7·2	52·9±8·0	102·7± 2·4	78·9*	17·1*
130	3	48·8±2·6	49·4±5·7	95·5± 9·4	71·1±7·1	25·2±4·3
140	3	52·2±3·5	50·2±5·1	91·2± 7·2	76·3±0·5	37·9±3·2

* Only one animal examined. † Only three animals examined.

Haematocrit determinations on the 70- and 80-day foetal blood samples were carried out using microhaematocrit tubes. All other samples were centrifuged in Wintrobe tubes for 1 hr at 1750 *g*. Three tubes were used for each sample.

III. Results

(*a*) *Prenatal*

The results of the examination of foetal blood from foetuses 70–140 days post-conception are shown in Table 1.

The foetal growth curve over the period during which the blood examinations were made was normal and is shown in Figure 1.

The packed cell volume increased significantly from a mean of 17·7% at 70 days to a mean of 52·2% at 140 days (Fig. 2). Linear and quadratic relationships were fitted to these data and both were significant at the 0·1% level. The difference between them is significant at the 5% level.

The concentration of potassium in the whole blood increased over the same period from a mean of 22·5 m-equiv/l to 50·2 m-equiv/l (Fig. 3). Linear and quadratic relationships were fitted to these data and both were significant at the 0·1% level but the quadratic accounted for more of the variation and was significantly different to the linear at the 1·0% level.

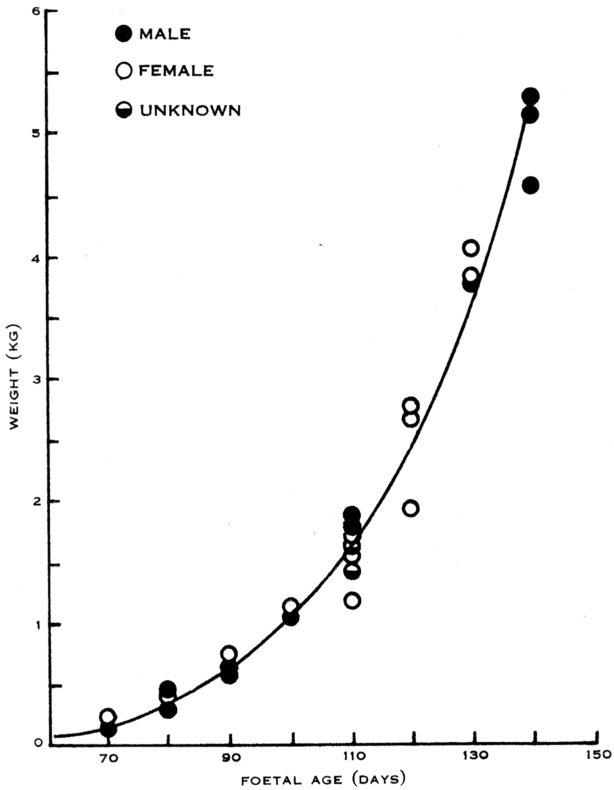

Fig. 1.—Foetal weights from 70–140 days postconception.

The concentration of potassium in the red blood cells decreased from 110·0 to 91·2 m-equiv/l over the 70-day period (Fig. 4). The linear relationship demonstrating the decrease is significant at the 1·0% level. This regression coefficient does not differ significantly from the relationship obtained by Widdas and can be expressed by the equation:

$$Y = 127{\cdot}59-(0{\cdot}23\pm0{\cdot}069)x \text{ m-equiv/l},$$

where x is the foetal age in days. A better fit is given by a quadratic relationship, however, and the curvature is significant at the 1·0% level. The variation accounted for by the curve is 57·0% of the total, and this is significant at the 0·1% level. The equation expressing the relationship is

$$Y = 64{\cdot}23+(1{\cdot}017x-0{\cdot}0059x^2) \text{ m-equiv/l},$$

where x is the foetal age in days.

The foetal data was examined for sex differences. There was no significant difference between male and female in the weights of the foetuses or between the means in the three blood parameters examined. The females showed more scatter, however, and this was significant at the 5% level in both packed cell volume and whole blood potassium concentrations. There were significant trends, at the 0·1% level, for both sexes in packed cell volume and whole blood potassium, but only in the male whole blood potassium data was the difference between the quadratic and linear relationships significant and then only at the 5% level.

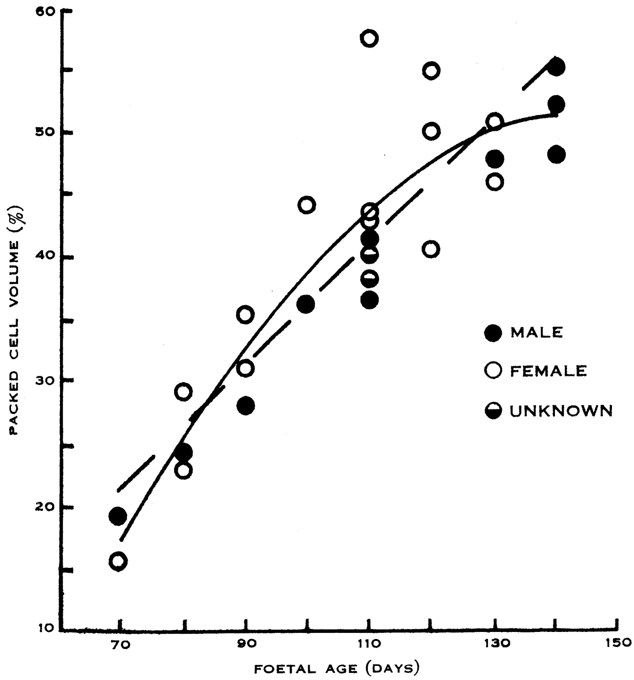

Fig. 2.—Packed cell volume (%) of the blood of foetal lambs from 70–140 days postconception.

(*b*) *Postnatal*

These data are shown in Table 2. The packed cell volume decreased from 56·0% at birth to 35·8% at 42 days and then rose to 40·0%. The concentration of potassium in whole blood also decreased from an initial value of 54·7 to 10·1 m-equiv/l at 15 weeks, while the red blood cell potassium fell from 91·4 m-equiv/l at birth to 15·5 m-equiv/l at 105 days (Fig. 5), and intracellular sodium rose from 34·9 to 87·2 m-equiv/l. The concentrations of potassium and sodium in plasma showed no significant trends during the first 15 weeks of life (Fig. 6).

Although the concentrations of potassium in the red blood cells in the foetus were higher than those obtained by Widdas (1954), the mean values at 140 days gestation and at birth in the experiments reported here are in very close agreement. This is also true of the other parameters examined in foetal and postnatal blood.

IV. Discussion

Barcroft (1946) has described the relationship of blood volume to foetal age in the sheep by demonstrating that from the 11th week of pregnancy onwards the volume in the cotyledons was almost constant whereas the volume in the foetus increased approximately in proportion to foetal age. The weights of the foetuses examined in this experiment are shown in Figure 1 and demonstrate that the changes described in this paper occurred against a background of normal foetal growth. It has been shown that the mean corpuscular volume and the mean corpuscular diameter

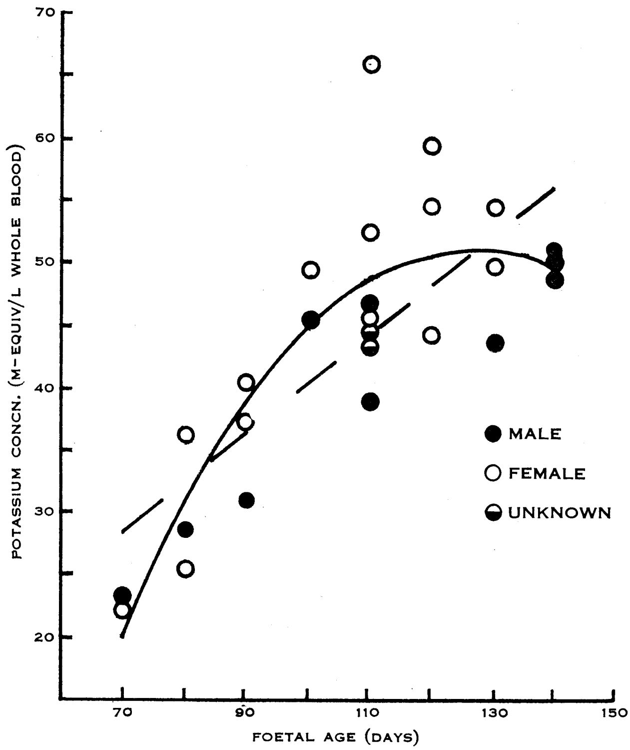

Fig. 3.—Concentrations of potassium (m-equiv/l) in whole blood of foetal lambs from 70–140 days postconception.

of foetal erythrocytes decreases with advancing foetal age in pig and man, and that the erythrocyte count increases over the same period (Wintrobe 1946). In the sheep, erythrocytes have been shown to decrease in size with advancing foetal age (Karvonen 1954). The increase in packed cell volume obtained in this experiment and illustrated in Figure 2 is most probably due therefore to a relative increase in the number of circulating cells. The increase in the concentration of potassium in whole blood (Fig. 3) tends to confirm this because the concentration of potassium in the plasma and red blood cells is relatively constant between 70 and 100 days post-conception. The foetal concentrations of potassium in the red blood cells show considerable scatter but as the linear regression for the total data for potassium concentrations in red blood cells is almost identical with that given by Widdas (1954) and the variation accounted for by the quadratic is significant at the 0·1% level and the quadratic gives a curve which fits the postnatal data, it seems likely that the potassium concentration in the foetal erythrocytes of potential *LK* sheep falls more rapidly in the last month of pregnancy.

Widdas (1954) was using foetuses from Welsh Mountain ewes, a breed with a gene frequency for *HK* of approximately 0·54 (Evans, Harris, and Warren 1958). Although a single linear relationship was fitted by Widdas to his data it is likely that at least 20–30% of the foetuses examined by him were potential *HK* animals. As the

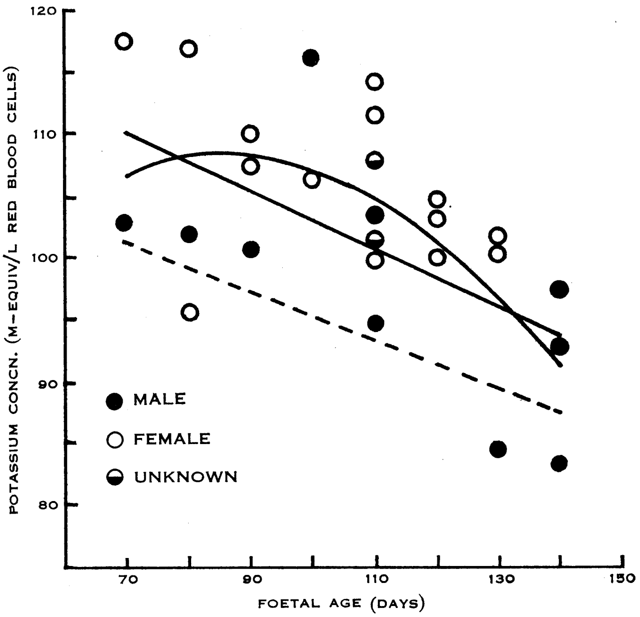

Fig. 4.—Concentrations of potassium (m-equiv/l) in erythrocytes of foetal lambs from 70–140 days postconception. ——— Least squares linear or quadratic fitted relationship. – – – – Linear fitted relationship (Widdas 1954).

foetuses in the present data were from Romney ewes, none of which was *HK*, the highest percentage of potential *HK* lambs among those examined would be about 10%. Since the concentrations of potassium in the red blood cells showed a more rapid decrease after 100 days in the experiments reported here, it is suggested that the difference between *HK* and *LK* animals may be evident prior to birth.

All the lambs which were examined postnatally developed into *LK* sheep, and gave a mean concentration of potassium in the erythrocyte of 15·5 m-equiv/l at 105 days. The concentrations of potassium at birth correspond closely with those in the foetus at 140 days (Figs. 5 and 6), and the general relationships of the curves agree. The concentrations of potassium which were recorded are higher and the concentrations of sodium lower than those described by Wright *et al.* (1958). These authors found values of 5 and 6 m-equiv. potassium per litre of red cells in two lambs at 42

TABLE 2

MEAN POTASSIUM AND SODIUM CONCENTRATIONS IN WHOLE BLOOD, PLASMA, AND ERYTHROCYTES AND PACKED CELL VOLUME IN BLOOD OF LAMBS FROM BIRTH TO 15 WEEKS

Six animals were examined for each criterion at each age, except at age 105 days when five animals were examined

Age (days)	Packed Cell Volume (%)	Potassium Concentration (m-equiv/l)			Sodium Concentration (m-equiv/l)		
		Whole Blood	Plasma	Erythrocytes	Whole Blood	Plasma	Erythrocytes
Birth	56·0±9·4	54·7±7·8	5·0±0·7	91·4±6·7	85·4±11·9	147·8±7·2	34·9±17·0
21	41·9±2·8	27·1±3·9	4·7±0·4	58·2±9·7	106·6± 6·0	148·5±4·0	49·0±11·6
42	35·8±3·5	13·7±1·8	4·8±0·4	29·9±8·2	121·9± 0·9	136·6±3·7	72·1±10·8
63	38·7±3·9	11·5±1·0	5·3±0·4	21·0±3·7	123·9± 2·6	147·9±2·0	86·9± 8·9
84	40·7±4·2	10·3±1·0	5·4±0·7	17·3±2·5	120·9± 2·4	145·8±4·2	85·2±10·6
105	40·0±1·5	10·1±1·2	5·2±0·4	15·5±2·9	120·8± 3·3	143·0±3·9	87·2± 5·0

days and 3 and 7 m-equiv/l in two animals at 60 days, which suggests that they were dealing with *LK* sheep whose concentrations of potassium in the erythrocytes were much lower than the mean concentrations for Merino sheep recorded by Evans (1960).

The higher concentration found in this experiment could be due to the fact that all lambs would be heterozygous *LK*.

Since completing this experiment we have been privileged to see the results of a similar experiment on the postnatal concentrations of potassium and sodium of Merino and Merino × Border Leicester lambs obtained by Koch and Turner (1960).

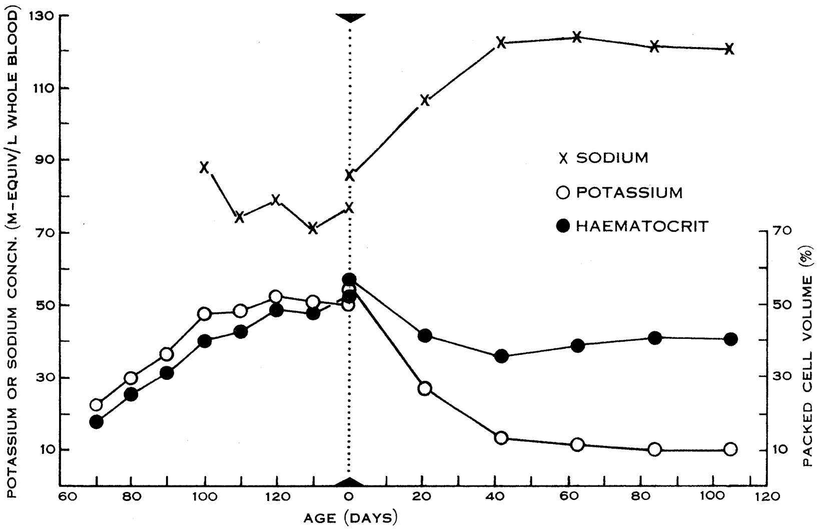

Fig. 5.—Means of whole blood concentrations of potassium and sodium (m-equiv/l whole blood) and haematocrit (%) in the erythrocytes of sheep from the 70th day postconception to 105 days after birth.

Their data shows very similar trends to those described here except that the concentrations of sodium in the red blood cells reach a peak about 10 m-equiv/l higher than recorded here at 40 days after birth and their concentrations of potassium are steady at 40–60 days at 10 m-equiv/l whereas those reported here only fall to 15·5 m-equiv/l. The differences in the final concentrations of potassium could be related to differences between animals; our animals being peculiar in that they are all heterozygous *LK*.

Similar haematocrit changes over the first few weeks of life have been reported in pigs and man by Wintrobe (1946). Wise *et al.* (1947) showed a decrease in the haematocrit of calves over the first 3 weeks of life which was followed by a rise. The sheep in this experiment (Fig. 6) showed a gradual fall up to 6 weeks of age and this was followed by a rise to a mean of 40%. This fall is probably an expression of the physiological anaemia described in other animals over the period when the growth rate of the body exceeds the rate of production of red blood cells.

Alterations in the degree of anoxaemia and the theory of a double cell population are the two hypotheses which have been evoked to explain the blood changes which occur at birth. Karvonen (1954) has demonstrated two cell sizes in the blood of foetal sheep using Price–Jones curves, but he could not accept the interpretation of a two-cell population because the transition did not reflect the change from foetal to adult haemoglobin, nor did the development of medullary haematopoiesis fit the same time scale. He suggests that the more likely explanation for the change is the difference in oxygenation. McCance and Widdowson (1956), working with pig and man, stress the difficulty of assuming that the mechanism which extrudes sodium, matures less rapidly than the one which takes up potassium because the reverse would have to be the case in the sheep.

Various authors, who have examined foetal and adult blood, have suggested that the cell population is not homogeneous (Widdas 1955; Goodwin 1956; Kutas

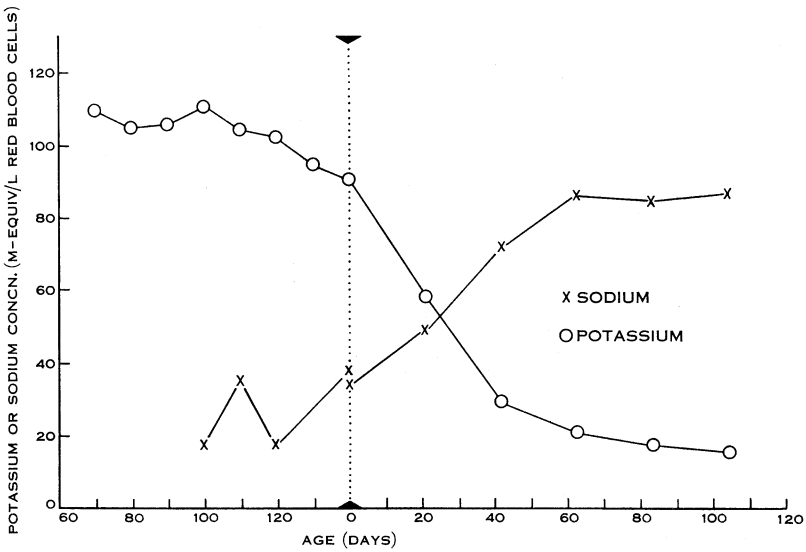

Fig. 6.—Means of the concentrations of potassium and sodium (m-equiv/l red blood cells) in the erythrocytes of sheep from the 70th day postconception to 105 days after birth.

and Stutzel 1957; Maizels 1958). Prankerd (1958) and Joyce (1958) have shown differences between young and old cells in their concentration of potassium and lipid content. Tucker (1958) has demonstrated that *HK* cells when transfused into *LK* sheep remain *HK*.

Although the difference between the oxygen dissociation curve of foetal and adult haemoglobins appears to be the result of the cell membrane rather than the type of haemoglobin (Haurowitz 1935; Hill and Wolvekamp 1936; McCarthy 1943), differences in the molecular structure of the haemoglobins have nevertheless been demonstrated (Kendrew and Perutz 1948). It is difficult to assume that one erythrocyte would contain both types of haemoglobin.

The percentage of foetal haemoglobin decreases up to birth in the human (Walker 1954) and in the sheep (Karvonen 1949; Barron 1951), and there is a more rapid decrease after birth in the human with negligible amounts of circulating foetal haemoglobin at 22 weeks (Jonxis 1949). These data give a curve which is similar to, but not identical with, the fall in potassium concentration in the present experiment, and suggest that a similar mechanism might be operating.

It can be shown that a change from one population of cells containing certain proportions of sodium and potassium to another population containing a different proportion can produce the type of changes in sodium and potassium concentrations observed in this experiment. If one assumes that one cell population contains 110 m-equiv/l of potassium and 30 m-equiv/l of sodium (foetal cells), and that another (the adult cell) contains 15 m-equiv/l of potassium and 90 m-equiv/l of sodium, and also that the rate of loss of foetal cells is equal to the rate of gain of adult cells, the proportions of foetal cells necessary to produce the concentrations of sodium and potassium described in this experiment, while adult cells are increasing, gives a curve almost identical with that of the decrease in potassium concentration which was found. While this does not prove an hypothesis it demonstrates that these changes could be brought about by alterations in cell populations.

The results obtained in this experiment enable an hypothesis to be erected which envisages that changes of red blood cell sodium and potassium could follow from two populations of cells of different composition, one of which is decreasing while the other is increasing. The results do not, however, do more than this.

Walker (1954) has suggested that the production of adult type haemoglobin and adult erythrocytes in the human is a function of age, and that foetal haemoglobin and red blood cells are produced at the low oxygen tensions of foetal life, and that when oxygen tension falls in late pregnancy foetal haemoglobin and red blood cell production is stimulated only to fail at the oxygen tensions of extra-uterine life. Foetal-type haemoglobin is known to be produced in adults in sickle cell anaemia, thalassaemia, and in acquired anaemias (Itano 1953). If the high concentration of potassium in the circulating red blood cells of the foetus is due solely to a high percentage of foetal type cells, and if foetal-type cells increase in the sheep, as in the human, towards the end of pregnancy one would expect a rise in potassium just prior to birth. This rise was not recorded in this experiment and the fall from 100 days postconception which was obtained suggests that changes in oxygen tension if they do occur in the sheep just prior to parturition are not associated with a rise in the concentration of potassium in the red blood cells.

The number of animals examined is, however, too small to allow valid conclusions and further experiments are planned to investigate these aspects of foetal haematology.

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