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A COMPARISON OF FŒTAL AND MATERNAL HÆMOGLOBINS IN THE GOAT.

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RECENT investigations have shown that the oxygen dissociation curves of fœtal and maternal bloods are not identical. Comparisons on the blood of goats have been published by Huggett [1927] and on human blood by Haselhorst and Stromberger [1931]. The affinity of hæmoglobin for oxygen depends upon the concentrations of hydrogen ions and other electrolytes, and in order to determine whether the differences in the dissociation curves are due to differences in the hæmoglobins, experiments have been made in this work on purified solutions of maternal and fœtal hæmoglobins, in which the concentrations of hydrogen ions and salts have been defined by the dialysis of the protein solutions against relatively large volumes of a standard buffer solution. In addition, measurements of osmotic pressure and of the membrane potential have been made in order to determine whether there is any difference between the molecular weights or the ionization of maternal and fœtal hæmoglobins[1].

Preparation of material. Samples of the blood of maternal and fœtal goats were used for the preparation of hæmoglobin. The corpuscles were washed three times with salt solution. The stromata were removed by the use of purified ether and sodium chloride, as described by Adair [1925]. The preparations were placed in collodion membranes and dialysed for 1 day with distilled water and then for periods from 4 to 10 days with the Sörensen phosphate buffer mixture containing 1/30 mol. of potassium dihydrogen phosphate and 1/30 mol. of disodium hydrogen phosphate per litre of solution. The pH value of this mixture is 6·81 at 20° and about 6·78 at 38° C. For the purposes of this work it seemed desirable to use the solution at pH 6·8 rather than at the physiological 7·4, or more alkaline reactions. In the first place, higher oxygen tensions are used for the

[1] Dilute solutions of hæmoglobin from the same series of goats were studied simultaneously by Prof. F. G. Hall using spectroscopic methods. The results were similar to those found in this paper and will be published shortly.

dissociation curve at 6·8, and the oxygen pressure at half saturation can be determined with greater accuracy from the gas analyses. A second and more important advantage is that the pH values of different hæmoglobin preparations are less variable after dialysis with solutions at pH 6·8 than at 7·4, because the phosphates have a higher buffer value at 6·8 and are less affected by traces of carbon dioxide. The effects of variations in the pH on the oxygen dissociation curve are smaller at pH 6·8 than at 7·4. Even if the pH values are identical at 0°, there may be variations at 38°, because the effect of temperature on the pH may be greater in the solutions with higher concentrations of hæmoglobin. The temperature correction is less important at pH 6·8 than at 7·4.

There is one disadvantage in working at pH 6·8; oxyhæmoglobin changes into methæmoglobin more rapidly at pH 6·8 than in more alkaline solutions. Estimates of the methæmoglobin formation were made by the following procedure. The refractive indices of the protein solution R' and of the dialysate R'' were measured and the protein concentration C was calculated by the formula $R' - R'' = 0{\cdot}001945\, C$ as described by Stoddard and Adair [1923]. The theoretical oxygen capacity was calculated on the provisional assumption that 1 g. of the goat's hæmoglobin is equivalent to 1·34 c.c. of oxygen, the value accepted for the hæmoglobin of the horse. The oxygen capacities were then determined and the results are recorded in Table I. Provisional estimates of

TABLE I.

	Date of pregnancy, weeks and days	Date dialysis started	Date dialysis ended	Date dissociation curve measured	Oxygen pressure in mm. at half saturation	n	Oxygen capacity	Oxygen p.c. of theoretical
Normal	—	March 25	March 31	April 7	47	2·12	14·01	92·3
Maternal	12	April 19	April 26	April 26	43	2·04 ± 0·2*	12·7	85·3
Maternal	13, 2	April 25	May 3	May 27	36	2·2 ± 0·2*	8·59	90·5
Fœtal	13, 2	April 24	May 2	May 2	31	1·7	6·76	82·8
Maternal	15, 1	May 5	May 10	May 19	39	2·25 ± 0·15*	13·36	84·7
Fœtal	15, 1	May 5	May 10	May 15	31·5	2·17	8·98	85·2
Maternal	16	June 1	June 8	July 22	42	2·35	6·87	74·7
Fœtal	16	June 1	June 8	July 13	24·5	2·17	7·18	—
Maternal	18	May 23	June 2	June 13	38	2·0	8·50	86·6
Fœtal	18	May 22	June 2	June 7	22·5	—	4·60	72·8
Maternal	19, 6	June 1	June 8	July 18	34	2·27	14·1	71·5
Fœtal	19, 6	June 1	June 8	July 17	26	2·5 ± 0·3*	8·15	73·6

* Probable error in the determination of n.

the percentage of the total hæmoglobin in the active form are recorded in Table I, column 9. These figures may require multiplication by a constant factor when goats' hæmoglobin has been subjected to further analysis, but from the results of colorimetric determinations of total hæmoglobin it appears unlikely that the factor will exceed 1·05. It appears therefore that some of the preparations contain over 20 p.c. of methæmoglobin.

It is by no means certain that the methæmoglobin formation is wholly due to the *p*H of the buffer mixture. In a programme of work on blood as well as on hæmoglobin, the blood must be dealt with first in order to minimize the risk of changes in *p*H and it may be necessary to defer work on the hæmoglobin. There is little doubt that higher percentages of oxyhæmoglobin could be obtained if the work could be finished in a shorter time.

Methæmoglobin interferes with certain methods for the study of the dissociation curve. The experimental results described below indicate that there is little difference between the dissociation curves of solutions containing varying amounts of methæmoglobin. Since the amounts of methæmoglobin formed in maternal and fœtal hæmoglobins, dialysed at the same time are the same, there is no reason to suppose that methæmoglobin formation vitiates the comparison of maternal and fœtal hæmoglobins.

Oxygen dissociation curves.

For the measurement of the oxygen dissociation curves, the hæmoglobin solutions were equilibrated at 37° C. in the new Barcroft saturator[1] with an enclosed double bulb pipette. Duplicate Haldane analyses were made on the gas phase, and the percentage oxygenation of the solutions was determined by the van Slyke manometric method.

The fœtal and maternal hæmoglobin solutions were obtained from goats in the 12th–20th week of gestation. The total period of pregnancy in the goat is 21 weeks. A non-pregnant goat was used as a control. The characteristics of the hæmoglobin solutions used are shown in Table I.

The oxygen dissociation curves of a number of preparations are shown in Fig. 1, and experimental data for the oxygen dissociation curves of additional preparations are recorded in Table II. The curve numbered 1 is a composite curve of all the maternal hæmoglobins. The range of variation covered by the points is comparatively small, and it appears that the oxygen dissociation curves of all the maternal hæmoglobins are nearly identical. The pair of curves, numbered 2 and 3, represent the fœtal and maternal hæmoglobin obtained at the 16th week of pregnancy. The fœtal and maternal hæmoglobins obtained at the 18th week of pregnancy are represented by curves 4 and 5, and those obtained at the 20th

[1] A description of this apparatus will be published later.

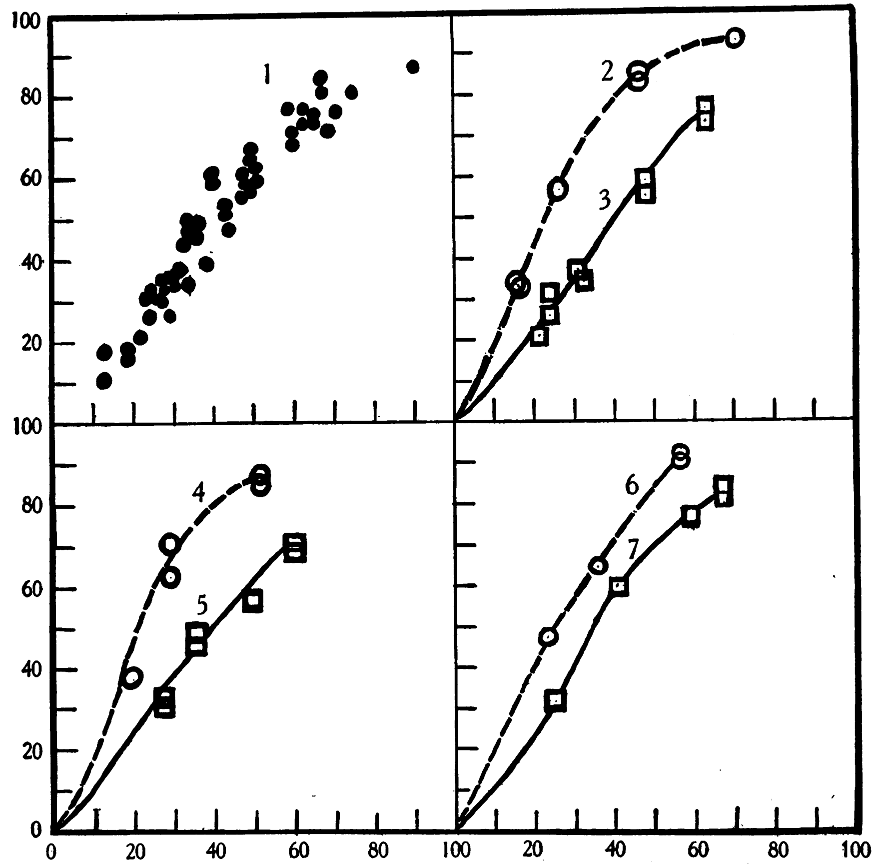

Fig. 1. Oxygen dissociation curves of hæmoglobin of goats. Ordinates: percentage oxygenation. Abscissæ: oxygen pressures, mm. of Hg. Circles: fœtal. Squares: maternal. Interpretation in text.

Table II. Percentage oxygenation and oxygen pressures in mm. of Hg.

Normal	Pressure in mm.	33·6	38·7	44·2	69·0	—	—
	Saturation p.c.	34·0	39·0	47·0	70·8	—	—
Maternal,	Pressure in mm.	29·0	43·5	51·0	90·5	—	—
12th week	Saturation p.c.	26·4	51·3	59·0	87·0	—	—
		35·6	53·0	62·0	—	—	—
Fœtal,	Pressure in mm.	26·4	13·2	50·0	80·7	—	—
13th week	Saturation p.c.	36·6	19·6	79·1	74·4	—	—
		45·8	21·4	66·0	85·9	—	—
Maternal,	Pressure in mm.	12·8	27·9	33·7	49·5	71·0	—
13th week	Saturation p.c.	10·10	32·9	47·2	64·5	75·5	—
		17·85	34·5	49·5	67·0	76·5	—
Fœtal,	Pressure in mm.	14·3	26·3	29·0	55·5	—	—
15th week	Saturation p.c.	13·0	37·4	42·2	78·0	—	—
		16·3	38·0	44·5	73·0	—	—
Maternal,	Pressure in mm.	19·3	30·3	32·8	65·4	48·0	75·2
15th week	Saturation p.c.	15·9	34·3	44·3	73·5	60·7	80·5
		17·5	35·4	44·6	75·0	—	—

week by curves 6 and 7. The goat from which these curves (6 and 7) were obtained died of asphyxia during operation. Subsequent investigation revealed a condition of pulmonary congestion.

It is evident that in all cases the curve for fœtal hæmoglobin is on the left of the maternal, so that fœtal hæmoglobin has a higher affinity for oxygen. In the second place there seems to be a slight difference in the shape of the curves, which can be represented by the calculation of n in Hill's equation, $y/100 = kx^n/(1 + kx^n)$, in which y represents percentage saturation, x oxygen tension and k is a constant. The mean value of n for the maternal hæmoglobins is 2·2 with a probable error of $\pm$ 0·3, and for fœtal hæmoglobins 2·0 with an error of $\pm$ 0·4. It is open to question whether the difference is significant, in view of the wide range of error in the determination of n, but it may be of interest, because a similar difference has been obtained in the bloods[1].

Human fœtal and maternal bloods have been studied by Haselhorst and Stromberger [1931]. They determined the relationship between the k of Hill's formula and the hydrogen-ion concentration, assuming that n is a constant. Although it is not possible to compare the values of n for the human hæmoglobins, the values of k for the human fœtal hæmoglobin are higher, in accordance with observations on goat's blood.

The observations recorded in this paper indicate that the differences in the bloods are due to differences in maternal and fœtal hæmoglobin rather than to any possible differences between the electrolytes and other substances present in maternal and fœtal corpuscles.

The osmotic pressures of maternal and fœtal hæmoglobins.

In view of the difference between the dissociation curves of maternal and fœtal hæmoglobins, a number of measurements of the osmotic pressure of the proteins were made, using the same phosphate buffer mixture. Osmometers with collodion membranes were used as described by Adair [1925]. The protein concentrations were determined by the refractometric method. The observations are recorded in Fig. 2. The continuous curve in Fig. 2 represents the osmotic pressure of the hæmoglobin of the sheep [Adair, 1928]. The circles represent observations on the fœtal hæmoglobin of the goat, and the squares on the maternal hæmoglobin. It appears that both of these forms have osmotic pressures in fairly close agreement with the data for sheep's hæmoglobin, which has a molecular weight of 68,000. Most of the points for fœtal hæmoglobin lie above the curve, but it is not unlikely that the relatively small

[1] Observations on blood made in this laboratory.

differences observed are due to experimental error. The results in Fig. 2 may be subject to a slight error, because the calculations of the protein concentration depend upon the assumption that the refraction value of a 1 p.c. solution is 0·001945, as in the case of the hæmoglobin of the sheep.

In addition to these measurements of osmotic pressure, a number of measurements of the membrane potential have been made, and from these the ratio E/C_v has been calculated as described by Adair and

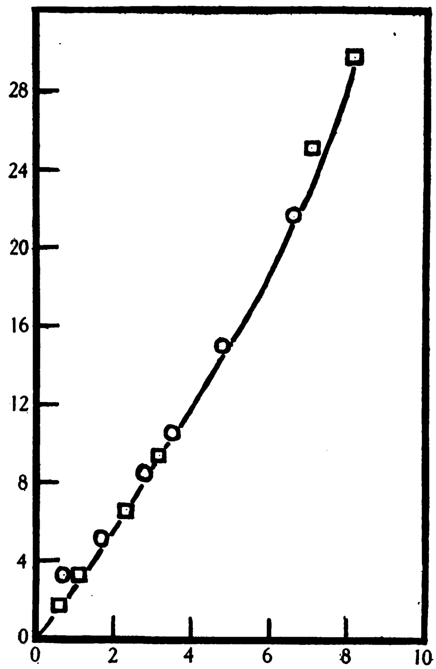

Fig. 2. Ordinates: osmotic pressure in mm. of mercury at 0° C. Abscissæ: concentrations in grams of protein per 100 c.c. of solution. The smooth curve is copied from the curve for sheep's hæmoglobin given by Adair [1928]. The circles are fœtal hæmoglobin, squares maternal hæmoglobin.

Robinson [1930]. E represents the membrane potential in millivolts and C_v the corrected concentration of the protein in grams per 100 c.c. of solvent. In the case of the maternal hæmoglobin the following values of E/C_v were obtained:

0·057, 0·055, 0·058, 0·075, 0·057.

In the case of fœtal hæmoglobin the ratios obtained were:

0·099, 0·077, 0·105, 0·091.

In all cases the protein solutions were negative, the protein acting as an anion in the buffer mixture at *p*H 6·9 at 0° C. It appears that the fœtal hæmoglobin has more negative charge than the maternal hæmoglobin at the same *p*H, but the potentials observed were so small that the difference might possibly be due to experimental error.

Summary.

1. Comparative investigations have been carried out on purified solutions of fœtal and maternal hæmoglobin of goats. Oxygen dissociation curves obtained from these solutions have shown that fœtal hæmoglobin has a greater affinity for oxygen than maternal hæmoglobin.

2. Determinations of osmotic pressure revealed no appreciable difference between the molecular weights of fœtal and maternal hæmoglobins.

This work forms a part of a study of the conditions of fœtal respiration planned by Prof. Barcroft, and I wish to thank him for his kind advice and help. I also wish to express my gratitude to Mr G. S. Adair for his generous and invaluable assistance in supervising the dialysis of solutions and the osmotic pressure determinations.

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