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ALKALINE RESISTANCE AND SPREADING VELOCITY OF FŒTAL AND ADULT TYPES OF MAMMALIAN HÆMOGLOBIN

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(*Received June* 4, 1936)

In a previous paper [Brinkman & Jonxis, 1935] in which the alkaline resistance and spreading velocity of human hæmoglobin was examined, we demonstrated the individuality of various kinds of hæmoglobin with this technique. We were not able, however, to find a distinction between any fœtal and adult hæmoglobin except that from man, although this might be expected from the researches of Barcroft and others on the dissociation curves of fœtal and maternal goat's hæmoglobin [Barcroft, 1935]. This was largely owing to technical difficulties in our method of determining the alkaline resistance of mammalian hæmoglobins (except for human blood), but we have now modified the method, so that any type can be examined accurately.

The result is that in the goat, cow and rabbit a distinction between fœtal and adult animal forms is clearly present. This statement is fully corroborated by the respective surface-pressure curves.

Method of making alkaline denaturation curves of animal hæmoglobin

About 1 c.c. of the blood is washed with isotonic saline in the centrifuge and a 40 vol. p.c. suspension of the corpuscles is made, which is hæmolysed by addition of some solid saponin. This is placed in a 37·0° C. bath, in which also a tube, containing 8 c.c. of the alkaline buffer solution is present. This buffer is a mixture of 0·15 *N* Na_2HPO_4 solution and *N* NaOH; its *p*H, and the *p*H after addition of the hæmoglobin solution and completion of denaturation are measured by means of the glass electrode, which could still be used in the range between *p*H 11 and 12.

A series of twenty 2 c.c. small centrifuge tubes was filled with 1 c.c. of a solution, composed of 500 c.c. saturated ammonium sulphate, 500 c.c. 0·15 *N* Na_2HPO_4 solution and 55 c.c. *N* KH_2PO_4 solution. This precipitates the denatured hæmoglobin, leaving the

undenatured in solution. 0·5 c.c. of the hæmoglobin solution to be examined is well mixed with 8 c.c. of the alkaline buffer solution at 37·0 and the stop-watch is started. Every minute (or every other minute) 0·5 c.c. of the denaturing solution is collected by means of a suitable syringe and injected into the 1 c.c. solution described above, which stops denaturation and precipitates the denatured product, with prevention of reversion to the native substance. After standing for 15 min. the tubes are centrifuged and the concentration of the not denatured hæmoglobin is determined.

The latter estimation must be made very carefully, as very small absolute concentrations are to be determined. We used a Hippel high-pressure mercury arc[1] and an orange filter which only transmits the 580 and 546 lines. Constancy of illumination is obtained by using a 100-watt generator, driven by a synchronous motor of 1500 rev. per min. A separate photo-electric cell (Weston), in series with a specially adapted pointer galvanometer, controls the stability and standard value of illumination, which can be corrected by a 400-ohm resistance in series with the mercury arc; the current taken is 150 mA.

The absorption by the solution examined is measured by a sensitive photo-electric "Sperrschichtcell" (Suddeutsche App. Fabrik, Nurnberg), the E.M.F. of which is measured by potentiometer. Calibration is made by making a dilution curve of the original Hb solution in each experiment; the dilution is made with the same salt solution in which the denatured hæmoglobin is finally present.

This method is very satisfactory, and accurate to about 0·1 p.c. of the original solution before denaturation. The surface-pressure curves were made with the technique described in our previous paper [Brinkman & Jonxis, 1935].

The results are given in Fig. 1 for goat's blood, which was taken because most of Barcroft's work was done with this species. The denaturation was made at *p*H 11·80, at 37·0° C., with a constant amount of hæmoglobin from washed corpuscles. It is seen that fœtal goat's hæmoglobin is distinguished by a reaction velocity which makes it much less alkali-resistant than the adult form. The blood 1 day after birth contains about 10 p.c. of the adult form, and this amount gradually increases, so that at the age of 4 weeks the percentage of hæmoglobin is about 30 p.c. The hæmoglobin of the adult and fœtal cow shows just the same difference in alkali resistance as the goat does. But fœtal rabbit's hæmoglobin is similar to the fœtal human pigment, its alkali resistance surpassing that of the adult form. In the cat no difference between very young and older hæmoglobin could be found. The results of spreading experiments lead to the same conclusion. The method is described in the previous paper, but a brief sketch may be inserted here.

A small amount of solution of protein, put carefully on a water surface, spreads to form a monomolecular film. The extent of this film depends on the kind of protein and the electrolyte properties of the carrier solution. On strong acid, base or salt solution the surface is at

[1] Schott u. gen., Jena.

once maximal, but on dilute buffer solutions the initial surface is much smaller and depends on the *p*H.

Only at the isoelectric point does the spreading become large or maximal again, and so the measurement of spreading velocity on dilute

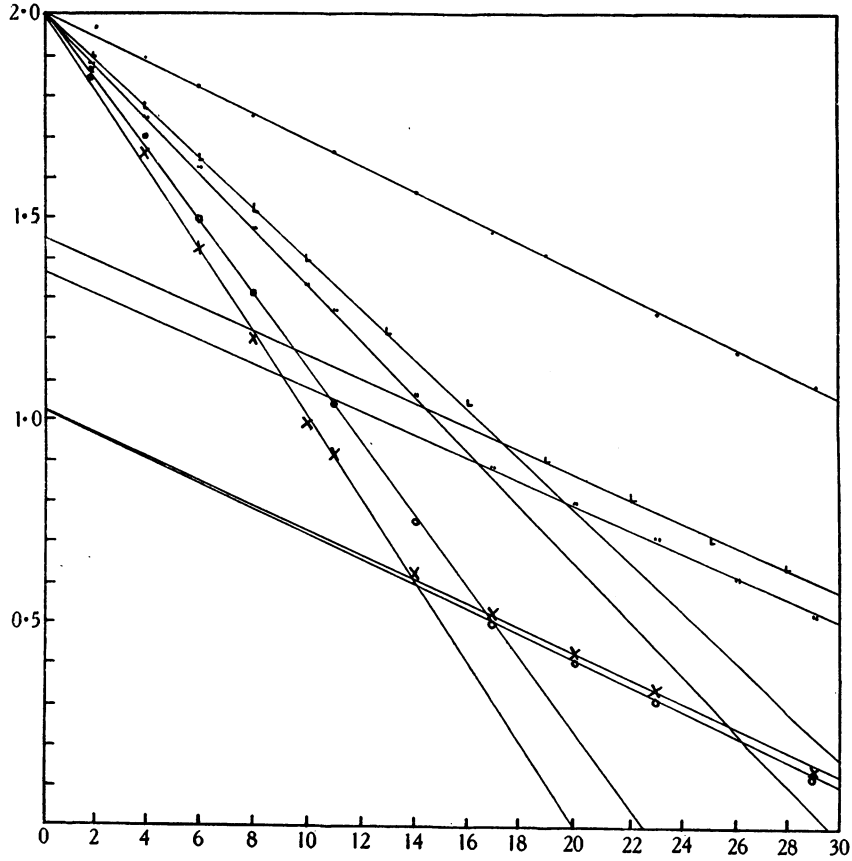

Fig. 1. Rate of alkali denaturation of adult and fœtal goat's hæmoglobin. ... adult hæmoglobin; × × × 2 days after birth; ooo 9 days after birth; : : : : 16 days after birth; LLLL 23 days after birth. Vertical axis: log percentage unchanged Hb. Horizontal axis: time in minutes.

buffer solutions of different *p*H is characteristic, and maximal in the isoelectric point of the protein examined. In Fig. 2 the spreading velocity curves of fœtal or adult hæmoglobins of various mammals are shown. The maximal surface is the same for all hæmoglobins, namely 1·15 square metres per mg. The spreading velocity at the isoelectric point is very

different however and it is characteristic of the species. In man, and in the rabbit, the isoelectric spreading velocity is larger in the adult, but smaller in the fœtal type. In the cow and the goat the situation is reversed, the fœtal type having the quicker spreading. The change from

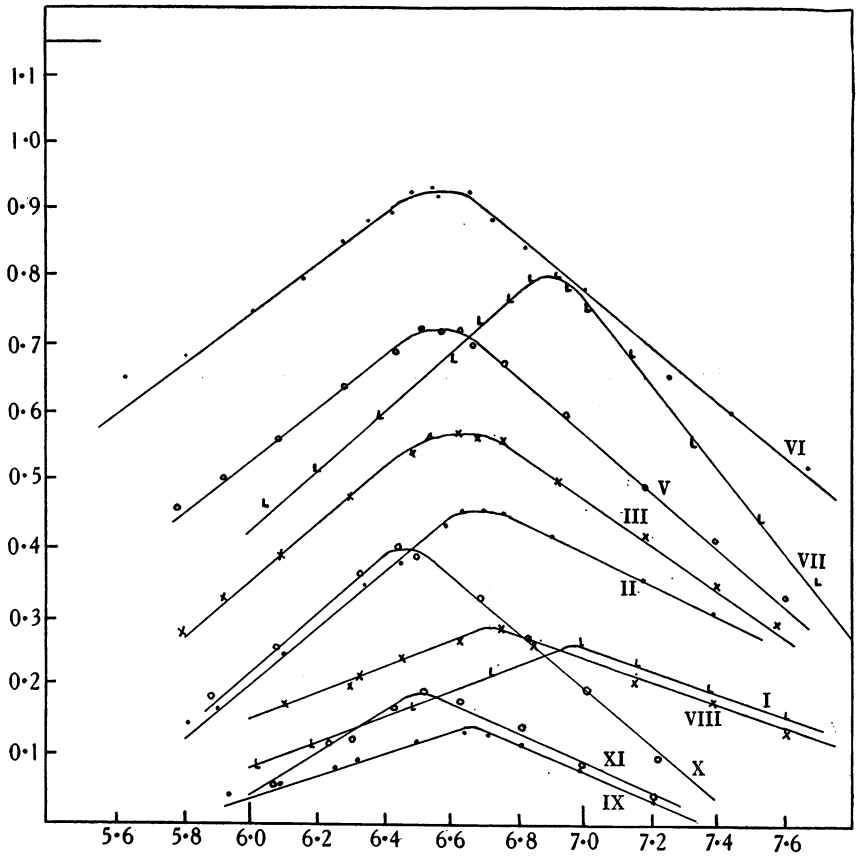

Fig. 2. Spreading of hæmoglobin of adult goat (I), fœtal goat (VI), 2 days old goat (V) 9 days old goat (IV), 16 days old goat (III), and 23 days old goat (II). Adult rabbit (VII), fœtal rabbit (VIII), cow (IX), newborn calf (X), and cat (XI). Vertical axis: surface of 1 mg. of hæmoglobin in square metres after 1 min. of spreading at 20° C. Horizontal axis: *p*H of buffer solutions.

the fœtal type to the adults can also be observed. In the cat no differences between fœtal and adult hæmoglobin can be seen.

It will be clear that these results correspond very well to the conclusions from denaturation curves, and further that a parallel may be drawn with the distinction of fœtal and adult oxygen dissociation curves.

In man the fœtal oxygen dissociation curve of pure hæmoglobin is to the right of the adult form, in the goat the relative positions are reversed, just as it is described now for the above mentioned properties.

SUMMARY

By measuring the reaction velocity of alkali denaturation and the spreading velocity in the isoelectric point, it has been shown that in various kinds of mammalian blood the fœtal and adult hæmoglobin are different, as was formerly found for human blood.

This confirms the same conclusion drawn already from the respective dissociation curves by Barcroft.

REFERENCES

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