THE SPECIFICITY OF HÆMOGLOBINS INCLUDING EMBRYONIC HÆMOGLOBIN

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Many attempts have been made in the last fifty years to establish finally the specificity of the hæmoglobins of different animals. These hæmoglobins derived from various sources manifest their variations by differences in:—

- i. Crystalline form (Reichert and Brown).
- ii. Solubility.
- iii. Serological precipitation (Bordet reaction).
- iv. Affinity for CO and CO₂ (Boor and Hektoen¹).
- v. Time of decomposition by acids and alkalies.

The present investigation is mainly concerned with the fifth point, which is illustrated by the application of von Kruger's reaction to the various bloods under discussion.

Korber in 1866 had already made some experiments to prove that the blood pigment of different species of animals was decomposed at varying speeds by acids and alkalies, but deficiencies in his methods failed to convince the scientific authorities of his time, and it was left to von Kruger to work out a satisfactory practical method for the demonstration of the time factor by means of the direct-vision spectroscope. Through this instrument he viewed dilute solutions of the oxyhæmoglobin of different animals and estimated the time of its decomposition by dilute sodium hydroxide, by simply noting the time of the disappearance of the two typical absorption bands. The change over of oxyhæmoglobin into alkaline hæmatin is also accompanied by a physical colour change from bright red to yellow, but this does not give a sufficiently definite end-point for accurate experiments, especially with the more lengthy and drawn-out rates of decomposition. By spectrophotometric methods² these times of disappearance of the α and β bands of oxyhæmoglobin (Centres 578λ and 540λ) can be more exactly noted, and it has been found that the time factors obtained in von Kruger's reaction by a skilled observer, corresponds roughly to a 90-100 per cent. change of oxyhæmoglobin into alkaline hæmatin. If independent exact values are desired the readings at a 90 per cent. change can be constantly obtained by means of precision spectrographs, but for the experimental work described below the simplicity of the original direct-vision spectroscope method is to be preferred since it is quite adequate to demonstrate the essential differences discovered.

As a preliminary, tests were made on the oxyhæmoglobin of the human adult, the sheep, the cow and the rabbit. Afterwards oxyhæmoglobin from patients with various pathological conditions was taken and tested, and finally oxyhæmoglobin from the human placenta and from the new-born infant.

METHOD.—About 1 c.cm. of blood is withdrawn and hæmolyzed by putting it into 10 c.cm. distilled water. The percentage of hæmoglobin is estimated at the same time separately in a Sahli-Leitz hæmoglobinometer. By calculation the hæmolyzed solution is diluted to a .1 per cent. concentration of hæmoglobin and filtered. Five c.cm. of the diluted solution is then poured into a clean test-tube which is clamped into position in front of the direct vision spectroscope previously adjusted to show the complete solar spectrum.

The α and β absorption bands are then sharply focussed before beginning the experiment.

One c.cm. of N/4 sodium hydroxide is then added to the solution without removing it from its clamp, and the moment of addition is measured on a stop-watch.

The fading of the two absorption bands is then watched narrowly through the spectroscope, and the final moment of disappearance is read off on the stop-watch. The end-point is very definite in the cases of quickly decomposed oxyhæmoglobin, but is rather indefinite in some of the lengthier observations. In these cases it is sometimes convenient to keep a tube at hand in which the reaction has been completed, and to substitute the tube for a moment or two as a control for comparison with the experimental tube.

Results.—For normal adult human blood the decomposition is complete in less than a minute and can be observed in its entirety; but for the oxyhæmoglobin of other types of animals, in which the decomposition takes anything from forty minutes to more than twenty-four hours, observations can only be taken at stated intervals. Some of these results are shown in Table 1.

TABLE 1.
TESTS ON VARIOUS TYPES OF BLOOD.

Type of blood	Time of decomposition
Normal adult human blood	40-60 seconds
Rabbit's blood	20-28 minutes
Sheep's blood	More than 5 hours
Placental human blood	40-60 minutes
Blood from patients with pernicious anæmia	40-60 seconds
Blood from patients with various pathological conditions, diabetes, nephritis, meningitis, etc.	40-60 seconds

All the readings shown in Table 1 were taken with daylight as the source of light. Readings taken with artificial light were more indefinite and were finally discarded.

For adult human blood, about 20 specimens were taken and the average of these readings (40 to 60 seconds) taken as a range for normal standard.

PLACENTAL BLOOD.—With the placental blood specimens, the end-point was difficult to fix. Readings were made from 26 specimens and were found to vary from 40 to 70 minutes, with an average of 53 minutes. In every case, whether the readings were 40 or 70 minutes, the decomposition takes definitely a much longer time than is the case of normal adult human blood, thus indicating that in the embryonic condition the blood-pigment of the human differs radically from the normal adult human blood pigment.

Whether the different human hæmoglobin, which for the sake of distinction we may designate as hæmoglobin-*a*, constitutes the whole of the blood pigment of embryonic blood or only a certain proportion, cannot be definitely settled by means of the experiments described.

Haurowitz³ has already advanced the view that the hæmoglobin-*a* is a percentage only of the total pigment present in placental blood, but until experiments are devised for its isolation, separation and identification by means of any of its specific characters, the proportion cannot be regarded as definitely proved.

MATERNAL BLOOD.—The next step after satisfactorily proving the presence, even if not the proportion, of hæmoglobin-*a* was to find out if it was contained in the maternal blood.

In none of the cases examined, however, was the mother's blood found to differ from that of the normal human adult in its response to the von Kruger's reaction (Table 2). Its average reading was 40 seconds.

TABLE 2.
COMPARISON OF PLACENTAL AND MATERNAL BLOOD.

Case No.	Time of decomposition	
	Placental blood	Maternal blood
1	40 minutes	45 seconds
2	40 "	38 "
3	50 "	45 "
4	40 "	35 "
5	50 "	38 "

BLOOD OF NEW-BORN.—After this, it was decided to test the blood of new-born babies in order to see if hæmoglobin-*a* appeared in early extra-uterine life, and to see if possible at what age approximately the hæmoglobin-*a* disappeared from the circulation and was entirely replaced by the normal adult human type of blood pigment.

The experimental details were slightly modified as follows:—

Twenty mm. of blood were withdrawn from the heel of the child and placed into 1 c.cm. of distilled water. At the same time the hæmoglobin percentage was estimated separately as before, and finally by calculation the hæmolyzed solution was diluted to a .1 per cent. concentration of hæmoglobin. One-fifth volume of sodium hydroxide was added as before and the time of decomposition noted with the spectroscope.

TABLE 3.
READINGS OF NEW-BORN INFANTS' BLOODS.

Name	Age	Time of decomposition
Hale	2 hours	45 minutes
Carney	4 hours	60 minutes
Harborne	1¼ hours	45 minutes
Green	16 hours	40 minutes
Hinckley	18 hours	30 minutes
Wharton	19 hours	45 minutes
Howard	24 hours	36 minutes
Perry	24 hours	60 minutes
Silvester	2 days	30 minutes
Poole	3 days	55 minutes
Davies	4 days	49 minutes
Deeley	5 days	47 minutes
Jones	6 days	43 minutes
Key	7 days	48 minutes
Wiltiey	8 days	50 minutes
Green	16 days	40 minutes
Green	31 days	38 minutes
Kingston	1½ months	35 minutes
Hughes	4½ months	55 seconds
Fellowes	5 months	40 seconds
Thomas	6 months	40 seconds

It will be seen by the figures given in Table 3 that hæmoglobin-*a* persists in the circulation of the new-born child for some time after birth, definitely for one month at least. By 4½ months, however, there is no trace remaining and all the hæmoglobin is of the adult human variety.

According to Haurowitz³, the differences in these various hæmoglobins depends on the specificity of the globin components and not on the different linkages of the prosthetic group. Barcroft has also advanced views in complete accordance with the theory, but the discussion of this problem lies rather outside the scope of this experimental investigation.

BLOOD DISEASES.—It was thought that perhaps in some of the pathological blood conditions—notably pernicious anæmia in which disease a theory has been advanced of a return to embryonic blood conditions—traces of the hæmoglobin-*a*, or other types of hæmoglobin, would be discovered, but such has not been the case.

In conclusion, I have to thank the Staff of the Maternity Block at Dudley Road Hospital for their courtesy in allowing me access to their patients, and for their assistance in helping me to obtain specimens of blood for investigation.

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