INTRAVASCULAR HEMAGGLUTINATION

EXPERIMENTAL AND CLINICAL OBSERVATIONS, WITH SPECIAL REFERENCE TO THE PATHOGENESIS OF KERNICTERUS

By Richard Day, M.D., and Elise Perry, A.B.

ERYTHROBLASTOSIS fetalis is different from all other hemolytic diseases in one important way: severe damage to nervous tissue, i.e., kernicterus¹ is a frequent complication. At autopsy the basal ganglia, hippocampal gyri and other areas of gray matter are stained yellow. Microscopic inspection in cases where death occurred at 3 or 4 days of age may reveal no changes from the normal, but in the occasional infant who lives ten days or more one can see degenerating nerve cells in the pigmented areas.² Kernicterus is a postnatal phenomenon. It is not seen in stillborn infants, and the premortem clinical manifestations are invariably absent at birth. For some hours before death, however, one sees opisthotonous, spasticity and slow respirations. There is little or no correlation between the degree of anemia and the occurrence of kernicterus. Of infants who survive erythroblastosis fetalis, about 10 per cent have spastic paralysis and mental deficiency.³ In our series, more than half the autopsied cases of erythroblastosis fetalis were complicated by kernicterus. Since about one newborn infant in 200 develops erythroblastosis, the condition rivals in importance many others which have received more attention.

The pathogenesis of kernicterus is entirely unknown. One theory that has received much attention is that of Wiener.⁴ He postulates that the Rh agglutinin produces intravascular agglutination of red cells which in turn impairs capillary circulation and causes tissue anoxia. The distribution of the lesions in the brain is somewhat similar to that in anoxia resulting from carbon monoxide poisoning. Although Wiener described one infant in whom small thrombi were seen at autopsy, it would not be necessary to expect actual thrombi. Rather, the intravascular agglutination could consist of loose clumps of cells which can break up, resulting in a situation analogous to the sludged blood described by Knisley⁵ in malaria and other diseases. Since the only known property of the Rh agglutinin is that of agglutinating red cells, the theory is especially attractive. According to Wiener,⁴ it forms the basis for the rationale of exchange transfusion.

Although our results are negative, thus failing to support the agglutination theory, they are presented because of the importance of the subject and because of their possible bearing on other diseases in which sludged blood,⁶ analogous to hemagglutination, does occur.

We have two types of information to present which bear on the subject. Experimentally, we have found that intravascular hemagglutination in the jaundiced rat does not result in kernicterus. Because of species differences such a failure can-
not be considered decisive. However, observations with a microelectrode disclose no significant fall in tissue oxygen tension in these rats. Clinically, in a human subject (an 11 month old infant with acquired hemolytic anemia) conspicuous intravascular hemagglutination was not associated with any evidence of mental or central nervous system damage. Also, we failed to demonstrate significant hemagglutination in a 4 day old infant dying with kernicterus.

Observations on Rats

Technic

Preparation of the serum. Normal rabbits were given three intravenous injections per week for two weeks of 0.5 ml. of a 50 per cent saline suspension of thrice-washed rat erythrocytes.

Dosage of serum. The anti-rat rabbit serum was administered intravenously to the rats to produce intravascular red cell agglutination. The same lot of serum was used for all the rats. It had a titer of 1:3000. In order to allow for the varying weights of the rats, our dosage is expressed in arbitrary units. These units represent the titer that one would expect if the administered serum were diluted in neutral serum equal in volume to the estimated total blood volume.

Preparation of the rat for tissue oxygen measurement. The rats were anesthetized with 4.5 mg. of Nembutal per 100 grams of body weight. The scalp was removed without bleeding in order to expose a suitable capillary bed. The head was selected for the observations only because it can easily be immobilized, and not because kernicterus occurs in the brain. A 20 power dissecting microscope was placed so that the exposed tissue and the placement of the platinum electrodes could be observed.

Oxygen tension measurements. For this purpose we used the platinum electrode method developed by Davies and Brink for measuring the oxygen tension of the brain of cats during metrazol convulsions. 6 Our electrode was 50 microns in diameter. All but the smooth, flat tip of the wire was insulated by a glass sheath. According to Davies and Brink, only oxygen is electrolyzed when a potential of 0.7 volts is used. Variations in the supply of oxygen to the electrode result in variations in the current. The latter are indicated by changes in the galvanometer reading. The supply of oxygen is dependent upon the oxygen tension, the diffusion characteristics of the tissue for oxygen and the area of platinum exposed to tissue. Of these factors, only the oxygen tension varies significantly in a single experiment. The type of electrode used does not permit an absolute calibration. According to Davies, 7 however, changes in galvanometer reading occur with a linear relation to changes in oxygen tension. We have used a biologic calibration, expressing galvanometer readings in per cent of the baseline galvanometer deflection. We found that when the rat breathes a 10 per cent oxygen 90 per cent nitrogen mixture the galvanometer deflection regularly falls to about half the baseline reading. When 100 per cent oxygen is administered by face mask the deflection increases to two and one-half times the baseline deflection. Such a large change is in accord with the fact that the instrument is responding to oxygen tension and not to total dissolved oxygen.

The oxygen of the air was prevented from influencing the electrode by placing the tip of the electrode in a small saline filled hole drilled in the calvarium down to but not through the dura.

The platinum tip could not be visualized to see how close it might lie to arterioles or venules. Since there was little variation in readings when the electrode was shifted in position, it is likely that an average sample of tissue was measured with an electrode of this size. It could be argued that the apparatus might not record a change in tension in the event that the tension should at the same moment rise in arterioles and fall in tissue spaces. If such a situation actually does arise, one would expect a decline in the delivery of oxygen to tissues, and therefore a decline in the total oxygen consumption of the rat. We have therefore also measured the total oxygen consumption in other rats given similar doses of agglutinating serum.

Total oxygen consumption. For this measurement we followed the advice of Grant 8 and set up a spirometer chamber similar to that described by Hanan. 9 The extent of intravascular agglutination was observed in the conjunctival vessels after the administration of the serum and again after removal of the rat from the chamber.
Results of the Observations on Rats

Effect of the serum on survival and on the blood. Table 1 indicates that about 30 units of agglutinating serum on our arbitrary scale is the dividing line which separates the usually fatal from the usually nonfatal doses. Kernicterus was not seen in these rats nor in others which were rendered very jaundiced by the injection of bilirubin in addition to serum. Some jaundice of the skin and sclerae was noted even without the added bilirubin. So far as anemia was concerned, our results paralleled those of Dameshek and Schwartz. Table 2 relates the anemia to the dose of serum.

Table 1.—Survival with Different Doses of Anti-rat Red Cell Rabbit Serum Injected Intravenously into Adult Rats

<table>
<thead>
<tr>
<th>Dosage range</th>
<th>Number of rats</th>
<th>Number surviving</th>
</tr>
</thead>
<tbody>
<tr>
<td>units</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 to 25</td>
<td>23</td>
<td>13</td>
</tr>
<tr>
<td>25 to 30</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td>30 to 35</td>
<td>9</td>
<td>3</td>
</tr>
<tr>
<td>35 to 40</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>40 or more</td>
<td>6</td>
<td>0</td>
</tr>
</tbody>
</table>

Table 2.—The Effect on the Red Blood Cell Count of Different Nonfatal doses of Anti-rat Red Cell Rabbit Serum Injected Intravenously into Adult Rats

<table>
<thead>
<tr>
<th>Rat Number</th>
<th>Dose of serum</th>
<th>Control RBC (millions per mm.³)</th>
<th>Minimum RBC</th>
<th>Day of minimum RBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>units</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.</td>
<td>8</td>
<td>7.4</td>
<td>6.7</td>
<td>10th</td>
</tr>
<tr>
<td>2.</td>
<td>9</td>
<td>7.6</td>
<td>6.5</td>
<td>6th</td>
</tr>
<tr>
<td>3.</td>
<td>13</td>
<td>7.9</td>
<td>4.3</td>
<td>4th</td>
</tr>
<tr>
<td>4.</td>
<td>16</td>
<td>7.8</td>
<td>4.3</td>
<td>4th</td>
</tr>
<tr>
<td>5.</td>
<td>16</td>
<td>7.0</td>
<td>3.7</td>
<td>2nd</td>
</tr>
<tr>
<td>6.</td>
<td>17</td>
<td>7.9</td>
<td>2.5</td>
<td>3rd</td>
</tr>
<tr>
<td>7.</td>
<td>20</td>
<td>6.6</td>
<td>1.1</td>
<td>3rd</td>
</tr>
<tr>
<td>8.</td>
<td>23</td>
<td>8.3</td>
<td>1.5</td>
<td>3rd</td>
</tr>
</tbody>
</table>

An important feature of the injected rats was their invariably rapid sedimentation rate and the fact that their red cells were seen to be agglutinated in vitro when suspended in their own serum.

Despite the in vitro autohemagglutination and the intravascular hemagglutination noted during life, which will be described below, postmortem examination of the tissues of serum injected rats did not disclose any thrombi either in the brain or elsewhere.

Intravascular hemagglutination. We have observed intravascular hemagglutination in the sclerae, in the tissue exposed by the removal of skin and in the mesentery.

* We have not been able to reproduce the results reported by Dereymaeker who found yellow staining of the brains of kittens and newborn rats injected with anti-red cell rabbit serum.
It seems justifiable to assume that it is present in the entire vascular tree. Figure 1 is a photomicrograph of the mesentery of a normal anesthetized rat, taken at 1/500 of a second. The vessels are filled with a continuous stream of cells which move in a manner fulfilling the criteria of normality stressed by Knisely. Figure 2 illustrates the scene after a rapidly fatal dose (80 units) of serum was administered intravenously. In some of the capillaries flow ceased and in others became intermittent. Large gaps are seen in the red cell columns in vessels of all sizes in the photograph. These groups of cells were seen to move as a plug.

We have used a 4 point scale to grade the severity of the intravascular hemagglutination. The degree of severity shown in figure 2 would be rated as +++++.

On our scale, + agglutination is present when flow can be seen clearly with 20 powers of magnification. We find normal flow to be difficult or impossible to see with this magnification because of the rapidity and steadiness of the stream. On our scale of severity, ++ and +++ indicate intermediate degrees of agglutination. As we interpret the work of Knisely, who used a more powerful microscope, he would consider our + degree of agglutination to correspond to "sludging" of severe degree. Whether sludging in the sense used by Knisely is physiologically the same thing as the intravascular agglutination seen in the injected rats is, of course, not certain. The microscopic appearance in the two conditions is very much the same.

Tissue oxygen tension. Figures 3, 4 and 5 are samples of the results obtained with different doses of antiserum. In figure 3, the rapid decline in oxygen tension and
Fig. 2.—A section of mesentery of the same rat as that illustrated in figure 1. A large dose (80 units) of anti-red cell rabbit serum has been given intravenously. Other vascular areas present the same appearance.

Fig. 3.—The changes in oxygen tension in the tissues as measured by the method of Davies and Brink. In the case of the rat illustrated, a large and rapidly fatal dose of anti-red cell serum produced a profound fall in tissue oxygen tension. The sharp peak and trough in the curve prior to the injection of serum were the result of the administration first of 100 per cent oxygen by face mask and then of 10 per cent oxygen and 90 per cent nitrogen.
the diminished response to administered oxygen were succeeded so quickly by the death of the animal that interpretation is difficult. Since the animal was moribund it is possible that the changes in oxygen tension were only indirectly the result of the serum. In the experiment illustrated in figure 4, the nonfatal dose of 23 units

![Graph](image)

**Fig. 4.**—The effect of 23 units of anti-red cell serum on tissue oxygen tension. Such a dose is not quite large enough to be fatal, but always produces marked sludging. The sludging continued despite the fact that tissue oxygen tension returned to normal. A second dose of 12 units had no effect on the oxygen tension.

![Graph](image)

**Fig. 5.**—The course of tissue oxygen tension in a rat given a succession of 16 unit doses of anti-red cell serum.

was followed by a prompt but transitory and slight decline in oxygen tension. Agglutination of grade +++ to ++++ was present, and continued during the prompt recovery of the oxygen tension to baseline values. Even when a second dose of 12 units was given, raising the total dose to a fatal level, there was no further change in tension or in response to administered oxygen. With such a total dose (35 units) death usually occurs in some twelve to twenty-four hours. In this
experiment, the animal was sacrificed by means of an overdose of ether in order to illustrate the decline of oxygen tension to zero at time of death. In figure 5 it can be seen that a series of doses each of which alone is sufficient to produce some

Table 3.—The Effect on Tissue Oxygen Tension of Different Doses of Anti-rat Red Cell Rabbit Serum Injected Intravenously into Adult Rats

<table>
<thead>
<tr>
<th>Rat Number</th>
<th>Dose of serum</th>
<th>Sludge</th>
<th>Reduction in tissue O₂ tension below that at the start (in per cent)</th>
<th>Recovery of tissue O₂ tension</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>12</td>
<td>++</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>2.</td>
<td>23</td>
<td>++</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3.</td>
<td>25</td>
<td>+</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>4.</td>
<td>17 given 3 times at 1-minute intervals</td>
<td>+++ at start</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td></td>
<td>51 U total</td>
<td>++</td>
<td>30</td>
<td>To 10% below normal in 10 minutes</td>
</tr>
<tr>
<td>5.</td>
<td>36</td>
<td>++++</td>
<td>30</td>
<td></td>
</tr>
<tr>
<td>6.</td>
<td>38</td>
<td>++++</td>
<td>30</td>
<td>To normal in 100 minutes</td>
</tr>
<tr>
<td>7.</td>
<td>66</td>
<td>++++</td>
<td>35</td>
<td>To normal in 20 minutes</td>
</tr>
<tr>
<td>8.</td>
<td>30</td>
<td>++++</td>
<td>35</td>
<td>Died</td>
</tr>
</tbody>
</table>

Fig. 6.—The oxygen consumption of normal rats anesthetized with Nembutal. There is often some variation during the course of three hours, which is usually related to the depth of anesthesia.

agglutination has no effect on the tension of oxygen until just before death. Sludging appeared after the first dose and became more pronounced after each injection of serum.

Table 3 presents a summary of the results in similar observations on other rats.
It is concluded from the observations on rats that severe degrees of sludging can be tolerated without any fall or with only a slight and transitory fall in tissue oxygen tension. Rats given rapidly fatal doses of agglutinating serum show a profound decline in tissue oxygen tension some minutes before death. Rats given bilir...
rubin as well as serum do not develop jaundice of the basal ganglia or other parts of the brain.

**Total oxygen consumption.** Figures 6, 7, 8 and 9 illustrate typical observations of total oxygen consumption. It is apparent from inspection of these curves that the total oxygen consumption responds to agglutinating serum in the same way that tissue oxygen tension does. The declines are absent, slight or transitory except in the case of a rat about to succumb. Sludging of grades ++ or +++ is tolerated well.

![Diagram of total oxygen consumption and oxygen tension](attachment:diagram.png)

**Fig. 9.**—The effect of anti-red cell serum in small dosage. All 3 of the rats showed sludged blood and all lived. The rise in oxygen consumption in Rat J was thought to be the result of inadequate anesthesia.

**Observations on Infants**

*Case 1. J. H., an 11 month old girl with chronic acquired hemolytic anemia who had intravascular agglutination but did not develop kernicterus. Her illness began at 9 months. Her blood was characterized by moderate spherocytosis, a strongly positive Coombs test, indirect bilirubinemia varying in degree from week to week, severe anemia and moderate thrombocytopenia. For the first few weeks of her illness she failed to show evidence of even normal blood regeneration but later she displayed an exceedingly high level of reticulocytes. Her serum had the ability to agglutinate weakly all types of human red cells in albumin or serum but not in saline. Her own red cells showed spontaneous agglutination in her own serum and in albumin. Her erythrocyte sedimentation rate was 115 mm. in one hour. It was thought that this elevation in rate was the result of in vitro erythrocyte aggregation. Splenectomy was without effect.

Capillary microscopy was performed when she was in quite good condition except for pallor and a slightly icteric skin. Her mentality was normal, and there were no manifestations of abnormality of the nervous system. The network of small surface vessels of the sclerae and of the auricle revealed red cell agglutination of +++ severity on our scale. Plugs of clumped cells were seen to travel separated from other clumps by clear areas. Occasionally, at a branching, a plug would stick for a moment and then go on through. One cannot describe the appearance without using the same language as Knisely in his description of the most severe sludging as seen in cerebral malaria.
Case 2. K. B., a 4 day old infant with kernicterus who did not have significant intravascular hemagglutination. She was first seen at 2 days of age because of severe jaundice which had developed at 12 hours of life. She had a normal older brother and sister. Her mother was Rh negative and had an anti-Rh titer of 1:32 in albumin and 1:2 in saline. The infant’s cells were Rh positive, and yielded a positive Coombs test, but did not agglutinate in albumin or in her own serum or plasma. Her erythrocyte sedimentation rate was only 1 mm. in one hour, a finding in contrast with the rapid rate in Case 1 and in the injected rats. She had an indirect bilirubin concentration in her serum of 40.1 mg. per 100 cc. At 48 hours of age, without any treatment having been given, her hemoglobin concentration was 16 grams per 100 cc. and her red cell count 5.3 million per cu.mm. Twenty-four hours later the hemoglobin was 10.5 grams and the red cell count 3.01 million. At that time she presented the physical signs of kernicterus: opisthotonos, spastic type of Moro response, slow respirations and loss of appetite. Later, bloody mucus came from her lungs. She died at 13 days of age. Autopsy revealed the usual findings in erythroblastosis fetalis complicated by kernicterus.

On the fourth day of life when the signs of kernicterus were pronounced, we were able to visualize the small vessels of the surface of her conjunctivae and in the skin of her auricle. Flow could be detected with the 20 power microscope, and therefore was categorized as + on our scale. The appearance of the flow was not different from that which might be seen in an infant severely ill with any disease; in other words, it could be characterized as showing some sludging, but it did not show anything like the agglutination seen in the rats and in the other patient. Further support for the opinion that such agglutination was not taking place lies in the failure of her cells to agglutinate in her own serum and in the slow sedimentation rate of her blood. In the rats and in the other patient where agglutination was present, the sedimentation rate was extremely rapid.

Comment

The intravascular hemagglutination theory of the pathogenesis of kernicterus implies that there is anoxia of the brain as a result of the agglutination. To confirm the theory it would be desirable to show that a fall in tissue oxygen tension does follow the occurrence of intravascular agglutination and that such agglutination does in fact take place in babies who develop kernicterus. In addition, it would be evidence against the theory if such agglutination of red cells were shown to be present in jaundiced animals or babies who failed to develop kernicterus. The observations presented in this communication bear on each of these questions, and in the case of each of them the results clearly fail to substantiate the theory. This negative evidence, of course, goes no further than circumstantial evidence of this type can go. We have not been able to devise a way to answer the following crucial question: What is the state of the supply of blood and oxygen to the basal ganglia in a newborn infant during the development of kernicterus? Until that question is answered, or until the cause of kernicterus is shown to lie in some entirely different mechanism, the pathogenesis of kernicterus will remain incompletely explained.

Summary

Severe degrees of intravascular red cell agglutination have been observed during life in rats injected with anti-rat red cell serum and in a patient of 11 months with chronic acquired hemolytic anemia. There was no significant fall in the tissue oxygen tension or in the total oxygen consumption of the rats. Neither the rats nor the infant developed kernicterus. In a baby dying with kernicterus no true hemagglutination was observed, although there was slight sludging such as is seen in many illnesses. Additional support for the belief that the red cells in
this case were not clumped lies in the fact that in vitro clumping was not observed; furthermore, the sedimentation rate was only 1 mm. in one hour. The blood of the injected rats and of the other infant who did not develop kernicterus sedimented extremely rapidly and displayed spontaneous agglutination in vitro. These observations indicate that intravascular agglutination has little if any bearing on the development of kernicterus in erythroblastosis fetalis.

REFERENCES

3. Wolf, Abner: Personal communication.
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