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Neonatal Hyperbilirubinaemia

by

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Neonatal hyperbilirubinaemia or severe jaundice in the newborn is important in that the bile pigments may damage brain cells giving rise to kernicterus resulting in death in 85% and physical and mental crippling in the remaining 15% (Wong, 1960a) in Singapore. The significance of this problem is further increased by the fact that after excluding prematurity, birth injuries and anoxia immediately after birth, kernicterus is the commonest cause of death in the first week of life in this country. Workers in the West generally find this fact hard to understand because haemolytic disease of the newborn due to Rh incompatibility is the commonest cause of neonatal hyperbilirubinaemia there and it is well known that the Rh negative rate is almost nil among the Malays and less than 1% among the Chinese and about 3-5% among Indians; while it is 15% in the Europeans. Referring to Table I which depicts the causes of neonatal hyperbilirubinaemia in 136 patients in a centre in Philadelphia (Boggs, 1960), compared to the causes seen in 139 patients in 1960 in Singapore, it will be seen that approximately ½ is due to Rh

Table 1

	Philadelphia (1959) 136 patients	Singapore (1960) 139 patients
Rh incompatibility	50%	3%
ABO incompatibility	27%	28%
Others	23% (mainly prematurity)	69%

incompatibility and ¼ each due to ABO incompatibility and prematurity, while about 70% of the cases seen here had no known cause, i.e. known in Western countries. This high incidence of hyperbilirubinaemia and kernicterus in Singapore was first stressed by Wong (1957), when he described 26 cases of kernicterus not due to Rh or ABO incompatibility or prematurity. At that time he postulated that sepsis or hepatic dysfunction as possible causes of these then unknown type of kernicterus. Since then more work has been done in the Department of Paediatrics to elucidate the cause of the hyperbilirubinaemia in these infants.

Bile Metabolism:

To tackle the problem in a logical manner an understanding of bile metabolism is necessary. Although the existence of two types of bile pigments in blood had been suspected by Hijmans van den Bergh in 1916 when he devised the well known van den Bergh reaction, it was not till 40 years later in 1956 that almost simultaneously Schmid (1957), Billing, Cole and Lathe (1957) and Talafant (1956) deduced that the indirect bilirubin (insoluble) was derived from the breakdown products of haemoglobin which was then conjugated in the liver to the direct bilirubin or bilirubin glucuronide which is soluble and then excreted in the kidneys. The indirect bilirubin is the toxic pigment which damaged the brain cells in kernicterus while the direct bilirubin is non-toxic so that the liver is a detoxifying organ in this respect.

The final essential process of conjugation in the liver is shown in the following reactions:—

Uridine diphosphate glucose (UDPG)
+
2 Diphosphonucleotide (DPN)
|
UDPG Dehydrogenase
|
V
UDP Glucuronic acid + 2 DPNH + $2H^+$

UDP Glucuronic acid + Bilirubin

↓ Glucuronyl transferase

Bilirubin diglucuronide + UDP

There is first an oxidation of UDPG with the aid of the enzyme UDPG dehydrogenase to form UDPG glucuronic acid which is the essential substrate, which then reacts with indirect bilirubin, which in turn is conjugated with the aid of the enzyme glucuronyl transferase to direct bilirubin or bilirubin diglucuronide. This enzyme glucuronyl transferase (Schmid, 1957) is resident in the microsomes of the hepatic parenchymal cells. This glucuronide conjugating mechanism is also responsible for the detoxification of a number of pharmacologic drugs such as salicylates, chloramphenicol, N-acetyl-p-aminophenol (Tempra) etc. and the fact that newborn infants especially prematures may suffer toxic effects from these drugs is essentially due to the immaturity of this glucuronide conjugating mechanism. Similarly, the so-called "physiological jaundice" of the newborn is the result of this hepatic immaturity (Brown and Zuelzer, 1958; Lathe and Walker, 1958).

Physiological Jaundice:

This term was coined to describe the mild jaundice seen in many newborns appearing on the second day of life and persisting for 2-3 days and subsiding by the sixth day. The incidence varies in different hospitals but in the Kandang Kerbau Hospital observation of over 700 newborns for a period of one week revealed that nearly 100% of them had visible jaundice (Wong, 1960 b.) This so-called physiological jaundice occurs because the Newborn liver is unable to clear the bilirubin in contradistinction to the fully matured liver. Approximately 1 Gm. of haemoglobin on breakdown yields 34 mg. of bilirubin (Crosby, 1955). In the human about 1% of the Hb mass is destroyed per day. A 7 lb. newborn infant may have a Hb of about 18 Gm. per 100 ml.; but it has a blood volume of about 300 ml.; therefore its Hb mass is 54 Gm., so that there is destruction of 1% of 54 Gm., i.e. about 0.5 Gm. of Hb per day. Hence, the newborn infant with physiological jaundice has a liver that can handle approximately 17 mg. of bilirubin per day (i.e. 0.5 x 34 mg.). By comparison the adult liver can deal with about 250 mg. of bilirubin a day.

Therefore, this physiological jaundice can be increased under the following conditions:—

(a) Greater destruction of haemoglobin, i.e. **Haemolytic Disease.**

(b) Greater immaturity of the glucuronide conjugation mechanism, i.e. conditions which interfere with this already immature system.

Therefore in any consideration of hyperbilirubinaemia of the newborn, excessive destruction of red cells is not the only cause but the liver has to be taken into consideration. Because of the failure of recognition of this liver group of causes, it has been assumed that if haemolytic disease can be excluded, then all jaundice of the newborn is "physiological" and because it is "physiological" no harm can occur, and since Rh disease is rare in this area, therefore kernicterus should be rare. It is this false reasoning that has made the acceptance of kernicterus as an important problem so difficult by many doctors in this country.

Concept of Aetiology of Hyperbilirubinaemia and Kernicterus:

Hyperbilirubinaemia can occur if there is a greater destruction of erythrocytes or if there is a depression of the glucuronide conjugating mechanism over and above that of the normal transient immature liver system. Implicit in this concept therefore kernicterus will occur only if the serum bilirubin is high, usually more than 20 mg.%. However, it was soon realised that a few cases of kernicterus occurred with serum bilirubin of 15 mg.% or less and these cases had a history of cerebral anoxia during delivery so that cerebral anoxia may facilitate transference of indirect bilirubin across the blood-brain barrier or that anoxic brain cells are more easily damaged by exposure to smaller concentration of bilirubin than healthy cells.

Therefore, hyperbilirubinaemia and hence kernicterus can occur as a result of pathology at one of 3 levels:—

(a) Erythrocytic
(b) Hepatic
(c) Cerebral

The rare cerebral cause has been mentioned above and will not be discussed further. The erythrocytic and hepatic causes were investigated as fully as possible in an attempt to elucidate the aetiology in this country.

Erythrocytic causes:

(a) **Rh incompatibility**: This is well known but rare in this country. It occurs in an Rh

positive infant with an Rh negative mother. The Rh positive foetal cells cause the formation of Rh antibodies which recross the placenta and haemolyse the Rh positive cells of the foetus with consequent hperbilirubinaemia and haemolytic anaemia. Here, the first-born usually escapes as sensitization takes time unless the Rh negative mother had been given Rh positive blood in previous transfusions. The baby may be still-born with hydrops foetalis, or develop kernicterus as a result of the hyperbilirubinaemia, or if mild present as a problem in neonatal haemolytic anaemia.

Diagnosis is made from the possible previous history of involved babies, stillbirths, kernicterus, etc. Racially, Europeans and Indians are the ones involved, occasionally in Chinese and almost never in the Malays. However, care must be taken in case of "Malays" who have Malay or Muslim names but are in effect Indian Muslims, and of course, mixed marriages must be taken into consideration in this racial context. Clinically, jaundice appears on the very first day together with pallor. Haematologically, there will be evidence of a haemolytic anaemia such as a palpable spleen, high reticulocytosis, normoblastaemia. The diagnosis is clinched if the mother is Rh negative, the infant Rh positive and the Direct Coombs Test is positive.

(b) **ABO incompatibility:** Here, the mother is usually Group O, and baby Group A, B or AB. Just as in Rh incompatibility, the presence of different blood groups in mother and infant does not necessarily result in haemolytic disease, so also in ABO incompatibility.

The jaundice here also appears on the first day, and the characteristic "pallor with jaundice" appearance can be spotted for what it is by experienced doctors. All races in this country are prone to this disorder. Again, haematologically, there are evidences of a haemolytic anaemia, but the distinguishing feature is the presence of **microspherocytes** in the peripheral blood in contradistinction to Rh incompatibility. Grouping of mother's and infant's blood will show possibility of incompatibility, and incubation of mother's serum and baby's cells will elicit a high haemolytic titre. The Direct Coombs Test is nearly always negative and if positive is only weakly so.

(c) **Glucose-6-phosphate dehydrogenase deficiency**: This erythrocytic enzyme is necessary for the reduction of triphosphopyridine nucleotide (TPN) in the red cell (reaction 2 below), and the reduced TPN, i.e. TPNH reduces glutathione (reaction 3 below) to reduced glutathione which is necessary for the integrity and stability of the erythrocyte (Fig. 1). Therefore, if the enzyme glucose-6-phosphate dehydrogenase

1. Glucose + ATP
   | hexokinase
   V
   glucose-6 phosphate + ADP
2. Glucose-6-phosphate + TPN
   | glucose-6-phosphate dehydrogenase
   V
   6-phosphogluconate + TPNH
3. Glutathione + TPNH
   | glutathione reductase
   V
   reduced glutathione + TPN

Fig. 1. Relevant biochemical reactions in erythrocyte.

(G-6-PD) is absent or deficient in a newborn, haemolysis may occur because the cells are less stable. For this to occur any trigger (Wong, 1961) in the form of Western drugs, native herbs and drugs, sepsis, trauma, etc. may start off the haemolysis, so that hyperbilirubinaemia with kernicterus may supervene (Smith and Vella, 1960).

The diagnosis of this condition may be made from the following characteristics. Like Rh and ABO incompatibility, G-6-PD deficiency is genetically determined and as such there may be a previous history of jaundice or kernisterus. Racially, Chinese and Malays are more commonly involved than Indians and it is almost never seen in Europeans in this country because they come mainly from U.K. However, this deficiency does occur in Southern Europeans such as Jews, Italians and Greeks. The incidence of the deficiency of this enzyme in the 3 main races in Singapore is shown below where random cord bloods were taken from newborns in Kandang Kerbau Hospital (Table 2). It can therefore be seen that

Table 2

Race	Normal	Deficient	Total	% deficient
Chinese	201	4	205	1.9
Malays	197	3	200	1.5
Indians	199	1	200	0.5

the incidence is about 2% in Chinese and Malays The genetics of this enzyme deficiency (Wong, 1963) is such that it is sex-linked in such a manner that heterozygous males are all deficient, while heterzygous females may be intermediate, normal or occasionally deficient, while homozygous females are totally deficient in the enzyme. Hence, more males are involved than females. The exact trigger mechanism in these cases of kernicterus with G-6-P.D. deficiency is still pursued. The jaundice in these babies, however, comes on the second to third day and mounts rapidly even in the space of 24 hours, so that kernicterus can supervene after the appearance of jaundice is noticed over a period of 1 or 2 days. The haematological signs of a haemolytic anaemia, however, are less striking than those in Rh or ABO incompatibility, but the results of G-6-P.D. estimation in the patient and members of the family will clinch the diagnosis.

Table 3 summaries some of the differentiating points in the diagnosis of Rh, ABO incompatibility and G-6-P.D. deficiency in any case of hyperbilirubinaemia with an erythrocytic cause.

Table 3

Characteristics	Family history	Onset of jaundice	Haemolytic anaemia signs	Direct Coombs Test	G-6-P.D.	Race
Rh disease	+	1st day	+++	+	Present	Europeans & Indians
ABO disease	+	1st day	++	—	Present	All
G-6-P.D. deficiency	+	2-3rd day	+	—	Absent	Chinese, Malays

Liver causes:

(a) **Prematurity:** It is conceivable that the liver of a premature infant is more immature than that of a full-term infant and hence there is a diminution in activity of the glucuronyl transferase enzyme and hence decreased ability in conjugating the toxic bilirubin to the non-toxic soluble bilirubin glucuronide, with consequent hyperbilirubinaemia and possible kernicterus. Here a serum bilirubin of 18 mg.% is taken as the working dangerous level as the brain is more immature also and hence more prone to kernicterus.

(b) **Sepsis:** The commonest infection in the newborn which may affect the liver is umbilical sepsis because of the relations of the umbilical vein to the liver, so that infection can travel and cause a hepatitis. This, in turn, could produce depression of glucuronyl transferase activity with resultant hyperbilirubinaemia.

In both prematurity and sepsis, there can be the added factor of erythrocytic haemolysis as the red cells of the premature infant are less stable than those of the full-term infant, and septicaemia is a well-recognised cause of haemolysis.

(c) **Liver "immaturity":**

If one accepts the concepts outlined above, and in a particular newborn with hyperbilirubinaemia, the following are excluded, viz.

1. Any haemolytic anaemia,
2. Rh, ABO incompatibility,
3. Deficiency of G-6-P.D.
4. Prematurity,
5. Sepsis,

then, the only possible cause is a depression of the liver enzyme activity. There are two problems that confront one immediately:—

1. How to prove the presence of this enzyme disability;
2. If proved, then why does it occur in certain patients.

The difficulty in assaying the enzyme activity is that at present liver tissue slice have to be obtained (Lathe, 1962) if the enzyme is to be directly estimated, while the indirect method as advocated by Vest (1958) involves the use of drugs which persumably are exclusively conjugated in the liver in exactly the same manner as bilirubin is. But evidence seems to point to the contrary (Lathe, 1962). The problem is still being tackled here and when the first difficulty is overcome, Chinese drugs will probably be found to be the culprit in the second poser above.

These liver "immaturity" cases are full-term infants, and after the first week of life, jaundice disappears and it seems that this immaturity is only transient and is different from the Crigler-Najjar Syndrome.

Results:

On the basis of the above concept, consecutive cases of hyperbilirubinaemia with or without kernicterus admitted to the Department were investigated. 139 patients in 1960 and 98 patients in 1961 were encountered, and Table 4 summarises the incidence of the various causes in newborns with severe jaundice with or without kernicterus. It can be seen that Rh incompatibility is rare and all were in English babies, however, 32% were due to ABO incompatibility and 28% due to G-6-P.D. deficiency while another 25% were due to liver "immaturity".

Table 4

	1960 (139 patients)	1961 (98 patients)	Total	%
Rh incompatibility	4	3	7	2.9
ABO incompatibility	39	37	76	32.0
G-6-P.D. deficiency	31	37	68	28.7
Prematurity	2	4	6	2.6
Sepsis	16	3	19	8.0
Liver "immaturity"	47	14	61	25.8
	139	98	237	100.0

However, if the cases of kernicterus were separated from those with severe jaundice who were saved by exchange transfusion, Table 5 is obtained.

Table 5

	1960	1961	Total	%
Rh incompatibility	0	0	0	0
ABO incompatibility	6	5	11	12.6
G-6-P.D. deficiency	17	21	38	43.7
Prematurity	2	2	4	4.6
Sepsis	11	0	11	12.6
Liver immaturity"	18	5	23	26.5
	54	33	87	100

These cases of kernicterus were adimitted too late already with brain damage due to late referral. Reference to Table 5 reveals that 43% of kernicterus cases is due to G-6-P.D. deficiency. This high incidence is due to the fact that jaundice appears later and mounts rapidly brooking no delay in its referral and management. Again about 25% of the cases is due to liver immaturity.

Perusal of Tables 4 & 5 and comparing them with Table 1 demonstrates at once that the causes of hyperbilirubinaemia and kernicterus here are totally different from those in England and America. This has to be firmly grapsed if the problem of management is to be tackled efficiently. It is not claimed that the above is a complete list of operative causes in this country but that it, at least, provides a springboard from which more research may be done. One is unhappy about the "immaturity" group in that it is labelled so long the process of exclusion rather than by positive proof. It may be a conglomeration of true liver "immaturity" and other still undiscovered causes.

Management:

The operative word in management is **prevention** because once kernicterus has set in, the battle has been lost. There would be no difficulty at all if all babies born in Singapore could be observed closely by experienced personnel for a period of 7-10 days since this neonatal period is the critical one from the point of view of kernicterus. Almost no infants begin to develop kernicterus after the 10th day of life. If this arrangement is possible, cases with jaundice judged

clinically by experienced observers to be over 20 mg.% can have the serum bilirubin estimated and exchange transfusion carried out if necessary. However, with about 70,000 births a year, evidently such an ideal cannot be achieved. Kandang Kerbau Hospital deals with about 100 births a day and keeping them under observation for 10 days in hospital means facilities both of space and personnel for 1,000 infants!

It may be said that some form of medical supervision is already available in that direction in that midwives or nurses visit the patients' homes for a period of about a week after discharge from hospital and therefore are in a position to review the depth of jaundice daily. However, unfortunately, it takes an experienced paediatrician to gauge almost correctly the serum bilirubin level from the depth of jaundice. Skin jaundice does not vary exactly proportionately with the serum bilirubin since there is a time lag in the first 2-3 days of life before the skin is stained yellow by the jaundiced blood and by the same token near the end of the first week the depth of skin jaundice is more intense than the level of serum bilirubin at that time. Furthermore, when the skin pigment colour of the Chinese, Indians and Malays are taken into consideration, it will be appreciated that an intelligent guess of the serum bilirubin by looking at the skin colour is very difficult of achievement. Therefore, we still receive infants too late for exchange transfusion because kernicterus has already set in. Would plastic skin icterometers as used for European infants be of use here? The answer is no, because of the interplay of racial pigment colour; but it would not be beyond the ingenuity of someone with a flair for colour matching to design icterometers which may be suitable for local infants in the near future.

Faced with such a problem, what can be done in the meantime to lower the mortality and morbidity from this disease? The following steps have been undertaken:—

1. Doctors, nurses and midwives are being informed of the peculiar circumstances causing kernicterus in this country and they have been told that as long as they think a certain depth of skin jaundice is intense, they are to refer for further opinion and observation.

2. Mothers whose infants had been investigated for hyperbilirubinaemia with or without kernicterus and the cause found to be Rh or ABO incompatibility or due to G-6-P.D. deficiency are reminded about the possibility of future infants being so involved and they should inform the obstetrician delivering their future babies of this fact and to hand over to the obstetrician a letter from the Department of Paediatrics about this so that these selected few infants could be observed carefully for 10 days for possible hyperbilirubinaemia.

3. All mothers of babies with hyperbilinaemia are informed not to take any doubtful drugs while breast feeding their infants in the first week of life or give such concoctions to the infants during this period. This is relevant not only to deficient G-6-P.D. cases but for all cases because as stated above depression of glucuronyl transferase activity of the liver may occur as a result of taking such drugs and hence aggravating a "physiological" or "pathological" jaundice.

4. Rapid and accurate methods for estimation of serum bilirubin in the Department.

5. An efficient method and experienced team for exchange transfusion.

6. Continuing research into the whole problem of hyperbilirubinaemia in this country.

Finally, a word on exchange transfusion. A full description of the technique as practised here is out of place in this context. But, in our experience, the mortality from this procedure is almost nil if exchange is carried out before kernicterus has set in. This is in contrast to the figure of about 2-4% in Western countries. This difference in mortality is not due to differences in technique but that Rh incompatibility is much commoner in Western countries and infants with this disease are already anaemic and some of them on the verge of cardiac failure. Exchange transfusion under such circumstances may be fraught with danger. Also repeat exchanges may have to be done on the same patient before he is out of danger and this increases the hazards even more. On the other hand, severe anaemia is not seen in our patients and a single exchange transfusion is all that is necessary to prevent kernicterus.

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