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Serum Bilirubin Level in Full-Term and Premature Infants after Exchange Transfusions

By

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During exchange transfusion a considerable amount of bilirubin passes from the extravascular into the intravascular compartment of newborn infants, and it is due to this so-called rebound phenomenon that the dye level decreases at a rate slower than that at which the red corpuscles are exchanged [2, 10]. The flow of bilirubin continues after termination of the transfusion so that the rebound phenomenon has two phases: a first one, coinciding with the exchange transfusion, and a second one occurring after the intervention [14]. The latter phase can be divided into two subphases, *viz.* a) early rebound, occurring within 12 hours following transfusion; b) late rebound, occurring thereafter.

The dye level is a result of the interaction of various factors during both phases. In the second phase, in addition to the diffusion of bilirubin between the extra- and intravascular compartments, haemolytic factors and the secretory activity of the liver are at play. A correct assessment of these factors is of considerable importance.

The primary purpose being the prevention of kernicterus, it must carefully be observed whether after the exchange the bilirubin level rises to the danger zone and whether, thereafter, a second transfusion will have to be performed.

It was hoped that the study of post-transfusion dye levels might facilitate the solution of these practical and theoretical problems.

Material and Method

I. *Serial post-transfusion estimations of serum bilirubin in mature newborns and prematures*

(Behaviour of the early rebound phenomenon)

A total of 29 full-term newborns has been examined 1, 3, 6, 9 and 12 hours after the exchange transfusion. In order to avoid excessive stress, blood from the same baby was withdrawn three times at the most. Exchange transfusion was performed in 13 cases on account of Rh incompatibility, in 8 cases of ABO incompatibility associated with icterus gravis, and in 8 cases of hyperbilirubinaemia. Coombs' reaction was positive in 11 cases. Average body weight of the infants was 3150 g. On the average, 2.7 times the circulating blood volume, regarded as representing 10 per cent of body weight [13], was exchanged at a rate of 130 ml/kg body weight/hour.

In addition, in 33 premature infants bilirubin concentrations were estimated 3, 6 and 12 hours following blood exchange. Exchange was performed on account of

hyperbilirubinaemia associated with immaturity in 28 cases, on account of Rh incompatibility in 2 (of which one was Coombs positive), and because of ABO incompatibility in 3 cases. Distribution according to body weight was

1250 to 1500 g	5 cases
1501 to 1750 g	11 cases
1751 to 2000 g	13 cases
2001 to 2500 g	4 cases

with an average of 1680 g. On the average, 1.7 times the volume of circulating blood was exchanged [18, 24] at a rate of 100 ml/kg body weight/hour.

Bilirubin estimation was made with the method of JENDRASSIK and GRÓF [7], while the samples of blood collected in the first hours after the intervention were examined with a Polytest Zeiss colorimeter. The latter method is, essentially, one of diazotization. It has the advantage that not more than twice 0.2 ml serum are needed in the cases with the highest bilirubin values; it was therefore enough to withdraw 1 ml blood even when high haematocrit values were expected.

With a view to finding out whether the results obtained with the semi-micro Polytest method were suitable for being inserted in the series yielded by the method of JENDRASSIK and GRÓF, we performed 150 parallel measurements which were supplemented by the triplicate examination of 18 serum samples containing different amounts of bilirubin. Mathematical evaluation revealed no significant difference between the bilirubin levels determined in the same sample of serum by either method.

II. *Connection between the parameters expressing the efficiency of exchange transfusions, and the extent and time of the post-exchange rebound*

The peak post-exchange bilirubin level was determined on the basis of one or two estimations performed daily, according to the clinical picture. Re-elevation has been expressed in per cents of the pre-exchange level and is termed "percentual rebound" in the following. The diagrams in Figs 1 and 2 show the degree of rebound in mg per 100 ml as well.

Evaluation of the percentual rebound seems to be more reliable in signalling the dangerous degree of re-elevation whereas the elevation expressed in mg per 100 ml is more dependent on the bilirubin level at the end of the intervention. The results of both methods show somewhat distorted values if the level of bilirubin is low at the beginning of the exchange transfusion. There were 6 such cases of Rh incompatibility in our material.

It has been attempted to establish a correlation between the post-exchange maxima (percentual rebound) and the following factors:

(i) exchanged blood volume in ml/kg body weight;

(ii) fall of bilirubin level during the intervention, expressed in per cent of the initial level;

(iii) amount of bilirubin removed from the organism during exchange transfusion, in mg/kg body weight;

(iv) amount of bilirubin removed from the extravascular compartment during exchange transfusion, in mg/kg body weight;

(v) period from the end of the exchange until the peak of re-elevation.

Values for (iii) and (iv) were estimated by a method described earlier [16], consisting in a serial determination of dye concentrations during the intervention, and the integration of the data so determined. Estimations of this nature were made in 74 full-term newborns.

Values for (i) and (ii) were studied in 100 full-term and 61 premature newborns. The serological distribution of this material was as follows.

Full-term infants

Rh incompatibility	35
ABO incompatibility	23
Double incompatibility	9
Hyperbilirubinaemia	33

Prematures

Rh incompatibility	4
ABO incompatibility	6
Hyperbilirubinaemia	51

Distribution according to volume of exchanged blood was,

Volume of exchanged blood, ml per kg body weight	Full-term newborns	Premature newborns
150 or less	2	30
151—200	16	19
201—250	26	12
251—300	43	—
301 or more	13	—
Total	100	61

Results

I. Early rebound

Fig. 1 shows bilirubin levels in full-term babies after blood exchange. The mean values are characterized by an attenuating exponential function. The initial steep segment of the curve represents the first 3 hours during which the elevation of bilirubin level averaged 1.7 mg per 100 ml per hour. The second segment of the curve represents the last 9 post-exchange hours. During this period re-elevation averaged 0.5 mg per 100 ml per hour.

Fig. 2 illustrates corresponding values for prematures. The curves are conspicuously flat, indicating the slight degree of rebound. A comparison with the dotted line (mean values for full-term infants) shows the difference very distinctly. Mean re-elevation in the premature group amounted to 0.5 mg per 100 ml per hour during the first 3 hours, and to 0.2 mg per 100 ml per hour in the next 9 hours.

II. Late rebound

(i) Fig. 3 shows the correlation between exchanged blood volume and peak dye levels. Only in cases of hyperbilirubinaemia did the rebound decrease in proportion to the increase in the volume of exchanged blood. The coefficient of this correlation was -0.549. The minus sign of the coefficient expresses an inverse relation between the respective quantities plotted on the ordinate and the abscissa. The diagram presents moreover the straight line of regression which conveys a more accurate idea of the direction of the inverse relationship; its formula is $y = -0.238x + 121$. Such a relationship was not demonstrable in cases of ABO incompatibility and still less in Rh incompatibility. It is, thus, not always possible in such cases to reduce the extent of rebound by increasing the volume of exchanged blood.

The majority of prematures was subjected to exchange transfusion on account of hyperbilirubinaemia, and — like in the full-term group of similar aetiology — the volume of exchanged blood varied inversely with the degree of re-elevation (Fig. 4). While out of a total of 30 it was in 11 cases that the extent of rebound surpassed 30 per cent when less than 150 per cent of the circulating blood was exchanged, this happened only in 3 out of 31 cases when the volume of exchanged

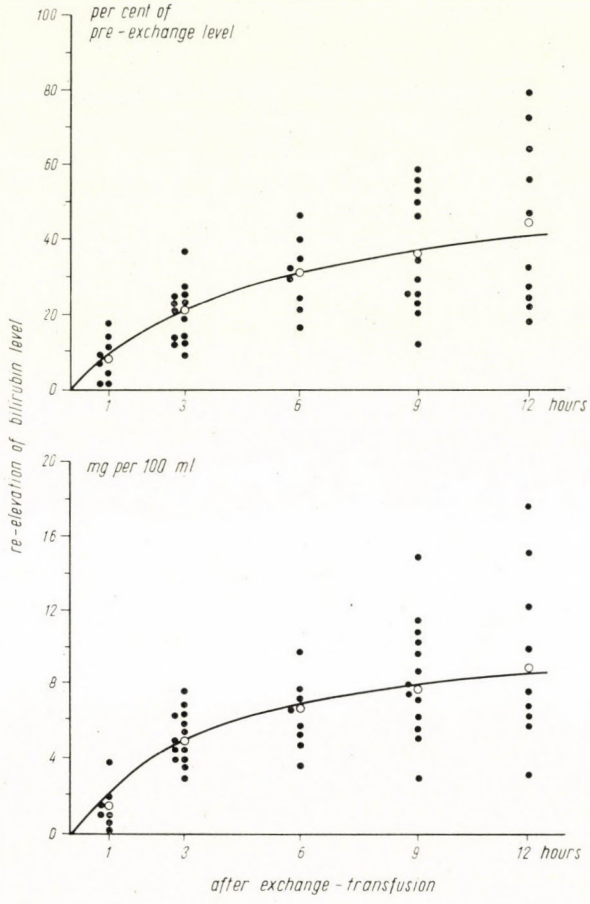

FIG. 1. Elevation of bilirubin level in full-term infants after blood exchange, in mg per 100 ml per hour in the lower part of the diagram, and in per cents of the initial level (percentual rebound) in the upper part. Circles indicate mean values

blood was larger. The chi-square test showed this difference to be significant ($\chi^2 = 6.28$, $p < 0.05$).

(ii) A correlation between the fall of the dye level during exchange transfusion and the degree of rebound was demonstrable likewise only in cases of hyperbilirubinaemia. The coefficient of the correlation was as under (i).

(iii) Comparisons between percentual rebound and the amount of removed bilirubin in full-term babies proved that the rebound was slight in cases of hyperbilirubinaemia and moderate in those of ABO incompatibility. Results in cases of Rh incompatibility were variable.

(iv) We were unable to find a correlation between the amount of dye removed from the extracellular compartment and the behaviour of post-exchange rebound.

(v) Re-elevation maxima suggest-

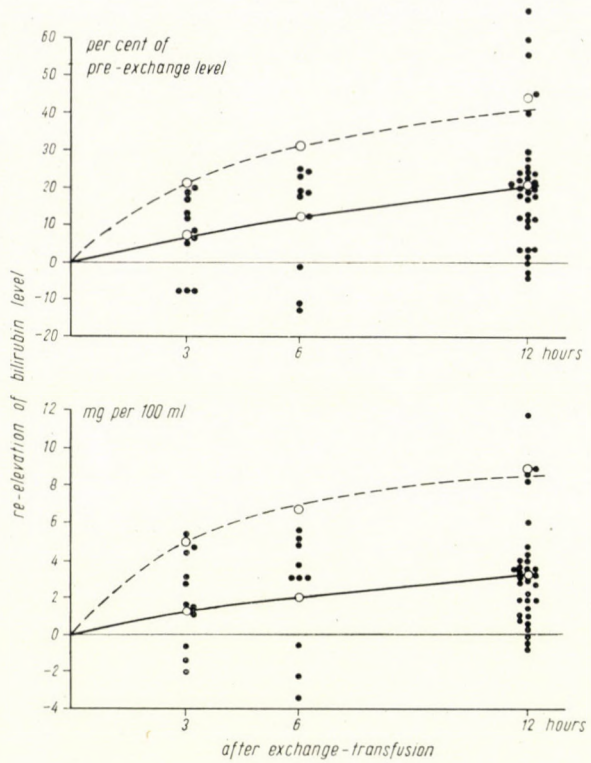

FIG. 2. Elevation of bilirubin level in prematures after blood exchange. Construction of the diagram is like in Fig. 1. The dotted line shows dye concentrations in full-term babies, as also the pertinent exponential curve

ing the necessity of a second exchange took 18 to 24 hours to appear after the first intervention. Development of such peaks took 36 to 72 hours in cases of ABO incompatibility and hyperbilirubinaemia.

The range of indications for exchange transfusions in non-immune haemolytic cases has been increasingly restricted in the last 2 to 3 years. As a consequence of this policy of wait-and-see the babies were somewhat older at the time of the intervention, and the critical bilirubin levels at which the transfusions seemed to become imperative were accordingly higher. The percentual rebound in general, as also with reference to the volume of exchanged blood and to the fall of the dye level during exchange, was less during the last two years than before.

DISCUSSION

As to the behaviour of the bilirubin level during the 12 hours following exchange transfusion, VALAES [25] holds that it takes only one half hour for a new equilibrium to be established

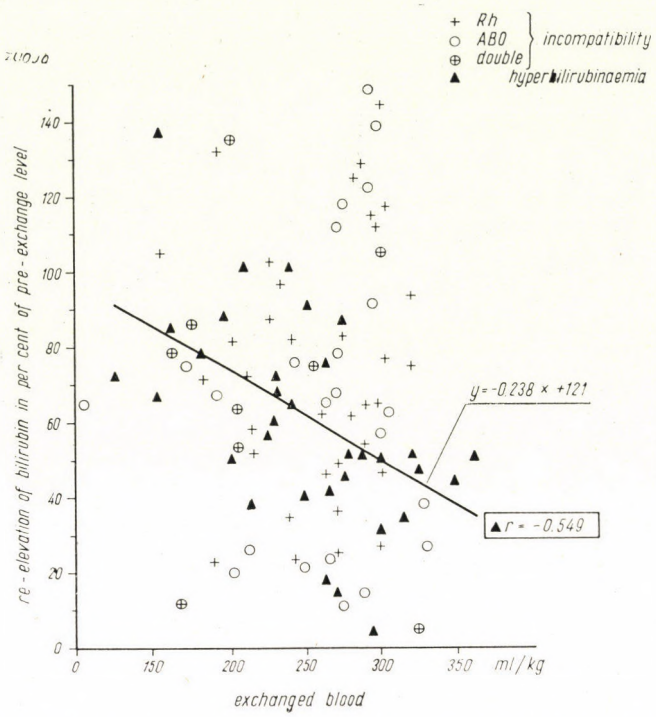

FIG. 3. Correlation between volume of exchanged blood and maximum of subsequent rebound expressed in percentage of initial dye-level, "percentual rebound"

between the intracellular and extracellular dye concentrations. The extent of the exchange reached in his cases 1.2 to 2.0 times the circulating blood volume. USHER [23], exchanging twice the volume of circulating blood, assumes that the post-exchange migration of bilirubin does not last more than one hour. KEUTH [9], studying the optimum conditions of two-stage exchange transfusion, found that the rebound terminated 3 hours after the first intervention, and recommended therefore to begin the second exchange at that point of time. GOLDFARB [6] performed exchange transfusions with 0 group erythrocytes suspended in AB plasma, and observed an intensive rebound during the next 4 hours, and a moderate one thereafter.

We have found that the establishment of post-exchange equilibrium is a process consisting of several phases.

The rate of re-elevation was rapid in the first 3 hours after exchange, amounting in full-term infants to 1.7 mg per 100 ml per hour on the average. Re-elevation was enhanced by copious and/or quick exchanges accompanied by a marked drop of the bilirubin level.

The bilirubin level during the first 3 post-exchange hours depends in the first phase on the quantity of bilirubin flowing in from the extracellular

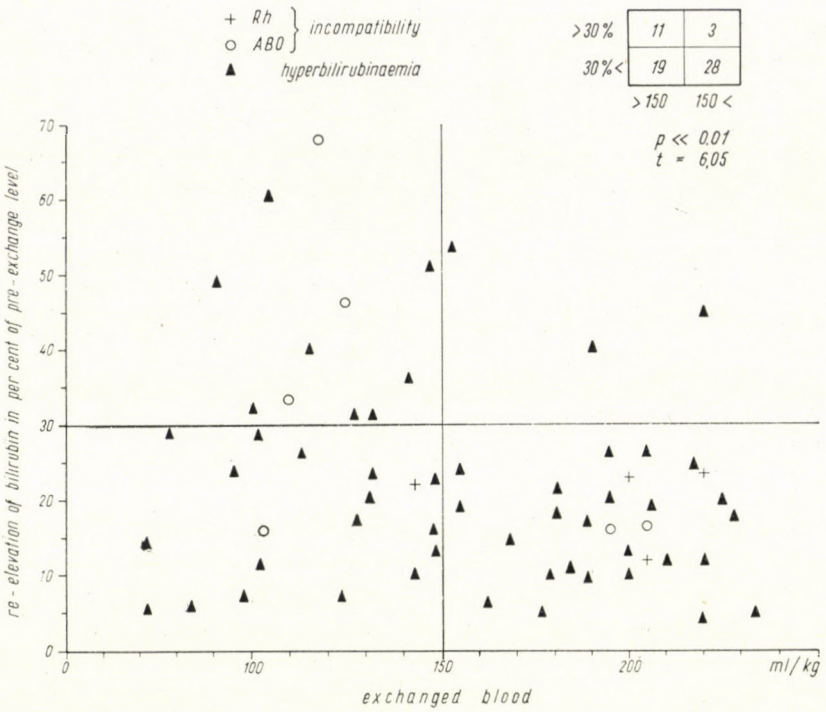

FIG. 4. Correlation between exchanged blood volume and post-exchange rebound in prematures

space. Since this influx is subject to the laws of diffusion [22], the process can be expressed by an exponential function.

Accordingly, the steepest segment of the rebound curve coincided with the first post-exchange hour in the present cases also. Those parts of the extracellular space which probably are contiguous to the blood path contain more bilirubin at the termination of the exchange than more distant parts do. This initial steep concentration gradient between the vessels and their surroundings explains the rapid rate of early rebound. However, 30 or 60 minutes do not seem to suffice for the establishment of bilirubin equilibrium between the intracellular and extracellular compartments. We share the view that at least 3 hours are necessary for the equilibrium to develop [3, 26].

The rebound curve runs a flat course in the next 9 hours. It is during this time that reversibly bound intracellular bilirubin passes into the blood stream and the jaundice loses in intensity.

There are numerous observations to show that the rebound occurring after the 12th hour is mostly due to haemolysis.

It has been claimed by KAUDER [8] that the necessity for a repetition of exchange transfusions is significantly

more frequent if a shortening of the haptoglobin half-time points to the presence of haemolytic breakdown products.

Various factors are known to be involved in haemolysis.

The role of sensitized erythrocytes remaining in the circulation at the end of the exchange is obvious. POLACEK [11] observed intensive rebound where the level of foetal haemoglobin remained high. It is commonly known that injected donor cells of identical blood group [5] or cells of citrated blood are particularly vulnerable [4]; a great part of the latter is destroyed within 48 hours.

The significance of sensitized red corpuscles to be found in the foetal haemopoietic tissues is more doubtful. The explanation advanced by SISSON [19] for the success of exchanges performed with sedimented erythrocytes was that the high erythrocyte count and the corresponding high haemoglobin level at the end of the intervention inhibited the outflow of such cells.

In the present experiments, post-exchange bilirubin values determined at 9 and 12 hours in ten Coombs positive newborns were 48 per cent higher than in their adequately matched hyperbilirubinaemic counterparts. It was evident that in cases of immune haemolysis, and especially in those of Rh incompatibility, not even a threefold exchange of the circulating blood volume was capable of keeping the rebound within the desired limits. Since the ratio of residual cells may amount to about 5 per cent in such cases, it is safe to assume that further haemolytic factors have contributed to the undesired extent of rebound. The flat rebound curve of prematures and the moderate rebound in mature hyperbilirubinaemic infants were further proofs to show that, in the absence of immune haemolysis, not even the imperfect glucuronizing activity, characteristic of prematures, need to bring about a dangerous re-elevation of the bilirubin level. Earlier investigations in immature babies [15] pointed likewise to the fact that it is rather by an increase of the rebound during blood exchange that the effect of extracellular dye stores manifests itself.

Results of this kind make it necessary to reconsider the efficiency of exchange transfusions.

It has been demonstrated in a previous study [16] that the extent of bilirubin removal is proportional to the volume of exchanged blood. SPROUL's subsequent isotope experiments [20] have confirmed our findings. It is, however, doubtful whether an increase in the volume of exchanged blood retains its rebound-diminishing effect after the intervention. The material of SPROUL consisted of immune haemolytic infants, and the extent of post-exchange rebound showed no correlation with the amount of removed bilirubin or its extravascular fraction. Our cases of immune haeemolysis were similar in this respect to those of SPROUL.

Some time ago we have justified the exchange of the threefold volume of circulating blood by the resulting

favourable degree of bilirubin removal [16]. Let us examine whether now, that the significance of haemolytic factors has been recognized, matters look different in this respect.

The evacuation of extravascular dye-depots is undoubtedly promoted by a generous exchange of blood in cases of hyperbilirubinaemia and weak sensitization. This applies to cases of immune haemolysis as well, and an ample exchange of blood inhibits moreover intravascular haemolysis. Results that can be achieved without risk by increasing the volume of exchanged blood should not be renounced on account of haemolytic factors that are hardly manageable and many of which are of unknown origin.

One of the advantages of a generous exchange is the deferred reappearance of dangerous dye concentrations. If the volume of exchanged blood was adequately large, not even in cases of Coombs-positive Rh-incompatibility was a second intervention necessary earlier than 18 to 24 hours after the first one. This interval was still longer in cases of ABO incompatibility and hyperbilirubinaemia. Besides, gain of time augments the chances of dispensing with the second intervention. Our experience justifies the statement that, after an adequately generous exchange, the bilirubin level need not to be re-examined before 12 hours and a second exchange performed before 24 hours.

How often a gain of time makes it possible to avoid a second exchange is shown by the fact that we had to repeat the intervention in not more than 4 per cent of our cases in the last $3\frac{1}{2}$ years. Not one single premature baby had to be subjected to a second intervention since the method of exchanging the twofold volume of circulating blood had been adopted. Exchange transfusion had to be repeated in 7 per cent of 118 babies with weights exceeding 2000 g. Our data include cases where a second intervention became necessary because the first had been interrupted owing to some technical reason. Our statistics will be better appreciated if we mention that the now usual ratio of blood exchange repetitions amounts to 12 to 20 per cent [17, 26, 27].

A restriction of the range of indications has doubtlessly contributed to the reduction in the number of repeated exchanges. It has been mentioned that the re-elevation of the dye level was more modest when the concentration of bilirubin had been allowed to attain a higher degree before the intervention. The role of evacuated stores of bilirubin was in such cases (hyperbilirubinaemia for the most part) presumably more decisive than that of haemolysis. These factors, however, do not yet explain why our quota of repeated exchanges is so much below that of other institutes [26, 27].

We conclude by proposing to continue the present practice of exchanging 2.5 to 3 times the volume of circulating blood. Further improvement of the conditions of exchange transfusions and therapeutical methods will allow to deal with the

haemolytic factors many of which still escape management.

Acknowledgement

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Summary

It has been found possible to characterize by an exponential function the mean bilirubin levels serially measured after the exchange transfusion in 29 full-term newborns. The rate of re-elevation averaged 1.7 mg per 100 ml per hour during the first 3 post-exchange hours, and 0.5 mg per 100 ml per hour during the next 9 hours. The corresponding figures for 33 prematures were 0.5 and 0.2 mg per 100 ml, respectively.

Influx of bilirubin from the extravascular spaces is the decisive factor in early, and liberation of the dye by haemolysis in late, rebound.

Re-elevation of the dye level has been studied following exchange transfusions performed in 100 mature and 61 premature infants. Increase in the volume of exchanged blood delayed the re-appearance of dangerous dye concentrations. The extent of rebound was found to have significantly diminished after a threefold (full-term) or twofold (prematures) exchange of the circulating blood volume. A single exchange, however copious, did not invariably yield the desired result in cases of immune haemolysis.

The ratio of repeated blood exchanges amounted in the present material during the last 3½ years to 4 per cent in general, and to 7 per cent in babies weighing more than 2000 g.

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