14420

Nucleated Erythrocytes in Newly-born Infants in Relation to Maternal Rh Compatibility.

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Studies on the incidence of nucleated erythrocytes in newly-born infants have not taken into account the possible incompatibility between mother and child as to the recently described Rh factor. When this blood factor[1-4] is present in the infant but not present in the mother's blood, the mother may develop antibodies against it, and abortion, jaundice, erythroblastosis fetalis and ill-defined conditions may sometimes develop.[3,5] It seemed expedient, therefore, to determine the normal expectancy of nucleated erythrocytes in viable newly-born infants when the mother and infant were compatible, *i.e.*, when both were either Rh-negative or both Rh-positive.

Materials and Methods. Human anti-Rh sera were obtained through the kindness of Dr. Phillip Levine and the technic recommended by him was followed. Smears and Rh determinations on the mothers were made routinely within 24 hours after deliverv until 116 had been obtained. The smear was stained with Wright's stain. The Jefferson Hospital material was used because this hospital contains only private patients who were assumed to be generally better nourished than charity patients.

The method employed consisted of determining the number of red blood cells in various high power fields until 10,000 were obtained.† Since the high power field is a circle and the area of a circle is $3.1416\ (d/2)^2$ it was only necessary to determine the diameter in each field. The number of red blood cells in the high power field was determined by considering the number in a diameter of the field as the number of red blood cells which a dividing hair touched. The number in the field was considered to be half this diameter squared, multiplied by 3.1416. The diameters of the fields in the 116 smears studied ranged from 12-26 on the various slides. It was found that on a given slide the various diameters had an almost insignificant variation. Consequently in only every fifth field was the diameter determined. Calculation was facilitated by having at hand a chart showing the theoretical number of red blood cells in the field corresponding to each diameter from 12-26. In this manner 10,000 red blood cells were estimated on each slide. The use of the method was validated by actual area counts and approximations within the limits of sampling error were obtained.‡

* With the assistance of the laboratory staff.

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† The enumeration was first done by using a pointer and counting all nucleated and non-nucleated erythrocytes which the tip of the pointer touched in systematic excursions across the slide. This proved too time-consuming and a simpler method was adopted. It was not believed that the calculation of the number of red blood cells per hundred white blood cells was sufficiently informative to be of much value; if used such a method would require, in addition, very accurate total red and white cell counts.

‡ Theoretically in the extreme case of one cell large enough to occupy a full diameter some 3,000 fields would have to be examined provided the cells were in closest approximation in order to obtain 10,000 red blood cells, since the times when one cell would exactly cover the field would be negligible and inconsequential. To test this possibility a number of circular objects of the approximate size

TABLE I.
Number of Nucleated Erythrocytes per 10,000 Red Blood Cells in 116 Infants with Known Rh Values in Mother and Child.

No. of nucleated red cells per 10,000	Mother Rh+ Infant Rh+	Mother Rh— Infant Rh—	Subtotal	Mother Rh+ Infant Rh—	Mother Rh— Infant Rh+	Subtotal	Grand total
0	59 x	8	67	4	11	15	82
1	15	1	16	0	2	2	18
2	2	1	3	2	1	3	6
3	0	0	0	1	2	3	3
4	0	0	0	1	0	1	1
5	1 i	0	1	0	0	0	1
6	0	1	1	0	0	0	1
7	0	0	0	0	0	0	0
8	1 d	0	1	0	0	0	1
9	1 d	0	1	0	0	0	1
10	1 d	0	1	0	0	0	1
11	0	0	0	0	0	0	0
12	0	0	0	0	0	0	0
13	1 d	0	1	0	0	0	1
Total	81	11	92	8	16	24	116

x—2 died. i—ill. d—died.

Results. Of the 116 pairs of mothers and infants there were 81 instances in which both parent and child were Rh-positive, 11 instances in which both were negative, 16 instances in which the mother was Rh negative and the infant Rh positive, and 8 instances in which the infant was Rh-negative and the mother Rh-positive. The distribution of the nucleated erythrocytes for each 10,000 red blood cells is presented for the four categories (Table I).

Among the 92 infants which had the same Rh value as their mother, 6 died within a few days. Among the 86 viable and Rh compatible infants only 33 nucleated erythrocytes were encountered among an estimated 860,000 red blood cells or an average of 0.39 nucleated red cells per 10,000 red cells. More nucleated red cells than 2 per 10,000 should occur less often than in one of each 100 newly-born normal infants. It will be seen that the infants with the 4 highest nucleated erythrocyte counts died within a few days. It is probable that routine nucleated erythrocyte counts on the newly-born would detect many dyscrasias sufficiently early to institute prompt treatment. By the method described counts of nucleated erythrocytes can be made as quickly as a total white cell count.

Discussion. Among the 24 infants which had a different Rh value than their mother, 7 had two or more nucleated cells in the 10,000 counted; whereas among the 92 infants having Rh values identical with their mother, only 9 had two or more nucleated per 10,000 red blood cells. This difference between the Rh compatible and the Rh incompatible was statistically significant ($X^2 = 6.15$, $n = 0.01$, significant). From the published reports one might assume that this increased percentage of nucleated cells was due to the group in which the mother was Rh-negative and the infant Rh-positive. However, although the tendency was in this direction in both, the real preponderance was in the group in which the child was Rh-negative and the mother Rh-positive. Four of the 8 in this group had 2 or more nucleated red cells per 10,000 as compared with 9 of the 92 with Rh compatibility. Some 30 additional observations would be necessary to test the significance of this tendency.

Summary. A method of making nucleated

of a field were kept in closest approximation and 20 attempts at random sorting made. From 1-3 cells were found to touch the dividing hair in the 20 instances and the actual number of cells touching the field were 3-4, averaging 3.75. The theoretical number averaged 3.37 and the difference, 0.38 ± 0.65, was not significant. It did not seem worthwhile to make the 3,000 additional samplings necessary to bring the total cells counted to 10,000.

erythrocyte counts per 10,000 red blood cells is presented. Routine counts within 24 hours of delivery are recommended on newly-born infants and more than 2 nucleated red blood cells per 10,000 are to be considered abnormal. Regardless of Rh compatibility the mortality among children with 5 or more nucleated cells per 10,000 was 67% in our small series, although among the Rh incompatible infants there were more nucleated red blood cells than among the Rh compatible group. The possibility of ill effects when the mother is Rh-positive and the infant Rh-negative is suggested by the data.

14421

A Microbiological Method for the Determination of Tryptophane.

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The widespread use of bacteria for the assay of the B-complex vitamins has drawn attention to their requirements for amino acids and their possible use as a basis of measurement of these substances. Detailed studies have been made of the amino acids required by *Lactobacillus casei*[1] and *Lactobacillus arabinosus* 17-5.[2] Bacterial methods of assay for the amino acids, especially tryptophane, have been under investigation in this laboratory and during the progress of this work there have appeared publications from other laboratories in which procedures have been outlined[3,4] for the use of *L. arabinosus* 17-5 in the assay of cystine, iso-leucine, methionine, leucine, valine, glutamic acid, threonine, and tryptophane. It seems desirable at this time to present the results of studies on the specificity of *l*-tryptophane for *L. arabinosus* 17-5 and methods for the preparation of proteins for assay. The importance of such studies is apparent since Snell[5] has recently found that indole or anthranilic acid may replace tryptophane for *L. arabinosus* 17-5, a fact which has also been observed in this laboratory.

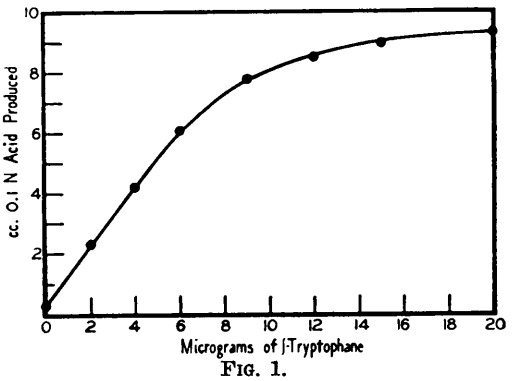

FIG. 1.

Although both *L. casei* and *L. arabinosus* 17-5 require tryptophane, the latter organism was selected for further study because its requirements appeared to be simpler. The Snell and Wright[6] niacin assay provided a technic readily adaptable to the assay of tryptophane. The basal medium differed only in the omission of tryptophane and the inclusion of niacin at a level of two micrograms per assay tube. The preparation and suitable combination of supplements have been described elsewhere.[6,7]

Specificity of Response to Tryptophane. The response of *L. arabinosus* 17-5 to pure *l*-tryptophane (Merck) is shown by a standard curve (Fig. 1) based on about 50 inde-

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