Riboflavin in Red Blood Cells in Relation to Dietary Intake of Children

VIRGINIA A. BEAL, M.P.H.* AND JOHN J. VAN BUSKIRK, B.S.†

THE LEVEL of riboflavin in red blood cells has been suggested as a sensitive and practical index for evaluating the riboflavin nutrition of the individual[1] although there have been few reports in the literature on the use of this technic. There is evidence that in severe dietary deficiency the level of riboflavin in red blood cells is lowered.[1,2] However, there is a paucity of data on well nourished individuals.

MATERIAL AND METHODS

In order to increase our understanding of the growth and development of a group of healthy children enrolled in the Child Research Council, an investigation of dietary intake was started in 1946; determinations of riboflavin in plasma and red cells were added to the program of blood study in 1948. Determinations of plasma riboflavin were discontinued in 1954 when analysis of the data showed that under the conditions of this study these determinations added little to our understanding of the physiologic status of individual children. At that time there were indications that the riboflavin in red blood cells might be more meaningful, therefore this procedure was continued in order to provide more data for later re-evaluation.

The children enrolled in this study came from upper middle class families in the Denver area and were primarily of North European extraction. Examinations were conducted at stated intervals from the day of birth to evaluate the physical, physiologic and psychologic status. No therapy was given by the Council staff. Nutrition histories were taken at monthly intervals during the first year of life and at intervals of three months thereafter. Nutrient intake was calculated from tables on food values. Details of this technic have been published.[4]

The method for determination of riboflavin in red blood cells by Burch, Bessey and Lowry[5] was used during an eleven year period by five different workers. The principle of the method is the estimation of riboflavin by the measurement of its fluorescence before and after reduction. No important changes have been made in the method as originally described but, because of variations in power supply, it has been found advisable to prepare a new standard curve for each day's analyses. In most cases determinations were made in duplicate or triplicate. Duplicate analyses have agreed within 5 to 15 per cent. This degree of precision seems insufficient for longitudinal studies since apparent individual variations may be due to errors inherent in the technic rather than to true differences in the state of riboflavin nutrition. An important theoretic source of error is the possibility that red blood cells may contain substances other than riboflavin whose fluorescence depends on the state of oxidation.

Blood was drawn for study at monthly intervals from three days to three months, at three-monthly intervals from three months to three years and thereafter at intervals of six months; determinations of riboflavin in red blood cells were not always done at each age on each child. The 25, 50 and 75 percentiles,

From the Child Research Council, University of Colorado School of Medicine, Denver, Colorado.

* Nutritionist, Child Research Council, and Assistant Clinical Professor of Pediatrics, University of Colorado School of Medicine; † Assistant Biochemist, Child Research Council, University of Colorado School of Medicine.

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TABLE I
Red Cell Riboflavin (μg. %)

Age	Boys						Girls					
	No. of Cases	Minimum	25 Per Cent	50 Per Cent	75 Per Cent	Maximum	No. of Cases	Minimum	25 Per Cent	50 Per Cent	75 Per Cent	Maximum
3 days ±	12	21.4	25.4	29.8	32.4	37.0	15	23.0	27.4	29.1	33.1	46.0
1 mo.	17	14.9	27.8	31.2	34.5	46.0	16	22.0	27.6	29.8	34.2	37.0
2 mo.	11	21.1	30.0	33.4	37.0	41.0	14	20.0	27.7	30.4	34.8	35.3
3 mo.	14	25.4	32.4	34.8	39.0	26.0	12	26.0	27.8	30.6	35.2	40.3
6 mo.	15	27.7	29.4	32.3	36.6	47.6	14	23.7	27.8	31.2	35.7	38.4
9 mo.	17	21.0	28.0	30.9	35.2	42.8	16	21.9	27.6	31.3	35.7	46.8
1 yr.	16	25.1	27.1	30.0	34.2	46.0	12	26.1	27.1	30.7	35.1	46.2
1¼ yr.	13	19.3	26.4	29.3	33.3	51.0	13	23.5	26.4	29.6	33.8	33.8
1½ yr.	18	18.2	25.7	28.6	32.4	37.0	13	19 5	25.4	28.7	32.8	33.3
1¾ yr.	14	17.9	25.1	28.1	31.8	27.3	18	18.6	24.4	28.0	32.0	33.0
2 yr.	12	19.0	24.7	27.7	31.3	35.4	17	17.5	23.7	27.3	31.3	35.0
2¼ yr.	12	21.4	24.3	27.2	30.8	34.3	17	17.9	23.1	26.8	30.7	36.4
2½ yr.	13	21.0	24.0	26.9	30.3	34.4	16	17.3	22.6	26.4	30.2	33.4
2¾ yr.	10	21.7	23.6	26.6	29.9	36.4	18	15.1	22.0	26.0	30.1	29.7
3 yr.	12	21.8	23.3	26.2	29.6	32.2	15	18.7	21.9	25.6	29.9	30.5
3½ yr.	14	18.0	22.7	25.7	28.9	27.8	18	19.3	21.3	25.0	29.6	38.6
4 yr.	12	20.3	22.3	25.3	28.3	30.0	16	16.0	20.9	24.5	29.4	42.0
4½ yr.	7	22.9	22.0	24.9	27.9	29.9	18	16.9	20.6	24.1	29.3	34.8
5 yr.	9	19.8	21.8	24.8	27.6	28.0	14	18.6	20.4	23.8	29.2	34.4
5½ yr.	18	16.9	21.7	24.7	27.2	29.4	17	15.9	20.3	23.7	29.1	35.5
6 yr.	17	13.4	21.7	24.7	27.2	32.2	15	17.0	20.2	23.6	29.1	36.6
6½ yr.	14	19.0	21.6	24.5	27.2	31.9	16	14.5	20.2	23.6	29.0	34.2
7 yr.	13	18.2	21.5	24.4	27.0	31.9	14	13.8	20.2	23.6	29.0	29.3
7½ yr.	7	15.8	21.4	24.2	27.0	29.0	15	17.6	20.2	23.5	29.0	29.3
8 yr.	8	17.4	21.3	24.2	27.0	30.1	14	14.6	20.2	23.4	28.9	34.2
8½ yr.	8	14.7	21.3	24.0	27.0	24.2	16	14.6	20.2	23.4	28.8	32.7
9 yr.	11	18.8	21.2	24.0	27.0	38.8	14	15.0	20.2	23.3	28.5	32.0
9½ yr.	9	16 1	21.2	24.0	27.0	27.1	11	15.2	20.2	23.3	28.2	26.1
10 yr.	15	16.1	21.2	24.0	27.0	36.3	11	17.2	20.1	23.2	27.9	31.8
10½ yr.	7	21.0	21.1	23.7	27.0	29.0	12	17.9	20.1	23.2	27.8	34.0
11 yr.	7	20.4	21.0	23.6	26.9	42.3	23	12.2	20.1	23.1	27.7	35.7
11½ yr.	8	18.8	20.9	23.4	26.8	39.6	22	17.0	20.1	23.1	27.6	32.6
12 yr.	19	16.3	20.8	23.1	26.6	36.6	17	16.4	20.1	23.0	27.5	28.0
12½ yr.	14	13.1	20.6	22.9	26.3	34.0	15	17.1	20.1	23.0	27.4	31.9
13 yr.	9	16.3	20.4	22.7	26.0	29.8	15	17.9	20.0	22.9	27.3	26.8
13½ yr.	12	18.1	20.3	22.3	25.7	29.2	21	12.2	20.0	22.8	27.2	30.5
14 yr.	14	15.2	20.2	21.9	25.3	28.5	20	17.2	20.0	22.8	27.1	30.4
14½ yr.	17	16.0	20.0	21.7	25.0	27.2	18	13.9	20.0	22.7	27.0	32.4
15 yr.	17	16.9	19.9	21.5	24.6	32.4	11	14.5	..	22.7	..	28.0
15½ yr.	14	13.9	19.8	21.4	24.2	32.8	2	19.4	..	22.7	..	27.1
16 yr.	13	12.8	19.7	21.3	23.8	38.0	12	16.4	..	22.7	..	29.7
16½ yr.	7	19.9	19.5	21.2	23.4	27.4	3	15.4	..	22.7	..	22.9
17 yr.	14	16.6	19.2	21.1	23.0	27.7	7	19.4	..	22.7	..	27.3

which have been visually smoothed, and the observed minimum and maximum values from three days to seventeen years of age are presented in Table I. Only those children were excluded who were known to have a serious illness at the time of the examination. As indicated in Table I, the number of determinations at each age is relatively small; no attempt was made to establish quartile levels on the infrequent values after seventeen years of age. However, these data are more extensive than any previously reported in the literature.

Because the determinations of riboflavin in red blood cells were done on a larger number of children but with less regularity than nutritional histories, the data used for correlations are those on children for whom there were concomitant determinations in both areas of study. The dietary figures represent the average daily intake during the preceding month or three months or six months, the time interval increasing as the child becomes older; the blood level was determined at the end of that time interval. There have been 282 pairs of determinations on thirty-two boys and 334 pairs on thirty-six girls, ranging from one month to eighteen years of age.

RESULTS AND COMMENTS

Total dietary intake increases with age while the riboflavin in red blood cells, after a rise

TABLE II
Intake of Riboflavin (μg./kg. Body Weight)

Age (Year and Month)	Boys						Girls					
	No. of Cases	Minimum	25 Per Cent	50 Per Cent	75 Per Cent	Maximum	No. of Cases	Minimum	25 Per Cent	50 Per Cent	75 Per Cent	Maximum
0-0 to 0-1	20	91	197	237	277	334	19	152	207	242	276	305
0-1 to 0-2	24	136	240	281	327	338	24	109	224	278	307	376
0-2 to 0-3	26	125	200	248	278	340	24	50	217	250	278	336
0-3 to 0-4	27	160	192	222	243	306	27	51	211	238	267	307
0-4 to 0-5	28	141	185	212	235	284	29	67	202	228	253	372
0-5 to 0-6	28	124	178	208	229	279	31	58	192	218	236	348
0-6 to 0-9	28	145	170	198	221	265	34	142	176	200	216	332
0-9 to 1-0	32	122	156	184	208	236	34	131	155	176	198	270
1-0 to 1-3	31	86	137	165	185	256	34	87	140	160	186	247
1-3 to 1-6	30	82	118	141	165	248	34	88	128	147	175	211
1-6 to 1-9	28	55	98	128	148	248	34	83	118	134	163	207
1-9 to 2-0	28	46	91	116	140	213	36	59	108	123	150	198
2-0 to 2-3	27	46	87	110	135	207	35	45	102	116	135	197
2-3 to 2-6	28	64	86	107	132	177	34	67	95	109	125	170
2-6 to 2-9	28	40	85	104	130	184	31	74	89	103	118	144
2-9 to 3-0	27	64	84	103	129	181	31	56	85	99	114	167
3-0 to 3-3	27	58	83	101	128	204	31	56	81	96	110	148
3-3 to 3-6	28	68	82	100	126	178	31	53	79	94	108	138
3-6 to 3-9	26	39	81	99	124	163	32	43	77	93	107	141
3-9 to 4-0	25	61	80	98	121	162	29	43	76	92	106	135
4-0 to 4-3	26	59	80	97	118	170	29	53	75	91	106	134
4-3 to 4-6	26	59	80	96	115	172	27	42	75	90	106	134
4-6 to 4-9	27	54	79	95	112	153	27	57	74	89	105	123
4-9 to 5-0	26	53	79	94	110	149	27	48	73	88	105	120
5-0 to 5-3	27	52	79	93	108	129	27	44	72	88	105	181
5-3 to 5-6	26	58	78	92	107	151	27	37	71	87	104	181
5-6 to 5-9	26	56	78	91	106	136	26	54	70	87	103	151
5-9 to 6-0	26	65	77	90	106	140	24	50	70	86	102	151
6-0 to 6-3	26	58	77	89	105	127	24	56	70	85	101	148
6-3 to 6-6	24	63	76	88	104	127	24	54	70	84	99	127
6-6 to 6-9	23	62	75	87	103	130	25	56	70	83	97	128
6-9 to 7-0	23	59	74	86	102	113	26	43	68	81	96	122
7-0 to 7-3	22	54	73	85	100	110	27	53	67	80	94	117
7-3 to 7-6	20	54	72	84	99	107	27	51	65	79	92	117
7-6 to 7-9	18	44	70	83	98	115	28	45	64	78	90	128
7-9 to 8-0	17	52	69	82	97	128	28	42	62	76	88	115
8-0 to 8-3	17	53	..	81	..	109	26	49	61	74	85	111
8-3 to 8-6	16	57	..	80	..	112	25	44	59	73	83	108
8-6 to 8-9	17	48	..	79	..	112	24	47	57	71	81	96
8-9 to 9-0	17	53	..	78	..	113	23	45	56	69	79	107
9-0 to 9-3	14	55	..	77	..	102	23	45	54	67	77	100
9-3 to 9-6	13	41	..	76	..	116	22	43	53	65	75	98
9-6 to 9-9	12	45	..	75	..	107	23	38	51	63	74	91
9-9 to 10-0	12	52	..	74	..	108	22	38	49	62	73	91

in the first three months of life, decreases rapidly to six years and then more slowly throughout this age span. As a result, a negative correlation was found between the red cell level and total dietary intake of riboflavin. Therefore, dietary intake per kilogram of body weight was used for all further correlations. The smoothed percentiles and observed extremes of dietary intake of riboflavin per kilogram of body weight from birth to ten years of age are presented in Table II. Data over ten years are still inadequate in number and will not be presented here.

Since vitamin supplements were taken by some children in varying amounts, these were added to dietary intake for the initial correlations. The correlation coefficients for riboflavin in red blood cells and intake per kilogram from dietary sources plus vitamin supplements ($+0.44 \pm 0.06$ for boys and $+0.42 \pm 0.05$ for girls) were slightly lower than those for red cell level and intake per kilogram from dietary sources only. Since this is a group of healthy children whose intakes of total riboflavin[6] tend to be higher than the Recommended Allowance of the National Research Council,[7] it may be assumed that intake of riboflavin from diet alone is adequate for physiologic needs and that supplementation by vitamin preparation is superfluous. Further analysis

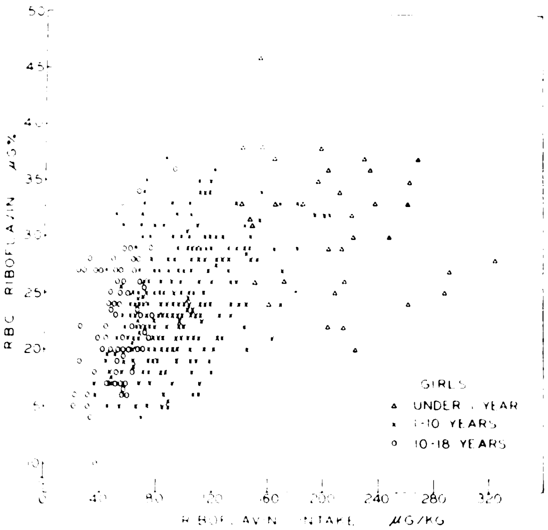

Fig. 1. Values for riboflavin in the red blood cells of girls of various ages (see text).

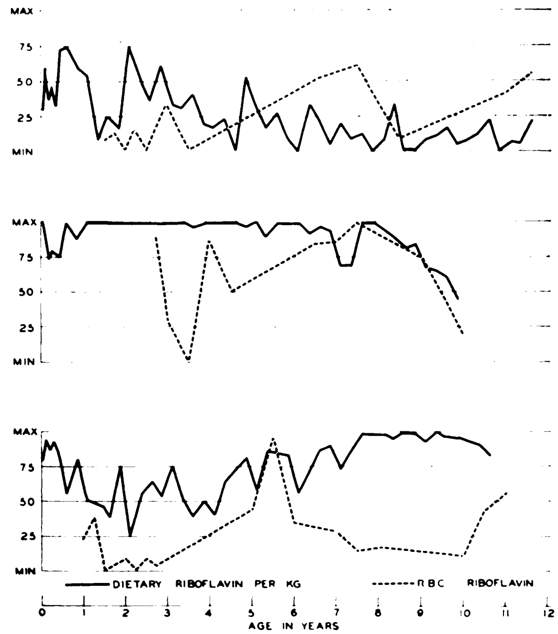

Fig. 2. Graphs for three children represent values obtained for both red cell riboflavin and dietary intake per kg. of weight, plotted on the rectilinear percentiles for age.

of the data was done with the exclusion of the supplements.

When red cell level was compared to dietary intake of riboflavin per kilogram of body weight, the correlation coefficients were +0.47 for boys and +0.46 for girls if all ages from one month to eighteen years were included. Inspection of the data showed that infants tend to have higher levels of riboflavin in both dietary intake and red blood cells while children over ten years of age tend to have lower levels. This may be seen in the graph of values for girls (Fig. 1) and suggests an artifact due to the similarity of decrease with age of both of these factors rather than a real effect of dietary intake upon the blood level. When smaller age groups were analyzed, the correlations between dietary intake and blood level ranged from zero to +0.39 (Table III), indicating relatively little relationship. A wide range of red cell values was observed at each dietary intake level while a wide range of dietary intakes was found in children whose blood values were similar.

Analysis of the records of individual children showed no consistent relationship between dietary intake and the level of riboflavin in red blood cells. As with the group data, there was greater disparity in blood level of the individual when vitamin supplements were added to diet than when dietary intake alone

Table III
Correlation Coefficients of Dietary Intake of Riboflavin per Kilogram of Body Weight with Red Blood Cell Riboflavin

Age	Boys		Girls	
	Correlation Coefficient	No. of Cases	Correlation Coefficient	No. of Cases
Under 1 yr.	+0.14 ± 0.12	65	0 ± 0.15	41
1–6 yr.	+0.18 ± 0.08	140	+0.26 ± 0.08	154
6–10 yr.	+0.07 ± 0.14	53	+0.30 ± 0.12	71
10–18 yr.	+0.05 ± 0.20	24	+0.39 ± 0.12	68
Total Group	+0.47 ± 0.06	282	+0.46 ± 0.05	334

was considered. An attempt to determine whether there might be a level of intake which could be interpreted as a saturation level in relation to the blood values was fruitless. The inconsistencies between intake and blood levels were so great that one child with a fairly constant intake level might show wide fluctuations in the level of riboflavin in red blood cells or another child with marked changes in intake might have fairly constant red cell values. Examples of the findings in three children are shown in Figure 2, in which values for both red cell riboflavin and dietary intake per kilogram of weight are plotted on the rectilinear percentiles for age.

Basal metabolic rates and patterns of growth in height and weight seem to bear little relationship to the levels of riboflavin in red cells and were not helpful in explaining the discrepancies between intake and blood levels. No significant relationship was found between riboflavin levels in red blood cells and reticulocyte counts of fifty-eight children under ten years of age.

CONCLUSION

It must be concluded that in this group of healthy children, although both dietary intake of riboflavin per kilogram of body weight and the riboflavin level in red blood cells decrease with age, the level of dietary intake seems to bear no significant relationship to the level of riboflavin in red blood cells within the intake range observed.

SUMMARY

As part of a study of the growth and development of children, determinations of riboflavin in red blood cells and in the diet were done at stated intervals for several years on the same children. This paper presents the quartile values and the minimum and maximum levels of riboflavin in red blood cells and of the dietary intake of riboflavin per kg. of body weight. A correlation coefficient of +0.47 was found in 282 paired determinations on thirty-two boys and a coefficient of +0.46 in 334 paired determinations on thirty-six girls, ranging from one month to eighteen years of age. Infants tend to have higher levels of riboflavin in both dietary intake and red blood cells while children over ten years of age tend to have lower levels. Within each age group correlations indicate relatively little relationship between the dietary intake of riboflavin and the amount of that substance found in the red blood cells.

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