

Anemia in Experimental Protein Deficiency in the Rhesus Monkey with Special Reference to Iron Metabolism

By S. K. SOOD, M. G. DEO AND V. RAMALINGASWAMI

ANEMIA is an important manifestation of Kwashiorkor,¹⁻⁵ a syndrome of protein malnutrition. Woodruff,⁵ on the basis of his studies in Africa, emphasized the difficulty of assessing the role of protein malnutrition per se in the genesis of anemia in Kwashiorkor, since the human syndrome was associated with multiple nutritional deficiencies and often complicated by intercurrent infections and parasitic infestations. This anemia responds slowly to high protein therapy alone unless the diet is supplemented with specific hematinics such as iron⁶ and folic acid.⁷ However, recently some workers have reported a good response of the anemia of Kwashiorkor to a high protein diet without other hematinics.⁸

The importance of dietary protein in the synthesis of hemoglobin was recognized several years ago by Whipple and Hooper⁹ and Jenks.¹⁰ These workers demonstrated that regeneration of hemoglobin in laboratory animals made anemic through phlebotomy is enhanced if the diet is supplemented with protein. Rats kept on a protein-deficient diet consistently develop anemia which is microcytic and hypochromic.¹¹

The reports on the morphologic type of anemia in Kwashiorkor are variable. Normocytic normochromic anemia is reported most commonly,^{3,7,12,13} but hypochromic⁵ and megaloblastic types are also reported.^{7,14} Disturbances in iron absorption, and a fall in serum iron and iron binding capacity have been found in human^{15,16} and experimental protein malnutrition.^{17,18} Changes in serum folic acid and vitamin B₁₂ have been also observed.^{19,20} The role of these changes in the pathogenesis of anemia in protein malnutrition is not known.

We have been able to induce in rhesus monkeys a syndrome very similar to human Kwashiorkor by tube-feeding them a diet deficient only in protein but adequate in all other nutrients and calories.²¹⁻²³ Using this experimental primate model, we attempted to elucidate the role of protein deficiency per se in the pathogenesis of anemia. In this communication we present the results of this study with special reference to the disturbances in iron metabolism.

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Table 1.—Composition of Diets

Group	Constituents in Gm.						Total Calories
	Sago*	Sugar	Casein	Peanut Oil	Salt Mixture	Water Soluble Vitamin† Mix.	
Group I (Low protein)	34	46	—	5	4	1	365
Group II (High protein control)	34	31	15	5	4	1	365

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*Sago consists of small globules or pearls made from starch obtained from tapioca (*Manihot utilisima*).

†Each monkey also received daily 400 I.U. vitamin A; 100 I.U. vitamin D, and 0.5 mg. of α -tocopherol.

MATERIAL AND METHODS

Twenty-four young growing rhesus monkeys were used. All except 3 were males. Their body weights ranged between 2 Kg. and 4 Kg. at the beginning of the experiment. They were divided into two groups. Group I consisted of 17 animals which were rendered protein-deficient by tube-feeding them a diet containing negligible amounts of protein but adequate in other nutrients. Group II consisted of 7 animals which served as controls. They were fed a diet identical in all respects to the diet given to group I, except that it contained 15 per cent of casein. Each animal in both groups received a diet equivalent to 100 calories per Kg. of body weight daily. The composition of diets is given in table 1. The care of the animals, the details of the mineral and vitamin supplements, the preparation of the diets and the technics of feeding have been described earlier.²¹ It may be mentioned that each animal in both groups received 10 mg. of iron in the form of ferrous sulfate, 1 μ g. of vitamin B₁₂ and 0.5 mg. of folic acid per day as a part of the vitamin and mineral supplements.

The following baseline investigations were performed before the animals were placed on the experimental diet and repeated at intervals throughout the experiment.

1. *Hematologic investigations*: Hemoglobin by the cyanmethemoglobin method, hematocrit and erythrocyte count.²⁴

2. Serum iron: (Se.Fe) Ramsay's²⁵ technic using 2:2 dipyrindyl as the coloring reagent. In some earlier studies, a modified technic of William and Zak²⁶ was used. Several simultaneous determinations on different samples by both technics gave comparable values.

3. *Serum unsaturated iron binding capacity*: (UIBC) (Ressler & Zak).²⁷

4. *Total serum proteins*: Biuret technic.²⁸ Fractionation by electrophoresis using Beckman's Spinco electrophoresis apparatus.

5. *Iron tolerance*: Animals were fasted overnight and during the test. Ferrous sulphate equivalent to 25 mg. of nonradioactive elemental iron was given orally. Serum samples were obtained before administering the dose of iron and at 1, 3- and 7-hour intervals and serum iron estimated.

6. *Radioiron absorption*: This was measured using the Fe⁵⁹ fecal recovery method of Bonnet et al.²⁹ modified for the monkey. Radioiron (Fe⁵⁹) of high specific activity (1 μ g. = 4000 μ c), in the form of FeCl₃ solution, was obtained from the Atomic Energy Establishment, Trombay, Bombay, India. Two tenths μ c. iron was administered by stomach tube with 50 μ g. of carrier nonradioactive iron as ferrous sulfate and 300 mg. of ascorbic acid. No food or iron was given for the next 8 hours. The animals were kept in metabolism cages for the test. Stools were collected until the fecal radioactivity fell to below 2 per cent of the administered dose. This was achieved in most of the animals in 3-4

Table 2.—*Hematologic Values in Deficient Animals*

Investigation		Basal (14)*	Weeks on Low Protein Diet	
			8-10 weeks (14)*	15 weeks (4)*
Hematocrit (%)	Mean	42.2	34.9	34.2
	Range	(32-54)	(28-44)	(30-39)
Hemoglobin Gm. %	Mean	12.6	10.4	9.4
	Range	(10.1-14.6)	(7.5-13.4)	(8.5-11.5)
RBC (x 10 ⁶ /mm. ³)	Mean	5.9	4.59	3.86
	Range	(4.12-7.20)	(3.46-5.84)	(3.49-4.25)
MCV (μ^3)	Mean	73.4	78.7	88.7
	Range	(52.3-96.0)	(51.3-102.8)	(82.7-94.1)
MCH ($\mu\mu\text{g.}$)	Mean	21.4	23.5	24.4
	Range	(13.7-25.7)	(14.3-18.5)	(21.4-27.0)
MCHC %	Mean	29.3	30.0	27.5
	Range	(25.2-31.5)	(26.8-34.3)	(25.8-29.4)
t and P test		Hematocrit	Hemoglobin	RBC
Basal and 8-10 weeks		t = 4.586 n = 13 p < 0.001	t = 5.238 n = 13 p < 0.001	t = 3.279 n = 13 p < 0.01

*Number of animals.

days. Entire stools were transferred to a pyrex glass beaker and digested using concentrated nitric acid. A 2-ml. aliquot was taken in a standardized test tube and counted in a well-type scintillation center.

7. *Bone Marrow*: Specimens were obtained under sodium pentobarbitone (Nembutal) anesthesia from the iliac crest at the end of the experiment. The smears were fixed in methanol and stained by Leishman Stain and by Perl's reaction.³⁰

8. All animals were sacrificed at the end of the study and sections of the liver, spleen and bone marrow examined for stainable iron.

RESULTS

Hematologic Studies

All protein-deficient animals, except 1, developed moderately severe anemia between 8 and 10 weeks of deficiency (table 2). The hematocrit showed a significant fall in 12 out of 14 animals. The mean hematocrit value initially and at 8-10 weeks of deficiency was 42.2 per cent and 34.9 per cent, respectively. Hemoglobin concentration and erythrocyte count were also significantly decreased (table 2). Further observations in 4 animals at 15 weeks of deficiency indicated a continued fall in hematocrit, hemoglobin concentration and erythrocyte count (table 2). In 2 deficient animals which were re-fed a high protein diet for 8 weeks the anemia was completely corrected. The anemia encountered in the deficient animals was normocytic and normochromic. The MCHC remained almost constant throughout the period of experiment. MCV and MCH were slightly raised in protein-deficient animals; however, these changes were not statistically significant. The controls did not show any significant change in hematologic indices throughout the

Table 3.—*Hematologic Values in Control Animals*

Investigation		Basal (7)*	8-10 Weeks High Protein Diet (7)*
Hematocrit %	Mean	43.7	42.7
	Range	(39-50)	(38-45.0)
Hemoglobin Gm. %	Mean	12.7	13.3
	Range	(11.3-14.2)	(12.3-15.8)
RBC ($\times 10^6/\text{mm}^3$)	Mean	6.5	5.4
	Range	(5.45-8.42)	(3.59-6.75)

RBC $t_0 = 1.969$; $P < 0.1$

*Number of animals.

Table 4.—*Serum Iron and Iron Binding Capacity in Protein-Deficient Animals*

			Low Protein Diet	
		Basal (12)*	4 Weeks (6)*	8-10 Weeks (12)*
Serum iron $\mu\text{g. \%}$	Mean	172	113	111.4
	Range	(83-308)	(70-177)	(75-175)
U.I.B.C. $\mu\text{g. \%}$	Mean	269	234	174
	Range	(140-344)	(164-296)	(106-272)
T.I.B.C. $\mu\text{g. \%}$	Mean	441	346	285
	Range	(275-547)	(314-394)	(193-347)
<i>t and p Test Only for the Paired Observations</i>				
Basal and 8-10 weeks		Serum Iron $t_{(11)} = 3.388$ $P < 0.01$	U.I.B.C. $t_{(11)} = 4.596$ $P < 0.001$	T.I.B.C. $t_{(11)} = 7.228$ $P < 0.001$

* = Number of animals.

experiment (table 3). The marrow of deficient animals was normoblastic. There were no morphologic abnormalities in the erythroid, myeloid and megakaryocytic elements. No giant erythroblasts or metamyelocytes were seen. The marrow cellularity, as examined on smears and sections, was not appreciably altered. The M.E. ratio was slightly raised in the protein-deficient animals and is probably due to reduced erythroid activity.

Serum Iron, Iron Binding Capacity of Serum and Iron Absorption

Serum Iron: The fasting serum iron showed a progressive fall in the protein-deficient animals (table 4). All the deficient animals, except 1, showed a marked reduction in Se.Fe at 8-10 weeks of deficiency. The mean value of 111.4 $\mu\text{g. per cent}$ of Se.Fe at this stage was significantly lower than the mean basal value of 172 $\mu\text{g. per cent}$. This change could be reversed by refeeding the animals a high protein diet.

In comparison with this, the control animals showed no significant alterations in the level of Se.Fe (table 5).

Serum Iron Binding Capacity: The most consistent change in this experiment was observed in the total iron binding capacity (TIBC) of serum which showed a definite, uniform and progressive reduction in all the protein-de-

Table 5.—*Serum Iron and Iron Binding Capacity in Control Animals*

Investigation	Basal (6)*	8 to 10 Weeks High Protein Diet (6)*
Serum Fe ($\mu\text{g. \%}$)	179.3 (128–250)	211.1 (96–263)
U.I.B.C. ($\mu\text{g. \%}$)	292.3 (104–384)	289.8 (120–400)
T.I.B.C. ($\mu\text{g. \%}$)	471.6 (336–527)	509.0 (346–628)
	Serum iron : $t_{(5)} = 0.761$	$P < 0.5$
	T.I.B.C. : $t_{(5)} = 0.638$	$P < 0.5$

* = Number of animals.

ficient monkeys (table 4). The reduction in TIBC was severe; its value falling to almost $\frac{2}{3}$ of the basal value at 8–10 weeks of deficiency. The UIBC also showed similar alterations (table 4). These changes could be reversed in the 2 protein-deficient animals, refed a 15 per cent casein diet for 8 weeks. In contrast, the control animals showed no significant changes in TIBC and UIBC.

Plasma Iron Tolerance: In preliminary studies on healthy monkeys maintained on stock diet, the highest level of plasma iron after oral iron was observed between 1 and 3 hours. The level tended to fall to nearly basal value at the end of 7 hours. The rise in plasma iron was generally $1\frac{1}{2}$ to $3\frac{1}{2}$ times the basal value.

The iron tolerance curves tended to be flat in protein-deficient animals (fig. 1). The highest levels of plasma iron reached after the test dose in deficient animals were $258.3 \mu\text{g. per cent}$ and $204.5 \mu\text{g. per cent}$ on the average at 4 and 8 weeks of deficiency. These were significantly lower than the mean basal value of $388.7 \mu\text{g. per cent}$. On replenishing protein in the diet in 2 animals, the curves returned to normal in 8 weeks (fig. 1). The iron tolerance was studied in 3 control animals only, at similar periods of dietary regime. No significant alterations from basal values were seen.

Fe⁵⁹ Absorption: Absorption of iron using Fe^{59} was studied in 9 deficient and 4 control monkeys. At 8–10 weeks of protein deficiency, there was a fall of iron absorption ranging from 9 per cent to 23 per cent over the basal values in 7 out of 9 animals (table 6). In 1 animal, the absorption was not affected and in another there was a rise of 6 per cent at the end of 10 weeks of protein deficiency. The average value for iron absorption in the deficient group was 39.9 per cent as compared to the basal value of 50.8 per cent. The fall in absorption was significant statistically. In 4 control animals, which were fed a high protein diet for a comparable period, no significant changes were observed (table 7).

Serum Proteins: The changes in serum proteins were very striking. The protein-deficient animals showed a marked and consistent fall in total serum protein and in the albumin fraction (fig. 2).

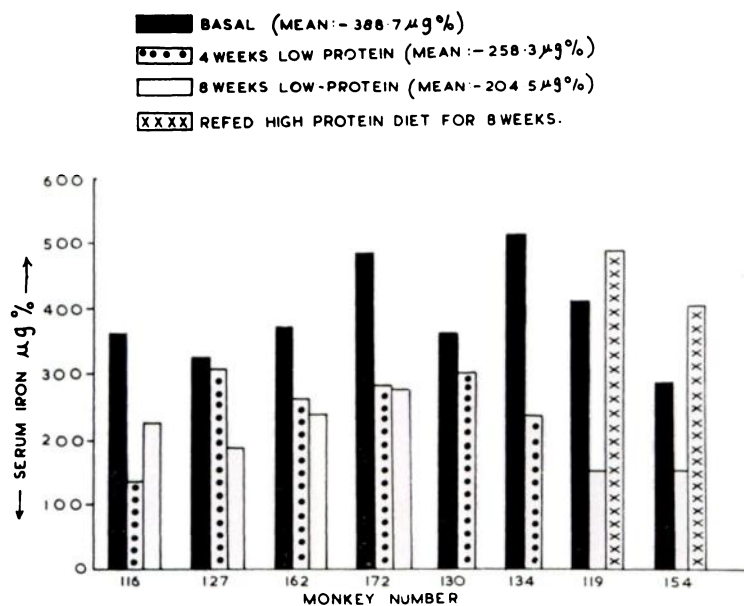


Fig. 1.—Iron tolerance in protein-deficient animals. Each bar shows maximum level of serum iron following a test dose of 25 mg. of oral iron. All animals showed lowering of peak levels at 4 and 8 weeks of protein deficiency. Refeeding of a protein rich diet to 2 animals (No. 119 and 154) resulted in restoration of these levels.

The fall was progressive as the duration of deficiency increased. At the end of 8 weeks, the concentration of total serum proteins and albumin had fallen from basal levels of 7.24 Gm. per cent and 3.87 Gm. per cent to 5.24 Gm. per cent and 1.21 Gm. per cent, respectively. There was a quantitative rise in total globulins solely due to a rise in gamma globulins. The alpha and beta globulins did not show any significant alteration. In 2 protein-deficient monkeys which were refed a diet containing 15 per cent casein, the total protein and albumin returned to original basal value, but the gamma globulin fraction remained high. Three animals used as controls did not show any remarkable variation from the basal value.

DISCUSSION

In the present experiment uncomplicated protein deficiency has been produced in rhesus monkeys by feeding them a diet adequate in calories and all other nutrients. Each monkey served as its own control, each animal having been studied before and at intervals during the feeding of test diets. Furthermore, the successful reversal of changes in 2 protein-deficient monkeys by replenishing their dietary protein, makes the observations even more significant. The controls on isocaloric and high protein diets did not show the changes observed in the deficient group. The changes observed in the deficient group may be reasonably attributed specifically to deficiency of dietary protein.

Table 6.—Absorption of Fe^{59} in Protein-Deficient Monkeys

Serial Number	Monkey Number	% of Orally Administered Fe^{59}	
		Basal	8-10 Weeks of Protein Deficiency
1.	284	54.7	35.0
2.	313	29.0	35.0
3.	348	34.0	22.7
4.	350	64.0	43.8
5.	351	40.0	36.8
6.	353	61.0	56.0
7.	358	58.0	35.7
8.	361	59.0	60.0
9.	370	58.0	45.0
Mean		50.8	39.9
		$t_{(8)} = 2.91$	$P < 0.02$

Table 7.—Absorption of Fe^{59} in Control Monkeys

Serial Number	Monkey Number	Basal	10 Weeks of High Protein Diet
1.	359	35.4	36.0
2.	360	65.0	56.0
3.	362	58.0	60.0
4.	379	30.0	36.0
Mean		45.6	47.6
		$t_{(3)} = 0.32$	$P < 0.8$

Anemia

Moderate anemia developed in a majority of animals at the end of 8 weeks of protein restriction. At this time, severe reduction in plasma albumin, marked atrophy of organs with high protein turnover such as the pancreas and gastrointestinal tract, and severe fatty change in the liver are present in these animals.²¹⁻²³ Compared to these changes, the anemia was not as marked. This is in conformity with Whipple's work which indicates that hemoglobin synthesis is maintained preferentially at the expense of other body proteins.³¹

In Kwashiorkor, the anemia is variable and can be of any morphologic type.^{3,6,7,12-14} The finding, in the present study, of only one type of anemia in monkeys fed a low protein diet which is adequate in all other hematincs indicates that protein deficiency per se causes only a normocytic normochromic anemia. These observations are in agreement with those of Cartwright and Wintrobe¹⁷ in protein-deficient swine but are at variance with those of Aschkenasy,¹¹ who reported microcytic hypochromic anemia in protein-deficient rats. This may be a species difference.

The reported therapeutic response of the anemia of Kwashiorkor to hematincs other than protein, like iron⁶ or folic acid,⁷ has led to a belief that protein deficiency plays, if at all, a minor role in the pathogenesis of anemia in this condition. However, it is now recognized that the anemia of Kwash-

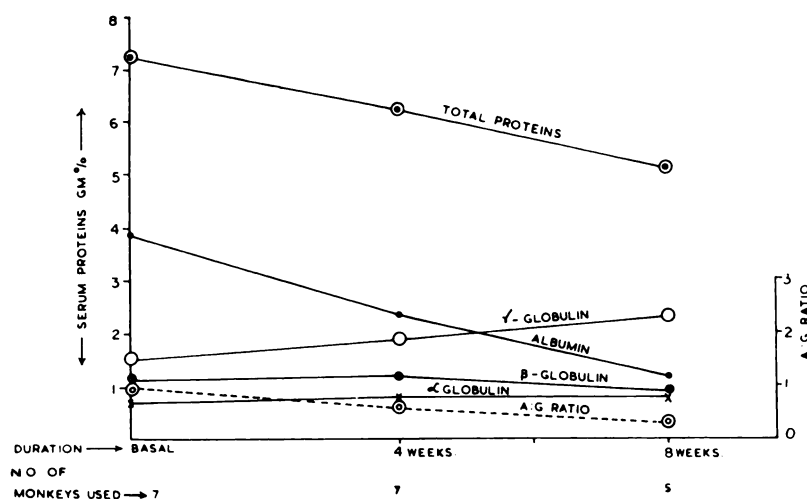


Fig. 2.—Mean serum protein levels in protein-deficient animals, initially and at 4 and 8 weeks of protein depletion. A progressive fall in total serum proteins, serum albumin and A:G ratio, and a rise in γ -globulin can be seen.

iorkor responds inadequately to treatment with iron, folic acid or vitamin B₁₂ unless the diet is supplemented with protein.⁵ Recently, therapeutic response of the anemia of Kwashiorkor to a high protein diet alone has been reported.⁸ In the present experiment, in 2 protein-deficient animals, anemia could be completely corrected by protein repletion. These observations bring into focus the importance of protein deficiency per se in the causation of anemia.

Iron Metabolism

It is interesting to note that the changes in Se.Fe and TIBC in protein-deficient monkeys are similar to those recently reported in Kwashiorkor.^{15,16} In human cases, the TIBC falls to about one-half to one-fourth of the normal values and levels as low as 78 μ g. per cent were reported by Lahey et al.¹⁵ The fall in Se.Fe was not as severe as the fall in TIBC and, therefore, the per cent saturation of transferrin was raised. In our protein-deficient monkeys, on the other hand, the per cent saturation of transferrin was not significantly altered due to a comparable fall in Se.Fe. The remarkable similarity between the results in monkeys and the reported data on Kwashiorkor strongly suggest a close inter-relationship between iron metabolism and protein deficiency. The relationship is difficult to define in the present stage of our knowledge. The fall in TIBC may be due to a diminished synthesis of the iron-binding protein (transferrin).³²

The fall in serum iron can be due to decreased absorption, poor iron reserves, failure of mobilization of iron from stores or increased erythropoiesis.³⁴ There was no evidence either of depletion of iron or of increased erythropoiesis in the present experiment.

Iron Absorption

The plasma iron tolerance curves were flat in the deficient animals. There are several limitations in the interpretation of these curves, since any point on the curve is a resultant of the rate of iron absorption and its clearance.^{35,36} The low plasma iron in the protein-deficient monkeys after an oral dose of iron, may be due either to a rapid clearance of iron from the plasma or low absorption. The common conditions which produce rapid clearance are infection³³ and increased erythropoiesis.³⁷ There was no obvious focus of infection in our deficient animals and erythropoiesis showed some evidence of depression. The uptake of Fe⁵⁹ by the bone marrow is known to be depressed in protein-deficient rats.³⁸ It would appear, therefore, that the flat curves indicate a lowered absorption in protein deficiency. The Fe⁵⁹ absorption studies support this hypothesis.

Considerable uncertainty exists in the literature regarding the effects of protein malnutrition on the absorption of iron. By feeding a low protein diet, enriched with iron salts, excessive deposition of iron in various tissues was reported by several workers.³⁹⁻⁴¹ On the basis of these observations, it was concluded that the absorption of iron was increased in protein deficiency.³⁹ These conclusions are based on indirect evidence and with diets containing excess of iron. They are, therefore, open to criticism. Bothwell et al.⁴² studied iron absorption in 4 cases of malnutritional siderosis, using the fecal recovery of Fe⁵⁹ as the method of measuring iron absorption. They concluded that iron absorption was lowered in these cases. More recently, Higginson et al.¹⁸ and Klavins et al.⁴³ presented evidence to indicate that iron absorption was, in fact, decreased in protein-deficient rats. These results are in agreement with the data presented here.

Mechanism of Anemia

The anemia observed in the protein-deficient monkeys was normocytic and normochronic. The marrow was normoblastic with reduced erythroid activity. It is unlikely to be due to a deficiency of iron, vitamin B₁₂ or folic acid. It has been shown in this laboratory that organs with high cell turnover such as the gastrointestinal tract, spleen and growing ends of bones of young animals undergo marked atrophy in protein deficiency probably as a result of decreased cellular proliferation.²³ The bone marrow is also an organ with high cell turnover. Bethard et al.⁴⁴ have shown drastic reduction in erythropoiesis in rats on protein-deficient diets. Decreased cellular proliferation may thus be an important mechanism of anemia in protein deficiency.

SUMMARY

1. This investigation deals with a study of the anemia of protein deficiency in Rhesus monkeys.
2. Protein deficiency was induced in 17 rhesus monkeys. Seven animals, given a protein-rich diet, served as controls. The diets of both the groups

were identical in all respects, except protein. All animals were tube-fed to ensure adequate caloric intake.

Hematocrit, hemoglobin, erythrocyte count, serum iron, serum iron binding capacity, plasma iron tolerance curves, and iron absorption using the Fe^{59} fecal recovery method were studied before and at intervals of the experiment in both deficient and control groups.

Protein-deficient monkeys consistently developed normocytic normochromic anemia of moderate severity. A striking fall in serum iron binding capacity, total proteins and albumin with a rise in gamma globulin was observed in all deficient animals. A significant and comparable fall in serum iron was also observed. The Fe^{59} absorption was depressed and there was flattening of plasma iron tolerance curves. Two deficient animals, refed a high protein diet, showed reversal of all these changes. The control animals did not show any of these changes.

The mechanism of anemia and decreased iron absorption observed in the protein-deficient animals and the relevance of these findings to those in Kwashiorkor are discussed.

SUMMARIO IN INTERLINGUA

1. Le hic-reportate studio esseva concernite con le anemia a carentia de proteina in macacas rhesus.

2. Carentia de proteina esseva inducite in 17 macacas rhesus. Septe altere animales recipeva un dieta ric in proteina e serviva como gruppo de controllo. A parte le proteina, le dietas del duo gruppos esseva identic. Le animales recipeva lor alimentation per intubation pro assecurar un adequate ingestion caloric.

Le hematocrite, le concentration de hemoglobina, le numeration erythrocytic, le nivello seral de ferro, le capacitate ferro-ligatori del sero, le curvas de tolerantia pro ferro del plasma, e le intensitate del absorption de ferro (con le uso del methodo a identification de Fe^{59} in le feces) esseva determinate ante le experimento e a intervallos durante illo, tanto in le gruppo a carentia de proteina como etiam in le gruppo de controllo.

Animales a carentia de proteina disveloppava uniformemente normocytic anemia normochromic de moderate grados de severitate. Un frappante declino del capacitate ferro-ligatori del sero, del total contentos seral de proteínas e de albumina, con un augmento del globulina gamma, esseva observate in omne le animales a carentia de proteina. Un significative e comparabile declino in le ferro seral esseva etiam observate. Le absorption de Fe^{59} esseva deprimite, e le curvas de tolerantia pro ferro del plasma esseva applattate. In duo animales a carentia de proteina, le transferimento a un dieta rica in proteina resultava in un reversion de omne le mentionate alterationes. Le animales de controllo non monstrava ulle de ille phenomenos.

Es discutite le mecanismo del anemia e del reduce absorption de ferro observate in le animales a carentia de proteina si ben como le signification de iste constatationes con respecto al tableau clinic in Kwashiorkor.

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REFERENCES

1. Brock, J. F., and Autret, M.: Kwashiorkor in Africa. W. H. O. Monograph Series No. 8, Geneva, W. H. O. 1952.
2. Trowell, H. C., Davies, J. N. P., and Dean, R. F. A.: Kwashiorkor 1st ed. London, Edward Arnold, 1954.
3. Adams, E. B.: Anaemia in Kwashiorkor. *Brit. Med. J.* 1:537, 1954.
4. Gopalan, C., and Ramalingaswami, V.: Kwashiorkor in India. *Indian J. Med. Res.* 44:723, 1955.
5. Woodruff, A. W.: Nutritional anemia with special reference to tropical regions. *In* Recent Advances in Human Nutrition. J. F. Brock, ed. London, J. A. Churchill Ltd, 1961, p. 415.
6. Trowell, H. C., and Simpkins, M. J.: Intramuscular iron in the treatment of the anemia associated with Kwashiorkor. *Lancet* 2:265, 1957.
7. Walt, F.: Anemia in Kwashiorkor. *J. Trop. Pediat.* 5:3, 1959.
8. Sandozi, M. K., Rajeshvari, V., Haguni, A. H., and Kaur, J.: Kwashiorkor. A clinicopathological study. *Brit. Med. J.* 2:93, 1963.
9. Whipple, G. H., and Hooper, C. W.: Blood regeneration after simple anemia II. Curve of regeneration influenced by starvation, sugar, aminoacids and other factors. *Amer. J. Physiol.* 45:576, 1918.
10. Jenks, Z.: Studies in regeneration of blood. *Amer. J. Physiol.* 59:240, 1922.
11. Aschkenasy, A.: On the pathogenesis of anemias and leucopenias induced by protein deficiency. *Amer. J. Clin. Nutr.* 5:14, 1957.
12. Altman, A., and Murray, J. F.: Anemia of malignant malnutrition (Infantile pellagra, Kwashiorkor); protein deficiency as possible etiologic factor. *S. Afr. J. Med. Sci.* 13:91, 1948.
13. Woodruff, A. W.: The natural history of anemia associated with protein malnutrition. *Brit. Med. J.* 1:1297, 1955.
14. Mehta, G., and Gopalan, C.: Hematological changes in nutritional edema syndrome (Kwashiorkor). *Indian J. Med. Res.* 44:727, 1956.
15. Lahey, E. M., Behar, M., Viteri, F., and Scrimshaw, N. S.: Values of copper, iron and iron binding capacity in Kwashiorkor. *Pediatrics* 22:72, 1958.
16. Edozien, J. C., and Udeozo, I. O. K.: Serum copper, iron and iron binding capacity in Kwashiorkor. *J. Trop. Pediatrics* 6:60, 1960.
17. Cartwright, G. E., and Wintrobe, M. M.: Studies on free erythrocyte protoporphyrin, plasma copper and plasma iron in protein deficient and iron deficient swine. *J. Biol. Chem.* 176:571, 1948.
18. Higginson, J., Grady, H., and Huntley, C.: The effects of different diets on iron absorption. *Lab. Invest.* 12:1260, 1963.
19. Satoskar, R. S., Kulkarni, B. S., Mehta, B. M., Sanzgiri, R. R., and Bamji, M. S.: Serum vitamin B₁₂ and folic acid (P. G. A.) levels in hypoproteinaemia and marasmus in Indian children. *Arch. Dis. Child.* 37:9, 1962.
20. MacDougall, L. G., and Ross, G. I. M.: Serum Vitamin B₁₂ concentration in Kwashiorkor and marasmus. *J. Pediat.* 57:589, 1960.
21. Deo, M. G., and Ramalingaswami, V.: Production of periportal fatty infiltration of the liver in the Rhesus Monkeys by a protein deficient diet. *Lab. Invest.* 9:319, 1960.
22. Ramalingaswami, V., Deo, M. G., and Sood, S. K.: Protein Deficiency in the Rhesus Monkeys. Publication No. 843. National Academy of Sciences—National Research Council Washington, D. C., 1961 p. 365.
23. Deo, M. G., Sood, S. K., and Ramalingaswami, V.: Pathological features

- of experimental protein deficiency in the rhesus monkey. *Arch. Path.* In press.
24. Dacie, J. V.: *Practical Haematology*, 2nd Ed., London, J. & A. Churchill Ltd., 1956.
 25. Ramsay, W. N.: Plasma iron. *Advances in Clinical Chemistry*, vol. I. H. Sobotka and C. P. Stewart, eds. New York, Academic Press Inc., 1958, p. 1.
 26. William, L. A., and Zak, B.: Standardization of hemoglobin. *Amer. J. Clin. Path.* 28:195, 1957.
 27. Ressler, N., and Zak, B.: Serum unsaturated iron binding capacity. *Amer. J. Clin. Path.* 30:87, 1958.
 28. King, E. J., and Wootton, I. D. P.: *Micro-analysis in Medical Biochemistry*, 3rd ed. London, J. A. Churchill Ltd., 1956, p. 57.
 29. Bonnet, J. D., Hagedorn, A. B., and Owen, C. A.: A quantitative method for measuring the gastrointestinal absorption of iron. *Blood* 15:36, 1960.
 30. Douglas, A. S., and Dacie, J. V.: The incidence and significance of iron containing granules in human erythrocytes and their precursors. *J. Clin. Path.* 6:307, 1953.
 31. Whipple, G. H.: *Haemoglobin, plasma protein and cell protein. Their production and interchange*. Springfield, Ill., Charles C Thomas, 1948.
 32. Edozien, J. C.: Serum proteins in Kwashiorkor. *J. Pediatrics* 57:594, 1960.
 33. Bothwell, T. H., and Finch, C. A.: *Iron Metabolism*. London, J. A. Churchill Ltd., 1962.
 34. Laurell, C. B.: Studies in the transportation and metabolism of iron in the body, with special reference to iron binding component in human. *Acta. Physiol. Scand.* 14 (suppl. 46), 1, 1947.
 35. Josephs, H. W.: Absorption of iron as a problem in human physiology. A critical Review. *Blood* 13:1, 1958.
 36. Hallberg, L., and Solvell, L.: Absorption of a single dose of iron in man. *Acta Med. Scand.* 168 (Suppl. 358), 19, 1960.
 37. Wetherley-Mein, G., and Hutt, M. S. R.: Radioactive iron studies in clinical pathology. *In Recent Advances in Clinical Pathology*. S. C. Dyke, ed. London, J. A. Churchill Ltd., 1960, p. 241.
 38. Bethard, W. F., Wissler, R. W., Thompson, J. S., Schroeder, M. A., and Robson, M. J.: The effects of acute protein depletion on the distribution of radio iron in rat tissues. *Blood* 13:146, 1958.
 39. Gillman, T., Canham, P. A. S., and Hathorn, M.: Experimental nutritional siderosis. *Lancet* 2:557, 1958.
 40. Kinney, T. D., Hegsted, D. M., and Finch, C. A.: The influence of diet on iron absorption I. The pathology of iron excess. *J. Exp. Med.* 90:137, 1949.
 41. Sriramachari, S., Srikantia, S. G., and Gopalan, C.: Cytosiderosis of liver in protein deficient monkeys. *Indian J. Path. Bact.* 2:226, 1959.
 42. Bothwell, T. H., Van Doorn-Wittkamp, H. van, W., Dupreez, M. L., and Alper, T.: The absorption of iron: Radio-iron studies in idiopathic hemochromatosis, malnutritional siderosis and transfusional haemosiderosis. *J. Lab. Clin. Med.* 41:836, 1953.
 43. Klavins, J. N., Kinney, T. D., and Kaufmann, N.: The influence of dietary protein on iron absorption. *Brit. J. Exp. Pathol.* 43:172, 1962.
 44. Bethard, W. F., Wissler, R. W., Thompson, J. S., Schroeder, M. A., and Robson, M. J.: The effect of acute protein deprivation upon erythropoiesis in rats. *Blood* 13:216, 1958.