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Foreword

According to the Recommendations of the World Health Organisation, the diagnosis of nutritional anemias should be based on a comparison between the hemoglobin level and reference values assimilated to the lower limits of the normal (W.H.O., 1968). Since such a definition allows only an arbitrary and incomplete evaluation of the prevalence of anemias, it has been suggested, either to use such a diagnosis only for patients whose level of hemoglobin increases during appropriate administration of certain nutrients (GARBY et al., 1971), or to evaluate the probability with which a given level of hemoglobin deviates from the distribution observed among normal subjects (COOK et al., 1971 ; VITERI and GUZMÁN, 1972). None of these definitions is based on functional considerations : in that perspective anemia should be defined as a condition in which a reduction of the hemoglobin quantity would limit the oxygenation of tissues without the intervention of compensatory mechanisms facilitating the transport of oxygen (OSKI, 1973).

There is no disease where the discrepancy between these definitions appears more clearly than in the syndromes of protein-energy malnutrition among children. The body pools are in fact considerably altered ; in particular, the blood volume and the active tissular mass are smaller before treatment than at the conclusion of refeeding (FONDU, 1977). Any comparison based on the measurement of the hemoglobin concentration is therefore more complex. Moreover, the increase in the quantity of hemoglobin observed during the period of refeeding does not prove that such a quantity was insufficient to ensure initially an adequate transfer of oxygen. The well-known expression "kwashiorkor anemia" is therefore strictly conventional : it only means that the hemoglobin level observed among patients is lower on the average than the level recorded among children free from malnutrition. The fact is so constant that anemia has been considered as one of the basic characteristics of kwashiorkor (BROCK & AUTRET, 1952).

The expression "kwashiorkor anemia" reveals a second ambiguity : one does indeed not know if the etiopathogenic factors responsible for the appearance of the major clinical and biological manifestations of this

syndrome are the same as those leading to anemia which is associated to them. The existence of a cause-effect relationship between malnutrition and anemia is considered probable on the basis of the data provided by animal experimentation. The situation is fundamentally different from that prevailing in several other nutritional anemias : far from taking as a basis accurate pathophysiological studies made on man, one assumes that the hematological picture observed in the patients is identical to that observed in animals in certain experimental conditions (FINCH, 1968 ; FINCH, 1975). Thus one refers generally to the observations made by WHIPPLE *et al.* (1942, 1947), BETHARD *et al.* (1958), HAXHE (1963, 1967) DELMONTE *et al.* (1964), ITO *et al.* (1964), REISSMANN (1964a, b), ITO and REISSMANN (1966), WOODRUFF (1968), STEKEL and SMITH (1969 a, b, 1970), ASCHKENASY (1971) and NAETS and WITTEK (1974). Submitted to a diet limiting the energy supply or the intake of proteins, the animal shows a slowering down of erythropoietic activity which is essentially attributable to a reduction of its oxygen tissular needs. There is no final argument to allow us *a priori* to extrapolate such results to malnourished children. In fact, in several animal experiments, the distinction has not been established clearly between the blood manifestations of three different entities : energy deficiency, deficiency in essential amino-acids and finally the restriction in proteins to which several other essential nutrients are maybe necessarily associated. Moreover it is clear that the sequence of pathological events which took place among patients could be only reproduced in an adequate manner if one had previously a sufficient knowledge of that sequence, which is not the case.

The third ambiguity of the expression "kwashiorkor anemia" derives from the fact that it might lead to assume that pure kwashiorkor *per se* is the only syndrome of protein-energy malnutrition which is complicated by anemia. Nothing can confirm such an assumption. In the Third World, protein-energy malnutrition presents extremely variable forms, although frequently present in association. Hence, in the highlands of Kivu, the clinical manifestation is most frequently marasmic kwashiorkor, that is the association in a same patient of a component "kwashiorkor" and a component "marasmus", each of these syndromes being more or less evident (VIS, 1969, 1975).

During the last decade we have undertaken a research program devoted to the hematological aspects of marasmic kwashiorkor in the region of Kivu. The use of accurate investigation methods, scarcely or even never applied in other previous studies made on the field, enabled us to define several intervening pathophysiological mechanisms. The

1. **Peripheral blood and bone marrow changes**

Marasmic kwashiorkor, as observed in the highlands of Kivu, is accompanied by a moderate reduction of the hemoglobin level. For identical age-groups, the level is 2-3 g/100 ml inferior to the level observed among well-fed children living in the same region (FONDU *et al.*, 1978a). On the average, the mean corpuscular volume (MCV) and the mean corpuscular hemoglobin concentration (MCHC) are within normal limits ; the percentage of reticulocytes is slightly increased (Table 1). The anisocytosis is marked, the curves of PRICE-JONES following generally a trimorphic configuration (VAN OYE, 1953). One frequently observes a

TABLE 1
Main hematological characteristics of marasmic kashiorkor in Kivu. (Mean ± SEM)

	Marasmic kwashiorkor (N = 188)	Local controls (N = 41)
Packed cell volume, %		
– all cases	$33.1^{**} \pm 0.4$	42.2 ± 0.7
– 2 to 6 years	$32.9^{***} \pm 0.5$	38.8 ± 0.5
VCM, FL	$93^{NS} \pm 0.7$	91 ± 1.6
MCHC, g/100 ml	$34,6^{NS} \pm 0.3$	35.6 ± 0.5
Aspect of smears	Anisocytosis	N
Reticulocytes/100 RBC's	$2.1^{*} \pm 0.1$ (N = 126)	1.5 ± 0.1
Platelets $\times 10^3/\mu l$	$238^{*} \pm 14$ (N = 50)	319 ± 17 (N = 16)
Leukocytes $\times 10^3/\mu l$	$9.8^{*} \pm 0.5$	7.7 ± 0.3
Polymorphonuclear neutrophils, %	$44.4^{**} \pm 1.1$	35.3 ± 1.7
Eosinophils, %	$3.6^{***} \pm 0.3$	8.5 ± 0.9
Basophils, %	$0.2^{NS} \pm 0.1$	0.2 ± 0.1
Lymphocytes, %	$50.7^{NS} \pm 1.0$	54.5 ± 1.6
Monocytes, %	$1.0^{NS} \pm 0.1$	1.3 ± 0.2
Percentages of positive parasitologic analyses feces	55^{NS}	62

^{NS} (non significant) : $P > 0.05$
* : $0.01 < P < 0.05$; ** : $0.001 < 0.01$; *** : $P < 0.001$

purpose of the present study is to describe the approach which led us to a coherent concept of anemia associated to protein-energy malnutrition in Kivu. Only the completion of such a study makes it possible to analyse the points of convergence between human observations and the results of animal models, or to understand the possible inadequacies of the later.

We wish to acknowledge all those who have enabled the realization of our studies, and notably Prof. H. L. Vis, Chairman of the medical section of CEMUBAC, and Dr. I. M. Mandelbaum for their helpful guidance and criticism.

slight increase in polymorphonuclear neutrophils and a small reduction in blood platelets. Thrombocytopenia cannot be explained by the existence of disseminated intravascular coagulation, since coagulation tests are usually normal, with the exception of an isolated lengthening of the thrombin-time (TSHIMPAKA & FONDU, 1979). One of the most remarkable hematological characteristic is the absence of increase in eosinophils despite the high frequency of intestinal parasitoses (FONDU *et al.*, 1978a).

The examination of myelograms often reveals anomaly of proliferation nor maturation. However, among approximately one third of the patients, some erythroblasts are abnormally small or exhibit a cytoplasm with irregular limits. In one fifth of the cases one can detect the presence of large erythroblasts whose aspect is transitional between that of normal erythroblasts and megaloblasts. The three aspects of erythroblasts are sometimes found in the same patient (FONDU *et al.*, 1973).

During refeeding the hematological evolution is characterized, among other factors, by a transitory reduction of the hemoglobin level, which reaches its lowest value after approximately two weeks and increases slowly afterwards. After two months of treatment, it is hardly above the value recorded on admission (Fig. 1). On the other hand, one observes a rapid increase of the percentage of reticulocytes which usually reaches its maximum value after a period of 3 to 8 days and remains high during 3-4 weeks. While the MCV and MCHC do not change, the morphology of erythroblasts alters : most of them become abnormally small (FONDU *et al.*, 1973).

The slight reduction of the hemoglobin level, the normocytosis, the normochromia, the frequently normal pattern of myelograms, as well as the transitory packed cell volume decrease and reticulocyte increase observed during refeeding, are rather common characteristics of the hematological pattern associated to syndromes of protein-energy malnutrition among children. One must avoid however to generalize : in some regions of the world, anemia can appear in variable forms, sometimes considerably different from the above-mentioned pattern (LIEN-KENG and TUMBELAKA, 1960 ; KONDI *et al.*, 1963 ; SANDSTEAD *et al.*, 1965 ; PEREIRA and BAKER, 1966 ; VITALE *et al.*, 1968 ; WOODRUFF, 1968 ; ADAMS, 1969, 1970 ; HALSTED *et al.*, 1969 ; BLOT *et al.* ; 1972 ; OMER *et al.*, 1973). It is therefore necessary to underline that the hematological aspects of protein-energy malnutrition are the results of the evolution of specific pathological deficiencies, occuring in populations with precise genetic characteristics, living in a given environment. Any pathophysiological study implies a previous knowledge of these local conditions. In order to clarify our

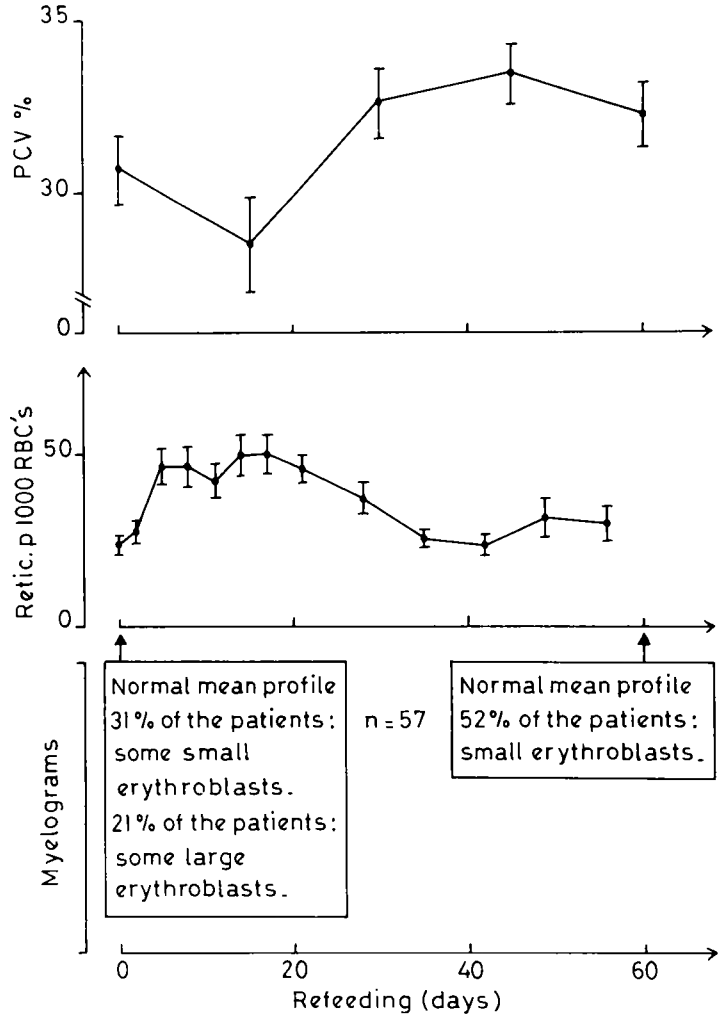

FIG. 1. – Repeated determinations of the packed cell volume (%), of reticulocytes counts (retic), and of myelograms during refeeding (mean ± SEM).

presentation, we will deal briefly with them in the subsequent paragraphs, according to the fact whether they are linked or not to marasmic kwashiorkor.

Many studies on the anemia associated with protein-energy malnutrition include only morphological descriptions, or pathogenic considerations based on the research of some vitamins or iron deficiencies. Such a methodological approach is insufficient. Through the use of various isotopic methods we have been able to evaluate in a satisfactory manner the intravascular volumes, the production of red cells and their destruction. We propose to summarize the results of these studies, as well as the assumptions relating to the evolution of the hematological changes associated to marasmic kwashiorkor. After describing the present state of knowledge of the causal relationship between kwashiorkor and the blood manifestations observed in the marasmic kwashiorkor of Kivu, we shall tackle a fundamental question, namely the analysis of the arguments which would enable us to accept or reject the hypothesis of an "adaptive anemia". Finally we shall determine to which extent the detailed study of the metabolism of erythroid cells enables us to understand better the profound mechanisms of the anemia associated with marasmic kwashiorkor in Kivu.

2. Factors independent from protein-energy malnutrition and susceptible to influence the hematological parameters of the children studies

2.1. Genetic factors

The children examined are most often Shi, seldom Havu and exceptionally Tutsi. The anthropometric data and the prevalence of genetic diseases are usually less clearly established for the Havu than for the Shi and Tutsi.

The prevalence of hemoglobinopathies is relatively small in that population. HIERNAUX (1952) noted a positive sickling test among 4.2% of the Shi. According to MOTULSKY *et al.* (1966), the frequency of sickle cell trait would be 6.5% among the Shi and 3.3% among the Tutsi. The observations made at Lwiro are closer to the estimation of Hiernaux than to that of Motulsky et al. : the sickling test is positive for 4.5% of the children admitted to the CEMUBAC Hospital (CEMUBAC Report, 1975). A systematic study of hemoglobins carried out on children admitted to the CEMUBAC Hospital did not enable to detect the existence of abnormal hemoglobins other than hemoglobin S (VAN STEIRTEGHEM, 1972).

The high prevalence of glucose-6-phosphate-dehydrogenase (G6PD) deficiency is a classical finding in Tropical Africa. The deficiency is present among 13.8% of the Shi males and 5.4% of the Tutsi males ; these values are considerably lower than those recorded in the West of Zaïre (MOTULSKY *et al.*, 1966). One usually considers that the G6PD deficiency does not lead to chronic anemia. However, it seems that in the absence of hemolytic attacks due to toxic agents or drugs, the deficiency could be a factor promoting or aggravating anemias resulting from other sources (VAN ROS, 1977).

Current literature does not include any epidemiological study dealing with the other congenital erythroenzymopathies in the Kivu region. In the light of the results submitted in the present report, their prevalence seems to us to be low in the Bushi and the Buhavu

2.2. Environmental factors

The geographical, demographic, socio-economic and nutritional characteristics of the populations of the Bushi and Buhavu regions as well

as the prevalence of various infectious diseases or parasitoses, have been reported in several publications (Vis *et al.*, 1969 ; CEMUBAC Report, 1970 ; CEMUBAC Report, 1975). We shall merely underline some specific factors susceptible to influence the distribution of the hemoglobin level in the population.

The area inhabited by the Shi and Havu people ranges from 1460 to more than 2000 meters, starting from the Lake Kivu and reaching on one side the Mitumba Mountains, and on the other the summits of the Idjwi Island. The most ancient geological stratum is composed of shale and quartzites usually covered either by granite or basalt. The chemical composition of the plants depends in each location on the nature of the superficial rocks. Therefore, the content of the plants in iron and manganese depends on the nature of the soil on which they are cultivated (Cornil *et al.*, 1974).

The average density of the population was evaluated to 90-100 inhabitants per square kilometer in 1969 (Vis *et al.*, 1969). It seems to be continuously increasing. The Shi's and the Havu's live generally in a subsistence economic system. Their nutrition is mainly based on stapple-crops. The perennal lack in energy supplies, the deficiency in proteins and its aggravation at certain times, the small intake of lipids, were described in details by Vis *et al.*, (1969). These date enable to better understand the seriousness of endemic malnutrition ; in a rural environment, 30% of the children exhibit biological symptoms of malnutrition (CEMUBAC Report, 1975).

Infantile mortality is very high and seems to be especially attributable to respiratory and digestive diseases. The prevalence of digestive parasitoses may exceed 60% ; it is almost identical for under or well-fed subjects (Fondu *et al.*, 1978a). Parasitoses leading to anemia remain rather rare. From 1970 to 1974 the prevalence of malaria (*Plasmodium falciparum*) varied from 1.7 to 4.2% among the children admitted to the Lwiro Hospital. A recrudescence (7.2%) of the cases) was observed in 1975 (CEMUBAC Report, 1975). Bilharziosis is restricted to a few limited centers around the Lake Kivu (CEMUBAC Report, 1970). Ancylostomiasis can be detected only in 0.6 to 2.4% of the children hospitalized at Lwiro (CEMUBAC Report, 1975).

The environment imposes therefore a certain number of constraints on the local population, due to

a) the altitude, which is relatively high ;

b) the nature of the soil, regulating the supply of several minerals ;

c) the quality and quantity of the diet ;
d) the prevalence of infectious diseases and parasitoses.

The impact of the diet on the "biological constants" can be seen for children who do not reveal any clinical or biological symptom of protein-energy malnutrition. We have emphasized previously the differences existing between the blood concentrations of certain vitamins between Shi children and Belgian children apparently in good health (LEJEUNE-LENAIN and FONDU, 1975 ; FONDU *et al.*, 1978a). The level of serum folates is higher among Shi while the levels of Vitamin B12, ascorbic acid and Vitamin E are lower. Plasma concentrations of iron do not differ (Table 2).

Table 2
Blood levels of iron and certain vitamins in children without symptoms of protein-energy malnutrition (mean ± SEM)

Parameters studied	Belgian children	Shi or Havu children
- IRON, µg/100 ml serum	$97^{NS} \pm$ (N = 20)	84 ± 5 (N = 23)
- FOLATES, µg/ml serum	8.1** ± 0.2 (N = 11)	12.8 ± 1.6 (N = 14)
- VITAMIN B12, pg/ml serum	780* ± 106 (N = 20)	450 ± 37 (N = 16)
- ASCORBIC ACID, mg/100 ml serum	0.96* ± 0.17 (N = 11)	0.60 ± 0.12 (N = 16)
- VITAMIN E, mg/100 ml serum	1.14*** ± 0.04 (N = 20)	0.81 ± 0.06 (N = 10)

Among all the environmental factors characteristic of the highlands of Kivu and which have a definite impact on hematological parameters, altitude is the most evident besides protein-energy malnutrition. Among healthy children of the same age, the hemoglobin level is higher in Kivu than in Belgium. For any give packed cell volume, the urine and plasma levels of erythropoietin are higher in Kivu than at sea-level (FONDU *et al.*, 1978 b).

In the present study it is therefore necessary to recall briefly the major repercussions of altitude on the metabolism of red cells. The study of these modifications has been the subject of several recent works. Without taking into account disorders linked to fluctuations in the frequency distribution of erythrocyte ages, three mechanisms have been identified : increase of the partial pressure of oxygen corresponding to a

saturation of hemoglobin in oxygen equal to 50% (P50), variations of the enzymatic activities, and finally blood peculiarities which some authors have tried to correlate to ethnic characteristics.

a) It is well established that the curve of dissociation of hemoglobin is shifted to the right for subjects living in altitude. The major cause of this shift to the right is an increase of 2,3-DPG* (LENFANT *et al.*, 1968 ; EATON *et al.*, 1969 ; LENFANT *et al.*, 1971). This event is observed both in the local population and among newcomers. It persists when the polycythemia is evident and is quickly reversed when the subjects are transfered to lower attitudes. The existence of increases in P50 and in the 2,3-DPG level among healthy subjects has been confirmed in Kivu (FONDU and MANDELBAUM, 1975).

The increase in the 2,3-DPG level which occurs within a few hours following the arrival in altitude seems to be mainly attributable to the alkalosis which follows hyperventilation (LENFANT *et al.*, 1071). According to RÖRTH *et al.* (1972, 1973) one must also take into account a redistribution of inorganic phosphates, with decreases in the plasma and erythrocyte pools. The lowered levels of phosphates would tend to modify the equilibrium constant of GAPD** in erythrocytes. That effect would be compensated by an influx towards the red cells of some poorly determined substances which are progressively liberated by the tissues. It is worth noting that these mechanisms, described for subjects who have arrived in altitude only a few hours ago, could be essentially different from those which prevail among people adapted to live in the mountains for longer periods.

b) any interpretation of adaptation to altitude must take into account the presence of erythrocytes having a modified enzymatic equipment (Table 3). We have demonstrated the existence of such a mechanism when studying certain enzymatic activities among healthy subjects adjusted to living in Kivu or in Brussels : in stable conditions the activities of G6PD, HK and PK are higher in Kivu (MANDELBAUM *et al.*, 1973). These data were partly confirmed in the Himalayan region (MORPURGO *et al.*, 1976). These facts suggest that hypoxia induces an adjustment of enzymatic syntheses and therefore a remodelling of enzymatic metabolism within red cells.

* 2,3-diphosphoglycerate .

** : glyceraldehyde phosphate dehydrogenase.

TABLE 3

Erythrocyte enzymes activities in well nourished subjects (mean ± SEM)

Ensymes studied[1]	BASHI			BELGIANS		
	I. living in Kivu (N = 32)	II. living in Brussels (N = 14)	tI-II	III. living in Kivu (N = 6)	IV. living in Brussels (N = 15)	tIII-IV
G6PD						
μM/g.Hb/min	4.7 ± 0.23	3.7 ± 0.30	2.588*	4.5 ± 0.16	3.4 ± 0.28	2.644*
μM/100 ml RBC's/min	168 ± 7.7	124 ± 10.0	3.275**	158 ± 6.2	115 ± 8.3	3.409**
μM/10^{10} RBC's/min	1.54 ± 0.076	1.09 ± 0.009	3.318**	1.47 ± 0.075	0.97 ± 0.075	4.167***
6PGD						
μM/g.Hb/min	3.6 ± 0.14	3.5 ± 0.14	0.646	3.4 ± 0.17	4.0 ± 0.22	1.844
μM/100 ml RBC's/min	130 ± 4.9	117 ± 4.8	1.534	119 ± 4.6	135 ± 6.8	1.843
μ%/10^{10} RBC's/min	1.19 ± 0.057	1.02 ± 0.049	1.913	1.11 ± 0.065	1.15 ± 0.061	0.363
HK[2]						
μM/g.Hb/min	1.23 ± 0.107	0.70 ± 0.055	3.520*	1.62 ± 0.211	0.65 ± 0.080	5.354***
μM/100 ml RBC's/min	44.1 ± 3.63	23.7 ± 1.86	3.954***	56.0 ± 6.29	21.8 ± 2.57	6.096***
μM/10^{10} RBC's/min	0.39 ± 0.031	0.20 ± 0.016	4.313***	0.52 ± 0.067	0.19 ± 0.023	6.176***
PK						
μM/g.Hb/min	14.9 ± 0.44	9.1 ± 0.78	6.747***	17.8 ± 2.74	11.4 ± 0.66	5.406***
$_{7}$M/g.Hb/min	533 ± 14.5	304 ± 25.0	8.331***	621 ± 90.8	383 ± 20.0	3.735***
μM/10^{10} RBC's/min	4.9 ± 0.18	2.7 ± 0.22	7.413***	5.8 ± 0.93	3.2 ± 0.17	4.185***

1 G6PD Glucose-6-phosphate dehydrogenase ; 6PGD = 6-phosphogluconate dehydrogenase ; HK = hexokinase ; PK = pyruvate kinase

2 For HK the number of Bashi studied in Lwiro is 26 and not 32.

c) Recent publications tend to demonstrate that the hematological characteristics of a healthy population living at high altitudes depend on ethnic factors. Their authors state that populations adapted to altitude since several generations, such as the Aymara Indians or the Nepalese from Tibetan ancestry, exhibit only a slight or no polycythemia (MOULIN, 1971 ; ADAMS and STRANG, 1975). Peruvian Indians have an increased Bohr effect (MORPURGO *et al.*, 1970) and the Sherpas living at very high altitude have a 2,3-DPG level equal to that of Europeans living at sea-level and a lower P50 value (MORPURGO *et al.*, 1976). According to MORPURGO, the observations made would confirm the intervention of an adaptative evolution, assuming different paths according to the ethnic group. Such a conclusion must be considered with care : it seems indeed that the influence of nutritional factors has not been analyzed in depth in these studies. It is well-known that the prevalence of nutritional deficiencies and in particular of protein-energy malnutrition is high in some of these regions (POURBAIX, 1974). Among healthy individuals belonging either to the Shi people or to the European population, living either in Belgium or in Kivu, the study of several biochemical parameters of the erythrocytes did not reveal the existence of genetic differences (MANDELBAUM *et al.*, 1973 ; FONDU and MANDELBAUM, 1975).

3. Eventual role of deficiencies in iron, vitamines and trace-elements in the hematological changes in marasmic kwashiorkor

3.1. Aspects of iron metabolism

The metabolism of iron in marasmic kwashiorkor has been analyzed by FONDU (1973, 1977) and by FONDU *et al.* (1973, 1977, 1978a). The main results are summarized in Table 4. The level of serum iron and the total iron binding capacity represent approximately two-thirds of the

TABLE 4
Iron metabolism (mean ± SEM)

Parameters studied	On admission	After 2 to 3 months of treatment	Controls
1. Serum			
– serum iron, µg/100 ml	60** ± 3	45*** ± 4	84 ± 5
– total iron binding capacity, µg/100 ml	208*** ± 9 (N = 116)	424* ± 14 (N = 47)	369 ± 17 (N = 23)
– percentage of siderophilin saturation	32* ± 2	12*** ± 1	23 ± 2
2. Marrow			
– sideroblasts p. 100 erythroblasts	*** 38 ± 3 (N = 68)	3 ± 1 (N = 62)	–
– iron in reticular cells	**		
a) O—((+))	N = 15	N = 15	–
b) >(+)	N = 36	N = 8	–
3. Liver			
– hepatocytes iron			
a) 0	N = 6	N = 9	–
b) +	N = 10	N = 7	–
– Kupffer cells iron			
a) 0	N = 6	N = 11	–
b) +	N = 10	N = 5	–
4. Intestinal absorption of iron**(%)	* 32 ± 7 (N = 8) NS	62 ± 9 (N = 7)	–

normal mean value. On the average, the percentage of saturation of siderophilin is slightly increased. The proportion of patients whose percentage of saturation is inferior to 16% does not differ from that observed in local controls or in healthy Belgian children. In the majority of cases, the histochemical reactions of iron are positive in the marrow reticular cells, in the hepatocytes and in the Kupffer cells. There is no obvious abnormality of the intestinal absorption of ferrous and ferric ions.

The reduction of the total capacity of saturation seems attributable to a decrease of the siderophilin synthesis. There is in fact a fairly good correlation between the total iron binding capacity and the albumin level (Fig. 2). For a given alumin level, the binding is however higher for patients whose histochemical iron reactions are negative (Table 5). We cannot therefore approve the opinion expressed by some authors, according to which the measurement of the siderophilin level would be a specific criterion enabling to evaluate the severity of protein malnutrition.

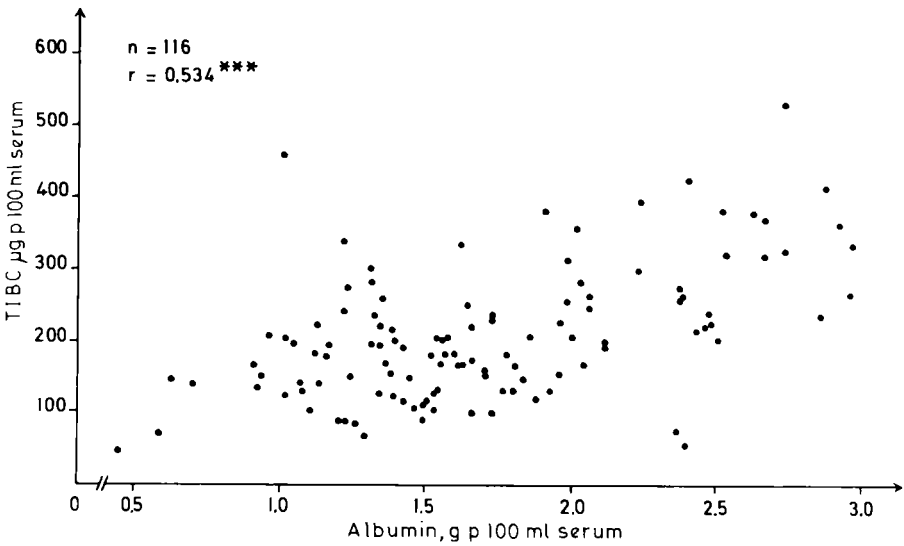

FIG. 2. – Correlation between total iron binding capacity (TIBC) and albumin level in the serum of patients studied before the start of refeeding (FONDU *et al.*, 1977).

There is no obvious relation between the serum iron level and the estimation of the iron stores. It seems probable that hyposideremia results essentially from a slowering down of the iron flow from the reticulo-endothelial system. Such a disturbance cannot be explained by the

intervention of mechanisms which are known to inhibit this transfer : there if no deficiency in ascorbic acid and the reductions in the concentrations of siderophilin or in ceruloplasmin are generally of limited importance. It is possible that the disturbance observed in the iron transport is associated to the inflammatory syndrome frequently reported in marasmic kwashiorkor, but the exact nature of the metabolic disorder cannot be defined in the present state of our knowledge.

TABLE 5
Relationships between iron stores, serum iron and total iron binding capacity in marasmic kwashiorkor (mean ± SEM)

Iron stores (marrow squashs)	0 to ((+)) (N = 15)	(+) to (+ + + +) (N = 36)
Serum iron, µg/100 ml	47^{NS} ± 6	58 ± 4
Total iron binding capacity, µg/100 ml of serum	268*34	203 ± 11
Saturation percentage of siderophilin	21* ± 3	30 ± 2

In spite of that uncertainty, it appears clearly that the iron supply to the erythroblasts is generally sufficient in the patients examined on admission.

During the period of refeeding, the patients develop an iron deficiency which is mainly characterized by a depletion of their iron stores, an increase in the intestinal absorption of ferrous ions, and a reduction in the percentage of sideroblasts.

Such a situation can be explained by the acceleration of the anabolic activities and more particularly by a considerable iron utilization for hemoglobin and myoglobin syntheses. The amount of iron necessary for the normalization of the red cell volume cannot be estimated accurately by taking into account the level of hemoglobin measured upon admission, since erythrocytes are distributed at that time in a volume smaller than the blood volume of normal children of the same height. The addition of a treatment with iron-dextran accelerates the normalization of the hemoglobin level and of the red cell volume (Fig. 3).

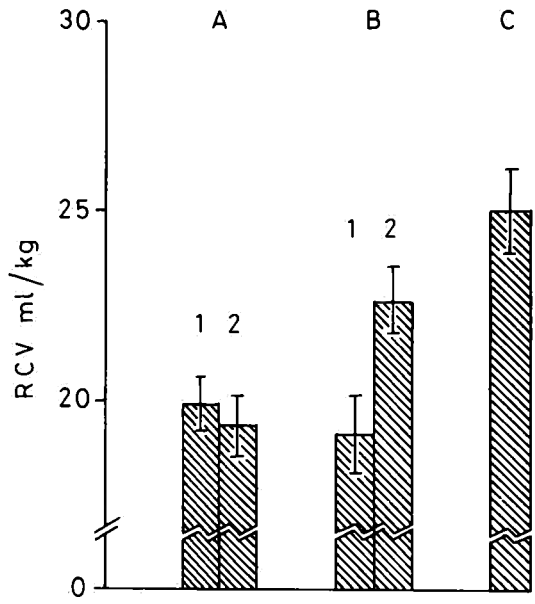

FIG. 3. – Red cell volume (RCV) on admission (1)and after 60 days of treatment (2)(mean ± SEM).
A : patients refed without addition of iron (n = 10)
B : patients refed in the same manner but receiving in addition 5 intramuscular injections of iron-dextran containing each 50 mg of iron (n = 10)
C : control subjects (n = 7)
The decision to include a patient in either group A or B was taken randomly.

The evolution of the metabolism of iron during treatment clearly shows that the application of the method of therapeutical trials may lead to erroneous interpretations in protein-energy malnutrition : a deficiency can become critical during the period of refeeding although it did not exist or had no hematological impact at the time of admission.

3.2. Blood content of certain vitamins

LEJEUNE-LENAIN & FONDU (1975) and FONDU *et al.* (1978a) have determined the blood content of certain vitamins in Shi and Havu children suffering from marasmic kwashiorkor (Table 6). Abnormally low levels of folates in the serum and erythrocytes were only exceptionally found. Such results are compatible with the absence of frank megaloblastosis in the Kivu region (FONDU *et al.*, 1973). The level of serum folates tends to decrease during refeeding. On the average, the plasma content of vitamin

TABLE 6

Blood levels of certain vitamins, selenium and ceruloplasmin (mean ± SEM)

Parameters studied	On admission	After 2 to 3 months of treatment	Controls
– serum folates, ng/ml	$10.3^{NS} \pm 1.1$ (N = 29)	$9.0^{**} \pm 1.7$ (N = 19)	12.8 ± 1.6 (N = 14)
– erythrocyte folates, ng/ml	$254.5^{NS} \pm 21.3$ (N = 29)	$215.9^{NS} \pm 21.5$ (N = 19)	270.4 ± 32.1 (N = 14)
– plasma vitamin B12 pg/ml	$1066^{**} \pm 133$ (N = 32)	$534^{NS} \pm 70$ (N = 19)	450 ± 37 (N = 16)
– total vitamin B12 binding capacity, pg/ml plasma	$3167^{NS} \pm 128$ (N = 28)	$3853^{**} \pm 169$ (N = 19)	2887 ± 342 (N = 13)
– serum ascorbic acid, mg/100 ml	$0.69^{**} \pm 0.06$ (N = 30)	$0.41^{NS} \pm 0.07$ (N = 20)	0.60 ± 0.12 (N = 16)
– serum vitamin E mg/100 ml	$0.47^{***} \pm 0.04$ (N = 24)	$0.47^{***} \pm 0.05$ (N = 12)	0.81 ± 0.06 (N = 10)
– serum selenium ng/ml	$42^{*} \pm 4$ (N = 20)	$75^{NS} \pm 5$ (N = 7)	59 ± 5 (N = 5)
– erythrocyte selenium, ng/ml	$112^{NS} \pm 7$ (N = 15)	$121^{NS} \pm 8$ (N = 16)	102 ± 10 (N = 9)
– serum ceruloplasmin mg/100 ml	$22.6^{*} \pm 2.0$ (N = 24)	$31.1^{NS} \pm 5.8$ (N = 12)	25.4 ± 1.3 (N = 10)

B12 is high ; it comes to the normal usually after more than one month of treatment. Such an anomaly might be attributable to hepatic steatosis. The total capacity of saturation of the plasma in vitamin B12 is normal on admission but increases during refeeding.

The serum content of ascorbic acid is higher in marasmic kwashiorkor than among african controls. It decreases in the course of treatment.

The plasma content of vitamin E is lowered on admission and remains such after two months of renutrition.

The measurement of the blood content of riboflavin does not allow to make a satisfactory diagnosis of the deficiency in that vitamin. The study of a flavin-enzyme such as GSSG-red* in the erythrocytes constitutes a more rational approach to that problem (SAUBERLICH *et al.*, 1972).

It seems therefore that vitamins deficiencies have less evident repercussions on the hematological condition observed in marasmic kwashiorkor of Kivu than in other states of protein-energy malnutrition described in the literature (FONDU *et al.*, 1978a). However, the accurate hematological significance of the reduction of the plasma content of tocopherols as well as the possible presence of a riboflavin deficiency will only be possible after studying the metabolism of red cells.

3.3. Eventual deficiencies in trace-elements

As indicated in Table 6 we noticed in marasmic kwashiorkor of Kivu a reduction in the plasma, but not in the erythrocyte, selenium content. The abnormality is compensated during refeeding (FONDU *et al.*, 1978a). This observation is in favour of a deficiency in selenium but does not however suffice to demonstrate its presence. One cannot in fact exclude the assumption of a redistribution of the trace element in the organism, due to a perturbation of the metabolism of the carrier proteins in the plasma.

On the other hand, clinical and biological studies made at present point to the existence of a copper deficiency in marasmic kwashiorkor (GOYENS, 1978). On this point it is worth recalling that the ceruloplasmin level is slightly lowered upon admission of the patients (FONDU *et al.*, 1977).

* glutathione-reductase.

The above-mentioned considerations do not enable to determine with certainty the eventual hematological impact of deficiencies in trace-elements. A more rational approach to this problem seems to us to reside in the study of the enzymes of erythroid cells, in the composition of which trace-elements actually intervene. This aspect will be dealt with in Chapter 7.3.

4. Isotopic studies in marasmic kwashiorkor

4.1. Red cell volume (Fondu, 1977)

Due to the diffusion of plasma tracers in edemas, the red cell volume is the only intravascular pool which can be accurately measured in patients with protein-energy malnutrition. The results can be expressed in percentages of the mean value observed in subjects of the same height or in ml/kg. In the latter case the weight considered must be the minimum weight observed during refeeding because the weight on admission can fluctuate considerably due to body hydration. Whatever the mode of expression chosen, the values are lower in marasmic kwashiorkor than for normal children. A significant correlation is observed on admission between the red cell volume and the packed cell volume, the regression line being however more flat than normal (Fig. 4). During refeeding, the

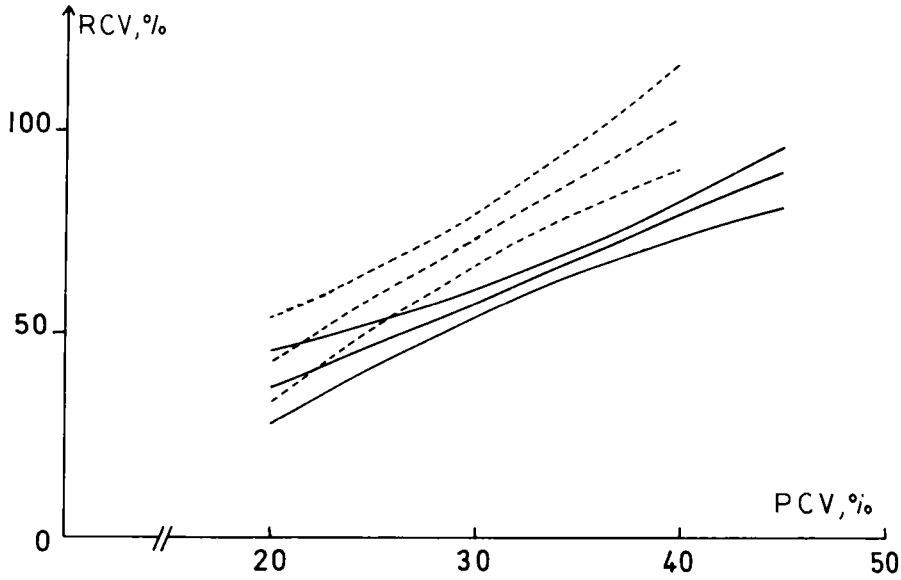

Fig. 4. – Regressions of the red cell volume (RCV) on the packed cell volume (PCV) measured simultaneously. Children are 28 patients studied on admission (continuous line ; r = 0.82) and 20 patients studied after 14 days of refeeding (dotted lines ; r = 0.82). RCV is expressed in percentages of the mean value observed in control subjects of the same height. – Confidence limits at risk P = 0.05 are also indicated.

plasma volume increases more rapidly than the red cell volume, which explains the transitory decrease of hemoglobin level (Fig. 5).

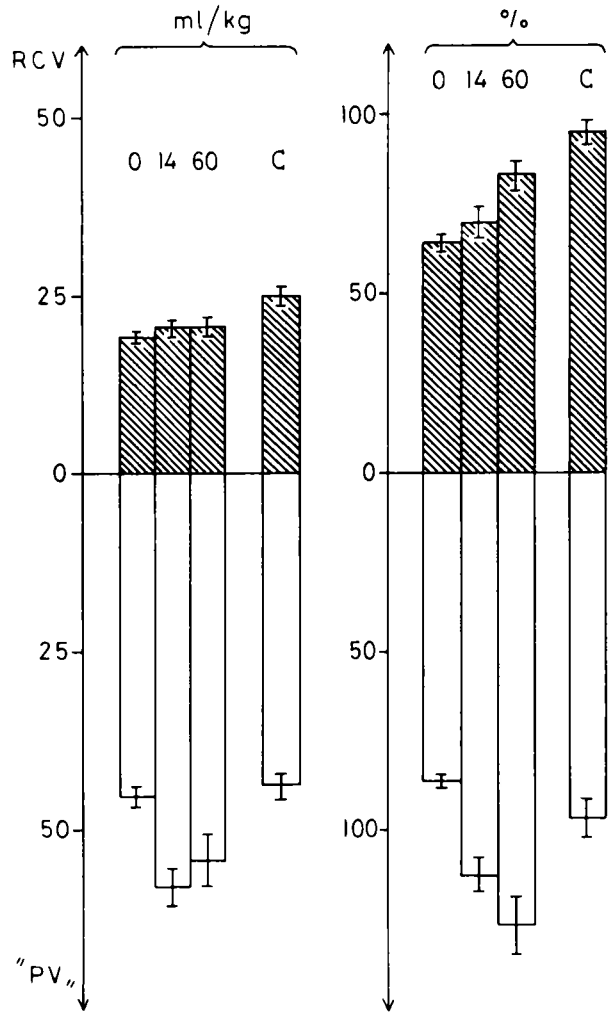

FIG. 5. – Evolution of the red cell volume (RCV) and "plasma volume" ("PV") at different times during refeeding. 0 = 0 day of treatment ; + 14 = 14 days of treatment ; + 60 = 60 days of treatment ; C = control subjects. PV was assessed indirectly from RCV and packed cell volume (PCV) assuming that the ratio between body PCV and venous is 0.90 in all the cases studied. The results are expressed 1) in relation to body weight (ml/kg), the weight chosen being in that case the minimum weight recorded during refeeding (0 and + 14) or the weight measured simultaneously (+ 60) ; 2) in percentage of the mean values recorded in control subjects of the same weight. Refeeding took place without parenteral iron supplementation.

4.2. Study of erythrocyte life span

The T50Cr* measured in auto-transfusion is 17.1 ± 1.2 days on admission ($n = 10$; range : 11.5 to 24.0 days) and 30.6 ± 1.5 days for patients refed for at least 2 months ($n = 10$; range : 25.0 to 38.0 days) (FONDU, 1973, FONDU *et al.*, 1978a). The normal values for children range from 25.0 to 42.0 days (STEKEL & SMITH, 1970) or from 24 to 31 days (MAGDOUGALL *et al.*, 1970). When the counts *in vivo* made during these studies are interpreted in the light of the recommendations of the International Committee for the Standardization of Isotopic Tests in Hematology (ICSH, 1975), the following values are obtained on admission : spleen/liver ratio at 0 day : 0.45 ± 0.02 ; maximum value of the standardized spleen/liver ratio : 1.49 ± 0.15 ; maximum value of the spleen excess counts : 120 ± 24 ; maximum value of the liver excess counts : 99 ± 29 ($n = 9$).

Such results are not in favour of the existence of a massive destruction of erythrocytes inside the spleen or the liver.

A reduction of T50Cr was also observed by other authors in patients suffering from kwashiorkor ZAMAR *et al.*, 1966 ; LANZKOWSKY *et al.*, 1967 ; ADAMS, 1969).

The interpretation of all these results is subject to some reservations, since the patients investigated were in the course of treatment, so that their red cell volume increased during the measurement of T50Cr. We have used two processes to alleviate that difficulty. In the first instance the erythrocytes of patients, labeled with radioactive chromate, were reinjected to voluntary healthy adults. The T50Cr thus measured is slightly diminished : 24.6 ± 1.2 days ($n = 10$; range : 18.0 to 29.0 days) (FONDU *et al.*, 1978a). There is therefore a moderate increase in hemolysis due to certain intrinsic abnomality(-ies) in red cells.

On the other hand, the probability of destruction of erythrocytes** was calculated in 20 patients whose red cell volume was measured on two occasions. The calculations were made using various equations corresponding each to a determined model of erythrocytes destruction. Whatever the model chosen, the results obtained indicate a moderate

* half-time of disappearance of the activity of ^{51}Cr chromate per ml of red cells.

** Under these terms we designate a parameter, the exact mathematical definition of which was given by BERGNER (1965) "average value of the cell death probability function in M(T)" ; it is equal to the initial slope of the standardized survival curve.

TABLE 7
Average red cell destruction probability at the time of labeling $\bar{\mu}$ *(T) (mean* ± SEM)

	Methods	$\bar{\mu}$ (T) ($-d^{-1}$)
Patients studied on admission	auto-transfusion (N = 10)	− 0.029 ± 0.003
	red cells transfused into healthy adults (N = 10)	− 0.015 ± 0.001
	two successive measurements of red cell volume (N = 20)	
	− Model 1 : $\frac{N_T}{N_o} = \frac{1 - T}{T}$	− 0.011 ± 0.001
	− Model 2 : $\frac{N_T}{N_o} = \frac{e^{-T/\lambda} - e^{-110/\lambda}}{1 - e^{-110/\lambda}}$	− 0.017 ± 0.002
	− Model 3 : $\frac{N_T}{N_o} = \frac{(1 - \frac{T^2}{C}) - (1 - \frac{110^2}{C})}{1 - (1 - \frac{110^2}{E})}$	− 0.014 ± 0.001
Patients refed for at least 2 months	auto-transfusion (N = 10)	− 0.011 ± 0.001

increase of the probability of destruction of red cells (Table 7). Nevertheless, we can reasonably assume that the destruction of erythrocytes, that is the product of the probability of destruction of the erythrocytes and the red cell volume (ml/kg), does not differ significantly from normal values (FONDU *et al.*, 1978a).

Since studies made in isotransfusion and repeated measurements of the red cell volume gave us estimates very close to the probability of erythrocyte destruction, the existence of an extra-corpuscular hemolysis seems to us improbable in the marasmic kwashiorkor of Kivu.

4.3. Study of erythropoiesis

With the exception of the study of KHALIL *et al.* (1969) including 3 patients, the only quantitative study of erythropoiesis in patients with

kwashiorkor or marasmic kwashiorkor is that carried out at the CEMUBAC mission (FONDU, 1973).

The ferrokinetic parameters of 20 patients determined on admission were related to their minimum weight. When the results are globally analyzed, the only significant differences between patients and the control group relate to red cell volume, which is lowered among patients, and to distribution volume of siderophilin labelled with radioactive iron, which is increased. The second disturbance is well known in patients presenting edema (NAJEAN *et al.*, 1970). The mean values of plasma iron turnover, of red blood cell utilization of iron, of erythrocyte iron turnover and of marrow transit-time do not differ clearly from those observed in the control group (Table 8).

TABLE 8
Ferrokinetics

Parameters studied	Patients studied on admission (N = 20)	Patients refed for at least 2 months (N = 10)	Controls (N = 7)
Serum iron, µg/ml	$40^{***} \pm 4$	$33^{**} \pm 9$	75 ± 8
^{59}Fe-labelled siderophilin-diffusion volume, ml/kg	$65.3^{*} \pm 2.4$	$63.1^{NS} \pm 4.3$	53.3 ± 2.0
Clearance rate, minutes	$47^{NS} \pm 6$	$39^{**} \pm 6$	67 ± 7
Plasma iron turnover, mg/kg/d	$0.58^{NS} \pm 0.05$	$0.46^{NS} \pm 0.08$	0.61 ± 0.07
Red blood cell utilization of ^{59}Fe%	$77^{NS} \pm 4$	$83^{**} \pm 5$	75 ± 1
Marrow transit time, days	$2.5^{NS} \pm 0.2$	$2.3^{*} \pm 0.2$	3.0 ± 0.2
Erythrocyte iron turnover, mg/kg/d	$0.47^{NS} \pm 0.05$	$0.37^{NS} \pm 0.06$	0.45 ± 0.05

Within certain limits, the measure of the erythrocyte iron turnover enables to evaluate the output of the red cells. If we express the results against the body weight, it appears that the output does not deviate significantly from normal values.*

* If the erythrocyte iron turnover is expressed in percentages of the mean value observed in control subjects of the same height, the results are $89.6 \pm 10.4\%$ on admission ($n = 20$) and $103.2 \pm 14.5\%$ for controls ($n = 7$); the mean values do not differ significantly ($t = 0.693$).

A positive correlation exists between the plasma iron turnover iron and the red blood cell iron utilization ; there is no significant correlation between the erythrocyte iron turnover and the serum iron level (Fig. 6).

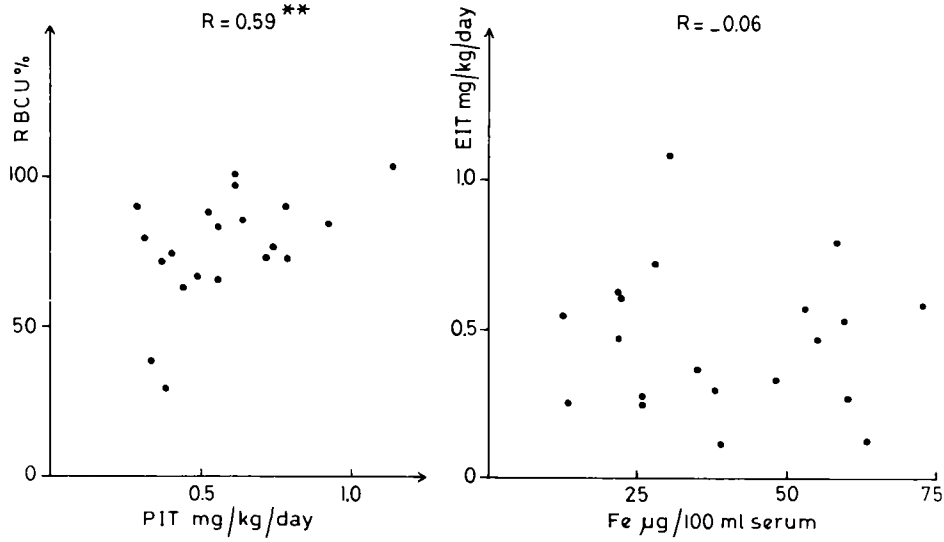

FIG. 6. – Left : correlation between red blood and cells utilization of radioactive iron (RBCU) and plasma iron turnover (PIT). – Right : correlation between erythrocyte iron turnover (EIT) and the serum iron level (Fe). – R : Spearman's correlation coefficients.

This last observation confirms that erythropoiesis is generally not limited by an inadequate iron supply to the erythroblasts, contrary to what occurs in anemias due to chronic disorders and in particular to certain infectious diseases (DOUGLAS & ADAMSON, 1975).

In vivo countings are normal when the red blood cell iron utilization is equal or exceeds the mean value of the control subjects ; when the percentage is lower than the mean value of the controls, a marrow hypocaptation and an early hepatic hypercaptation are sometimes observed. Splenic countings are always normal.

Accordingly, we assume that radioactive iron tests do not bring forward any argument in favour of the existence of an intramedullar hemolysis. They indicate that the proliferation of erythroblasts may vary significantly from one patient to another.

After two months of treatment, the ferrokinetic parameters show the characteristics which are usually described in anemias resulting from a limited iron supply.

4.4. Discussion and conclusions

When the parameters considered are related to the body weight, isotopic investigations indicate that the red cell volume is significantly lowered in marasmic kwashiorkor, while the production and destruction of erythrocytes do not differ from normal values. Therefore the production of red cells is almost equal to their destruction before refeeding is initiated. This conclusion enables us to cast some doubts on the hypothetical interpretation of kwashiorkor anemia and marasmic kwashiorkor proposed by VITERI *et al.* (1968). According to these authors, the onset of kwashiorkor or marasmic kwashiorkor constitutes always an acute event, whose direct hematological consequence is an impairment of erythropoietic activity leading to a rapid reduction of the red cell volume. Such a theory implies that the production of red cells would be inferior to their destruction in a group of children studied before refeeding.

The theory of VITERI *et al.* (1968) also implies that the median age of the erythrocytes population would be increased in marasmic kwashiorkor. However these authors have not studied in details biochemical parameters of erythrocytes which would enable to evaluate the median age.

As regards the results presented in this paragraph it is worth stressing that the expression of the data compared to the minimum weight has only a conventional value and only provides an opportunity for comparing different investigations carried out in patients having similar degrees of malnutrition. No specific functional significance can be attributed to these expressions ; this fundamental methodological problem will be detailed in paragraph 6.

5. Relations existing between hematological parameters and the severity of the kwashiorkor component

In conditions of protein-energy malnutrition, it is often possible to identify the existence of a marasmus component and of a kwashiorkor component whose respective clinical and metabolic characteristics were summarized by Vis (1975). The presence of these two distinct nosological entities has been the subject of several controversies (McCance & Widdowson, 1968 ; Vis, 1969 ; Waterflow & Alleyne, 1971 ; Olson, 1975). The experiments made by Platt (1968) suggested that marasmus would appear in subjects submitted to a global deficiency in energy supplies, while kwashiorkor would develop in subjects whose protein supplies would be drastically reduced, but not their energy supplies would be drastically reduced, but not their energy supplies in the form of carbohydrates.

Some doubts have been however expressed towards that purely dietetic theory of the genesis of the different forms of protein-energy malnutrition. It seems that the reasons for which a kwashiorkor component would or would not appear in malnourished children are manifold. Therefore the consideration of a single factor such as the composition of the diet can be misleading because numerous non-dietetic factors can alter the response of the individual living in an unfavourable environment (Coward *et al.*, 1977).

In Kivu, the marasmus component is best defined by the insufficiency of weight related to height and the kwashiorkor component by the existence of metabolic disturbances which are generally reversible (Vis, 1975). These two components are most frequently mixed.

The research of correlations between hematological parameters and some clinical or metabolic data characterizing either the marasmus component or the kwashiorkor component enabled us to bring forward some clarifications regarding the etiopathogeny of the blood manifestations of marasmic kwashiorkor (Table 9).

a) The calculation of partial correlations indicates that for a given weight insufficiency there exists a significant correlation between the packed cell volume and the albumin level. On the contrary, for a given

level of albumin, there is no significant correlation between the packed cell volume and the weight insufficiency.

TABLE 9
Correlations between some haematologial parameters and the severity of malnutrition

PCV (N = 118)	
r_{PCV} – Alb = 0.49***	
r_{PCV} – Alb, Wdev% = 0.45***	
r_{PCV} – Wdev%, Alb = 0.16^{NS}	
RCV (N = 27)	
r_{RCV}(ml/kg) – Alb = 0.49*	
r_{RCV}(%) – Alb = 0.45*	
EIT (N = 17)	
r_{EIT}(mg/kg/d) – uroc = -0.33^{NS}	
r_{EIT}(%) – uroc = −0.54*	

Symbols : PCV = packed cell volume (%)
Alb = serum albumin, g/100ml
Wdev% = weight deviation, %
RCV = red cell volume
EIT = erythrocyte iron turnover
uroc = urocanic acid excretion after L-histidine loading.
r = Spearman's correlation coefficient.

b) There exists a significant correlation between the level of plasma albumin and the red cell volume expressed either against the minimum weight or in percentages of the mean normal value for the height ; on the contrary there is no significant correlation between the red cell volume and the weight deficit (VIART, 1976 ; FONDU, 1977).

c) In a previous study, we demonstrated that, at a given stage of malnutrition, the metabolic consequences of the kwashiorkor component would be best reflected by the urocanic acid excretion in the urines after loading in L-histidine than by the dosage of albumin ; the more the component kwashiorkor is severe, the more important is the excretion (MANDELBAUM *et al.*, 1975). This phenomenon could be explained by the characteristics of the metabolism of albumin in cases of malnutrition : one knows that some compensatory mechanisms tend to maintain the level of albumin within rather narrow limits while its synthesis could be significantly altered (HOFFENBERG *et al.*, 1966 ; JAMES & HAY, 1968). On the basis of these observations, we have studied the correlation existing

between erythropoietic activity and the excretion of urocanic acid after an excess loading with L-histidine. We came to the conclusion that the more severe the kwashiorkor component is, the longer is the marrow transit time and the smaller is the erythrocyte iron turnover (FONDU, 1973).

The correlations observed between certain hematological parameters and the severity of the kwashiorkor component do not allow to derive any conclusion as far as "pure" marasmus is concerned. That form is practically non existent in Kivu and we do not have any personnal data on the subject. The hematological repercussions of chronic energy deficiency are rather well-known. One of the major hematological differences between kwashiorkor and marasmus would be in the life span of erythrocytes, which is slightly reduced in the first syndrome but which is normal or even increased in the second (STEKEL & SMITH, 1970 ; READ *et al.*, 1974).

6. Arguments in favour or against the existence of an "adaptive" anemia

According to HAXHE (1963) "adaptive anemia" is a reduction of the concentration of red cells, the volume of which nevertheless suffices to oxygenate normally the tissues without the intervention of compensatory mechanisms. Such an anemia thus exhibits the following characteristics :
1) there is no increase of P50, heart output or production of erythropoietin ;
2) the marrow response to erythropoietin is not diminished ;
3) the life span of red cells is not shortened.

Due to the significant changes of the body composition which occur during malnutrition, it is extremely difficult to find reference standards to express the values of the different compartments or body functions. This situation is well illustrated by the difficulty to interpret the measurements of oxygen consumption. It is established that oxygen consumption expressed in liters per minute is lower for an undernourished child than for control subjects of the same age or the same height (MONTGOMERY *et al.*, 1962 ; MÖNCKEBERG *et al.*, 1964 ; PARRA *et al.*, 1975). On the other hand, it is difficult to find out whether the respiratory metabolism of the active tissular mass is reduced or not.

The fact that the basic metabolism and the red cell volume are both inferior to normal values according to the age and height does not constitute a sufficient proof that the reduction of oxygen requirements is the single or the major cause of the reduction of the total amount of hemoglobin. Simultaneous measurements of these two parameters have never been made in cases of protein-energy malnutrition. One must consider woith skepticism the conclusions reached by VITERI *et al.* (1968), based on simultaneous determinations of the red cell volume and of the index of creatininurea, since we doubt that such an index truly reflects the value of the basal metabolism in undernourished children (FONDU, 1977).

Two investigations made at Lwiro might corroborate the assumption of the existence of an "adaptive anemia" in cases of marasmic kwashiorkor.

a) FONDU & MANDELBAUM (1975) have studied the affinity of hemoglobin for oxygen and the level of 2,3-DPG in cases of marasmic kwashiorkor. P50 and 2,3-DPG level are normal on admission and

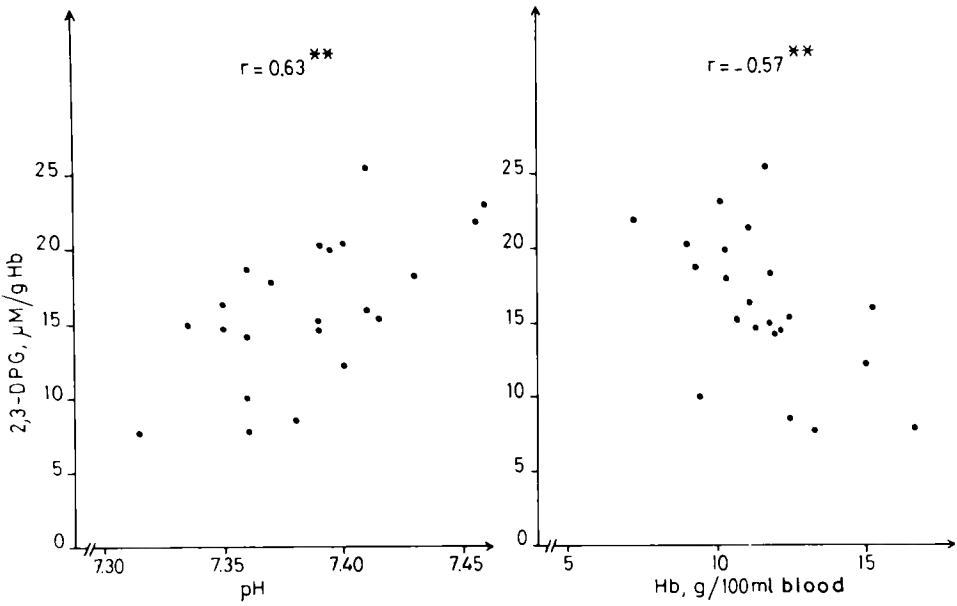

FIG. 7. – Correlations observed on admission between the erythrocyte content of 2,3-diphosphoglycerate (2,3-DPG) and either the hemoglobin content (Hb) or the pH of capillary blood.

increase during treatment. Significant correlations exist between the level of 2,3-DPG, the level of hemoglobin and the pH of capillary blood (Fig. 7). Several disturbances which would be in theory susceptible to inhibit the accumulation of 2,3-DPG, such as acidosis, hypoglycemia, hypophosphoremia or hypomagnesemia do not exist in the cases of marasmic kwashiorkor investigated (FONDU & MANDELBAUM, 1975 ; MANDELBAUM *et al.*, 1982). The absence of an accumulation of 2,3-DPG, which is rather exceptional in cases of anemia, was previously described in panhypopituitarism (RODRIGUEZ & SHAHIDI, 1971).

b) The studies made by VIART (1977, a, b) indicated that the majority of patients presented hemodynamic conditions comparable to those observed in cases of hypothyroidism : the circulatory time is increased and there is a tendency towards bradycardia and hypotension. The heart

* One cannot however certify that the hemodynamic condition represents an adequate adaptation to a restriction of metabolic activities. The existence of electrocardiphic abnormalities suggests a possible alteration of the myocardic function. Moreover, a hypovolemic shock can be observed in the most severe cases (VIART, 1977a, b).

output the systolic index and the function of the left ventricle are reduced. (*)

Other observations lead us to believe that the hematological picture observed in marasmic kwashiorkor does not correspond to the definition of "adaptive anemia".

a) As we have previously recalled, the probability of erythrocyte death is increased.

b) A second argument is to be found in the dosages of erythropoietin made in patients observed in the Kivu region. Because of the low ambient p02, any added hypoxic stimulation tends to create a marked increase of erythropoietin output ; therefore the dosage of the hormone can be done in particularly favourable conditions.

The level of erythropoietin in the plasma is higher in marasmic kwashiorkor than in the case of healthy children living at the same altitude ; it remains high after two months of treatment (Table 10, Fig. 8). Curiously enough, there is no positive correlation on admission between the levels of erythropoietin in the plasma and in the urine, while such a correlation is found in refed patients (FONDU *et al.*, 1978b).

TABLE 10
Erythropoietin (ESF) levels (mean ± SEM)

Parameters studied	Incoming patients	Refed patients	Controls
PCV	33.8* ± 0.8 (N = 7)	36.5* ± 0.9 (N = 12)	46.3 ± 1.4 (N = 7)
	NS		
Plasma ESF (% incorporation of Fe)	5.10 ± 0.71 (N = 15)	4.70 ± 0.93 (N = 12)	1.49 ± 0.26 (N = 7)
	NS		
Urine ESF (% incorporation of Fe)	1.76 ± 0.38 (N = 13)	1.48 ± 0.34 (N = 11)	–
Correlation between plasma and urine ESF levels	$R = 0.26^{NS}$ (N = 11)	R = 0.62* (N = 11)	– –

R = Spearman's correlation coefficient.

The significant increase in the levels of erythropoietin observed in marasmic kwashiorkor brings the anemia observed in that affection in opposition to that which accompanies chronic diseases (DOUGLAS & ADAMSON, 1975).

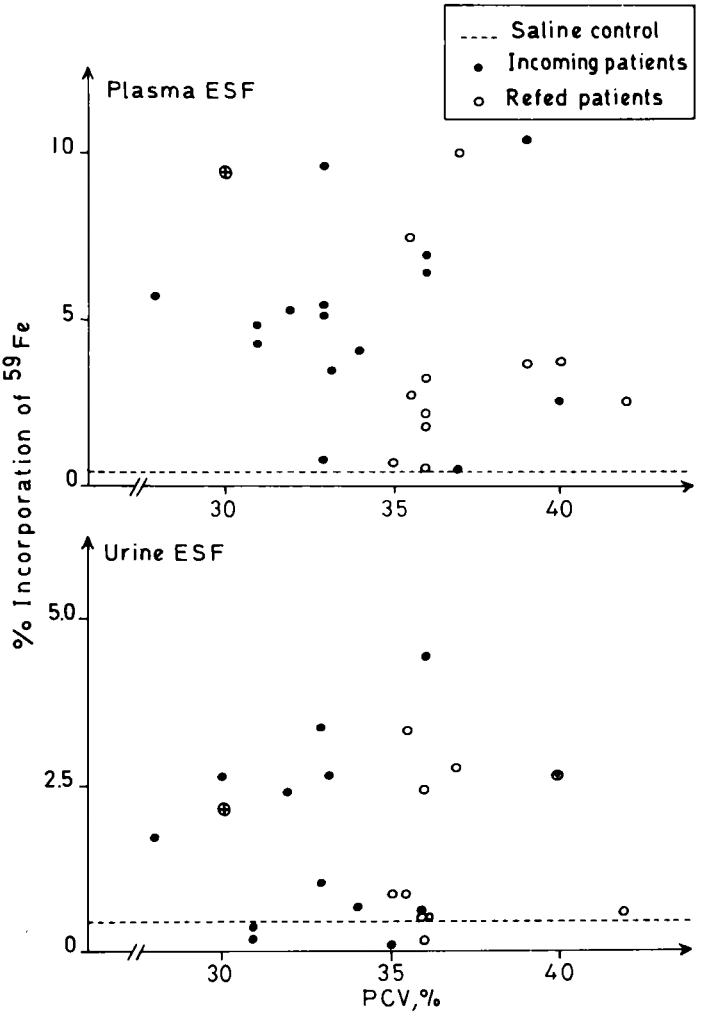

FIG. 8. – Uptake of 59Fe in mice injected with plasma and urine ESF from patients on admission (.), refed patients (o) and one refed patient with malaria (⊕), plotted against packed cell volume (PCV) (FONDU *et al.*, 1978b).

Therefore we can conclude that the anemia observed in the marasmic kwashiorkor of Kivu represents an autonomous hematological syndrome. The reduction of the red cell volume results both from an increase in the probability of erythrocyte destruction and from a reduction of the marrow responsiveness to erythropoietin.

The interpretation of that syndrome is however difficult. Although the increased levels of erythropoietin seem to point to the existence of hypoxia, other compensatory mechanisms which usually intervene in conditions of anemia are not detectable here : there is neither an accumulation of 2,3 – DPG in the red cells nor an increase of the heart output.

7. **Erythrocyte metabolism**

7.1 Introduction

The previous considerations indicate clearly that an understanding of the mechanisms of anemia observed in marasmic kwashiorkor can only be obtained through a detailed study of the metabolism of erythroid cells. In theory, erythroblasts and red cells should be the subject of such an endeavour ; in the present state of our knowledge only the study of red cells seems however possible.

More precisely, the biochemical study of red cells can provide an answer to four questions raised in the previous chapters :

a) the identification of erythrocyte disturbances susceptible to explain the increase in the probability of their destruction ;
b) the definition of the impact of eventual deficiencies in some nutrients on erythroid cells ;
c) the study of the adjustment mechanisms of erythrocytes to hypoxia ;
d) the evaluation of the median age of the erythrocyte population.

Red cells of a given age could present two types of disturbances in patients suffering from protein-energy malnutrition. In the first place, the synthesis of erythrocyte constituents could be altered during erythropoiesis. Therefore, one could assume the existence of disturbances of synthesis rate of certain proteins, especially of certain isoenzymes, leading to changes in the global activity, kinetic properties or life-time of enzymes intervening in a given metabolic reaction. It was never demonstrated that a deficiency in essential amino-acids might be followed by an alteration of the structure of a protein. However one knows that a restricted availability of some other nutrients may alter the properties of erythrocyte enzymes (Beutler, 1970 ; Boivin, 1970).

In the second place, an abnormal plasma environment might create alterations of the metabolism of nature red cells. These lesions would not necessarily be irreversible : normalization might take place before the renewal of the erythrocyte population.

A review of current literature provides only partial data on the metabolism of erythrocytes in protein-energy malnutrition. The interpretation is even more complex since deficiencies associated to protein-

energy malnutrition *per se* are not always defined and since no assumption based on specific experimental arguments enables us to obtain a picture of the evolution of the red cell volume as a function of time. The eventual changes of the distribution of the frequencies of erythrocyte ages, on which a considerable number of biochemical values depend, remain therefore totally unknown.

Erythrocyte glycolysis has never been studied in malnutrition. This fact is rather surprising since the Embden-Meyerhof pathway is the most important source of energy at the disposal of red cells, and since glycolytic abnormalities were described in other tissues in cases of malnutrition. For instance, the activity of pyruvate-kinase seems to be reduced in the muscles (METCOFF, 1975 ; METCOFF *et al.*, 1960, 1966) and in leukocytes (YOSHIDA *et al.*, 1967, 1968).

Some authors refer to the pentose-phosphate pathway in erythrocytes during malnutrition. A lowering of the level of GSH* was described in kwashiorkor by MODY & SMITH (1964) and confirmed by VERJEE & BEHAL (1976), although the same result was not obtained by SARACLAR & OZSOYLU (1966). In 1968, BATALDEN *et al.* observed a reduction of the level of GSH in a considerably emaciated adult ; the formation of Heinz bodies after incubation in the presence of acetyl-phenylhydrazine was increased. On the basis of animal experiments, these authors thought that the synthesis of glutathione was reduced, due to an insufficient dietary supply of methionine and cysteine. This explanation remains highly conjectural, as the level of GSH could be diminished for several other reasons. In 1975 MIKHAIL *et al.* indicated an increase in the glycine intake by red cells during kwashiorkor. One cannot however infer that the synthesis of glutathione is increased ; in fact it is well-known that the measurement of the glycine intake by red cells does not permit to evaluate the synthesis of glutathione (BJÖRNESJÖ, 1965). Transitional deficiencies in G6PD were described by VITERI *et al.* (1968), but not by VERJEE and BEHAL (1976). The latter authors reported on the other hand the existence of acquired deficiencies in GSSG-red** and in GSH-Px***. Consequently, the very scarce data published do not enable to put forward general pathophysiological rules relating to the functioning of the pentose-phosphate pathway and to the reduction mechanisms of activated oxygen.

* reduced glutathione
** glutathione-reductase
*** glutathione-peroxidase

The existence of an abnormality of the membrane was envisaged for the first time following the work of LANZKOWSKY *et al.* (1967), who reported an increase of osmotic resistance in kwashiorkor. Their findings seem to indicate that the ratio between the surface and the volume of red cells is increased and that such an abnormality can be accompanied by morphological peculiarities, such as the presence of target-cells or an increased incorpuscular diameter. As a matter of fact, one finds in the literature references to the existence of target-cells in kwashiorkor (VITERI *et al.*, 1968). Moreover, VAN OYE (1953), ADAMS (1954) and WOODRUFF (1968) underline the existence of an increase in corpuscular diameter but they possibly confuse macrocytosis (increase of VCM) and macroplany (expression proposed by WERRE *et al.*, (1970) to designate an increase in diameter with or without increase of VCM). In 1971, Coward reported an increase in the level of phosphatidylcholine in the membrane without being able to provide a satisfactory explanation for that phenomenon. Coward noted on the other hand that the time necessary for hemolysis *in vitro* in the presence of glycerol or thiourea was prolonged and attributed such a disturbance to a reduction in membrane permeability. However one knows that hemolysis time depends not only on permeability coefficients but also on the surface/volume ratio of red cells (WESSELS *et al.*, 1973). Therefore it is difficult to give an interpretation to the results obtained by COWARD.

Finally, BROWN *et al.* (1978) brought forward some arguments in favour of the existence of an increase of cholesterol in the membranes during protein-energy malnutrition but they could not explain the pathogenesis of these disturbances (FONDU *et al.*, 1979, a).

The erythrocyte contents of Na^+ and K^+ depend on the passive permeability of the membrane and on the activity of the sodium and potassium pump. High contents of Na^+ and low contents of K^+ have been described in erythrocytes during kwashiorkor (KHALIL *et al.*, 1974). On the basis of these results KHALIL *et al.* have assumed the existence of a defect of the sodium pump, assumption which is nevertheless in conflict with the measurements of the activity of $Na^+ - K^+$ – ATPase* carried out by KAPLAY (1978). The only other membrane enzyme which received some attention in protein-energy malnutrition is acetylcholinesterase ; its activity seems to be reduced and its kinetic characteristics altered (KAPLAY, 1975).

* Adenosine-triphosphatases of the erythrocyte membrane activated by Na^+ and K^+ ions.

In conclusion, the review of the data contained in the literature provides us with divergent results, difficult, if not impossible, to understand. One of the reasons is to be found according to us in the fact that the authors have studied only isolated aspects of disorders whose characteristics differ from one region to another (FONDU *et al.*, 1979a,b). It seemed to us more rational to approach the biochemical studies in a more global perspective.

7.2. Energetic metabolism of red cells (FONDU *et al.*, 1978c ; MANDELBAUM *et al.*, 1982).

Mature red cells obtain essentially their energy from the consumption of glucose through the Embden-Meyerhof pathway ; this pathway allows a gain of two molecules of ATP* per molecule of glucose consumed (Fig. 9). ATP is used in several endergonic reactions, a major one being catalysed by NA^+-K^+-ATP-ase. On the other hand, the maintenance of a high level of 2,3-DPG, which characterizes red cells, depends on the function of the RAPOPORT and LUEBERING shunt. The regulating mechanisms of that pathway are still largely nuclear at present.

When the erythrocytes of patients observed in Kivu are incubated in anaerobiosis, one notes that their consumption of glucose and their formation of lactate are normal or increased (Fig. 10).

The assumption of a stimulation of glycolytic activity in red cells was supported by two types of different investigations : the measurement of the maximum speed of certain enzymes and the measurement of the level of certain glycolytic intermediates.

As indicated in Table 11, the maximum velocity of HK**, pacemaker of the Embden-Meyerhof pathway, is increased on admission. On the contrary, the maximum velocity of PK*** is not altered.

The study of glycolytic intermediates reveals two important facts (Fig. 11) : on the one hand, the significant increase in the level of G6P**** confirms the increase of the activity of HK *in vivo*. On the other hand, one cannot confirm the existence of a cross-over at the level of the

* adenosine-triphosphate
** hexokinase
*** pyruvate kinase
**** Glucose-6-phosphate

reaction catalysed by PFK*. It is thus hardly conceivable that the balance of the action of effectors on PFK will lead to a slowering down of the conversion of F6P** into FDP***.

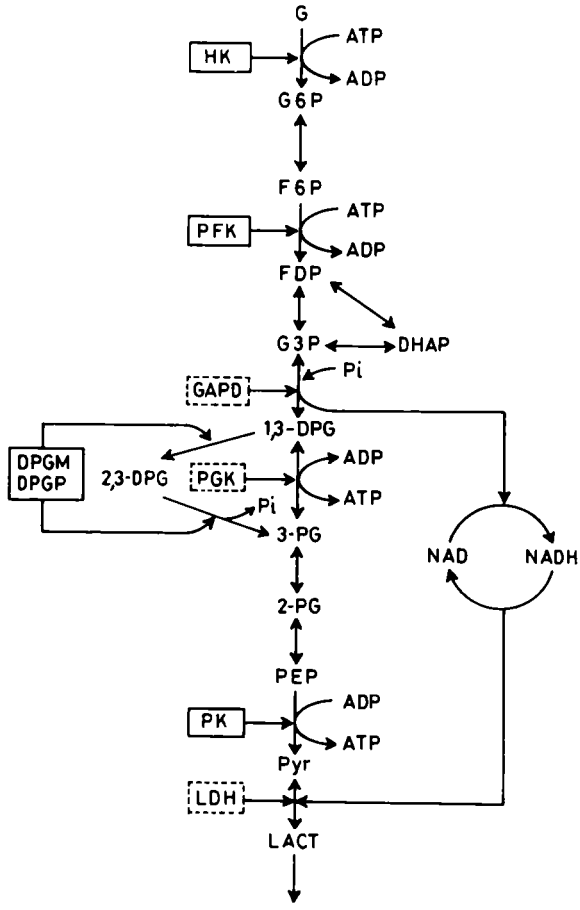

FIG. 9. – The glycoloitic pathway and the Rapoport-Luebering shunt. Hexokinase (HK), phosphofructokinase (PFK) and pyruvate kinase (PK) catalyse reactions characterized by important free energy changes. Glyceraldehyde phosphate dehydrogenase (GAPD), phosphoglycerate kinase (PGK) and lactic dehydrogenase (LDH) are "equilibrium enzymes". The regulation of the 2,3 dephosphoglycerate (2,3-DPG) pool depends on the properties of a multifunctional enzyme, possessing diphosphoglyceromutase (DPGM) and diphosphoglycerophosphatase (DPGP) activities.

* Phosphofructokinase

** Fructose-6-phosphate

*** Fructose-1,6-diphosphate

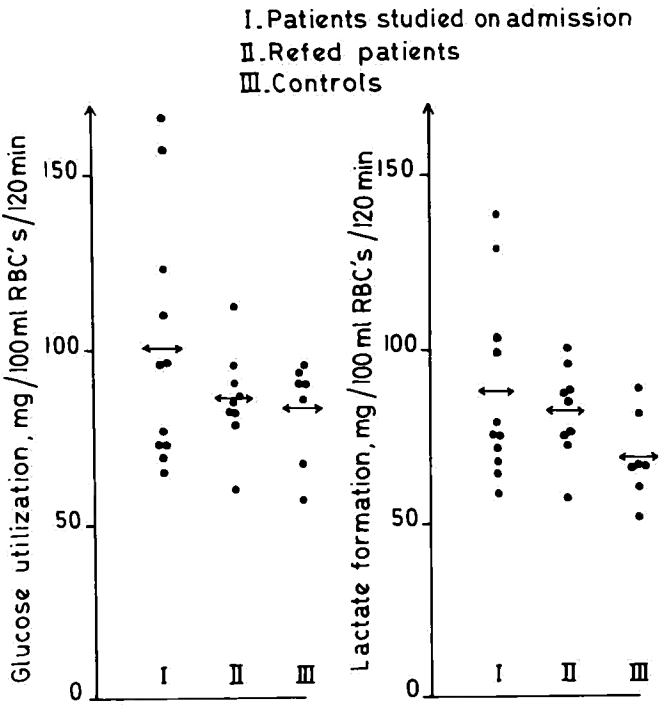

FIG. 10. – Glucose consumption and lactate formation by red blood cells.

TABLE 11

Activities of hexokinase (HK), pyruvate-kinase (PK) and Na^+-K^+-adenosine-triphosphatase (ATPase) in the erythrocytes (mean±SEM)

Parameters studied	Patients studied on admission	Refed patients	Controls
HK, µM/gHb/min	$1.69^{**} \pm 0.11$ (N = 33)	$1.41^{*} \pm 0.08$ (N = 18)	1.12 ± 0.14 (N = 19)
PK, µM/gHb/min	$15.9^{NS} \pm 0.8$ (N = 33)	$17.3^{*} \pm 0.9$ (N = 18)	14.3 ± 0.6 (N = 19)
Total ATPase, µMP/gHb/h	$36.4^{**} \pm 1.9$ (N = 12)	$25.2^{NS} \pm 1.3$ (N = 9)	19.3 ± 1.8 (N = 6)
s-ATPase µMP/gHb/h[1]	$23.4^{**} \pm 1.8$ (N = 12)	$14.5^{NS} \pm 0.9$ (N = 9)	12.1 ± 1.8 (N = 6)
i-ATPase µMP/gHb/h[2]	13.1 ± 0.8 (N = 12)	0.7 ± 1.2 (N = 9)	7.2 ± 0.4 (N = 6)

1 : Ouabaïne-sensitive
2 : Ouabaïne-insensitive

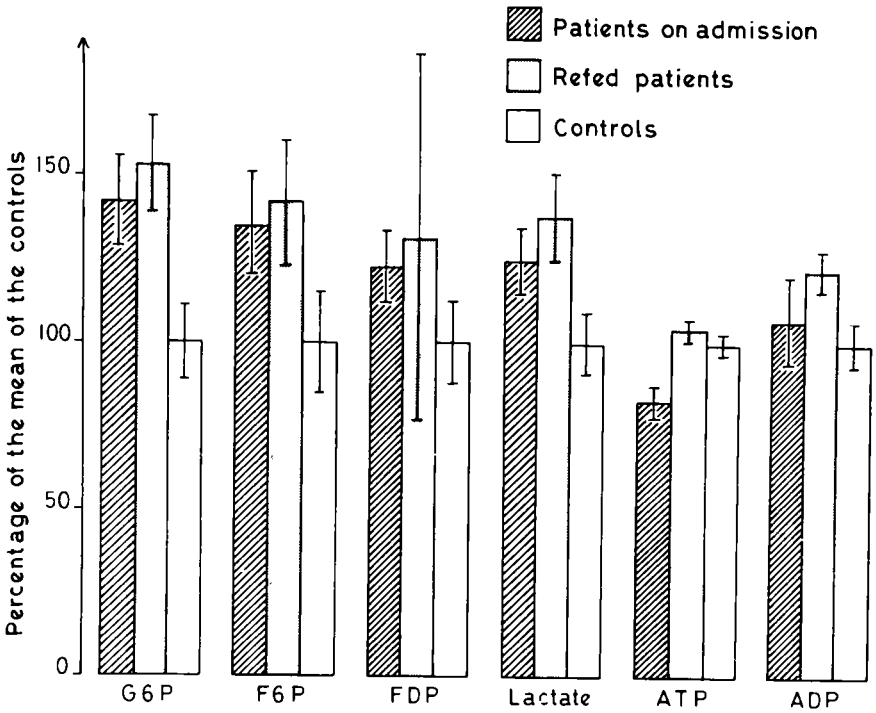

FIG. 11. – Levels of glycolytic intermediates and adenosine di- and triphosphate (mean ± SEM). – G6P : glucose-6-phosphate ; F6P : fructose-6-phosphate ; FDP : fructose-1,6-diphosphate ; ATP : adenosine triphosphate ; ADP : adenosine diphosphate.

All these data converge to indicate that the red cells of patients exhibit an increase of their glycolytic activity, which is the case for young erythrocytes (ULTMANN *et al.*, 1957 ; BERNSTEIN, 1959 ; BROCK *et al.*, 1966 ; CHAPMAN & SCHAUMBURG, 1967 ; BISHOP & VAN GASTEL, 1969 ; OSKI, 1969 ; NIESSNER & BEUTLER, 1973 ; ROGERS *et al.*, 1975).

It seems therefore probable that the median age of the erythrocyte population is reduced on admission. In a stationary system, this would suggest the existence of a hemolytic process in which hyperdestruction would be restricted to the oldest red cells. The facts recorded are essentially different from those observed in animals submitted to drastic fasting ; in that case the contents of several erythrocyte constituents are reduced (BISIANI, 1965 ; SANCHEZ DE JIMENEZ *et al.*, 1965 ; ITO & REISSMANN, 1966 ; ASCHKENASY, 1971).

If we refer to the above-mentioned observations, we note that two results seem *a priori* to be in conflict : the absence of increase in the levels

of 2,3-DPG and a moderate reduction, although significant, of the levels of ATP (Fig. 10). High levels could be observed in young cells. In certain pathological conditions, the incubation of blood in anaerobiosis reveals an instability of 2,3-DPG (Oski & Cittadino, 1977). Such a situation is not observed in marasmic kwashiorkor (Fig. 12). The insufficient state of our present knowledge of the regulation of the Rapoport-Luebering shunt makes it impossible to formulate a more accurate interpretation of the "paradoxical normality" of 2,3-DPG in the patients studied. Nevertheless it remains possible to provide a more general explanation. One considers generally that the accumulation of 2,3-DPG which takes place in conditions of hypoxia results from an increased deoxygenation of hemoglobin leading to erythrocyte alkalosis through Haldane effect. Alkalosis suppresses the inhibition of PFK and activates DPGM*, while inhibiting DPGP**, which would result in an increase of the 2,3-DPG

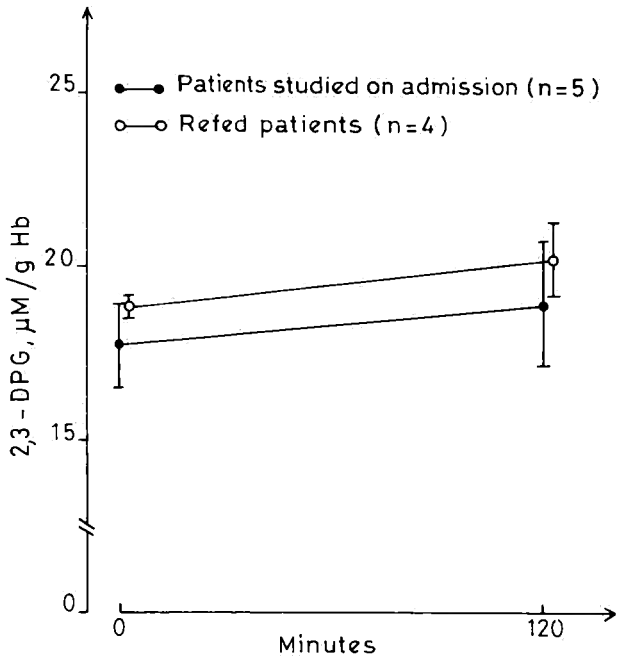

Fig. 12. – Variation of the erythrocyte level of 2,3-diphosphoglycerate (2,3-DPG) observed when blood is incubated in anaerobiosis (mean ± SEM).

* Diphosphoglyceromutase
** Diphosphoglycerophosphatase

pool (ROSE, 1973). In the light of our results, it is clear that the "paradoxical normality" of 2,3-DPG cannot be explained by an absence of stimulation of the key-reactions of the Embden-Meyerhof pathway, catalyzed by HK and PFK. We believe therefore that, contrary to the theory supported earlier by VALERI and FORTIER (1969), the dosage of 2,3-DPG does not always allow to evaluate with precision the partial pressure of oxygen in tissues.

The reduction of the level of ATP can be more satisfactorily explained if we consider that the use of the nucleotide during the reaction catalyzed by Na-K-ATP-ase is increased (Table 11). The increased activity of this enzyme is responsible for the changes of the erythrocyte levels of Na^+ and K^+ ; the first is reduced while the second is increased (Table 12).

The present studies of the energy metabolism of red cells do not provide us with any clear explanation of the increase of probability of erythrocyte destruction.

TABLE 12
Sodium (Na^+) and potassium (K^+) levels in the erythrocytes (e) and the plasma (p) (mean ± SEM)

Parameters studied	Patients studied on admission (N = 22)	Refed patients (N = 21)	Controls (N = 10)
Na^+e, meq/l RBC'S	$5.1^{***} \pm 0.2$	$6.0^{***} \pm 0.2$	7.9 ± 0.2
K^+e, meq/l RBC's	$118.4^{**} \pm 0.2$	$116.4^{NS} \pm 2.9$	107.4 ± 2.6
Na^+p, meq/l plasma	$133.2^{NS} \pm 1.1$	$134.2^{NS} \pm 1.3$	134.1 ± 0.8
K^+p, meq/l plasma	$4.7^{**} \pm 0.2$	$4.8^{***} \pm 0.1$	4.0 ± 0.1

7.3. Reduction of activated oxygen (FONDU *et al.*, 1978c ; VERTONGEN *et al.*, 1981)

The red cell is the seat of a constant formation of deleterious derivates of oxygen considered under the expression "activated oxygen" : superoxide radical, free hydroxyl radical, hydrogen peroxide and probably singlet. The reduction of activated oxygen requires the intervention of a well-defined sequence of enzymatic reactions, and a defect in any of them is able to lead to the so-called "oxidative hemolysis" (Fig. 13) (CARRELL *et al.*, 1975). The simplest method used to determine

the presence of oxidative hemolysis is to count the Heinz bodies produced by the oxidative injury of hemoglobin (GORDON-SMITH & WHITE, 1974).

After incubation in the presence of acetylphenylhydrazine, it appears that the formation of Heinz bodies is increased in marasmic kwashiorkor (Fig. 14). Therefore it is necessary to study the reduction mechanisms of activated oxygen. The results of the investigations made in that respect are presented in Figure 15 and can be explained as follows.

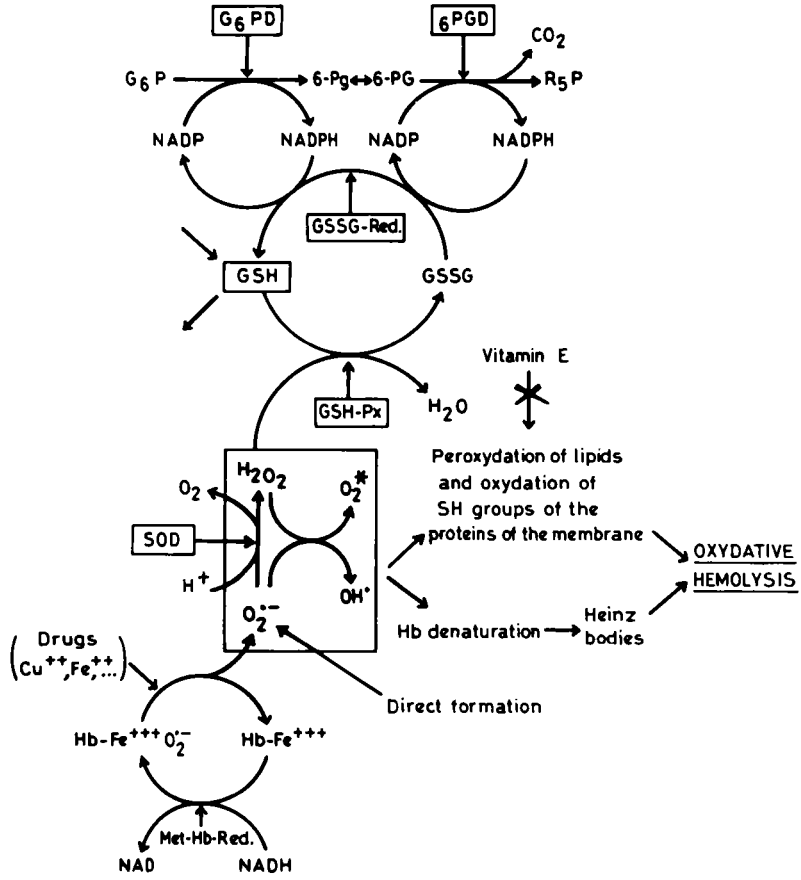

FIG. 13. – Main mechanisms of production and of reduction of the activated oxygen. The catalase and the synthetic and catalytic pathways of glytathione are not presented, nor is deoxyhemoglobin. The forms of activated oxygen (rectangle) are : $O_2^{\cdot}$: superoxyde anion : O_2^* : singlet ; $OH^{\cdot}$: Hydroxyle ; H_2O_2 : hydrogen peroxyde.

Some parameters studied are normal in marasmic kwashiorkor, such as the level of GSH and the activity of G6PD ; others are increased such as the activity of 6PGD*, or GSSG-red measured with or without addition of FAD** ; others are reduced such as the activities of GSH-Px or SOD***. Finally, the "activity coefficient" of GSSG-red, that is the activity measured in the presence of an *in vitro* addition of FAD and the activity measured without addition of FAD, is normal on admission, but is increased in refed children (Table 13).

The discussion of these results deals with three successive aspects.

First, the increase in the activities of 6PGD and GSSG-red is compatible with the assumption of a reduction of the median age of the erythrocyte population already mentioned in the paragraph 7.2.

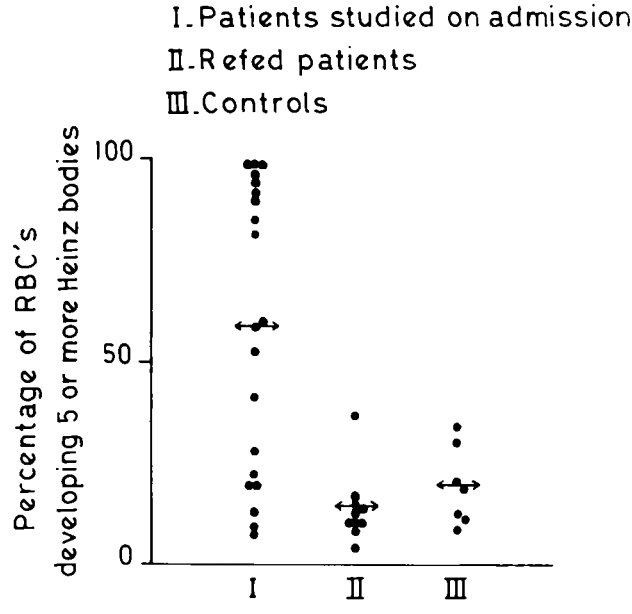

FIG. 14. – Percentages of Heinz bodies after incubation of red cells in the presence of acetylphenylhydrazine.

* 6-phosphogluconate dehydrogenase
** Flavin adenine dinucleotide
*** Superoxide dismutase

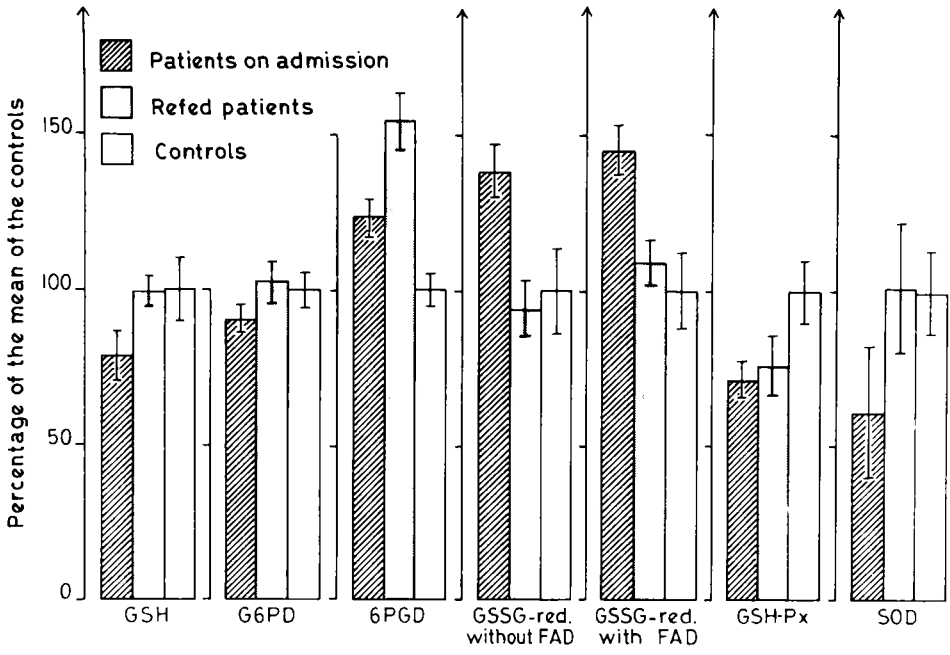

FIG. 15. – Major mechanisms intervening in the reduction of activated oxygen (mean ± SEM). – GSH : reduced glutathione ; G6PD : glucose-6-phosphatase dehydrogenase ; 6PGD : 6-phosphogluconate dehydrogenase ; GSSD-red : glutathione reductase ; FAD : flavine adenine dinucleotide ; GSH-Px : glutathione peroxidase ; SOD : superoxide dismutase.

TABLE 13
Erythrocyte glutathine-reductase assay[a]

Enzyme assay (μmoles/gHb/min)	PEM, on admission (n = 13)	Refed patients (n = 9)	Controls (n = 7)
Without added FAD (1)[b]	5.5* ± 0.34	3.7 ± 0.36 (NS)	4.0 ± 0.56
With added FAD (2)[b]	6.6** ± 0.36	4.9 ± 0.33 (NS)	4.5 ± 0.55
Activity coefficient (2/1)[b]	1.21 ± 0.043 (NS)	1.36* ± 0.080	1.16 ± 0.045

[a] Mean ± SEM.
[b] Comparisons between patients and local controls.

Second, the increase of the "activity coefficient" of GSSG-red in refed patients points to the appearance of a relative deficiency in riboflavin (GLATZLE *et al.*, 1968 ; BEUTLER, 1969 ; SAUBERLICH *et al.*, 1972). Such a deficiency does not increase the fragility of red cells to oxidants. This result is compatible with the data presented in the literature relating to deficiency in vitamin B2 (BEUTLER & SRIVASTAVA, 1970 ; PANIKER *et al.*, 1970). The appearance of a relative deficiency in riboflavin during refeeding can be attributed to the high nutritional needs accompanying an intensive period of anabolism.

Finally, it is worth noting that the only two erythrocyte enzymes for which decreased activities have been observed in the patients are precisely those in the composition of which trace-elements intervene : selenium in the case of GSH-Px, copper and zinc in the case of SOD. In the light of the data presented in the literature, it seems possible that a deficiency in these two enzymes could explain the oxidative hemolysis observed in marasmic kwashiorkor (NECHELES *et al.*, 1968 ; MACDOUGALL, 1972 ; FEE & TEITELBAUM, 1972 ; HOPKINS & TUDHOPE, 1973 ; RODVIEN *et al.*, 1974).

On the other hand, a series of arguments suggest that the reduction of activity of GSH-Px in erythrocytes reflects quite frequently a deficiency in selenium (SCHWARZ & FOLTZ, 1957 ; ROTRUCK *et al.*, 1972 ; HAFEMAN *et al.*, 1974 ; ALLEN *et al.*, 1975 ; OMAYE & TAPPEL, 1974 ; SMITH *et al.*, 1974 ; COMBS *et al.*, 1975 ; HOEKSTRA, 1975 ; BURK, 1976 ; GANTHER *et al.*, 1976 ; GROSS, 1976) ; in the same way as a reduction in the activity of SOD can result from a copper deficiency (BOHNENKAMP & WESER, 1976).

It seems therefore to us probable that marasmic kwashiorkor in Kivu is accompanied by a deficiency in selenium and copper or in zinc, and that these deficiencies in trace-elements disturb the metabolism of the erythroid cells. If it is true that the assumption of the existence of a deficiency in selenium, copper and zinc is not new in protein-energy malnutrition (LAHEY *et al.*, 1958 ; EDOZIEN & UDEOZO, 1960 ; SCHWARZ, 1961 ; CORDANO *et al.*, 1964 ; SMITH & PRETORIUS, 1964 ; SANDSTEAD *et al.*, 1965 ; MAJAJ & HOPKINS, 1969 ; HOLTZMANN *et al.*, 1970 ; LEVINE & OLSON, 1970 ; KUMAR and RAO, 1973 ; KHALIL *et al.*, 1974 ; GRAHAM & CORDANO, 1975), it is nevertheless directly confirmed here for the first time by the demonstration of the impact of these deficiencies on the activities of certain specific enzymes.

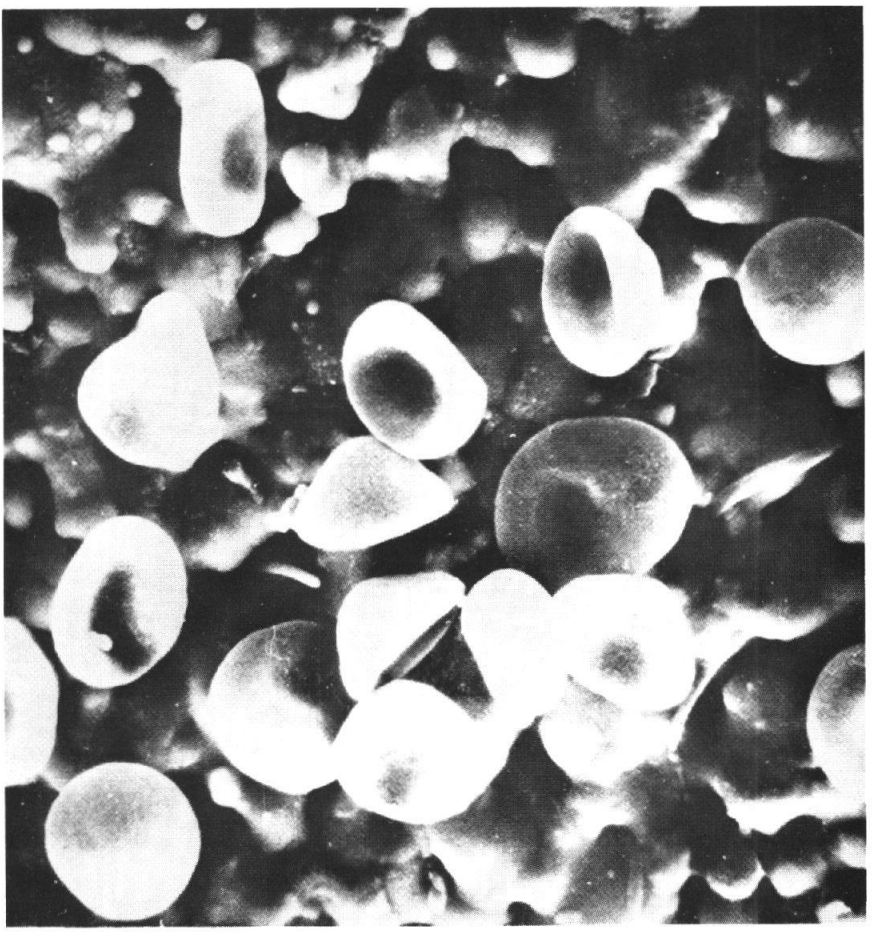

FIG. 16. – Scanning electronic microscopy of the red cells of a patient studied on admission. Most of the red cells are cup-shaped.

7.4. The membrane (FONDU *et al.*, 1978d ; 1979a, 1980)

7.4.1. Accumulation of cholesterol and phosphatidylcholine

Our interest for membrane lipids originates in the observation of an increase of the surface/volume ratio in the red cells of patients suffering from marasmic kwashiorkor. This observation is based on the following data (Table 14, Figure 16) :

- the mean halometric diameter is increased, while MCV is normal ;
- a high percentage of target-cells can be put in evidence ;
- scanning electronic microscopic shows that red cells often exhibit deformations in the shape of cup-cells ;
- finally, the average osmotic resistance is increased.

We were able to attribute the increase of the surface/volume ratio to an increase in the membrane contents of cholesterol and PC*, while the contents of the other lipids in the erythrocytes are normal (Fig. 17).

It is well-known that permanent exchanges of cholesterol and PC occur between the erythrocyte membrane and the plasma lipoproteins. Therefore it was necessary to find out whether the disturbances mentioned could account for the accumulation of cholesterol and PC in the patients' red cells.

TABLE 14

Studies of mean corpuscular volume (MCV), *mean corpuscular diameter* (MCD), *percentage of target cells and mean osmotic fragility (mean* ± SEM)

Parameters studied	Patients studied on admission		Refed patients		Controls	
MCV, fl	$93^{NS} \pm 2$	N = 25	$94^{NS} \pm 3$	N = 11	94 ± 2	N = 7
MCD, µm	$7.6^{*} \pm 0.1$		$7.2^{NS} \pm 0.1$		7.1 ± 0.1	
Target cells P 100RBC's	$5.8^{**} \pm 1.4$		$0.9^{NS} \pm 0.7$		0.04 ± 0.03	
Mean osmotic fragility, g NaCl/dl	$0.33^{**} \pm 0.01$ (N = 10)		$0.38^{NS} \pm 0.01$ (N = 7)		0.41 ± 0.01 (N = 5)	

* phosphatidylcholine

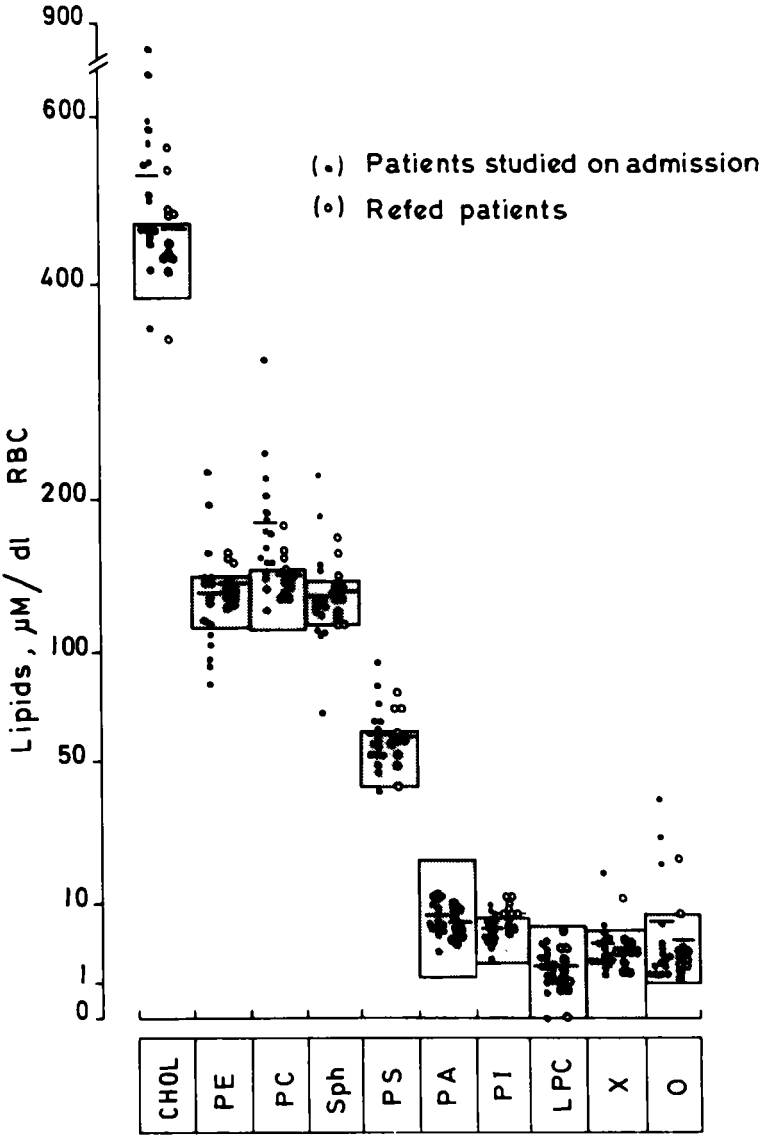

FIG. 17. – Levels of erythrocyte lipids before (.) and after (o) treatment, as well as in normal children (rectangles). In order to obtain a graphic representation of all the results, a square root scale was arbitrarily chosen. – Chol : cholesterol ; PE : phosphatidylethanolamine ; PC : phosphatidylcholine ; Sph : sphyngomyelin ; PS : phosphatidylserine ; PA : phosphatidic acid ; P : phosphatidic acid ; P : phosphatidylinositol ; LPC : lysophosphatidylcholine ; X : non identified lipid (globoside ?). – Origin : lipids remaining at the origin during phospholipid bidimensional chromatography.

Several abnormalities of plasma lipids are observed in marasmic kwashiorkor (Table 15). There exists a hypolipidemia and a reduction in the cholesterol content and, in particular, in cholesterol esters ; the levels of the various phospholipids are normal. The electrophoresis of lipoproteins reveals a decrease in the percentage of (preβ + β) lipoproteins.

Finally, the ultracentrifugation of lipoproteins indicates various abnormalities and in particular a reduction of the cholesterol/proteins ratio in LDL (density between 1.006 and 1.063). It also shows an increase in the phosholipids/proteins ratio in HDL (density between 1.063 and 1.210) (Table 16).

Two types of kinetic investigations were carried out in order to define whether an abnormal plasma environment plays a role in the accumulation of cholesterol and phosphatidylcholine.

In the first investigation, the red cells of a patient were labelled with 51-Cr-chromate and injected to a normal adult ; the evolution of osmotic resistance was then observed. A progressive normalization of this parameter was noted (Fig. 18).

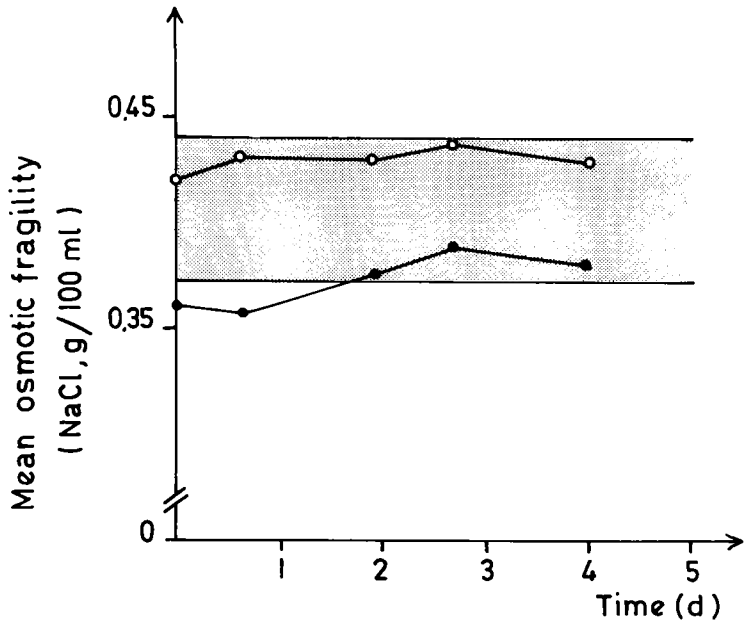

FIG. 18. – Evolution of the mean fragility when the red cells of a patient studied on admission are labelled with 51-chromate, then reinjected to a normal adult (.). Simultaneous study of the osmotic fragility of the erythrocytes of that adult (o). – The dotted area indicates the range of values determined in normal children.

TABLE 15
Plasma lipids

	Patients studied on admission	Refed patients	Controls
	Composition (MG/DL plasma)		
Total lipids	$500^{*} \pm 17$ (N = 49)	$566^{NS} \pm 22$ (N = 18)	627 ± 39 (N = 14)
Lipid phosphorus	$6.63^{NS} \pm 0.29$ (N = 24)	$5.92^{NS} \pm 0.31$ (N = 12)	6.39 ± 0.41 (N = 10)
Total cholesterol	$117^{*} \pm 5$	$141^{NS} \pm 7$	152 ± 6
Non-esterified cholesterol	$60^{NS} \pm 5$ (N = 24)	$47^{NS} \pm 3$ (N = 12)	49 ± 3 (N = 10)
Esterified cholesterol	$57^{***} \pm 5$	$94^{NS} \pm 8$	102 ± 7
	Chromatography of phospholipids (%)*		
	(N = 7)	(N = 5)	(N = 5)
PE	$4.5^{NS} \pm 0.5$	$4.4^{NS} \pm 0.5$	3.9 ± 0.4
PC	$73.6^{NS} \pm 1.7$	$69.0^{NS} \pm 1.0$	70.4 ± 1.4
Sph	$15.7^{NS} \pm 1.1$	$18.5^{NS} \pm 1.1$	17.1 ± 1.4
LPE	$0.3^{NS}0.1$	$0.8^{*} \pm 0.1$	0.4 ± 0.1
PI	$2.3^{NS} \pm 0.1$	$2.3^{NS} \pm 0.3$	2.6 ± 0.3
LPC	$3.5^{NS} \pm 1.0$	$4.9^{NS} \pm 0.6$	5.1 ± 0.8
O	$0.1^{NS} \pm 0.1$	$0.2^{NS} \pm 0.1$	0.3 ± 0.1

* Same abbreviations as in Fig. 17
LPE : Lysophosphatidylethanolamine.

TABLE 16
Composition of lipoproteins ($\bar{x}$; s)

	Density	Patients studied on admission N = 8	Refed patients N = 6	Controls N = 5	I-II	I-III	I-II&III
			mg/100 ml plasma				
Total cholesterol	VLDL	*6.3* ; 3.6	*12.1* ; 3.7	*7.4* ; 3.5	U = 7*	U = 15.5 (NS)	U = 22.5 (NS)
	LDL	*31.3* ; 19.0	*76.9* ; 20.4	*63.1* ; 17.2	U = 3**	U = 4*	U = 7**
	HDL	*31.8* ; 13.7	*52.8* ; 16.4	*62.7* ; 7.1	U = 9 (NS)	U = 3*	U = 12**
Lipid phosphorus	VLDL	*0.39* ; 0.29	*0.57* ; 0.20	*0.38* ; 0.18	U = 16 (NS)	U = 19 (NS)	U = 35 (NS)
	LDL	*2.34* ; 2.25	*2.12* ; 0.50	*1.82* ; 0.69	U = 15 (NS)	U = 15 (NS)	U = 30 (NS)
	HDL	*2.83* ; 0.91	*2.45* ; 0.48	*2.89* ; 0.77	U = 19 (NS)	U = 19 (NS)	U = 38 (NS)
			mg/g proteins in each fraction				
Total cholesterol	VLDL	*344* ; 73	*405* ; 52	*231* ; 89	U = 13 (NS)	U = 5*	U = 40 (NS)
	LDL	*540* ; 161	*825* ; 178	*865* ; 175	U = 5*	U = 3*	U = 16*
	HDL	*190* ; 62	*177* ; 79	*116* ; 27	U = 21(NS)	U = 5*	U = 25 (NS)
Lipid phosphorus	VLDL	*17.8* ; 5.7	*18.7* ; 2.8	*11.8* ; 4.9	U = 22 (NS)	U = 5*	U = 34 (NS)
	LDL	*34.1* ; 15.7	*8.4* ; 3.2	*5.5* ; 2.5	U = 2**	U = 0**	U = 2**

In the second experiment, we incubated normal red cells, the membrane of which had been previously labelled with tritiated cholesterol, in the presence of LDL or HDL of the patients examined before or after refeeding. We were able to show that the cholesterol flux between erythrocytes and LDL was abnormally low in some children studied before treatment (Fig. 19).

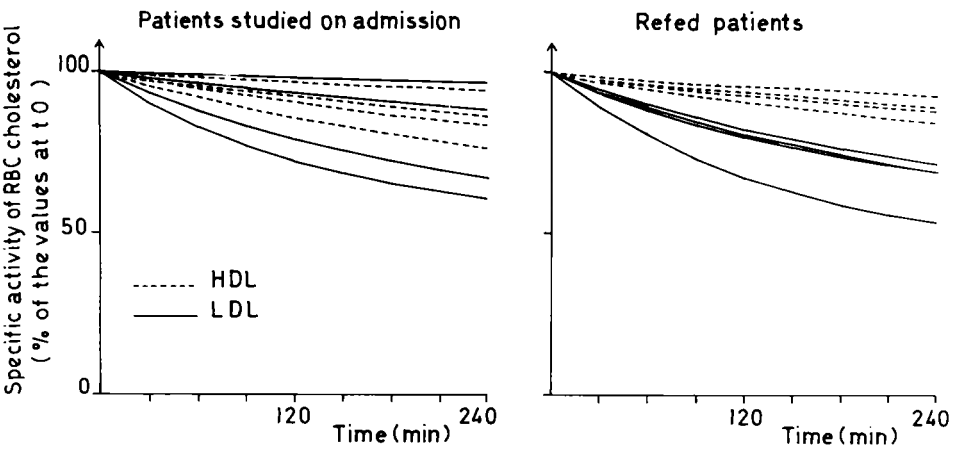

FIG. 19. – Evolution of the specific activity of erythrocyte cholesterol measured when the red cells of a normal adult, previously labelled with tritiated cholesterol, are incubated in the presence of low (LDL) or high (HDL) density lipoproteins obtained either from not yet refed patients or from recovered patients.

It seems therefore that complex disturbances of the plasma lipoproteins and, in particular, of LDL can be held responsible for the accumulation of cholesterol in erythrocytes, and also eventually for the accumulation of phosphatidylcholine.

A modification of the membrane content of cholesterol and/or phosphatidylcholine can have theoretically two functional impacts resulting in the premature destruction of red cells : a limitation of the deformability of the erythrocytes and/or an alteration of the membrane permeability. The first assumption is hardly conceivable in marasmic kwashiorkor : in fact it is generally when the surface/volume ratio is reduced that the filtrability of the red cells is diminished (WEED, 1975). The filtration time of red cells can also be prolonged when the ratio between the cholesterol content and the phospholipid content in the membrane reaches values which are considerably higher than those which we observed (COOPER *et al.*, 1975).

It is known that the permeability of the erythrocyte membrane, in particular to ions Na' and K^+, is a function of its cholesterol and phospholipids contents (MOORE, 1968 ; JAFFÉ & GOTTFRIED, 1968 ; KROES and OSTWALD, 1971 ; KROES *et al.*, 1972 ; SHOHET, 1972 ; SHOHET *et al.*, 1973 ; WILEY & COOPER, 1975). However, there does not seem to exist in marasmic kwashiorkor an exaggeration of the passive transfer of Na^+ and K^+. The erythrocyte levels of these two ions exhibit significant alterations (reduction in Na^+, increase in K^+) which are nevertheless opposed to what would be observed if there were an exaggeration of passive transfer not compensated by Na^+-K^+-ATP-ase (Table 12). Moreover, when ATP-ase is inhibited *in vitro* during the incubation of red cells in the presence of ouabain, the evolution of the levels of Na^+ and K^+ does not at all suggest the existence of an increase of passive transfer (Fig. 20).

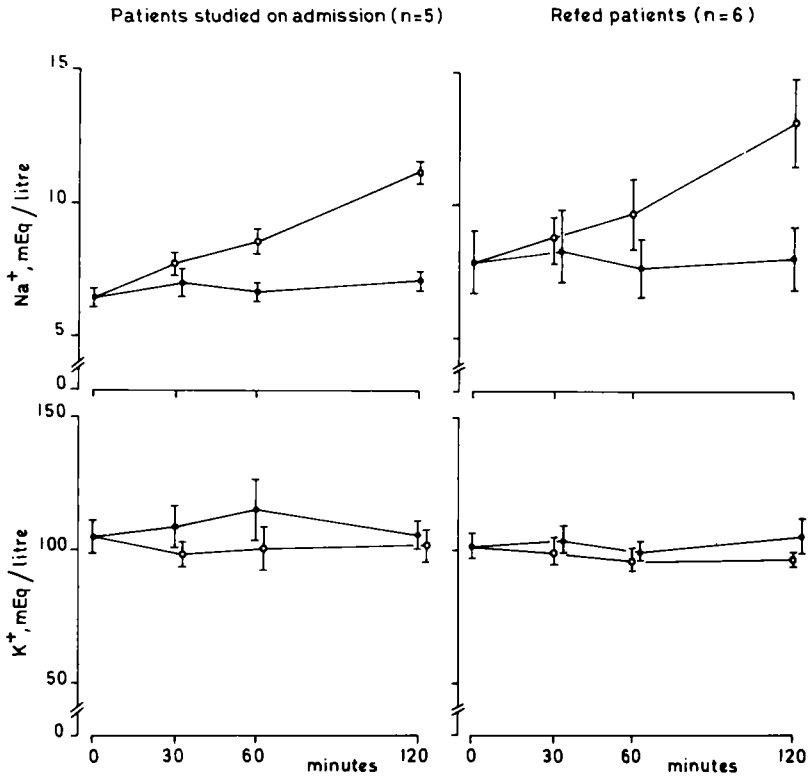

FIG. 20. – Incubation of red cells in the absence (o) or presence (o) of ouabaïn 10^{-4}M. Evolution of the erythrocyte levels of sodium (Na^+) and potassium (K^+). – Mean ± SEM of the values obtained in two groups of 4 patients.

In conclusion, the accumulation of cholesterol and phosphatidylcholine in red cells does not suffice in itself to explain the increase of the probability of destruction of erythrocytes. On the other hand, these lipid disturbances constitute a model of the disturbances which can occur in some plasma membranes during protein-energy malnutrition, together with the appearance of profound disturbances of the metabolism of lipoproteins.

7.4.2. Peroxidation of the fatty acids of the membrane

In a disease associated to oxidative hemolysis, the mechanisms leading to the premature death of red cells are either the formation of Heinz bodies or a peroxidation of the lipids as well as SH radicals of membrane proteins.

The demonstration of a peroxidation of membrane lipids is usually difficult. It is based either on the dosage of certain catabolites of lipoperoxides – in particular malonyldialdehyde – after incubation of the red cells in the presence of hydrogen peroxide, or on the study of the kinetics of fatty acids incorporated in the phosphatidylcholine (PC) and phosphatidylethanolamine (PE) of the membrane.

According to SHOHET (1972) the major steps of the transfer of fatty acids within the erythrocyte membrane can be summarized as follows (Fig. 21):

1) a fatty acid (FA) associated to plasma albumin is passively exchanged with a "superficial" erythrocyte compartment labeled F1

$$FA\ (Alb) \longrightarrow FA\ (F1)$$

2) the fatty acid is actively transfered to a "deep" compartment:

$$FA(F1) \rightarrow FA\ (F2)$$

3) in the presence of acyltransferases, adenosine triphosphate and coenzyme A, the fatty acid is fixed on lysophosphatidylcholine to regenerate PC:

$$FA\ (F2) + LPC \rightarrow PC$$

4) in the presence of transacylases the fatty acid is transfered to lysophosphatidylethanolamine (LPE) and regenerates PE:

$$PC + LPE \rightarrow LPC + PE$$

5) in the presence of phospholipases the fatty acid is finally transported in the plasma:

$$PE \rightarrow LPE + FA\ (Alb)$$

If one considers that LPC and LPE can constitute a final stage of the peroxidation of the chain of a polyinsaturated fatty acids of PC or PE, the

relationship existing between peroxidation and the mechenisms described above appears clearly. For instance, more important is the formation of endogenous LPE, more active will be the transfer of the fatty acid from PC to LPE. Therefore, the study of the recyclation of PC and PE makes it possible to estimate the peroxidation of these two phospholipids. This method has been previously applied to the study of different pathological conditions associated to oxidative hemolysis (JACOB & LUX, 1968 ; OLIVEIRA & NASON, 1968 ; LUBIN & SHOHET, 1970 ; WITTELS, 1970 ; MILLER & SMITH, 1976).

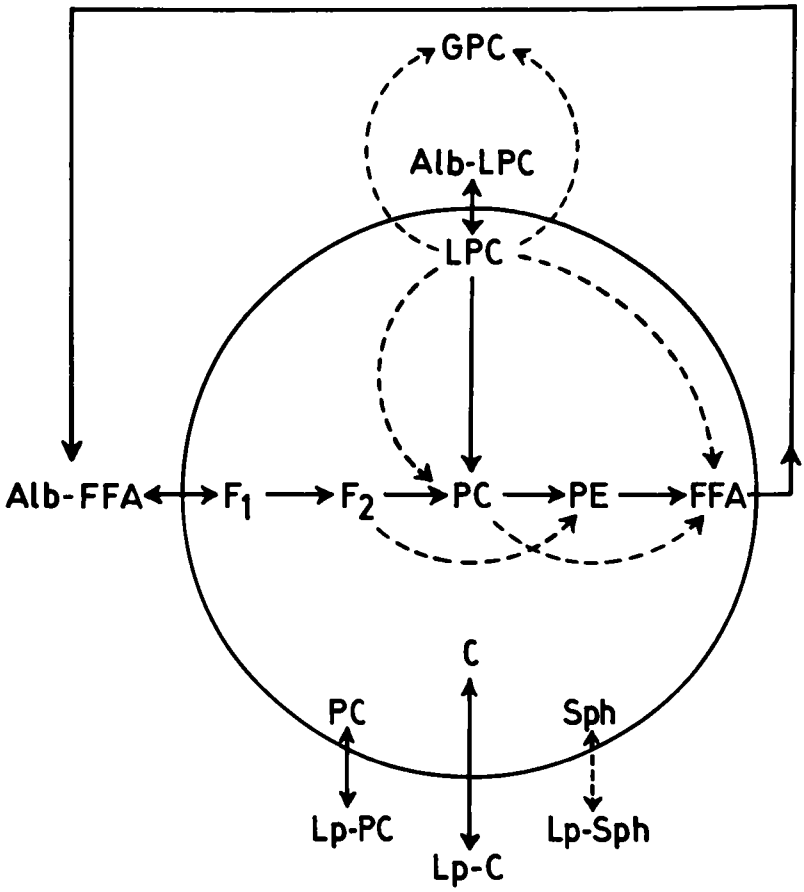

FIG. 21. – Metabolism of the lipids of the erythrocyte membrane. – FFA : free fatty acid ; F1 : "superficial" pool of fatty acids ; F2 : "deep" pool of fatty acids ; C : cholesterol ; PC : phosphatidycholine ; PE : phosphatidylethanolamine ; LPC : lysophophatidylcholine ; Sph : sphingomyelin ; GPC : glycerylphosphorylcholine ; Alb : albumin ; LP : lipoproteins.

Our experimental protocol included two successive incubations. The first was short and consisted essentially of a labelling of the compartments F2 and PC with radioactive linoleic acid.

The red cells were there reincubated in autologous serum for 22 hours and the evolution of the radioactivity as a function of time was measured in the PC and PE pools and in the serum.

The results obtained are included in Figure 22. Starting from the 9th hour, the radioactivity is higher in the PC or PE and lower in the serum of non refed patients than in refed subjects. This finding suggests the existence in patients not yet treated of an increased peroxidation of polyunsaturated fatty acids of PC and PE.

Three factors could influence the significance of the peroxidation of fatty acids in the membrane of red cells : its content in polyunsaturated fatty acids, its content in vitamin E and finally the whole series of intracellular factors regulating the production and reduction of activated oxygen.

The sensitivity of red cells to oxidative injuries is enhanced when the diet is enriched with fatty polyunsaturated acids. However, several arguments suggest that the supply of fatty polyunsaturated acids is low in children suffering from protein-energy malnutrition (BAKER & MAC DONALD, 1961 ; SCHENDEL & HANSEN, 1961 ; MACDONALD *et al.*, 1963 ; HAFIEZ *et al.*, 1971 ; TAYLOR, 1971 ; NAISMITH, 1973) and in particular in Kivu (VIS *et al.*, 1969).

Vitamin E intervenes as protecting agent of membrane peroxidations as it is considered as a constituent of the membrane. According to DIPLOCK & LUCY (1973), physico-chemical interactions seem to exist between the phytyl group of tocopherols and the chains of fatty poly unsaturated acids, in particular the chain of arachidonic acid.

The lowering of the plasma content of vitamin E is a constant finding in kwashiorkor (MAJAJ *et al.*, 1963 ; ASFOUR & FIRZLI, 1965 ; SANDSTEAD *et al.*, 1968 ; VITERI *et al.*, 1968 ; ADAMS, 1969 ; HALSTED *et al.*, 1969 ; KULAPONGS, 1975 ; FONDU *et al.*, 1978a). However, the dosage of vitamin E in erythrocytes – which encounters considerable technical difficulties – has never been made in malnutrition. Some indirect arguments lead us to believe, like HORWITT *et al.* (1969), that there is probably no depletion of the erythrocyte level of tocopherols :

1) there is no evident relationship between the reduction of the plasma content of vitamin E and hemolysis, since the level is the same before and after treatment, while T50Cr is corrected ;

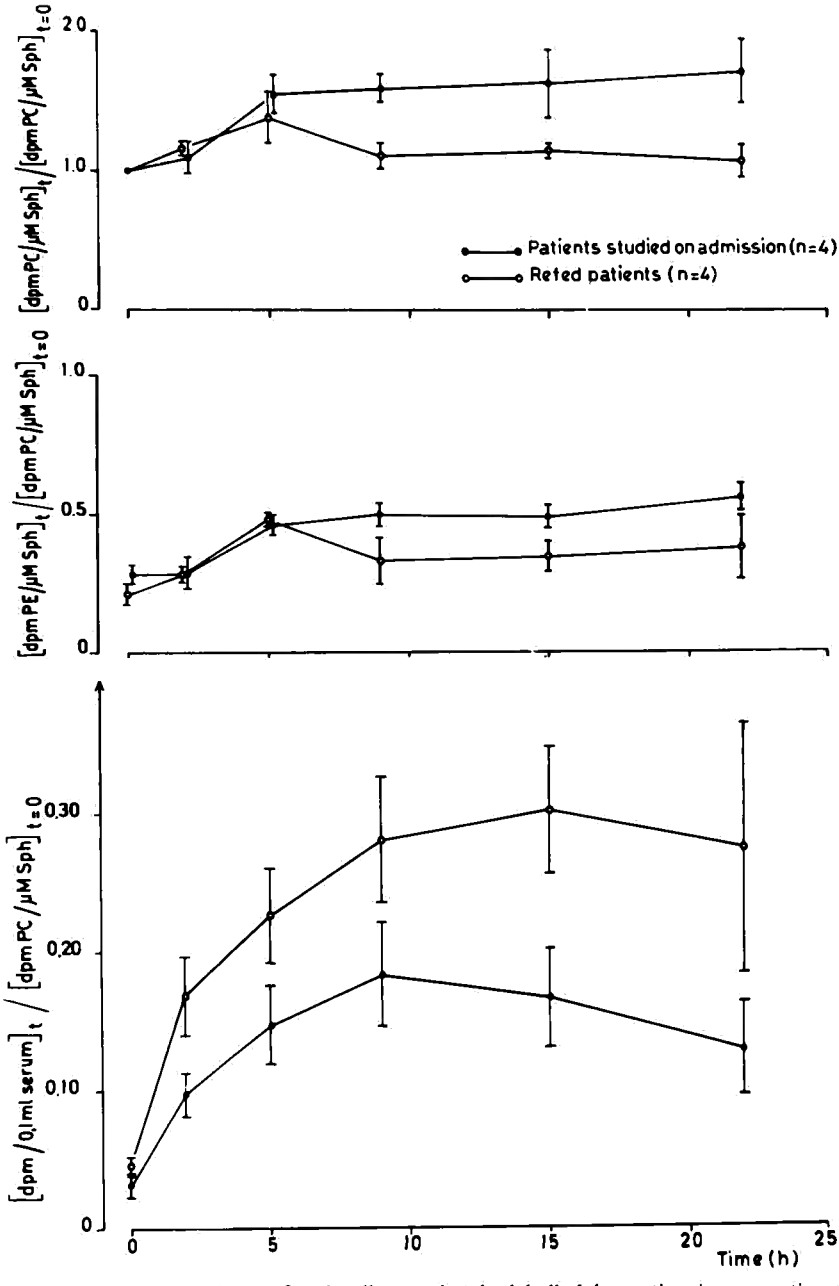

FIG. 22. – Incubations of red cells previously labelled by active incorporation of radioactive linoleic acid. The radioactivity (dpm) was measured in phosphatidylcholine (PC) and phosphadityl-ethanolamine (PE) in the erythrocytes as well as in the serum. The results obtained at time t per micromole (μM) of sphingomyelin (Sph) are plotted against the initial values. – Mean ± SEM of values obtained in two groups of 4patients studied before and after renutrition.

2) there is no significant correlation between the membrane content of PE and the plasma content of vitamin E (Fig. 23). Such a correlation could be found in a selective deficiency in that vitamin (GROSS and MELHORN, 1972) ;

3) the existence of a significant correlation between the plasma levels of tocopherols and cholesterol (Fig. 23) suggests that there is a certain similitude in the transport of these substances, a fact which is supported by several animal experimentations (DAVIES *et al.*, 1971 ; BJORNSON *et al.*, 1975 ; BIERI *et al.*, 1977) ;

4) the hemolytic syndrome attributable to a deficiency in vitamin E generally results from the appearance of a desequilibrium between nutritional supplies having a low content of vitamin E but a high level of polyunsaturated fatty acids (HASSAN *et al.*, 1966 ; BRIN *et al.*, 1974 ; BUNNEL *et al.*, 1975). Nutritional surveys indicate that the probability of such a desequilibrium is very low in marasmic kwashiorkor of Kivu (VIS *et al.*, 1969).

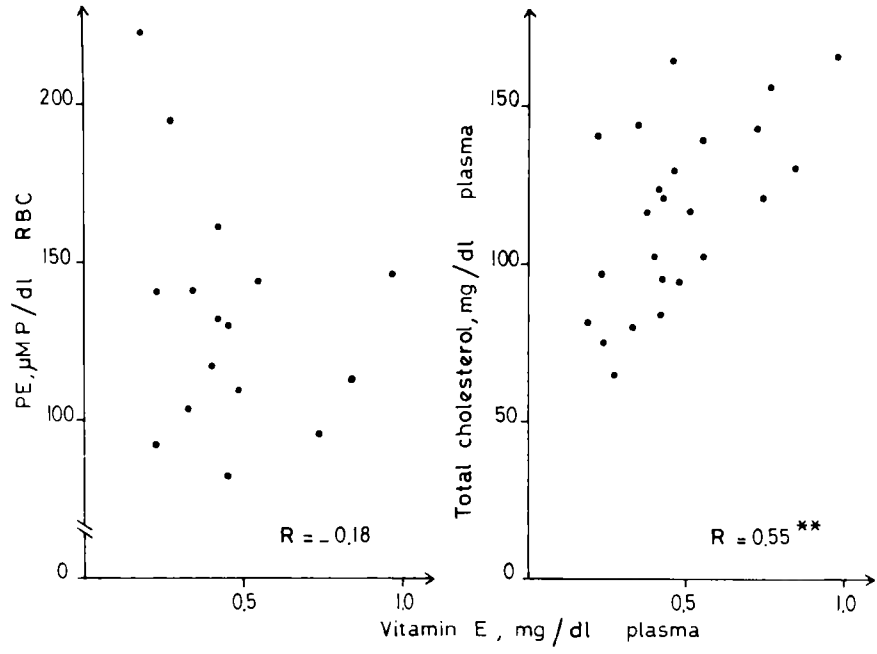

FIG. 23. – Correlations observed on admission between the plasma level of vitamin E and either the erythrocyte content of phosphatidylethanolamine (PE) or the total level of plasma cholesterol (chol). – R : Spearman's correlation coefficients.

The most rational interpretation of the abnormal acylation of membrane phospholipids is to be found, according to us, in what appears in paragraph 7.3, where it is stated that the reduction of the activity of GSH-Px and SOD increases the risk of deterioration of several constituents of erythrocytes.

In particular, an oxidative attack can lead to a destruction of polyunsaturated fatty acids, which are then replaced by saturated acids. At that stage, the rigidity and permeability of the membrane would show alterations leading to the destruction of red cells (SHOHET, 1972 ; RAZ & LIVNE, 1973). The simultaneous formation of Heinz bodies in contact with the membrane probably contributes to the rapid elimination of red cells. The oldest cells, with their reduced metabolic activities, could be the most exposed to such damages.

In marasmic kwashiorkor, the fatty acids of PC and PE are exposed to peroxidation. The situation differs from that described in the deficiency in vitamin E observed in membranes, in which PE is preferentially destroyed. Two considerations facilitate the understanding of the mechanism of PC peroxidation in marasmic kwashiorkor :

1) the increase of the PC content per volume unit of red cells could make PC more sensitive to peroxidation (RACHMILEWITZ *et al.*, 1976).

2) in patients receiving normal or high supplies of polyunsaturated fatty acids, PE is richer than PC in polyunsaturated acids and plasmalogens (DODGE and PHILIPS, 1967) ; therefore PE is more sensitive to oxidative attacks. Because of the depletion of the nutritional intakes of unsaturated acids, one can suppose that the content in fatty acids differ to a lesser extent in patients suffering from marasmic kwashiorkor.

We may therefore conclude that the recurrence of oxidative attacks increases the probability of destruction of red cells which, in marasmic kwashiorkor of Kivu, are insufficiently equiped to enable the reduction of activated oxygen.

In marasmic kwashiorkor, the administration of oxidant drugs is therefore as dangerous as in congenital anomalies of the pathway of pentose-phosphates (BEUTLER, 1971), in renal insufficiency (YAWATA *et al.*, 1973 ; ROSENMUND *et al.*, 1975), in acute hepatic diseases (SMITH *et al.*, 1975) and in the late anemia of prematurity (GROSS, 1976). Iron prescribed at high doses is among these dangerous drugs (MELHORN & GROSS, 1971). It is therefore reasonable to refrain from applying an iron treatment during the first days of treatment of marasmic kwashiorkor.

The prescription of vitamin E, considered as antioxidant agent, cannot fully correct that situation : located exclusively within the membrane, tocopherols cannot hamper the formation of Heinz bodies (ROTRUCK *et al.*, 1972).

Therefore, the therapeutic interest of vitamin E in marasmic kwashiorkor, which has been the subject of many controversies (MAJAJ *et al.*, 1963 ; MAJAJ, 1966 ; WHITAKER *et al.*, 1967 ; BAKER *et al.*, 1968 ; HALSTEAD *et al.*, 1969 ; KULAPONGS, 1975) seems to us of limited interest.

Summary and General Conclusions

The interpretation of anemia associated to syndromes of protein-energy malnutrition is usually based on the extrapolation to the child of data obtained from animal experiments. Submitted to a limitation of energy supplies, the animal shows no reduction in the life span of its red cells or in the marrow responsiveness to erythroprotein. The organism does not require the intervention of the compensatory mechanisms which intervene in conditions of hypoxia. It seems that the reduction of the red cell volume is an adaptation to a restriction of metabolic activities. The extrapolation to malnourished children is an assumption which has never been supported by convincing demonstrations. In particular, the studies carried out in Kivu do not allow us to share such an interpretation.

At the completion of the studies made in Kivu, it would also be wrong to assume that our conclusions can be directly extrapolated to all types of protein-energy malnutrition syndromes in children. The hematological aspects of malnutrition result from the evolution of a specific deficiency, appearing in a population with precise genetic characteristics and living in a given environment.

There is only one rational method to approach the study of these various hematological syndromes. It consists in an attempt to define the mechanisms at stake without assuming *a priori* the existence of an analogy with a specific model. In these studies, it is important to compare the results obtained in the patients with those obtained in local well defined controls. The investigations carried out in Kivu clearly demonstrate the importance of global studies covering simultaneously the morphological, kinetic and biochemical aspects of anemia resulting from malnutrition.

It is only at the cost of such an endeavour that one can hope to reach a coherent understanding of the hematological picture which accompanies malnutrition in a given region. So will it eventually become possible in the future to define the hematological charateristics common to all the syndromes of protein-energy malnutrition.

The following conclusions can be drawn from the results obtained in the Kivu area.

1) *Anemia seems to settle slowly during the evolution of marasmic kwashiorkor*

This concept is not based on a longitudinal study of the red cell volume, which could be extremely difficult to make in a developing region. It is based on the following observations :

a) isotopic studies indicate that, on admission, red cells production is almost equal to red cells destruction ;

b) the increase in the concentration of various enzymes (hexokinase, 6-phosphogluconate dehydrogenase, glutathione-reductase, Na^+-K^+-adenosine triphosphatase) or erythrocyte substrates (glucose-6-phosphate), as well as the modification of the level of erythrocyte cations (reduced Na^+, increased K^+) suggest that the median age of the erythrocyte population is diminished.

2) *The severity of the anemia is a function of the severity of malnutrition*

Such a conclusion is based on the observation of several significant correlations, in particular between red cell volume and plasma albumin level. It does not mean however that the deficiency in essential amino-acids is the single factor responsible for the appearance of anemia. Other nutrients associated to nutritional proteins can be absent in undernourished patients.

3) *The peripheral blood examination underestimates the severity of the anemia*

The blood volume observed on admission is indeed inferior to the volume measured in children of the same height or weight, free from malnutrition.

4) *The anemia cannot be explained by the existence of deficiencies in iron or vitamins*

The level of iron in the serum is reduced but the percentage of saturation of siderophilin is increased. Cytochemical and ferrokinetic studies indicate that erythropoiesis is not hampered by an insufficient marrow iron supply. The plasma contents of vitamin B12 and C are increased, while the plasma and erythrocyte contents of folates are normal. The reduction of the plasma content of vitamin E probably results from a redistribution of tocopherols following alterations of the metabolism of plasma lipoproteins. Finally, the study of the activity coefficient of glutathione-reductase does not suggest the existence of a deficiency in riboflavin on admission.

5) *The anemia of marasmic kwashiorkor constitutes an autonomous hematological syndrome*

Its characteristics are not only distinct from those observed in iron or vitamin deficiencies ; they also differ from those observed in the anemia of chronic disorders, since erythropoietic output is adequatly increased and since the marrow iron supply is not limitating – and from the hypothetical "adaptive anemia" – since the plasma and urine contents of erythropoietin are high. The paradoxical normality of the level of 2,3-diphosphoglycerate observed in that syndrome cannot be explained by the absence of hypoxia but by a disordered erythrocyte metabolism, the mechanism of which cannot be fully specified in the present state of our knowledge.

6) *The anemia results both from a reduction of the marrow responsiveness to erythropoietin and from a shortening of the life-span of red cells*

In spite of an intensive erythropoietic stimulation, red cell output, measured with the radioactive iron method, does not exceed normal values. The life span of red cells is difficult to measure in underfed patients ; the patients are indeed treated from the moment of their admission and are not in a steady state. However, specific investigations (tests in isotransfusion, repeated determinations of the red cell volume) enable us to confirm the existence of a moderate hemolysis linked to one (or several) abnormality(-ies) of red cells.

7) *Of the two major alterations of erythroid cells – (decreased responsiveness to erythropoietin and reduced erythrocyte survival) – only the second lends itself to a detailed study.*

8) *Some erythrocyte abnormalities – in particular the accumulation of cholesterol and phosphatidylcholine – result from biochemical changes in the plasma ; they have no evident impact on red cell survival.*

Changes in the pattern of lipoproteins and in particular of lipoproteins of low density lead to a redistribution of non-esterified cholesterol and phosphatidylcholine. The lipid build-up in the membrane explains the existence of perturbations such as the presence of target-cells (optical microscopy) or cup-cells (scanning electronic microscopy), as well as the increase of osmotic resistance. However, these abnormalities cannot lead to a diminished erythrocyte filtrability nor to an altered permeability of the membrane.

9) *Oxidative hemolysis is the major cause of the premature destruction of red cells.*

The reduction of the concentration of glutathione peroxidase and superoxide-dismutase is accompanied by a reduction of the resistance of

red cells to oxidative injuries. In certain experimental conditions, one can observe that the oxidative deterioration of hemoglobin is accelerated. Moreover, it is possible to demonstrate that the increased peroxidation of fatty acids in the membrane leads to an increase of their turnover within phosphatidylcholine and phosphatidylethanolamine.

10) *The study of erythrocyte enzymes containing trace-elements suggests the existence of a deficiency in these elements.*

The reduction of the activity of glutathione peroxidase suggests the existence of a deficiency in selenium ; the reduction in the activity of superoxide dismutase indicates a deficiency in copper and/or zinc. The results of dosages of selenium in the plasma and erythrocytes of patients are compatible with such an interpretation.

Deficiencies in trace-elements, although neglected in experimental models of protein-energy malnutrition, can lead to severe metabolic disturbances in malnourished children.

11) *The period of refeeding is characterized by a more rapid normalization of the blood volume than of the red cell volume*

Therefore, the hemoglobin level diminishes at the beginning of refeeding and reaches its lowest value approximately after two weeks.

The beginning of the period of refeeding is also characterized by a rapid increase of erythropoiesis due to several factors. The increase in the metabolism and the reduction of the hemoglobin level resulting from the expension of the plasma volume tend to increase the output of erythropoietin. Moreover, refeeding probably improves the marrow response to erythropoietin.

12) *Due to the intense anabolism which characterizes the period of refeeding, some relative deficiencies can appear at that time. The most important is iron deficiency.*

The study of glutathione reductase in erythrocytes points to the settlement of a riboflavin deficiency. We are however able to state that it is the progressive appearance of an iron deficiency which delays to all evidence hematological recovery. In fact, the addition of a parenteral iron treatment accelerates the normalization of the red cell volume. Taking into account the existence of an oxidative hemolysis, it is however preferable not to start that treatment from the beginning of refeeding.

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