A REGIONAL BASIC DIET FROM NORTHEAST BRAZIL AS A DIETARY MODEL OF EXPERIMENTAL MALNUTRITION[1]

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SUMMARY

The nutritional potentiality of the "Regional Basic Diet" (RBD) was assessed in albino rats. The diet, which was prepared according to data from food consumption surveys and was similar —in terms of quality and quantity— to those consumed by human populations in Northeast Brazil, was compared to a balanced diet (22% casein). The centesimal composition, aminogram, minerals content, NDpCal% and GCal%/PCal% ratio were determined. Vitamin contents were calculated from data in the literature. The RBD was shown to be imbalanced and poor in certain nutrients, mainly protein (7.8%). The growth curve of the RBD group was severely impaired; the naturally occurring sex-related differences in body weight were not detected. For fetuses and suckling pups, the weights of the liver and diaphragm paralleled body weight; brain weights were about 20% lower than those of controls, and its ratio was higher. RBD pregnant females presented, despite their higher food and energy intakes, lower weight gain as compared to the controls. During lactation food and energy intakes of RBD dams were lower than those of controls. The mortality rate among RBD pups was 24%. Our data indicate that RBD produces in the rat a type of malnutrition similar to that prevalent among children from Northeast Brazil, namely an association to nutritional dwarfism with some clinical signs of marasmus.

RBD is considered to be a useful experimental model for studies on human malnutrition in countries where basic food patterns are similar to those in RBD-consuming regions.

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INTRODUCTION

The nutritive value of regional foodstuffs as a source of a specific nutrient has been investigated in diets balanced in the other nutrients (1-4). On the other hand, the relative amounts of the foods as they appear in human diets have been experimentally modified (5).

The relationship between the consumption of a diet used by a population group and the prevailing type of malnutrition has not been sufficiently exploited. Protein-energy malnutrition (PEM), however, tends to present some different biochemical and functional manifestation from one region to another, where dietary patterns are varying (6, 7).

The use of experimental diets, similar in quality and quantity to those consumed by man, is attractive for assessing the effects of such diets on the nutritional status. This approach could be useful not only to minimize difficulties when the extrapolation of laboratory findings to humans is desired, but also to provide invaluable data for intervention programs to combat malnutrition, one of the most serious public health problems in underdeveloped and developing countries, including Brazil.

In some areas of Northeast Brazil, cumulative data show that more than 20% of the children suffer from 2nd and 3rd degree malnutrition (Gómez *et al.* classification) (8). The mortality rate in the first year of life, basically caused by malnutrition, was shown to increase progressively between 1972 (3.8%) and 1980 (4.8%). After a slight decrease in 1982 (3.8%), this rate increased again in 1984 (5.6%) (9).

The Brazilian economic system can be accounted for this situation; incomes are unevenly distributed, and very large segments of the population live under marginal conditions, particularly in Northeast Brazil. In Recife, for example, investigation (10) revealed that in May 1987, 80.8% of the laborer's salary (living wage) was spent for acquisition of the 12 food items included at the "family food basket". Furthermore, according to SIBAN VII th. (11), the less privileged groups have a low purchasing power and are not able to buy these 12 products.

The development of experimental malnutrition models will make possible a better knowledge of many biological aspects of human malnutrition prevailing in the northeastern population.

The present work describes such a dietary model and its effects on pregnant and lactating rats, as well as on the development and growth of their offspring.

MATERIAL AND METHODS

Animals

A total of 645 Sprague-Dawley rats from the Colony of our Department were used.

Diets

Experimental — The experimental diet, named Regional Basic Diet

(RBD), was prepared according to data from food consumption surveys (12-18) in 918 families: 5,939 persons from one of the three physiographic zones of Pernambuco State —the South "Mata" area, performed by the Laboratório de Nutrição em Saúde Pública of our Department, using the dietary recall method.

Taking into account their highest consumption and frequency, their lowest cost, availability in the market and use in the main daily meal, four foods were selected: beans *(Phaseolus vulgaris)*, manioc flour *(Manioc esculenta)*, dried and salted meat, and sweet potato *(Ipomaea batatas)*.

The foods were bought at the Central de Abastecimiento de Pernambuco (CEASA).

They were home-made, cooked, oven-dried (60-70°C) and ground, except manioc flour.

The four foods were used in RBD in the same proportions as detected by the surveys (Table 1). The mineral contents of the RBD were determined by the "Laboratório de Análises Minerais do Departamento de Recursos Humanos da Superintendência do Desenvolvimento do Nordestge (SUDENE)", according to Turman (19), Sandel (20), Snell and Snell (21) and Perkin-Elmer (22). Vitamins were calculated from the literature data (23).

TABLE 1

MEAN *per capita* CONSUMPTION (g/d) OF THE FOODS SELECTED FROM THE SURVEYS AND THEIR CORRESPONDING VALUES, IN THE REGIONAL BASIC DIET (RBD)

Foods	Mean *per capita* consumption (g/d)	RBD: Foods after moisture reduction (g %)
Beans 37.1	18.34	
Manioc flour*	67.4	65.16
Dried and salted meat	13.9	3.74
Sweet potato	32.0	12.76

* Without moisture reduction; it was used to make up 100 g RBD.

The centesimal composition of the foods, as well as that of the RBD and control diet, were determined by current techniques used in the "Laboratório de Experimentação e Análise de Alimentos" of our Department.

The amino acid contents of RBD, as well as those of the control diet, were established by the "Centro Interdepartamental de Química das Proteínas" from the "São Paulo" University, according to Spackman *et al.* (24); tryptophan was determined in alkaline hydrolysate, according to the Lucas and Sotelo method (25).

Control —The control diet (C) provided 22% casein and was balanced (26) (Table 2).

The two diets were pelletized.

TABLE 2

CENTESIMAL COMPOSITION OF DIETS

Diets	Ingredientes	g %	Centesimal Composition							
			Proteins	Carbohy-drates	Fats	Ash[a]	Fibers	Water-soluble vitamins	Fat-soluble vitamins	kcal%
RBD	Beans[b]	18.34	3.99	10.66	0.24	0.57	1.09			60.76
	Manioc flour	64.81	0.84	48.59	0.12	0.43	5.64			198.80
	Poor fat-dried and salted meat[b]	3.74	2.74	—	0.06	0.06	—			11.50
	Dried and salted meat fat	0.35			0.35					3.15
	Sweet potato[b]	12.76	0.30	9.99	0.03	0.20	0.48			41.43
	Total	100.00	7.87	69.24	0.80	1.26	7.21			315.64
Control	Comercial casein[c]	27.08	22.00							88.00
	Biscomil[d]	57.42	0.58	50.44	0.12	0.09				205.16
	Vegetable fat	7.50			7.50					67.50
	Water-soluble vitamin mixture[a]	1.00						1.00		
	Fat-soluble vitamin mixture[a]	1.00			1.00				1.00	9.00
	Salt mixture[a]	4.00				4.00				
	Filter paper	2.00					2.00			
	Total	100.00	22.58	50.44	8.62	4.09	2.00	1.00	1.00	369.66

a = According to the Nutritional Biochemical Corporation (23); b = Cooked and dried; c = 81.24% of proteins (Laboratório de Experimentação e Análise de Alimentos, Department of Nutrition, Federal University of Pernambuco, Brazil); d = Corn starch from Purinas Alimentos do Brasil.

Pregnancy and Lactation

After fertilization, the ability of females to develop successful pregnancy and lactation was determined in 55 RBD and 37 control dams. The body weight and food intake of the dams, as well as the number and body weight of the newborn and suckling pups, were determined. Furthermore, the general appearance and locomotor activity of the pups were observed every day.

Growth

The body weights of 553 animals (323 RBD and 230 controls) were determined at the following developmental stages: 157 fetuses at the 19th and 20th days; 182 suckling rats at 0, 4, 17 and 21 days of age; 214 rats at 38, 42, 63, 91, 119, 147, 182, 240 and 365 days of age. The weights of the brain, liver and diaphragm were determined in fetuses and suckling animals.

Procedure

Fertilization was detected by the presence of sperm in the vaginal washing of the mated females.

The fertilized dams were removed to individual cages and their respective diets were provided *ad libitum*.

The litter size was limited to eight pups. At weaning (21 days) the animals were transfered to individual cages, and the same diet of their mothers was provided *ad libitum*.

In order to weigh the organs, fetuses were removed by cesarean section, under ether anesthesia (Rhodia), starting from the free extremity of the uterus horn, so that blood supply disturbance to the other fetuses was minimized. Temperature was maintained at 38 ± 1.0°C by means of warm physiological saline.

Statistical differences between the dietary groups were assessed using the "t" Student's test, assuming 5% as the limit to reject the null hypothesis.

RESULTS

The energy adequacy of RBD (Table 2), when compared to the control diet, is 91%, but only 11% of that value come from fats (GCal% 2.5, versus 21.0 in the control diet); 31% of the total mineral salts and 35% of proteins (2.9 times less than in the control diet); carbohydrates and fiber contents are high (138 and 360%, respectively).

The mineral elements are not adequately distributed to meet the rat requirements (26). K, P, S, and Ca contents are below the requirements, while Na, Cl and Mg are above them. The trace elements are above the requirements, especialy Zn and Al; furthermore, Si Ni, Sr and Cr are present in minimal amounts.

The ascorbic acid, retinol, biotin, thiamin, riboblavin, niacin, paraaminobenzoic acid, pyridoxine, inositol, cyanocobalamin and choline levels are extremely low.

The RBD is poor in protein in terms of quantity (7.87%) and quality (96% provided by beans + roots and 4% by dried and salted meat). Its PCal% and NDpCal% are, respectively, 9.4 and 5.64.

Figure 1 shows the amino acid contents of the control and RBD diets, expressed in mg/100g of diet. The essential (E) and non-essential (NE) amino acid values in RBD representent, respectively, 25% and 24% of the control ones. Among the E amino acids, the Met level was the lowest (17% of the control) and Arg level was the highest (43% of the control). The remainder varied from 20% to 28%. Among the NE amino acids, proline had the lowest level (10%) and Cy-S. Cy had the highest value (83%).

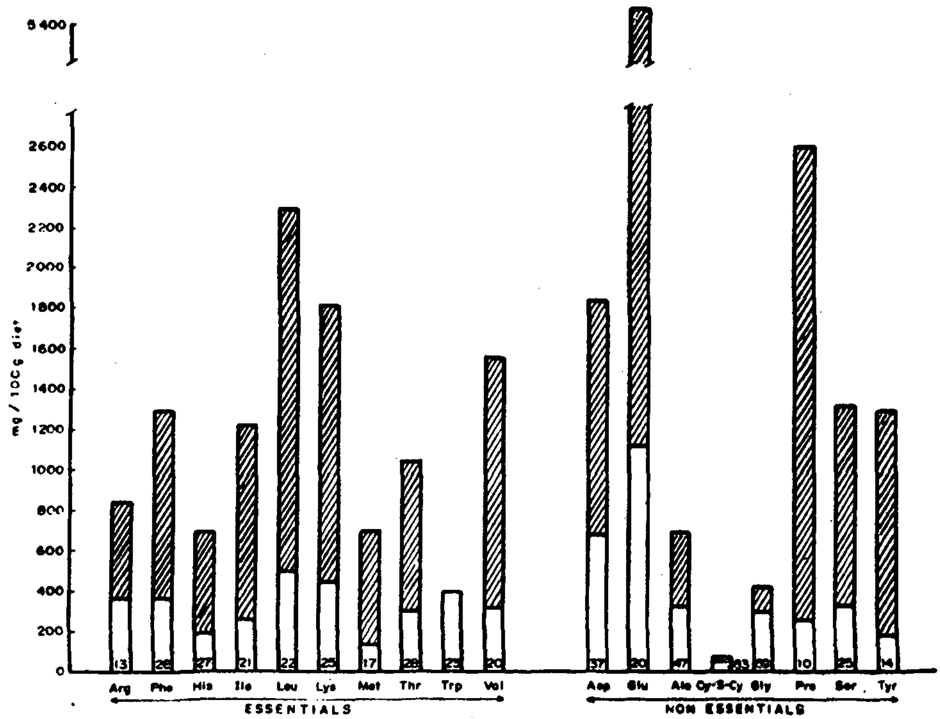

FIGURE 1

Amino acid contents of the RBD (7.87% proteins) and of the control diet (C) (22.58 g% casein), ☐ = RBD, ▨ = Control Diet, number in columns = RBD% of the C.

Taking the RBD amino acids in groups according to some metabolic functions, it was noted that the branched-chain amino acid contents (Val, Leu and Ile) corresponded to 21% of their values in the control diet; the sulfur amino acids, to 20%; Tyr and Phe, precursors for the synthesis of neurotransmitters or hormones, range from 17% to 28%, respectively. The Val/Gly ratio is 0.30 versus 3.6 in the control diet. Lys and Thr contents correspond to 25

and 28% respectively, of the control values.
The energy density (27) of the RBD is too low. The GCal%/PCal% ratio is 0.27, that is, 32% of the control one.

Effects upon Growth

The weights of the body, liver, diaphragm and brain of RBD rats were significantly different from those of controls. Concerning body weight, RBD fetuses were heavier at day 19 and lighter at day 20. This latter difference increases from birth until 365 days of age. The naturally occurring sex-related differences in body weight were not detected when the growth curve of the RBD males was compared with that of RBD females (Figure 2). The growth curve is altered, so that it is very difficult to identify both the start point to the catch-up and the point where the decrement of growth usually becomes evident.

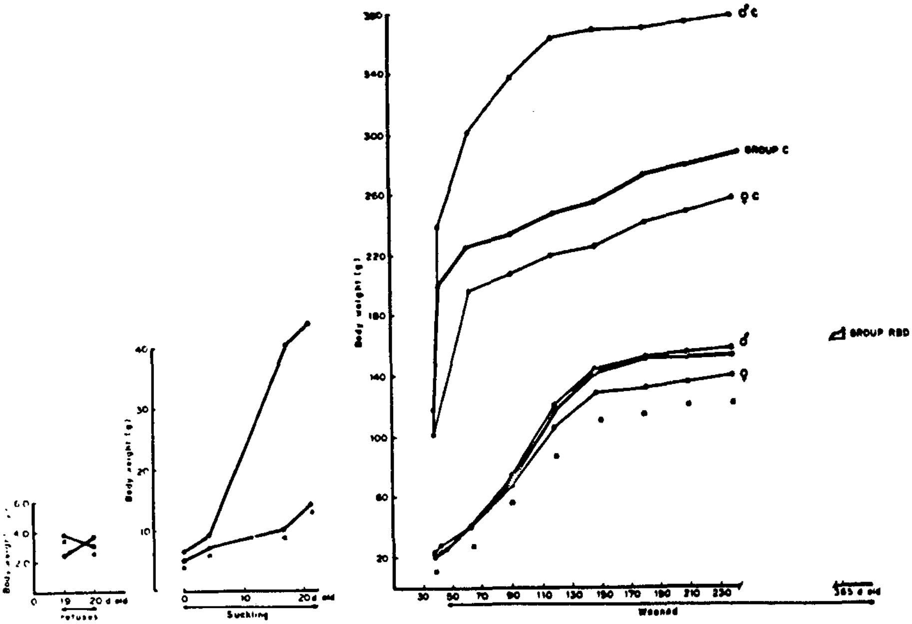

FIGURE 2

Body weight (g) of rats *in utero* (104 RBD and 53 C), during breast feeding (105 RBD and 77 C) and after weaning (114 RBD and 100 C), O = RBD, O = C, — = group, ♂ or ♀ , * = Significant difference (Student's "t" test) between RBD and Control (Group, ♂, ♀).

Also, as shown in Figure 3, the weights of the three organs were altered in the RBD rats. The RBD brain weighed about 80% of the controls during the suckling period. However, the liver and the diaphragm had 71% and 79%, respectively, of the control weights in the first postnatal week, in parallel to the body weight, which corresponded to 74-81% of the controls. This parallelism was maintained until the last week of suckling, with RBD liver and diaphragm weights corresponding to 20-29%, while the body weight was 25-32% of the control rats.

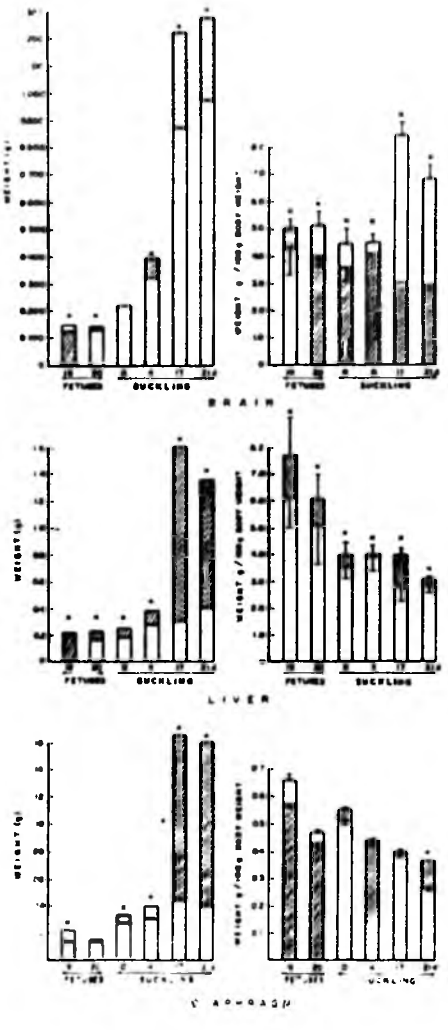

FIGURE 3

Organ weight (g) and organ weight/100 g body weight ratio of the brain, liver and diaphragm of fetuses and suckling rats. □ = RBD, ▨ = Control, * = significant difference (Student's "t" test) between RBD and c,⊤= SD.

When the weight of the organs was expressed per 100 g of body weight, the RBD brain presented higher ratios than the controls during all the period studied, except at the 19th day of gestation; the peak was observed at postnatal day 17. For the liver, this ratio was significantly lower than in the control, except at postnatal day 4, while for the diaphragm the ratio did not differ from the controls, except at the postnatal day 21, when the ratio was lower.

Effects upon Pregnancy and Lactation

Despite the fact that the food and energy intakes of the RBD pregnant rats were higher than those of controls (28% and 15% respectively), the weight gain of the former was lower than that of the control (Table 3).

No pups died in the control group, while the mortality rate among RBD pups was 24%. The causes of this mortality were either cannibalism by the mother, or a failure in the production of milk as checked by pressing the mammae.

During lactation, food and energy intakes of RBD dams were lower (59% and 63% of the controls, respectively) and the loss of body weight was 85% higher than that of the controls.

In RBD suckling rats, a disproportion between the sizes of the head and the rest of the body was evident, the former appearing excessively big; hairs were bristly, sparse and without the natural shining seen in the controls; their back was usually arched, and the tail presented stiffness (Figure 4); their locomotor activity was very high.

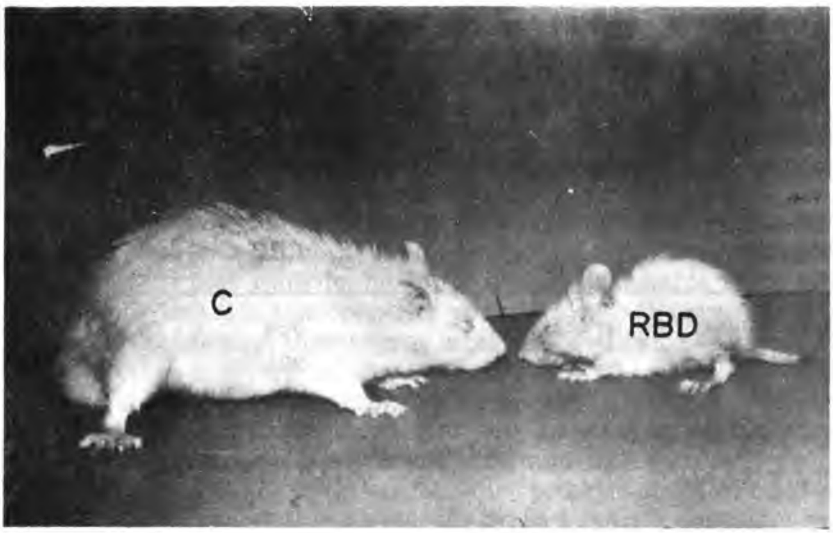

FIGURE 4

Rats 120 days old. C = Control;
RBD = Regional basic diet

TABLE 3

EFFECTS OF THE REGIONAL BASIC DIET (RBD) UPON SOME PARAMETERS OF PREGNANCY AND LACTATION IN FEMALE RATS COMPARED WITH CONTROL ONES

Pregnant	RBD	Control
Number of fertilized	55	37
Number of pregnancies	48	34
Daily food intake ($\bar{x}$ ± SD)	19.3 ± 2.8*	15.1 ± 2.4
Daily protein intake ($\bar{x}$ ± SD)	1.5 ± 0.2*	3.4 ± 0.6
Daily calorie intake ($\bar{x}$ ± SD)	64.6 ± 9.3*	56.0 ± 8.9
Body weight change during pregnancy	94.9 ± 21.0*	119.7 ± 16.1
Newborns	**RBD**	**Control**
Alive	435	288
Number of pups per litter ($\bar{x}$)	11.2	9.6
Body weight at birth g ($\bar{x}$ ± SD)	5.0 ± 0.9*	6.2 ± 0.7
Stillborn	2	—
Lactatings	**RBD**	**Control**
Body weight change, g ($\bar{x}$ ± SD)	-69.4 ± 18.7*	-37.5 ± 5.4
Daily food intake, ($\bar{x}$ ± SD)	12.6 ± 0.6*	29.4 ± 2.9
Daily protein intake, ($\bar{x}$ ± SD)	0.9 ± 0.0*	6.6 ± 0.7
Daily calorie intake, kcl ($\bar{x}$ ± SD)	40.0 ± 2.1	108.9 ± 10.8
Sucklings	**RBD**	**Control**
Standard number by litter (8)	384	272
Number at weaning	291	272
Average body weight, at weaning, g ($\bar{x}$ ± SD)	14.2 ± 1.3	44.2 ± 1.0
Number of defections	93	—

* = P < 0.001 ("t" Student's test).

DISCUSSION

Our data show that both the development and the growth processes were affected by the RBD, as assessed by the weight of the organs and by the growth curve of the animals under study.

Distortions were seen in the growth curve. The increment of body mass slowed down with ageing. The naturally occurring sex-related differences in body weight were not observed in adult animals. The same results have been reported by Stewart and Sheppard (28), immediately after weaning, in malnourished rats fed a diet with a NDpCal% very similar to ours (5.0 and 5.64, respectively). Furthermore, there was no remarkable point of acceleration or deceleration of growth rate.

Changes in body weight observed between fetuses aged 19 and 20 days, as well as at the day of birth (see Results), could be due to a substantial loss of water immediately before birth, since it is known that the undernourished newborn rat loses more water and has less dry matter than the controls. According to Portela *et al.* (29), rats fed an imbalanced diet present a higher body water content when compared to rats fed a low-protein diet balanced in all the other nutrients, and to that of the control ones. It is worthy noticing that RBD is imbalanced and has a low protein content.

Regarding birth weight, the present results do not agree with those from Resnick and Morgane (30) nor Turner (31), who did not detect any difference between control and malnourished pups. These authors induced malnutrition by feeding the dams with an 8% casein diet balanced in all other nutrients. Nevertheless, also Stewart and Sheppard (28), using a similar diet but with only 4% casein, found a significantly low birth weight.

The low birth weight found in our experiment cannot be attributed to an energy deficit, since the energy intake of RBD dams was higher than that of the controls (Table 3). Therefore, care must be taken by the nutrition intervention programs to prevent human low birth weight only by correcting the dietary energy deficits of the mothers.

During the lactation period, the RBD mothers showed a higher decrease in body weight than the control ones. This is in agreement with data notified by Widdowson and Cown (32) in lactating rats fed an 8% protein diet.

On the other hand, the deficiency in milk production does not seem to be due to histological changes in the mammary gland, since the glands of dams fed a low-protein diet were found to be undistinguishable from those of controls (31). It is, thus, reasonable to postulate that the inability of RBD lactating dams to increase their energy intake, as well as inadequate levels of protein and other nutrients of the diet, are involved in the deficient milk production observed in our experiment.

The present data on the organ weights indicate, as observed by Castellano and Olivero (33), that while the liver and the diaphragm parallel the progressive loss of body weight, the brain weight deficit remains about 20% during the breast-feeding period. For this reason, the brain weight/body weight ratio in the RBD rats is higher than in the controls during the suckling period, the peak being attained at the 17th day of age. Accordingly, Forbes *et al.* (34) observed that the brain-to-body-ratio is highest at the 16th day of age in malnourished rats fed an 8% casein diet.

This increased ratio is attributed to a "sparing" mechanism to protect the brain against the nutritional insult (30, 34, 35) differently from the body. Despite of this fact, some lasting irreversible sequelae of malnutrition are usual findings in the CNS (36). Thus, this brain "sparing" mechanism needs further investigation. It is important to keep in mind the possible participation of the different changes in water distribution in both the brain and the rest of the body (34-37). The fact that the chronograms of the brain and body are not the same and the heterogeneity of the brain regarding the ability of its different areas to use the nutrients, may also play a role on the effects observed in RBD rats. Furthermore, the effect of imbalanced diets upon the brain substrata (35, 36, 38) producing even impairment of many functions, including those that regulate hunger, appetite, behavior and so on, has to be taken into account (38).

The picture of the RBD induced malnutrition is not surprising, since the nutritional potentiality of the diet is too low. Thus, its nutritional deficiencies must be investigated through the contribution of each nutrient to its imbalance (4).

Analysis of the protein-energy potentialities of the four foodstuffs —just as they are consumed by the population (Table 1)— shows that RBD is nutritionally inadequate.

Our data indicate that RBD produces, in the rat, a type of malnutrition similar to that prevalent among children from Northeast Brazil: impairment of growth, as in the nutritional dwarfism associated to other clinical signs of marasmus.

Identical basic food patterns have been found in 38 countries of the Near and, the Middle East (39), Central America (4, 5) and Peru (3), where some clinical signs similar to those seen in RBD induced malnutrition have been described.

Our findings lead to the conclusion that an experimental model like this can provide valuable information for a better knowledge of malnutrition in countries where nutritional and socioeconomic conditions are similar to ours.

RESUMEN

UNA DIETA BASICA REGIONAL DEL NORDESTE DEL BRASIL COMO MODELO DIETARIO DE MALNUTRICION EXPERIMENTAL

Se evaluó, en ratas albino, la potencialidad nutricional de una dieta (Dieta Básica Regional - DBR) constituida, en términos de calidad y cantidad por cuatro alimentos que, encuestas dietéticas, revelaron ser preferenciales en el Nordeste del Brasil. Se comparó a una dieta balanceada, con 22% de caseína. En la DBR se determinó la composición centesimal, el aminograma y los elementos minerales, el NDpCal%, y la relación GCal% estimándose las vitaminas a partir de datos en la literatura. Se comprobó que la DBR era muy pobre, no era una dieta balanceada; además era deficiente en ciertos nutrientes, sobre todo en proteínas (7.8%). La curva ponderal de los animales que recibían la dieta fue severamente afectada; la diferencia entre dicha curva y la de los controles se acentuó con la edad, sin que se detectaran diferencias entre sexos. En los fetos y las ratas lactantes, el peso del hígado y el del diafragma eran muy bajos y paralelos al peso corporal y del encéfalo, que pesó un poco menos que el de los controles, pero con peso relativo (20%) mayor. Las hembras con DBR preñadas,

acusaron menos aumento de peso, aunque su ingestión fue mayor que la de las controles. Durante la lactancia, tanto el aumento de peso como la ingestión fueron menores que en las ratas control. La defunción entre los lactantes fue muy elevada. Los resultados configuran un cuadro de desnutrición propio de la asociación del enanismo nutricional con el marasmo, asemejándose al tipo de desnutrición prevalente en la Región.

Se considera así que con la DBR se dispone de un modelo dietético adecuado para profundizar el estudio experimental de la desnutrición. Su diseño experimental puede ser aplicado en países cuyos alimentos básicos sean comunes a los de la DBR, predominantemente vegetal, y de condiciones socioeconómicas de consumo similares a las de regiones donde dicha dieta es la habitual.

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