TRABAJOS DE INVESTIGACION

The Recovery of Rats from Protein Deficiency by feeding Proteins from different Sources

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SUMMARY

The study deals with the effect of various proteins (egg-white, casein, opaque-2 corn, common corn and gelatin) on rats in both normal and protein deficiency states.

Opaque-2 corn is as efficient a protein source as casein or egg-white. Within a 15 day recovery period from a deficiency state, none of the proteins caused the recovery to normal levels of all the parameters studied (plasma proteins, hepatic fat, weight of animals); opaque-2 corn was the most efficient in the recovery of normal body weight, plasma proteins, albumin and β-globulin. For the recovery of liver fat levels, egg-white, casein and gelatin were the most efficient.

The authors conclude that for recovery within a short period of time, although normal growth is attained, a single protein diet is not sufficient and an association of proteins is suggested to accelerate the process.

INTRODUCTION

This paper describes part of a program to study the effect of various proteins on the development of animals, the prophylactic effect against future protein depletion and the effect on protein recovery from a deficiency state. This information

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is of fundamental importance since it would contribute to the knowledge of how to proceed in cases of protein deficiency in humans, especially in children. For the world, in general, and particularly in various areas of Brasil, the incidence of human protein deficiency is high because of the inadequate intake of proteins that results from the wrong choice of proteins for human consumption (1).

The results presented refer to experiments carried out in rats kept on diets with the following protein sources: egg albumin, casein, opaque-2 corn, common corn and gelatin. Casein and egg albumin were used as standard proteins. Two types of corn were chosen because corn is commonly eaten by the population. Opaque-2 corn (MO-2) is of particular interest because it offers a much better amino-acid balance than common corn (CC) (2-7).

MATERIAL AND METHODS

Animals - young albino Wistar rats were used as the experimental subjects. The initial weight of the animals is indicated in the description of the various tests.

Diets - the following diets were used:
protein x g: peanut oil 4ml: cod liver oil 2ml; mineral salts 2g (8); vitamins 2ml (9); sugar q.s.p. (90 - x g).

Protein was supplied at a level of 7g/100g of ration by one of the following sources:

OV - egg white
CAS - casein
MO-2 - opaque-2 corn*
CC - common corn
GE - gelatin
PF - protein free

Control Methods:

(a) weighing of the animals on alternate days;
(b) plasma protein determinations (electrophoresis) (10);
(c) liver fat determinations (11).
Blood was obtained by cardiac puncture.

* Obtained from the Instituto Agronômico de Campinas.

EXPERIMENT I

Young rats of a 95g average body weight were kept for 15 days on a control diet (OV). After this period they were fed the protein free diet (PF) for 15 days**, and then by a protein recovery diet containing OV, MO-2, CC or GE, for 15 days. Ten rats were used as experimental controls for each diet. They were fed on the OV, MO-2, CC or GE diet for the entire 45 day period.

EXPERIMENT II

Young rats of a 75g average body weight were kept for 15 days on a CAS, MO-2, CC or GE diet. They were then fed the protein free diet (PF) for 15 days** and later a protein recovery CAS diet for 15 days.

The number of rats was always sufficient to allow for the sacrifice of 10 aimals at the end of each observation period.

RESULTS

Experiment I:

The variation in body weight is presented in Figure 1.

The greatest weight recovery was obtained with the MO-2. The GE was not efficient in the recovery. Table I shows the results for body weight, liver fat and plasma proteins.

Plasma albumin decreased significantly during the period of protein depletion. It recovered to normal values after 15 days on the MO-2 and OV diets, but not on the CC or GE diets. Recovery of α_1--globulin was attained with MO-2 and CV. The β-globulin level which increased during the period of protein depletion, returned to normal after 15 days on OV, MO-2 and GE, but remained lower on MO-2 diet. The recovery diet of 15 days on MO-2 produced a significant improvement of albumin, α_1 and β-globulin. Liver fat increased during the deficiency period. It returned to normal only on the OV diet while total plasma protein returned to normal after 15 days on both the MO-2 and OV diets.

** Five rats fed the OV diet were pair-fed with the groups which was fed the protein-free diet (PF).

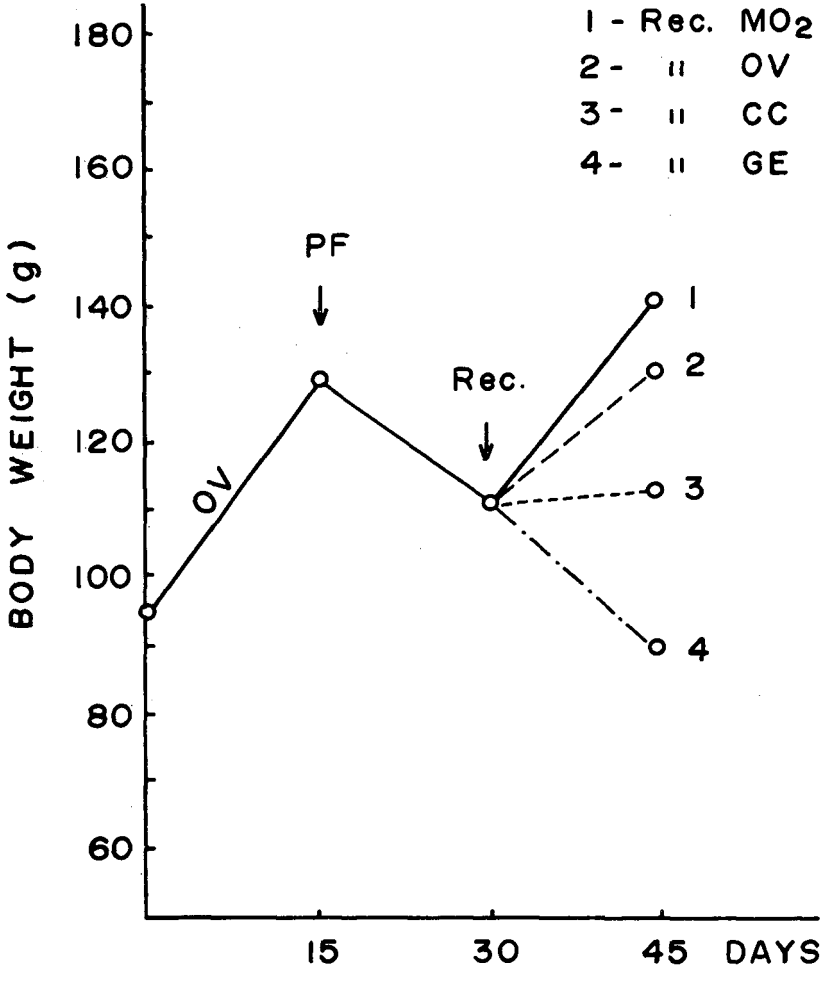

Figure 1. Variation in body weight of rats fed iniatially with Ovoalbumin (OV) (7g/100g diet) followed by 15 days on a protein free diet (PF) and then kept for 15 days on a diet containing as protein source opaque-2 corn or common corn (MO-2 or CC); or ovoalbumin (OV) or gelatin (GE). The protein content of the OV, MO-2, CC or GE diets was 7g/100g of ration. An average of 15 observations was made for each determination.

TABLE I

BODY WEIGHT, LIVER FAT AND PLASMA PROTEINS OF RATS(*) FED OVOALBUMIN DIET FOR 15 DAYS (OV), FOLLOWED BY 15 DAYS ON PROTEIN FREE DIET (PF) AND 15 DAYS BY RECOVERY WITH OVOALBUMIN (Rec. OV); OR OPAQUE-2 CORN (Rec. MO-2); OR COMMON CORN (Rec. CC); OR GELATIN (Rec. GE)

Diet	Body weight (g)	Liver fat g/100g(1)	PLASMA PROTEIN (g/100 ml)					
			Total	Albumin	GLOBULIN			
					$\alpha 1$	$\alpha 2$	β	γ
OV(2)	129,0±11,1	6,8±0,38(a)	6,1±0,45(a)(3)	3,41±0,41(a)	0,568±0,081(a)	0,293±0,031	0,312±0,051(a)	0,76 ±0,047(a)
PF(4)	110,0± 9,5	14,0±0,88(b)	5,1±0,25(b)	2,16±0,09(b)	0,435±0,091(b)	0,296±0,051	0,464±0,059(b)	0,74 ±0,100(a)
Rec. OV	130,5±08,8	7,5±0,29(a)	6,0±0,28(a)	3,10±0,52(a)	0,615±0,088(a)	0,390±0,028	0,360±0,048(a)	0,775±0,035(a)
Rec. MO-2	142,0±07,9	9,8±0,39(c)	6,2±0,40(a)	3.90±0,45(a)	0,559±0,052(a)	0,304±0,038	0,298±0,062(c)	0,670±0,041(b)
Rec. CC	112,0±12,4	8,0±0,29(a)	5,2±0,40(a)	2,71±0,80(b)	0,405±0,082(b)	0,374±0,068	0,415±0,041(b)	0,670±0,025(a)
Rec. GE	088,0±05,4	7,6±0,65(a)	4,3±0,55(c)	2,16±0,78(b)	0,450±0,101(b)	0,258±0,047	0,271±0,028(c)	0,640±0,009(a)

Mean ± standard error.

(1) - g/100g wet iver.

(2) - OV, ovoalbumin diet; PF, protein free diet; Rec. OV, recovery with OV diet; Rec. MO-2, recovery with MO-2 diet; Rec. CC, recovery with CC diet; Rec. GE, recovery with GE diet.

(3) - in each column, the same letter means not significanly different, $p<0,05$ in each column, different letter means significantly different from each other, $p<0,001$.

(4) - Pair fed OV with protein free (PF) fed rats attained 142±5,8 g of body weight.

N =10

The protein content of the OV, MO-2, CC and GE was 7g/100 diet. (Same situation as fig. 1).

(*) initial average body weight - 95g.

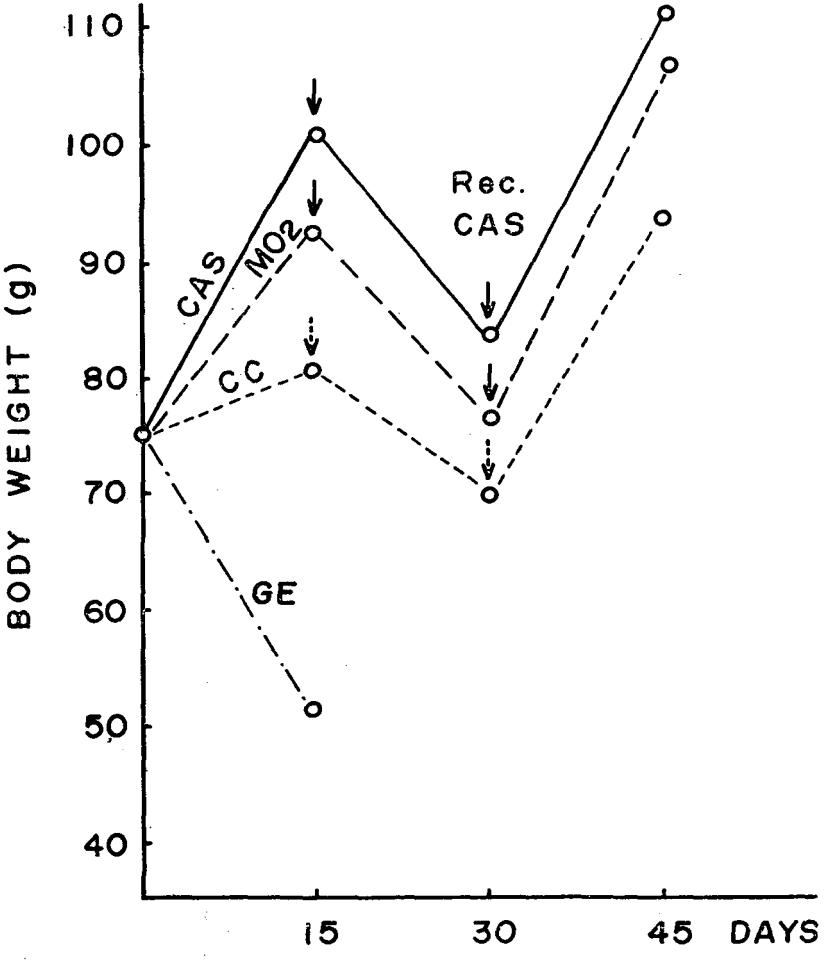

Figure 2. Weight variations of rats kept 15 days on diets containing various protein sources: casein (CAS); opaque-2 corn (MO-2); common corn (CC); or gelatin (GE) followed by a 15 days of protein free diet(PF), and fnally, a 15 day's recovery period on CAS. The recovery solpe was not significantly different for any of the diets ($p>0,05$).

TABLE II

BODY WEIGHT*, LIVER FAT AND PLASMA PROTEINS OF RATS FED CASEIN DIET (CAS); OR OPAQUE-2 CORN DIET (M)-2); OR COMMON CORN DIET (CC)

Diet	Body weight (g)	Liver fat g/100g(1)	PLASMA PROTEIN ((g/100 g)					
			Total	Albumin	GLOBULIN			
					$\alpha 1$	$\alpha 2$	β	γ
CAS	102±6,9	3,5±0,21(a)(2)	5,0 ±0,41(a)	2,75±0,18(a)	0,55±0,110(a)	0,260±0,034(a)	0,334±0,071(a)	0,625±0,108(a)
MO-2	93±9,4	4,9±0,28(b)	4,95±0,21(a)	2,58±0,22(a)	0,53±0,051(a)	0,290±0,019(a)	0,323±0,087(a)	0,620±0,201(a)
CC	80±8,1	5,0±0,27(b)	4,2 ±0,40(b)	2,02±0,35(b)	0,44±0,082(b)	0,315±0,052(a)	0,340±0,055(a)	0,625±0,098(a)

Mean ± standard error.

(1) - g/100 wet liver.

(2) - in each column the ame letter means not significantly different, $p<0,05$ in each column a different letter means significantly different from each other, $p<0,01$.

N = 10.

The protein content of the diets was 7g/100g diet. (Same situation as Fig. 2).

* Initial average body weight - 75g.

The control rats, fed the entire 45 days on the OV, MO-2 or CC diets, attained a weight of 130,6 ± 18,2g, 142 ± 28,1g and 82 ± 7,4g, respectively. This weight was not significantly different from the final weight after recovery of rats subjected to the 15 days protein deficient period (Figure 1).

Experiment II:

Rats of 75g mean body weight developed well on diets containing CAS or MO-2 as protein sources. The CC diet was inferior and the animals kept on the GE diet lost weight rapidly and finally died (Figure 2).

The recovery rate of these animals with Casein was not different for rats fed initially with CAS, MO-2 or CC. The results for liver fat, body weight and plasma proteins are presented in Table II.

The lowest liver fat value was obtained with CAS, suggesting that this protein is the best for protection against fatty liver. After 15 days on CAS or MO-2 diets, the results for plasma protein were not significantly different; however both were significantly different from the plasma protein values of rats on CC diet.

DISCUSSION

This study was carried out with 7% protein diets, because this is the level in common protein sources such as corn or rice. Protein deficiency was induced by a protein free diet without prevention of coprophagy. In previous study (unpublished results) we observed that with a protein free diet, the animals suffered a loss of appetite. At the 15th day, the mean daily intake was 7,2 ± 0,82g for rats fed a protein free diet and 10,0 ± 2,1g for a 25% casein diet. The loss of body weight was not only a result of the decrease of total caloric intake, since the animals of the pair fed group gained weight.

The results suggest that the best sources of protein tested for recovery from protein deficiences were opaque-2 corn, egg-white and casein. Opaque-2 corn was the most efficient for recovery of body weight.

After 15 days on a protein free diet the rats presented: high liver fat and β-globulin plasma level and decreased to-

tal plasma proteins, albumin and α_1-globulin. They recovered well when fed ovalbumin or opaque-2 corn from most of the symptoms with the exception of the fatty liver. In the case, gelatin was more efficient than opaque-2 corn.

When the proteins were given from the beginning as a maintenance diet, casein was more efficient than opaque-2 corn for the protection against fatty liver; but for maintaining the plasmatic protein level, they were similar. Common corn was not an adequate protein for growth, total plasmatic protein or albumin. However, common corn was better than opaque-2 corn for the maintenance of low liver fat levels. As regards to γ-globulin, the real plasmatic concentration was not different for any of the situations studied. The relative quantity of γ-globulin to total plasmatic protein was very enhanced in rats fed the protein free or the gelatin diet. β-globulin (g/100g total protein or g/100ml plasma) increased significantly in protein free and common corn fed rats.

As neither opaque-2 corn or casein are completely efficient for growth, liver fat maintenance and plasma proteins level, it is possible that an association of these two sources of protein would improve the results.

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RESUMO

Deficiência proteica

Recuperação com milho opaco-2, milho comum e proteinas controles. Estudo do peso corporal, proteinas plasmáticas e gordura hepática.

Estuda-se o efeito de diversas proteinas (ovoalbumina, caseina, milho opaco-2 e milho comum e gelatina) na manutenção ou recuperação de deficiência proteica.

O animal utilizado nas experiências é o rato.

Proteina de milho opaco-2 foi tão eficiente quanto a caseina ou ovoalbumina.

Recuperação por 15 das não foi eficientemente obtida com qualquer das proteinas testadas, e isto, para todos os parâmetros estudados (peso corporal, proteinas plasmáticas e gordura hepática).

Milho opaco-2, como única fonte proteica foi a mais eficiente para a recuperação do peso corporal, proteina plasmática total, albumina e β-globulina; no entanto, para e recuperação da gordura hepática, as proteinas mais eficientes foram: caseina, ovoalbumina, e gelatina.

Os autores concluem que para a recuperação total, dentro de um curto prazo, embora se tenha conseguido atingir um crescimento normal, uma única fonte proteica não foi suficiente, de tal forma que sugerem uma associação de proteinas, as quais provavelmente acelerariam o processo de recuperação.

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