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Sparing Action of Protein on Pantothenic Acid Requirement of Rat. III. Fibrin as the Protein Component.*

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The sparing action of protein on the pantothenic acid requirement of the rat was first demonstrated by Nelson and Evans[1] with purified diets, deficient in pantothenic acid, containing 64% instead of 24% casein. When intermediate levels (24%, 34%, 44%, 54%, and 64%) of casein were used, the sparing action as judged by growth, survival and urinary excretion of pantothenic acid of deficient rats was proportional to the casein level.[2] The presence of small amounts of "combined" pantothenic acid in the casein used (and in all types of purified casein tested) prompted a study of the effects of 24% casein diets supplemented with the amounts of pantothenic acid occurring in the casein of the 44% and 64% casein diets. The results showed that the pantothenic acid content of the purified casein used, while exercising an effect, could not fully account for the protective action of the high casein diets.[2]

However, it was felt highly desirable that a protein free from or considerably lower in pantothenic acid than casein be used in the further investigation of this problem. Preliminary assays indicated that beef blood fibrin, purified by washing with water or with dilute acid, was extremely low in pantothenic acid. The present communication reports the growth and survival of pantothenic acid deficient rats maintained on diets containing two levels of purified fibrin, 24% and 48%, and on the corresponding casein-containing diets.

Experimental Procedure. Dried crude beef blood fibrin (Armour and Company) was purified by washing with water for one week.† One kg of crude fibrin was placed in a 20-gallon crock and 15 gallons of water (either tap or distilled) added to it. The mixture was stirred thoroughly and allowed to settle. The supernatant liquid was siphoned off 2-4 times daily and fresh water added. Ten cc of chloroform were used with each change of water as a preservative. After 5-7 days of washing, the supernatant was drained off and the fibrin filtered through a large Buchner funnel. Suction was maintained for several hours or overnight if necessary to dry out the fibrin cake. It was then broken up by sieving and dried for 24 hours in a low temperature oven (50°C). After grinding, the fibrin is ready for use in the diets.

Microbiological assays for pantothenic acid were carried out by the method of Hoag, Sarett, and Cheldelin[3] with crystalline B vitamins substituted for the rice bran extract and autolyzed yeast in the assay medium. Enzymatic digestion with clarase or mylase P for 24 hours at 37°C[4] followed by the 24-hour assay procedure was used for the purified fibrin and casein. The water-washed fibrin used in the diets averaged 0.5 μg pantothenic acid per g whereas the alcohol-extracted casein contained 1.5-1.8 μg per g. There was considerable variation in the pantothenic acid content of different lots of fibrin after washing and additional purification was sometimes

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1 Nelson, M. M., and Evans, H. M., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 319.

2 Nelson, M. M., van Nouhuys, F., and Evans, H. M., *J. Nutrition*, 1947, **34**, 189.

† The procedure for washing the fibrin was that used by D. H. Copp (Department of Physiology, University of California).

3 Hoag, E. H., Sarett, H. P., and Cheldelin, V. H., *J. Ind. Eng. Chem., Anal. Ed.*, 1945, **17**, 60.

4 Ives, M., and Strong, F. M., *Arch. Biochem.*, 1946, **9**, 251.

necessary. Other methods of purification are being investigated in order to prepare fibrin completely free of pantothenic acid for balance studies.

The basal diet contained 24% alcohol-extracted casein or water-washed fibrin, 64% sucrose, 8% hydrogenated cottonseed oil (Crisco) and 4% salts No. 4.[5] The proportion of casein or of fibrin in the higher protein diets was increased to 48% at the expense of the sucrose. All diets contained the following crystalline vitamins per kg diet: 5 mg 2-methyl-1,4-naphthoquinone, 5 mg thiamine HCl, 5 mg pyridoxine HCl, 10 mg riboflavin, 10 mg p-aminobenzoic acid, 20 mg nicotinic acid, 400 mg inositol, and one g choline chloride. Each rat received weekly a fat-soluble vitamin mixture containing 650 mg corn oil (Mazola), 800 U.S.P. units vitamin A, 115 Chick Units vitamin D, and 6 mg alpha-tocopherol. The basal diet containing 24% casein is the high-carbohydrate diet used in previous studies[1,2] with the addition of 2-methyl-1,4-naphthoquinone to the vitamin mixture. The previous weekly allowance of the fat-soluble vitamins has been doubled.

In the first series of animals, 20 litters of male rats (Long-Evans strain) were divided into 4 equivalent groups and placed on the pantothenic acid deficient diets containing 24% or 48% casein or fibrin at weaning. An additional series of 10 litters of male rats were divided into equivalent groups and placed on the 2 fibrin diets at weaning. All animals were maintained in individual cages provided with screens to prevent coprophagy.

Results and Discussion. The growth of littermates (20 rats per group) maintained on 24% and 48% casein or fibrin diets deficient in pantothenic acid is shown in Fig. 1, while Table I presents the data on growth and survival for the combined series of animals. The sparing effect on growth and survival of doubling the casein level (24% to 48%) is evident although the effect is not as great as it would be in a longer experimental period or with higher casein levels.[2] The substitution of purified fibrin in the 24% casein diet resulted in a marked accentuation of the deficiency, shown in the greatly decreased growth and survival in comparison to all other diets. Despite the severity of the deficiency, doubling the fibrin level (48% fibrin diet) resulted in significantly increased growth and survival. In fact, the differences in growth and survival were greater than those between the 24% and 48% casein diets during the short experimental period.

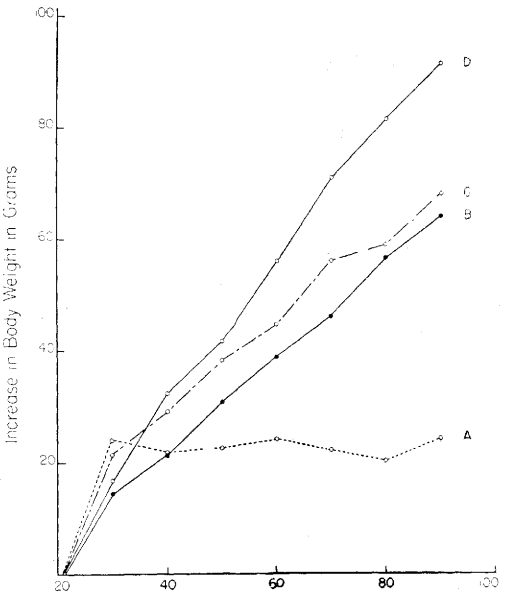

FIG. 1.

Growth of pantothenic acid deficient rats maintained on purified diets varying in casein or fibrin level.
A. 24% fibrin diet.
B. 48% " "
C. 24% casein "
D. 48% " "

The accentuation of the deficiency observed on the 24% fibrin diet was not due to the lack of any essential factors in the purified fibrin. Control rats receiving the 24% fibrin diet supplemented with calcium pantothenate grew slightly faster than littermates receiving the supplemented 24% casein diet. This is in agreement with the findings of Risser[6] that fibrin, both crude and pharmaceutical grades, was somewhat higher in biological value than casein. The decreased pantothenic acid con-

[5] Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1941, **138**, 460.

[6] Risser, W. C., *J. Nutrition*, 1946, **32**, 485.

TABLE I.
Effect of Casein and Fibrin Levels on Growth and Survival of Pantothenic Acid Deficient Rats.

Dietary protein	No. of rats	Survival Day 30 %	Survival Day 45 %	Survival Day 60 %	Avg body wt Day 60 g	Survival Day 75 %	Survival Day 90 %	Avg body wt Day 90 g
24% fibrin	30	83	60	23	72	3	3	73
48% "	30	97	77	57	86	43	33	111
24% casein	20	100	80	60	93	50	40	117
48% "	20	100	95	85	105	55	45	132

tent of the washed fibrin is the obvious factor responsible for the accentuation, although other possibilities such as the presence of accentuating factors, decreased intestinal synthesis, or the lack of biotin and folic acid[7,8] cannot be eliminated without investigation. Sarma, Snell and Elvehjem[9] have reported an accentuation of pyridoxine deficiency when fibrin-containing diets were used and believed that the accentuation was greater than could be accounted for by the decreased pyridoxine content of the fibrin diets.

Fibrin and casein are similar in amino acid content[10] but are markedly different in regard to phosphorus content. Fibrin is extremely low in phosphorus and is frequently used for studies of phosphorus deficiency, while casein a conjugated phosphoprotein, contains somewhat less than 1% phosphorus. Doubling the proportion of fibrin in the diet does not significantly affect the phosphorus content whereas doubling the proportion of casein markedly increases the phosphorus concentration of such diets. The phosphorus content of the diets used (calculated from the phosphorus analyses of purified fibrin and casein‡ and the composition of the salt mixture) are as follows: 24% fibrin diet—0.6%; 48% fibrin diet — 0.6%; 24% casein diet — 0.7%; and 48% casein diet—0.9%. The fact that an increased phosphorus content is unnecessary for the sparing action of high fibrin diets indicates that phosphorus can be eliminated as a possible factor in the sparing action of protein on the pantothenic acid requirement of the rat.

Summary. The sparing action of protein on the pantothenic acid requirement of the rat has been confirmed by using a protein considerably lower in pantothenic acid, washed beef blood fibrin, instead of casein as the protein component of the diet. Despite the accentuation of the pantothenic acid deficiency produced by substituting purified fibrin for casein at the 24% level, doubling the fibrin level resulted in significantly better growth and survival. The differences in growth and survival between the two protein levels in the fibrin-containing diets were more pronounced than those observed for the casein-containing diets. The protective effects of the high fibrin diet indicated also that phosphorus can be eliminated as a possible factor concerned in the sparing action.

7 Wright, L. D., and Welch, A. D., *Science,* 1943, **97**, 426.

8 Wright, L. D., and Welch, A. D., *J. Nutrition,* 1944, **27**, 55.

9 Sarma, P., Snell, E. E., and Elvehjem, C. A., *J. Biol. Chem.,* 1946, **165**, 55.

10 Block, R. J., and Bolling, D., *The Amino Acid Composition of Proteins and Foods,* 1945, Chapter X, p. 300, Charles C. Thomas, Springfield, Ill.

‡ The phosphorus analyses were carried out by Miss Genevieve Beach through the courtesy of D. H. Copp. The water-washed fibrin contained 0.045% and the alcohol-extracted casein 0.61% phosphorus.