TABLE IV. Effect of Intravenous Injection of Different Substances on Blood Sugar of Rabbits.

Wt, g	Treatment	Blood sugar (mg/100 cc blood) Hr after inj. of substances 0	½	1	1½	2	3
1500	Inj. with 0.2 mg dihydroergotamine	124	—	115	—	94	99
1500	Inj. with 20 μg epinephrine	135	175	221	240	—	—
1500	Inj. with 0.2 mg dihydroergotamine followed by inj. of 10 μg epinephrine ½ hr and 1¼ hr after inj. of dihydroergotamine	88	88	95	97	85	63
2000	Same as above	83	95	95	104	90	63
1100	Inj. with 0.2 mg dihydroergotamine followed ½ hr later by inj. of dehydroascorbic acid (1.1 g)	96	107	89	—	84	

oxidation of glutathione and reduction of dehydroascorbic acid to ascorbic acid in the body. Persistent hyperglycemia and glycosuria could not be observed in any one of the rabbits injected with dehydroascorbic acid. The rabbits also did not show persistent diabetic type of glucose tolerance curve. No histological changes could be observed in the pancreas. All these findings indicate that dehydroascorbic acid is not diabetogenic in rabbits.

Summary. Intravenous injections of dehydroascorbic acid in rabbits in the dose 1 g/kg or 1.5 g/kg could not produce either persistent hyperglycemia or persistent diabetic type of glucose tolerance curve. Rabbits so treated neither excreted sugar in the urine nor showed any histological changes in the pancreas, suprarenal and pituitary. All these findings indicated that unlike rats dehydroascorbic acid is not diabetogenic in rabbits.

1. Patterson, J. W., *J. Biol. Chem.*, 1950, v183, 81.
2. Patterson, J. W., and Lazarow, A., *J. Biol. Chem.*, 1950, v186, 141.
3. Patterson, J. W., *Am. J. Physiol.*, 1951, v165, 61.
4. Patterson, J. W., and Mastin, D. W., *Am. J. Physiol.*, 1951, v167, 119.
5. Merlini, D., *Acta Vitaminol.*, 1951, v5, 67.
6. ———, *Bull. Soc. ital. Biol. sper.*, 1950, v26, 1005.
7. Banerjee, S., *Nature*, 1943, v152, 329.
8. Banerjee, S., and Ghosh, N. C., *J. Biol. Chem.*, 1947, v168, 207.
9. Banerjee, S., *Nature*, 1944, v153, 344.
10. Banerjee, S., Debb, C. C., and Belavady, B., *J. Biol. Chem.*, 1951, v195, 271.
11. Hagedorn, H. C., and Jensen, B. N., *Biochem. Z.*, 1923, v137, 92.
12. Woodward, G. E., and Fry, E. G., *J. Biol. Chem.*, 1932, v97, 465.
13. Komrad, E. L., and Loew, E. R., *Am. J. Physiol.*, 1951, v165, 66.

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Unidentified Factors Capable of Reducing Stress in Iodinated Protein-Fed Rats.* (20288)

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Liver preparations have been shown capable of partially relieving the stress produced in animals by a considerable number of experimental conditions. These studies have been summarized recently by Ershoff(1). The deleterious effects resulting from the administration of thyroid-active materials may be controlled under certain circumstances by yeast(2) and by several semi-purified protein materials(3,4) as well as by liver fractions when fed in conjunction with diets consisting

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of highly refined ingredients. The use of an assay for vit. B_{12} activity employing hyperthyroid rats in this laboratory(5,6) directed our attention toward a study of the differences in response of rats to vit. B_{12} when receiving highly purified and crude diets containing iodinated protein or other thyroid stimulants. Several investigators have observed that vit. B_{12} reduces the toxic effects of thyroactive materials when administered to animals receiving diets containing such crude constituents as yellow corn meal, soybean meal, cottonseed meal or dextrin(7-9) while the vitamin produces only slight beneficial effects when the animals receive purified diets (10,11). It is also significant that relief from other stress circumstances is given by vit. B_{12} when crude(12,13) but not when highly purified diets are employed(14). Work reported earlier(15) has demonstrated that either corn or soybean meal added to an otherwise purified diet in place of appropriate amounts of protein and carbohydrate makes possible a very significant response to vit. B_{12}. Further studies directed toward elucidating the reasons for the dissimilar responses of hyperthyroid animals to the two types of diets indicated that differences in production of vit. B_{12} or vit. B_{12f} could not account for the observed variations(16,17).

In experiments preliminary to the present work in which levels of iodinated casein as high as 0.5% were employed, two facts became evident: a) Survival time of either adult or young rats was a more accurate measure of protection against such toxic amounts of this stress agent than growth or maintenance of body weight; and b) Certain diets containing crude constituents, notably pork or beef and high levels of corn and soybean meal, were capable of increasing the survival time of the animals even in the presence of adequate amounts of vit. B_{12}. While not the most refined measurement, survival data have yielded useful information concerning the protection afforded by the various dietary regimens while other indices were being evaluated. Studies arising from these observations are reported herein.

Procedure. In the experiments to be described, male weanling albino rats of the Sprague-Dawley strain with a weight range of 40-50 g were divided into identical groups and housed in individual suspended, mesh-bottomed cages. Food and water were supplied *ad libitum* and weights of the animals were recorded weekly. The basal rations employed contained 20-24% casein,† 0.3% cystine, 4% salts IV(18), 5% corn oil, 0.15-0.5% iodinated casein,‡ vitamins and sucrose to 100%. The water soluble vitamins were employed at 5 times the levels described previously(16) with the exception of riboflavin used here at 25 and vit. B_{12} at 0.1 mg/kg. Fat soluble vitamins were administered as 4 drops weekly of a 1:1 haliver oil-corn oil combination containing 1 mg menadione and 2.5 mg α-tocopherol per ml of mixed oil. Except in cases which will be noted individually, the protein and fat levels of the supplemented diets were kept constant with those of the basals by appropriate reduction in the casein and corn oil levels. The cystine content of the various diets was reduced in proportion to the reduction in the casein level. Although several investigators(3,10,11) studying protection against the toxic effects of iodinated protein or thyroid material have added supplements at the expense of the carbohydrate of the diet, in view of the reports of protection afforded by increased levels of protein(19) or fat(20,21), such a practice was deemed inappropriate until adequate studies on the effects of these nutrients had been conducted. The corn-soybean diet employed in the first study reported in Table I contained equal quantities of yellow corn meal and soybean meal replacing the entire amounts of casein and sucrose of the basal diet. Ground whole pork was included in the third ration in this experiment at the expense of the casein and sucrose in an amount sufficient to produce a 21% protein level (N x 6.25). The meat was prepared according to previously reported methods(22).

Results and discussion. The preliminary findings indicating a high level of protection by the corn-soybean and pork containing

† Vitamin test casein, General Biochemicals, Chagrin Falls, Ohio.

‡ Protamone, Cerophyl Laboratories, Kansas City, Mo.

TABLE I. Influence of a Variety of Natural Materials on Growth and Survival of Rats Receiving Toxic Levels of Iodinated Protein (6 Animals per Group).

Exp.	Diets employed	Avg growth per wk (3 wk)*	Avg survival (days)†
1.	0.4% protamone		
	20% casein basal	23	26.4 ± 4.1‡
	21% pork protein	32	38.0 ± 5.2(1)
	Corn-soybean	30	56.2 ± 3.4(4)
2.	0.5% protamone		
	Ether extract of pork	25	22.2 ± 2.1
	Alcohol " " "	25*	20.5 ± 1.6
	Water " " "	26*	19.8 ± 1.3
	Extracted residue	26	40.8 ± 6.9(2)
	Dried whole pork	31	43.0 ± 4.9(1)
	20% casein basal	24	20.5 ± 1.1
3.	0.5% protamone		
	45% ground whole wheat	22	18.2 ± 1.7
	45% cooked fresh eggs	22	14.3 ± 0.3
	45% haddock	22	23.0 ± 3.6
	33.3% milk powder	15	14.0 ± 1.2
	20% alfalfa leaf meal	24	39.7 ± 5.6(1)
	20% barley malt	19	17.7 ± 1.7
	20% crude casein	24	17.8 ± 2.2
	15% yeast	23	29.3 ± 7.0(1)
	Basal + 10% cellulose	20	15.8 ± 1.2
	20% casein basal	16	14.0 ± 0.6
4.	0.5% protamone		
	20% alfalfa leaf meal	28	42.8 ± 6.4(1)
	Corn meal-soybean meal (10%+10%)	27	19.5 ± 1.6
	20% corn meal	23	23.4 ± 0.7
	20% soybean meal	27	24.8 ± 2.8
	19.8% haddock (dried)	23	21.0 ± 1.2
	20% yeast	30	35.3 ± 2.6
	20% eggs (dried)	29	25.3 ± 1.8
	20% whole liver (dried)	30	31.7 ± 2.1
	5% liver concentrate§	25	25.7 ± 3.2
	19.3% pork (dried and defatted)	25	49.7 ± 4.7(1)
	19.2% mutton (dried and defatted)	29	45.5 ± 6.6
	20% casein basal	25*	18.6 ± 1.9

* 2 wk growth reported in cases where fewer than half of animals survived 3 wk.

† Experiments terminated at end of 60 days. For those animals surviving entire experiment, 60 days has been used in the calculations of average survival. No. in parentheses indicate No. of animals surviving for 60 day period.

‡ Stand. error of mean, $\sqrt{\frac{\Sigma d^2}{n(n-1)}}$, where d = deviation from mean and n = No. of observations.

§ NF Powder, Wilson Laboratories.

diets are substantiated by the results of exp. 1. The difference between the survival of the animals receiving the basal and the pork rations is highly significant ($p < .01$) while even more protection is afforded by the corn-soybean mixture. Later work has indicated that the fat level of the ration containing whole pork (15%) can account for only part of the protection given by this diet.

Since meat may be separated into several fractions by simple procedures, in the next experiment an attempt was made to remove the protective activity from the major bulk of pork muscle. For this study the pork was dried, extracted with ethyl ether, then extracted with 95% ethanol and finally extracted with water. As a positive control dried whole pork was fed at a protein level comparable with the casein basal, and the various fractions were fed at levels comparable to their content in the whole pork ration. In each case the nitrogen level was increased to that of the basal with additional casein. The data indicate that little if any of the protective activity was removed by the extraction procedures. This observation agrees with the work of Ershoff(10) and Graham *et al.*(3,23) in which the "antithyrotoxic" properties of liver and beef muscle have been found to remain in the extracted residue.

In the last two studies reported in Table I a variety of materials have been assayed for their ability to prolong the life of rats receiving large amounts of iodinated casein. The most active materials found, listed in approximate order of decreasing potency, are mammalian muscle, alfalfa leaf meal, yeast and whole liver. Although Graham *et al.*(3) found whole egg protein to be active in maintaining growth and survival in their assay, the two trials reported here indicate a rather low content of the anti-stress activity for this material. Both the eggs and the haddock were dried before incorporation into the rations for Exp. 4 since it seemed possible that their high moisture content in the previous experiment might make the protamone more available or more toxic to the animals. While the eggs increased somewhat in activity when dried, neither material can be considered to be highly protective. Whole wheat, milk powder, crude casein, corn or soybean meal, when they constitute only 20% of the diet, or liver concentrate, NF powder, are

TABLE II. Influence of Protective Materials on Growth and Organ Size of Young Rats.

Regimen* and exp. period	Surviving rats/Total No.	Avg gain of surviving rats	mg/100 g body wt — Thymus	Spleen	Adrenals	Kidneys	Seminal vesicles	Testes
No protamone—3 wk exp.								
Normal controls	10		303 ± 14	369 ± 15	18.7 ± 0.8	835 ± 10	56.9 ± 3.3	1196 ± 42
No protamone—5 wk exp.								
Casein basal	6/ 6	196 ± 9.2						
Basal + pork	6/ 6	209 ± 12.2						
.5% protamone—3 wk exp.								
Casein basal	8/11	62 ± 4.2	244 ± 22	333 ± 20	37.8 ± 2.8	1688 ± 29	21.3 ± 9.3	1096 ± 46
Basal + pork	12/12	77 ± 5.7	274 ± 20	455 ± 27	35.5 ± 1.7	1466 ± 33	34.4 ± 4.5	1252 ± 39
" + alfalfa	5/ 6	90 ± 4.1	322 ± 24	462 ± 10	37.2 ± 1.5	1397 ± 23	31.2 ± 5.1	1135 ± 77
" + yeast	6/ 6	88 ± 5.1	290 ± 20	519 ± 59	29.5 ± 2.6	1393 ± 43	21.9 ± 6.5	1289 ± 41
.15% protamone—4 wk exp.								
Casein basal	4/ 5	111 ± 6.3	214 ± 17	355 ± 16	38.3 ± 3.9	1.65 ± .34†		1.40 ± .09†
Basal + alfalfa	5/ 5	129 ± 8.4	300 ± 33	476 ± 64	29.9 ± 4.6	1.27 ± .08		1.25 ± .09
" + pork	5/ 5	136 ± 3.6	281 ± 14	496 ± 19	27.6 ± 3.7	1.38 ± .35		1.27 ± .07

* Basal diets in these experiments contained 24% casein. Supplements were added at 20%. † Grams.

also poor sources of the protective factor(s). Cellulose added to the basal diet also gave insignificant protection, indicating that the bulk content of the crude diets does not account for the protective action.

To ascertain whether our survival assay could be correlated with the growth assays of other investigators, the experiment employing 0.15% protamone shown in Table II was initiated. With this relatively low level of protamone, the growth data correspond very closely to the degree of protection by pork and alfalfa as measured by the survival assays (Table I). Growth data have frequently not correlated closely with survival data in the experiments employing 0.4-0.5% protamone. Also shown in Table II are the weights of several organs for normal rats and for animals receiving both 0.5 and 0.15% iodinated protein. Under both conditions the thymus is atrophied in the basal groups but is maintained at almost the normal size in the protected animals. In these studies the spleens of the protected animals are larger than those of the unprotected animals, while the adrenals of the rats receiving 0.15% protamone are hypertrophied less in the groups receiving alfalfa and pork than in the basal group. The kidneys of all the groups receiving iodinated casein are enlarged but are consistently reduced in size by the protective substances. Contrary to the findings of Ershoff(10) and Meites(13), no significant reduction in size of the testes is observed in the rats receiving iodinated casein. Differences in diet and susceptibility of the animals may account for the discrepancies. The seminal vesicles are partially restored to normal in some of the protected groups.

According to the descriptions by Selye(24) of the manifestations of general stress, the thymuses, spleens, adrenals, kidneys, and seminal vesicles of the rats show protection by some or all of the active materials against the stress induced by iodinated casein administration. The presence of vit. B_{12} in our diets may prevent the hypertrophy of the spleens in unprotected hyperthyroid animals as reported by Graham, *et al.*(23). The prevention of such hypertrophy by vit. B_{12} observed by these workers supports such a pro-

posal. In view of the results of these studies, it is suggested that the presence of anti-stress factors in crude diets makes possible the previously considered vit. B_{12} response in hyperthyroid or otherwise stressed animals while similar animals receiving purified diets are deficient in both vit. B_{12} and these uncharacterized factors and therefore cannot respond to vit. B_{12} alone.

Summary. 1. A variety of natural products have been examined by means of a rat-survival assay for their ability to relieve the stress induced by iodinated casein administration. Mammalian muscle and alfalfa have been found to be excellent sources of the protective material(s) being measured, with liver and yeast also containing significant amounts. 2. Materials most active in the survival assay have been demonstrated to reduce thymus and seminal vesicle atrophy and kidney enlargement. When low levels of iodinated protein are employed, the adrenals are also partially restored to normal by the protective substances. Under the conditions employed, the spleens are significantly larger in the protected than the unprotected animals and the testes are not reduced in size by the thyroprotein administration.

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1. Ershoff, B. H., *J. Nutrition,* 1952, v47, 289.

2. Ershoff, B. H., and Hershberg, G., *Am. J. Physiol.,* 1945, v145, 16.

3. Graham, C. E., Reichstein, I. P., Watson, W. J., and Hier, S. W., PROC. SOC. EXP. BIOL. AND MED., 1953, v82, 7.

4. Overby, L. R., Frederickson, L. R., and Frost, D. V., *Fed. Proc.,* 1953, v12, 425.

5. Register, U. D., Ruegamer, W. R., and Elvehjem, C. A., *J. Biol. Chem.,* 1949, v177, 129.

6. Tappan, D. V., Lewis, U. J., Register, U. D., and Elvehjem, C. A., *Arch. Biochem.,* 1950, v29, 408.

7. Emerson, G. A., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 392.

8. Nichol, C. A., Dietrich, L. S., Cravens, W. W., and Elvehjem C. A., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 40.

9. Frost, D. V., Fricke, H. H., and Spruth, H. C., *J. Nutrition,* 1953, v49, 107.

10. Ershoff, B. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v73, 459.

11. Betheil, J. J., and Lardy, H. A., *J. Nutrition,* 1949, v37, 495.

12. Meites, J., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 692.

13. ———, *Metabolism,* 1952, v1, 58.

14. Ershoff, B. H., PROC. SOC. EXP. BIOL. AND MED., 1951, v78, 836

15. Lewis, U. J., Tappan, D. V., Register, U. D., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 568.

16. Lewis, U. J., Tappan, D. V., and Elvehjem, C. A., *J. Biol. Chem.,* 1952, v194, 539.

17. ———, *J. Biol. Chem.,* 1952, v199, 517.

18. Hegsted, D. M., Mills, R. C., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.,* 1941, v138, 459.

19. Abelin, I., *Biochem. Z.,* 1930, v228, 165.

20. Greenberg, S. M., and Deuel, Jr., H. J., *J. Nutrition,* 1950, v42, 279.

21. Ershoff, B. H., *J. Nutrition,* 1949, v39, 259.

22. Henderson, L. M., Schweigert, B. S., Mozingo, A. K., and Elvehjem, C. A., *J. Nutrition,* 1948, v36, 479.

23. Graham, C. E., Reichstein, I. P., Watson, W. J., and Hier, S. W., PROC. SOC. EXP. BIOL. AND MED., 1952, v80, 657.

24. Selye, H., *The Physiology and Pathology of Exposure to Stress,* 1950, Acta, Inc., Montreal, Can.

Received March 31, 1953. P.S.E.B.M., 1953, v83.