American Biology Teacher. The sixth segment will appear in the January issue of that journal. Seven thousand copies of the complete report, entitled, "The Teaching of Biology in Secondary Schools of the United States—A Report of Results from a Questionnaire," are in press, and arrangements have been completed for mailing copies to biologists, educators and others throughout the United States.

Dr. Oscar Riddle, Carnegie Institution, Cold Spring Harbor, and Dr. Walter F. Loehwing, University of Iowa, were appointed to serve as representatives of the Union of American Biological Societies upon the recently formed "Cooperative Committee on Science Teaching." This latter committee also has representatives from the American Association of Physics Teachers, the American Chemical Society, the Mathematical Association of America and the National Association for Research in Science Teaching. This joint committee plans to consider certain problems (see SCIENCE, Vol. 95, p. 38, 1942) relating to the teaching of science in secondary schools.

The following officers were elected to serve for the Union of American Biological Societies during 1942: *President,* A. J. Carlson, University of Chicago; *Secretary,* F. A. Brown, Jr., Northwestern University; *Treasurer,* D. H. Wenrich, University of Pennsylvania; *Executive Committee,* B. M. Duggar, University of Wisconsin; A. P. Hitchens, University of Pennsylvania; G. W. Hunter, III, Wesleyan University.

FRANK A. BROWN, JR.,
Secretary
UNION OF AMERICAN BIOLOGICAL SOCIETIES

SPECIAL ARTICLES

THE PROCARCINOGENIC EFFECT OF BIOTIN IN BUTTER YELLOW TUMOR FORMATION[1]

EARLY in 1940 during the course of investigations which led to the demonstration[2,3,4] that biotin is identical with the anti-egg white injury factor (vitamin H), and that biotin is thus functionally involved in animal metabolism, experiments were initiated to ascertain whether or not biotin is a dietary factor present in liver and yeast which is protective against primary carcinoma of the liver induced in rats by the administration of N,N-dimethylaminoazobenzene (butter yellow). Protection against butter yellow by liver and yeast supplements had been reported by the Japanese workers.[5,6]

In two preliminary experiments two very crude biotin preparations, one from liver and one from yeast, were used as supplements to the butter yellow-brown rice-carrot basal diet which alone gave regularly a high incidence of tumors, namely, 96 per cent. at 150 days. Indications of a protective effect were obtained.

Other studies on protection against butter yellow revealed at this time that the addition of 200γ of riboflavin and 18 per cent. casein to the brown rice-carrot basal diet gave marked protection.[7] The tumor incidence at the end of 150 days was decreased from 96 per cent. to 7 per cent. This effect, though marked, was not as complete as that obtained by supplements of dried liver or yeast. Accordingly, the effect of the

TABLE I

DIETS EMPLOYED

	Diet A*	Diet B	Diet C*
Brown rice	82 per cent.	..	81.5 per cent.
Casein (vit. free)	18 " "	15 per cent.	18 " "
Crisco	...	10 " "	..
Sucrose (C.P.)	...	60 " "	..
Egg white (dried)	...	10 " "	..
Cystine	...	1 " "	0.5 " "
O. M. salt mixture	...	4 " "	..
Supplements—Amounts per 100 grams of diet			
Choline	...	250 mg	160 mg.
Riboflavin†	1 mg	2 "	2 "
Thiamin	0.5 "	0.5 "	..
Pyridoxine	1 "	0.5 "	..
Nicotinic acid	5 "	2 "	..
Inositol	...	100 "	..
Pantothenic acid	2.5 "	1 "	..
Vit. A (concentrate)	...	4000 units	..
Vit. K (Me Naphthoquinone)	0.5 "	0.1 mg	..
Vit. E (*dl*, α-tocopherol)	...	1.0 "	..
Irradiated ergosterol	...	400 units	..
Ergosterol	100 "	...	..
N,N-dimethylaminoazobenzene	60 "	100 mg	60 "

* When diets A and C were used 1 gram of carrot was given per rat per day as used by R. Kinosita, *Trans. Japan. Path. Soc.,* 27: 665, 1937.

† 0.5 mg added in Diet B instead of 2 mg from 1st through 45th day.

[1] Acknowledgment is made with full appreciation to Dr. Paul György for the crude liver concentrates used in these experiments and to the S.M.A. Corporation for supplies of biotin concentrates which made this investigation possible. A grant in support of this work to one of us (C.P.R.) from Standard Brands, Inc. is also gratefully acknowledged.

[2] P. György, D. B. Melville, D. Burk and V. du Vigneaud, SCIENCE, 91: 243, 1940.

[3] V. du Vigneaud, D. B. Melville, P. György and C. S. Rose, SCIENCE, 92: 62, 1940.

[4] P. György, C. S. Rose, K. Hofmann, D. B. Melville and V. du Vigneaud, SCIENCE, 92: 609, 1940.

[5] W. Nakahara, K. Mori and T. Fujiwara, *Gann,* (*a*) 32: 465, 1938; (*b*) 33: 13, 1938; (*c*) 33: 406, 1939; (*d*) 33: 57, 1939.

[6] T. Ando, *Gann,* 32: 252, 1938.

[7] C. J. Kensler, K. Sugiura, N. F. Young, C. R. Halter and C. P. Rhoads, SCIENCE, 93: 308, 1941. On the basis of independent investigations, P. György, C. E. Poling and H. Goldblatt (*Proc. Soc. Exp. Biol. and Med.,* 47: 41, 1941), have also obtained a protective effect of casein in the presence of riboflavin.

addition of other members of the B complex vitamins plus ergosterol and vitamin K, as given in Diet A, Table I, was next studied. Results shown in Table II indicate that these additional vitamins did not improve significantly the protection afforded by the riboflavin-casein supplement to the brown rice diet. In the hope of obtaining still more complete protection, several groups of animals were fed in addition to Diet A, 0.3 and 1.0γ of biotin respectively in the form of two concentrates, one 0.1 per cent. pure and the other (biotin ester) 25 per cent. pure. These animals appeared, however, to be less protected. Tumors were found in only 1 of the 9 animals on Diet A, whereas tumors were found in 13 out of 34 rats which had been given the biotin supplements in addition to Diet A.

TABLE II

EFFECT OF BIOTIN ON THE PRODUCTION OF HEPATIC TUMORS BY N,N-DIMETHYLAMINOAZOBENZENE

Exp. No.	Diet	Biotin prep. used	Amount of biotin added daily	No. days on diet	No. rats surviving	No. rats with hepatic tumor
I	A	...	...	150–230	9	1
	A	0.1 per cent. conc.	0.3 γ	" "	8	3
	A	25 " " " (ester)	" "	" "	9	3
	A	0.1 " " "	1.0 "	" "	9	3
	A	25 " " " (ester)	" "	" "	8	4
II	B	.....	...*	180	5	0
	B	crystalline	2.0 "	"	5	3
III	C	.....	...	150–210	14	0
	C	crystalline	2.0 "	" "	11	6
		SUMMARY OF RESULTS				
Exp. I, II, III		Controls	.....		28	1
		Crude and crystalline biotin	0.3–4.0 γ		50	22
Exp. II, III		Controls	.....		19	0
		Crystalline biotin	2.0–4.0 γ		16	9

* 4.0 γ crystalline biotin were fed from the 45th to the 115th day.

In view of this unexpected result, a further study was made of the effect of biotin added to highly protective diets. Crystalline biotin was used. Since only a limited amount of this material was available, the study was restricted to a small number of animals. A supposedly highly protective diet containing casein supplemented with cystine and choline was employed and sufficient egg white was added to produce a borderline biotin deficiency. It is to be noted that this diet did not contain the brown rice. The other constituents of the diet are shown in detail under Diet B, Table I. One group of animals was fed this diet alone and another group received in addition 2.0γ of crystalline biotin daily except from the 45th to the 115th day where 4.0γ were given daily. As is shown in the table, no tumors were found in the low biotin group at 6 months, whereas hepatomas were found in 3 out of 5 rats which had received the crystalline biotin.

While this experiment was in progress another experiment was made with the brown rice-carrot diet as the basal ration. Riboflavin, casein, cystine and choline were added (Diet C, Table I). No liver tumors were present at 150 to 210 days.[8] On the other hand, 6 animals out of 11, which in addition to Diet C had been given 2γ of crystalline biotin daily throughout this experimental period, showed well-developed neoplasms when sacrificed.

Thus in 3 sets of experiments in which rats were maintained on diets affording a high degree of protection, crystalline biotin and two biotin concentrates were found to "break through" the protection.[9] Moreover, this effect was obtained on two radically different diets; in one experiment no rice was used and in two a rice diet was employed. A combination of the data of these 3 experiments shows that only 1 rat out of the 28 control animals developed a liver tumor. However, when biotin (0.3 to 4.0γ) was added to these 3 diets, 22 rats out of a total of 50 developed liver tumors. A combination of the data of the 2 experiments in which crystalline biotin was used shows that, whereas there were no tumors in the controls (19 rats), 9 out of 16 rats fed the biotin had tumors. The preliminary experiments in which some protection was provided by the crude biotin preparations suggest the possibility that these crude preparations may also contain a factor which affords a small amount of protection which becomes apparent when a largely non-protective diet is used.

We feel that the results presented here justify the conclusion that biotin can exert a procarcinogenic effect when butter yellow is fed to rats receiving a highly protective diet. Whether biotin can break down the protection against butter yellow afforded by a yeast or liver supplement; whether the procarcinogenic action we have observed under our experimental conditions is a unique property of biotin; whether the effect can be extended to other carcinogenic agents and other species; and whether the reversed effect can be produced by a biotin deficiency (egg-white, avidin or other means), must all await further experimentation. The present data throw no light on these ques-

[8] A detailed report of our experiments investigating the protection afforded by riboflavin, casein, methionine, cystine and choline will appear at a later date.

[9] We have learned through private communication that György, Landy and Goldblatt have obtained with the biotin concentrate from liver (0.1 per cent. pure) a similar procarcinogenic biotin effect using a significantly protective diet. They found in an experiment involving 50 test and control rats that when 0.8 to 3.5 γ of biotin were administered daily to rats on a diet consisting of cooked polished rice and carrot supplemented daily with 20 γ of riboflavin, B_1, and B_6, tumor incidence was increased at 150 days from 33 to 52 per cent.

tions. We forego any discussion of the possible significance of the finding reported herein until further experimental data on these and related aspects are forthcoming.

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PRELIMINARY OBSERVATIONS ON "EGG WHITE INJURY" IN MAN AND ITS CURE WITH A BIOTIN CONCENTRATE[1,2]

IT seemed important to determine whether any of the manifestations of spontaneous avitaminosis in human beings might be related to biotin deficiency. To this end a small group of volunteers ate a diet planned to contain a minimal amount of biotin; to this was added desiccated egg white in amounts sufficient to furnish approximately 30 per cent. of the total caloric intake. The diet was composed of 125 gm of polished rice, 80 gm of patent white flour, 75 gm of farina, 205 gm of cane sugar, 32 gm of lard, 10 gm of butter washed five times in hot water and 25 gm of lean beef. To this was added 200 gm of dehydrated but otherwise unmodified egg white. The basal components contained approximately 387 gm of carbohydrate, 31 gm of protein and 32 gm of fat with a caloric value of 1,960; the egg white contained 160 gm of protein and 32 gm of fat with a value of 928 calories. Such a diet is extremely poor in vitamins except riboflavin which is present in egg white in amounts approximating 10 mg per 100 gm. The daily vitamin supplement was:

Thiamin chloride	6 mg
Riboflavin	9 mg
Nicotinic acid	75 mg
Pyridoxine	5 mg
Ca. pantothenate	5 mg
Ascorbic acid	75 mg
Vitamin A	5,000 units

In addition, 1 gm of ferrous sulfate and 1 gm of calcium lactate were given daily.

It was possible to continue four subjects under observation to a satisfactory conclusion; three were white men, one a Negro woman. During the third and fourth weeks all four developed a fine scaly desquamation without pruritus. This disappeared spontaneously in seven to ten days. Thereafter nothing of significance was noted until the seventh week when one man developed a maculosquamous dermatitis of the neck, hands, arms and legs. During the seventh and eighth weeks all showed a pronounced grayish pallor of the skin and mucous membranes which was out of all proportion to the blood picture. During this same period the white patients showed atrophy of the lingual papillae, it was notable that the tongues remained pale with none of the capillary engorgement seen in pellagra or ariboflavinosis. During the ninth and tenth weeks all subjects showed increasing dryness of the skin with marked reticulation and a return of fine branny desquamation. No ocular or genital lesions were observed.

After the fifth week symptoms were prominent; these were strikingly like those noted in experimental thiamin deficiency. Mild depression progressed to extreme lassitude, somnolence and in two instances a mild panic state. Muscle pains, hyperesthesia without demonstrable neurologic changes and localized paresthesias were complained of by all. After the tenth week anorexia occurred with occasional nausea. Two patients complained of precordial distress and showed definite electrocardiographic changes.

Examinations of the blood at frequent intervals showed a definite diminution in hemoglobin content, the number of erythrocytes and the volume of packed red cells, there was slight increase in bile pigments and striking rise in the serum cholesterol. Determinations of biotin excretion in the urine showed that after seven to eight weeks of the experimental regimen the subjects excreted from 3.5 to 7.3 micrograms of biotin in 24 hours as compared with 29 to 52 micrograms excreted by individuals taking a normal diet.[3]

Treatment with an injectable biotin concentrate[4] has been completed in three of the four volunteers. The daily dose, administered in three aliquots, has varied from 75 to 300 micrograms; 150 micrograms seemed the minimal amount required for prompt relief. Depression, muscle pains, precordial distress and anorexia were abolished on the third to fifth days of treatment. Active distaste for the diet was replaced by willingness, even eagerness to eat it, although there was no significant increase in the amount consumed. The striking ashy pallor of skin and mucous membranes disappeared in four days. The elevated level of serum cholesterol was significantly reduced after

[1] From the University of Georgia School of Medicine and the University Hospital, Augusta, Ga., and the Division of Chemotherapy, National Institute of Health, Bethesda, Md.

[2] This investigation was made possible by grants-in-aid by the John and Mary R. Markle Foundation and an anonymous donor and by donations of vitamins by many manufacturers. We gratefully acknowledge the constant help and valuable suggestions of Dr. R. E. Butler and the technical assistance of Mrs. Marjorie Bee.

[3] Biotin determinations made by Dr. H. Isbell, of the National Institute of Health.

[4] Biotin concentrate supplied by the S.M.A. Corporation, Chagrin Falls, Ohio.