

of similarly high activity by animal passage. These conclusions stand unaltered by the present findings. Whether the substances present or formed in these different instances are identical, remains to be demonstrated. Activation of pyridoxine in this manner by heating *in vitro* is not an effect of amino acids alone. Such substances as thioglycollic acid, ammonia, and others also promote the change.

One other strain of *S. lactis* tested behaves the same as that used in this study. Yeast is not dependent upon such a change in the pyridoxine molecule before it is able to use it. Other organisms have not been studied in this regard. Landy and Dicken² have recently recommended *Lactobacillus casei* as a test organism for the assay of pyridoxine in natural materials. In our experience, this assay is subject, in a lessened degree, to the same disturbing influences previously outlined¹ and those mentioned above. For ex-

ample, in one assay carried out according to their procedure, Difco yeast extract contained 2.0, 0.6 or 0.35 mg of pyridoxine per gram as determined after autoclaving the test for 10, 20 or 40 min, respectively. Yeast assay³ gives 0.015 mg pyridoxine per gram of this material. It is evident that much more investigation into forms of pyridoxine which are physiologically active for animals is required before any organism can be used routinely for the estimation of "vitamin B₆" in natural materials.

Summary. Synthetic pyridoxine is almost inactive as a growth factor for *S. lactis* R in a pyridoxine-free medium if heat sterilization is avoided. Autoclaving media which contain pyridoxine greatly increases its activity for this organism. The same effect was achieved in varying degrees by autoclaving pyridoxine at neutrality with individual amino acids. Cystine and glycine were most effective in producing this change.

² Landy, M., and Dicken, D. M., *J. Lab. Clin. Med.*, 1942, **27**, 1086.

³ Williams, R. J., Eakin, R. E., and McMahan, J. R., *Univ. Texas Pub.*, 1941, No. 4137, 24.

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Biotin Deficiency in the Rat.

GLADYS A. EMERSON AND JOHN C. KERESZTESY.

From the Merck Institute for Therapeutic Research, Rahway, N.J., and Research Laboratories of Merck & Co., Inc.

A number of investigators have reported the occurrence of depigmentation of the pelage of rats maintained on egg-white containing rations. György and co-workers^{1,2} noted a brownish discoloration of the black fur, or single gray hairs interspersed in the fur without generalized graying, or both. Sullivan and Nicholls³ also observed a diffuse, partially decreased pigmentation in some animals receiving egg-white containing ra-

tions. Symmetric patterns of grayness such as produced by a deficiency of pantothenic acid were not observed. In the present study the occurrence of a symmetrical achromotrichia is described in black rats maintained on dried, fresh egg-white containing rations after curative therapy had been initiated by the feeding of biotin. The pattern was the reverse of that observed in pantothenic acid deficiency.

A comparison was made of several diets in respect to the production of the biotin deficiency syndrome and the alleviation of symptoms with biotin therapy.

Experimental. Littermate weanling male rats were segregated into each of 6 dietary

¹ György, P., in Pfaundler, A., and Schlossmann, M. v., *Handbuch der Kinderheilkunde*, 1935, **10**, 55.

² György, P., and Poling, C. E., *Proc. Soc. Exp. Biol. and Med.*, 1940, **45**, 773.

³ Sullivan, M., and Nicholls, J., *Arch. Derm. and Syph.*, 1942, **45**, 295.

TABLE I.
Composition of Diets.

Ingredients in diet	G per 100 g					
	19	20	21	22	23	24
Egg-white*	50	50	30	30	15	15
Casein (Harris)	—	—	—	—	15	15
Sucrose	34	24	54	44	52	54
Crisco	10	10	10	10	10	10
Yeast (brewers')	—	10	—	10	—	—
Salts No. 4	4	4	4	4	4	4
Cod liver oil	2	2	2	2	2	2
Dried whole liver	—	—	—	—	2	—

Addenda Diets: 19, 21, 23 and 24.

	Mg per 100 g
Thiamin	0.8
Riboflavin	1.6
Pyridoxine	0.8
Ca pantothenate	5.0
Nicotinamide	10.0
Choline Chloride	100.0

*Dried fresh flake—Henningsen Bros., 60 Hudson Street, New York City.

groups (10 animals in each lot).

The diets were shown in Table I.

The first evidence of biotin deficiency (loss of the guard hairs leaving a soft, downy mole-like coat) was noted in all animals after 3-4 weeks on diet. An exfoliative dermatitis of the abdomen followed, progressing to a more or less general alopecia. The "spectacle eye" was observed in a large percentage of the animals, particularly those maintained on diets 23 and 24. The abnormality in gait, typical of the deficiency, was seen in 8 of the rats and again 6 of the 8 cases were with the animals receiving diets 23 and 24. This abnormality of locomotion, a type of hopping gait, developed within 4 weeks. Nielson and Elvehjem⁴ noted this type of gait only after their animals, on 10% egg-white containing rations, had been on experiment for 9-12 weeks. These workers were able to produce this condition in 9 weeks only if the diets were low in fat. The presence of high amounts of fat in their diets slightly delayed the onset of the paralysis.

As is seen in Table II, the most satisfactory growth occurred with the group of rats receiving 15% egg-white and 15% casein but

TABLE II.
Gain in Weight of Rats After 40 Days on Egg-white Containing Diets.

Diet	Gain in wt, g
19	94
20	125
21	105
22	110
23	147
24	116

with 2% of dried liver incorporated in the ration (diet 23). The rapid growth of the animals may account for the development of a more complete deficiency picture. The apparent superior growth of the rats receiving diet 20 is deceptive in that 4 of the initial 10 rats died during the second or third week of the experiment.

After approximately 35 days on test, the weights plateaued for the several groups and supplementation with crystalline biotin was initiated. The rats receiving 50% and 30% egg-white were segregated into 2 like groups (as judged from weight and severity of the dermatitis etc.) and were dosed orally by stomach tube with 1 or 2 μ g of free biotin 6 times weekly. The animals on the 15% egg-white diets were given 0.5 or 1 μ g. After 3 days of supplementation a marked improvement was evident; the heretofore erythematous areas were blanched and the animals were clean in appearance. The "wash-board"-like scaly dermatitis of the abdomen had largely disappeared. The abnormality in gait was no longer evident after one week of therapy; a finding in agreement with that of Sullivan and co-workers⁵ who found that rats likewise affected were relaxed and assumed a normal posture and gait after 3 or 4 days of biotin treatment. These investigators were unable to find any anatomical change in the central nervous system. They suggested that the hypertonicity might be due to irritability and that the rigidity was not myotonic in origin. Nielson and Elvehjem⁴ noted a complete cure after 3 to 4 weeks of biotin therapy. A marked growth response was noted in all animals.

⁴ Nielson, E., and Elvehjem, C. A., *J. Biol. Chem.*, 1942, **144**, 405.

⁵ Sullivan, M., Kolb, L., and Nicholls, J., *Bull. Johns Hopkins Hosp.*, 1942, **70**, 177.

TABLE III.
Growth Response to Biotin.

Biotin 6 times weekly	No. of rats	Diet	Wt gain 30 days, g
1	5	19	79
2	5	19	74
1	3	20	74
2	3	20	66
1	4	21	44
2	4	21	74
1	5	22	54
2	4	22	72
0.5	4	23	53
1	4	23	64
0.5	4	24	54
1	5	24	68

The average weight gains are shown in Table III.

The quantities of biotin fed were, in all probability, sub-optimal since improvement was not continuous *e.g.*, the spectacle eye condition persisted in some animals after 5 weeks of biotin therapy. Employing different diets, Nielsen and Elvehjem⁶ produced a complete regression of this condition after 2-3 weeks of biotin administration.

The most striking observation was in the

⁶ Nielsen, E., and Elvehjem, C. A., *Proc. Soc. Exp. Biol. and Med.*, 1941, **48**, 349.



FIG. 1.
Lateral graying of the pelage. Biotin-depleted rat—fed biotin.

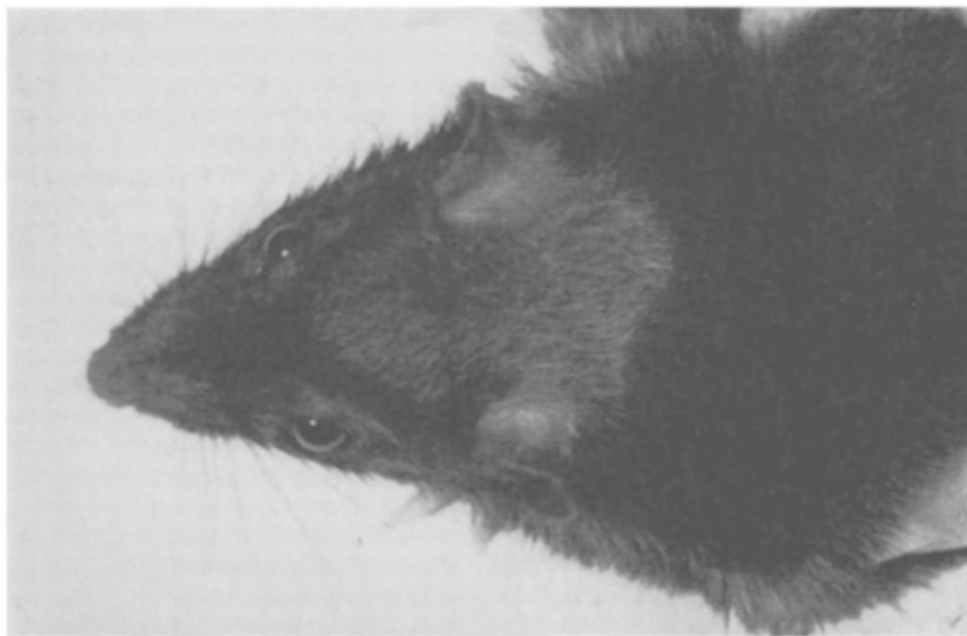


FIG. 2.
Diamond shaped depigmented area on head. Biotin-depleted rat—fed biotin.

pelage, for 29 out of 30 black rats, irrespective of diet, showed a symmetrical graying of the ingrowing fur, after 12 days of supplementation. The gray pattern outlined the lateral aspect of the head and trunk and in most cases extended from the tip of the nose to the base of the tail (Fig. 1). A graying or rusting of a triangular or diamond-shaped area on the top of the head was observed in several cases (Fig. 2). It is of interest to note that the distribution of the pattern is the reverse of that observed in rats deprived of, or receiving sub-optimal intakes of pantothenic acid. The graying persisted to some extent even after 5 weeks of therapy and was not infrequently accompanied by rusting.

Summary. It is possible to reduce materially the time necessary to produce in the rat a fully, developed syndrome of biotin deficiency by feeding a diet containing in addition to 15% dried egg-white and 15% casein, 2% dried liver. On this diet most of the signs develop within less than 4 weeks.

Biotin depleted animals fed small doses of biotin curatively showed a characteristic, symmetrical lateral graying of the pelage that persisted to some extent even following 5 weeks of biotin supplementation.

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The Proteus X Bacilli and the Weil-Felix Reaction.

A. POMALES-LEBRÓN AND P. MORALES-OTERO.

From the Department of Bacteriology, School of Tropical Medicine, San Juan, Puerto Rico.

The probable existence of typhus fever of the endemic type in Puerto Rico^{1,2} has recently aroused much interest among the medical profession concerning the reliability of the Weil-Felix reaction, under local conditions, in the diagnosis of this disease.

During the course of an investigation of the proteus group of bacilli, 72 cultures of proteus X organisms utilized in the diagnosis of certain rickettsial diseases, kindly supplied to us by 30 Public Health laboratories in the United States, have been studied.

Sixty-seven out of the 72 cultures investigated gave typical biological and agglutination reactions, but 5 cultures reacted atypically in one way or another.

The carbohydrates (20% aqueous solutions) were sterilized by filtration and added to the sterile medium (Difco's phenol red broth base) to obtain a final concentration of approximately 1%. For the production of

H₂S, indol and gelatin liquefaction, lead acetate agar, tryptophane broth and nutrient gelatin (all Difco products) respectively were used. Beef extract broth to which methylene blue was added to give a final concentration of 1:5000 was utilized in testing for the reduction of this dye. Rabbits were immunized by intravenous injection of fresh living suspensions.

Fermentation and other reactions, except indol, were read after 18 hr, 48 hr and one week. The production of indol was tested for after 48 hr. Alcohol-treated suspensions were used in all the agglutination tests.³

No difference was noted in the action on gelatin among the different cultures of X type bacilli studied. It was interesting to note the complete absence of agglutination, with a known typhus serum of high titer, of 21 out of the 23 proteus X-2 cultures, and the slight agglutination of 3 out of the 14 proteus XK cultures tested.

All the X-2 and X-19 cultures fermented saccharose, maltose, dextrose, salicin and galactose with the production of acid and gas and xylose and glycerol with the production

¹ Pons, J. A., *Bol. Asoc. Méd. de P. R.*, 1940, **32**, 196.

² Riera López, S., Watt, J., and Doull, J. A., *P. R. Jour. Pub. Health and Trop. Med.*, 1942, **17**, 216.