13317

Cure of Spectacle Eye Condition in Rats with Biotin Concentrates.*

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The "spectacle eye" condition in rats has been described by several investigators, namely, Goldberger and Lillie,[1] Bourquin and Sherman,[2] Robinson and Newton,[3] Lepkovsky, Jukes and Krause,[4] Sjollema,[5] Karrer, *et al.*,[6] Mackay and Barnes,[7] Oleson, *et al.*,[8, 9] and Pavcek and Baum.[10] During studies on the effect of pantothenic acid on rats reared on various rations it was observed that this typical denudation around the eyes resulted when egg white was added to our basal ration. The eye condition was well defined and this deficiency seemed worthy of further study.

Experimental. The unmodified basal ration had the following percentage composition: sucrose 73, Labco casein 18, salts IV 4, and corn oil 5. Choline hydrochloride was added to this basal at 1 g per kg of ration. The B complex vitamins were fed daily in supplement dishes at the following levels: thiamin hydrochloride 20 γ,

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riboflavin 20 γ, pyridoxine 25 γ and calcium pantothenate 100 γ. Two drops of haliver oil were given each rat per week. The egg white used in these studies will be referred to as "untreated," if the commercial dried egg white was used and "heated," if treated in the following manner: 300 g of dried egg white was stirred in 2 liters of water and the suspension was heated in a steam oven for 18 hours, dried and ground in a mill to a fine powder.

Twenty-one-day-old albino and piebald rats from our colony were used throughout all the experiments. On the above ration the rats grew well and did not develop any symptoms even if left on the ration for 8 weeks. When the casein was completely replaced by "heated" egg white all the animals developed spectacle eye at the end of 5 weeks. On this ration 1 litter of 8 rats developed the eye condition at the end of 3 weeks; however in all the other groups the symptoms were not noticed until the end of 5 weeks.

When 60% of "heated" egg white was incorporated into the ration at the expense of the casein and part of the sucrose, the eye condition did not develop more rapidly. However, a growth variation resulted and it was observed that the animals which grew better exhibited the more severe eye denudation.

In another series 4 animals were placed on the basal containing 18% of "heated" egg white and with the B vitamins 10 mg of inositol (Pfanstiehl) per day was given as suggested by Pavcek and Baum.[10] All animals in this series developed spectacle eye at the end of 5 weeks. Rats showing the syndrome were given 10 mg of inositol per day for 4 weeks and showed no improvement.

Since the heat treatment seemed to be ineffective, in reducing the egg white effect 10% of the casein was replaced by "untreated" egg white. The eye denudation was again produced as quickly and effectively as with the higher levels of "heated" egg white. Four animals were placed on the basal in which 5% of the casein was replaced by "untreated" egg white and at the end of 5 weeks 2 animals showed spectacle eye while at the end of 7 weeks only 1 of the remaining 2 rats showed slight denuding. When 1% of the casein was replaced by "untreated" egg white none of the animals exhibited denuding at the end of 8 weeks.

In order to study the effects of corn oil and pyridoxine in this deficiency a group of rats was placed on a basal diet in which 10% of the casein was replaced by "untreated" egg white. In a group of 4 rats the usual 5% of corn oil was raised to 10% and at the end of 5 weeks complete denuding was again noticed. In another series of 4 animals the pyridoxine was increased to 125 γ per day and no

protection was observed. An additional 4 rats were placed on a ration containing 10% of "untreated" egg white, 10% of corn oil and 125 γ of pyridoxine, and no protection was noted. One deficient animal was given 500 γ of pyridoxine per day for 4 weeks and no improvement was observed. Inositol was also inactive when fed in conjunction with 10% "untreated" egg white.

TABLE I.
Spectacle Eye Incidence.

No. of rats	% egg white	Treatment	Modification of ration	Incidence
14	18	Heated		14
10	60	"		10
4	18	"	10 mg Inositol	4
7	10	"Untreated"		7
4	5	"		2
3	1	"		0
4	10	"	10% Corn Oil	4
4	10	"	100 γ Vitamin B_6	4
3	10	"	10% Corn + 100 γ B_6	3
2	10	"	10 mg Inositol	2
3	5	"	2 γ Biotin (orally)	0
3	5	"	2 γ " (inj.)	0
3	10	"	2 γ " (orally)	0

Pantothenic acid did not have any protective effect on the development of this syndrome. The egg white content of the diet was varied and 25, 50 and 100 γ levels of pantothenic acid were fed. In a few cases the improvement in growth resulted in more severe denuding, but the onset of the deficiency was not materially shortened.

Many variations in the original basal ration have been studied, namely, replacing the sucrose by lactose, dextrin, starch, cerelose and polished rice, increasing and decreasing the levels of fat and casein but in all cases no spectacle eye was noted. In one series a ration containing cerelose as the carbohydrate and deficient in pantothenic acid showed slight spectacle eye when 3 mg of para-aminobenzoic acid was fed daily over a 7-week period.

Since the presence of egg white in the ration allowed this deficiency to develop it was suggested that biotin might be involved. The rôle of biotin was studied from 3 approaches. In one group of 3 rats showing definite spectacle eye, the egg white was taken out of the ration and cures were noted at the end of 3 weeks. Thus it appeared that the ration contained an appreciable amount of biotin which was unavailable to the animals when egg white was present. We had at our disposal a biotin concentrate (S.M.A. Corporation) No. 5000, and this was injected at a level to supply 2 γ of biotin per day and

after 5 days slight pigmentation was noted at the edge of the eyelids. The normal restoration of the hair was complete after 2-3 weeks of treatment. At present 14 rats have been cured in this manner without removing the egg white from the ration. Before we noted that 5% of "untreated" egg white only allowed half of the animals to show spectacle eye, an attempt was made to prevent the development of this symptom. In this group 3 animals were given 2 γ of biotin per day orally and 3 animals were given the same level by injection and at the end of 8 weeks all the group was normal. Since this level of egg white appeared to be minimum for the production of spectacle eye, another group of rats receiving 10% "untreated" egg white was given 2 γ of biotin per day from the start of the experiment and complete protection was observed.

With 5% of the ration as "untreated' egg white the injected animals grew on an average of 13 g more than the ones receiving the same level of biotin orally over a 7-week period. On a ration containing 5% of "untreated" egg white the weight gain of the rats receiving 2 γ of biotin per day over the control group is very significant. For a 7-week period the average weight gain of 4 control animals was 100 g and an average weight gain for 4 animals receiving biotin was 172 g or an increase of 72 g over the control group.

After spectacle eye has developed and if the animals are left on the ration an additional 2-4 weeks there develops a bilateral denudation over the hind quarters and extending up the midline of the back to the head. This further denudation was also noted when 2 γ of biotin was administered in our curative technic with 10% of "untreated" egg white in the ration.

Summary. The spectacle eye syndrome in rats has been produced very consistently by the inclusion of egg white in a synthetic diet which allows good growth in the absence of egg white. Pantothenic acid at levels up to 100 γ per day does not prevent the onset of this condition. Corn oil and pyridoxine or the combination of the two are without effect. Inositol has neither a preventative nor a curative action under these conditions. A biotin concentrate at a level of 2 γ of biotin per day is specific for the prevention and cure of this syndrome. The cure is first noticeable after 5 days and complete restoration of the hair results after 2-3 weeks of treatment. A definite weight gain is noted with rats receiving the biotin concentrates.