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Spectacled Eye Condition in Rats.*

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In a previous paper we reported the occurrence of a specific deficiency syndrome in rats which was termed "spectacled eye condition".[1] This condition was differentiated from acrodynia, paralysis and hemorrhagic disease. The eye erosions were cured by the fullers earth filtrate of liver extract but not by the eluate or crystalline vitamin B_6. Corn oil appeared to interfere with the production of the spectacled eye symptoms. In this paper we wish to report further studies on this condition.

The ration used previously (ration J_{29}) had the following composition: sucrose 78%, purified casein 18%, and salts IV 4%, supplemented with 1.2 mg thiamin, 2.0 mg riboflavin, 300 mg choline and 300 mg nicotinic acid per kilo of diet. Two drops of haliver oil were given each rat per week.† The treatment of the animals and preparation of the diet were as previously described.[1] This ration was not entirely satisfactory for these studies as many rats failed to survive long enough to develop the specific symptoms desired. Consequently we sought a suitable supplement which would afford survival and a certain amount of growth and yet permit the development of severe deficiency symptoms.

Two materials were tested at several levels, a dehydrated cereal grass‡ and a liver extract prepared by Dr. Klein.§ The results are shown in Table I and include the average weights at 5 weeks and the deficiency symptoms observed. These symptoms are identical with those already described in detail.[1] In addition to the symptoms listed

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1 Oleson, J. J., Bird, H. R., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1939, **127**, 23.

† The thiamin, nicotinic acid and vitamin B_6 were supplied by Merck and Company, haliver oil by Abbott Laboratories and the alpha-tocopherol by Hoffman-LaRoche Company.

‡ This is a dehydrated cereal grass furnished by the Cerophyl Laboratories, Kansas City, Missouri.

§ This liver fraction (No. 36872) was furnished by Dr. David Klein, Wilson Laboratories, Chicago, Illinois.

TABLE I.
Effect of Liver Extract and Grass on Growth and Symptoms.

Ration	Supplement	No. of rats	Initial wt, g	Wt at 5 weeks, g	Symptoms
J_{29}	1% L.E. 36872	2	41	71	Dermatitis, spectacled eye, paralysis, scaly ears, hemorrhagic disease.
J_{29}	2% L.E. 36872	2	41	104	Dermatitis, sl. spectacled eye
J_{29}	4% L.E. 36872	2	40	124	No symptoms
J_{29}	1% grass 1109-5B	2	37	46	Dermatitis, paralysis
J_{29}	2% " 1109-5B	2	43	57	Dermatitis, paralysis, 1 dead
J_{29}	4% " 1109-5B	2	37	57	1 sl. dermatitis 1 spectacled eye
J_{29}	6% " 1109-5B	2	36	64	No symptoms
J_{29}	8% " 1109-5B	2	38	59	" "

in the table, all of the rats showed severe scaly tails and paws, typical of linoleic acid deficiency and also the testicle atrophy in males and delayed vaginal openings in females reported by McKibbin, *et al.*[2] The rats receiving the liver extract grew considerably better than those on the grass, which evidently lacks certain factors present in liver. Since the rats receiving ration J_{29} plus 1% of the liver extract showed severe symptoms, this level was used for all of the assays reported in this paper.

Rats receiving ration J_{29} plus 1% liver extract developed acrodynia and the spectacled eye condition in about 6 weeks. The addition of 10 gamma of crystalline vitamin B_6 per rat per day cured the acrodynia in about 3 weeks but the eye erosions remained unchanged. This further demonstrates the inactivity of vitamin B_6 in curing the spectacled eye syndrome. If vitamin B_6 crystals were fed from the beginning, the acrodynia was prevented and the onset of the eye condition was delayed somewhat, due to the increased food consumption of the rats, but when the condition developed it was as severe as in the other cases.

Of the materials tested for their ability to cure the spectacled eye condition, corn oil proved to be the most active. Two or 3 drops of corn oil per day would heal severe eye erosions in 3 weeks. This supplement would also cure the scaly tails and cause the testes of the rats to return to normal. Alpha tocopherol was completely inactive at 50 gamma per week with respect to the eye condition or testes.[2]

The fuller's earth filtrate of liver extract was again found to be active, although the cures were slower than in the case of corn oil.

[2] McKibbin, J. M., Oleson, J. J., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 25.

Healing took place in 4 to 6 weeks when the filtrate, made by our usual method,[1] was fed equivalent to 250 mg liver extract per rat per day.

A purified concentrate of pantothenic acid was fed at a level furnishing 100 gamma of pantothenic acid per rat per day. This concentrate, treated with alkali to completely inactivate the pantothenic acid, was also fed at the same level. The preparation of these fractions was described by Oleson, *et al.*,[3] and their pantothenic acid content determined by bacterial assay.[4] Both of these supplements showed equal activity, but appreciably less potency than the liver filtrate. Healing took place in about 8 weeks. This definitely eliminates pantothenic acid as the factor concerned. The results are given in Table II.

From the evidence presented in this and our earlier work, we conclude that there is present in liver extract a factor which prevents the characteristic erosions about the eyes in rats. Its relation to other factors which we have studied, namely pantothenic acid,[3] factor W,[5] and the factor preventing nutritional achromotrichia,[6] can be at least indicated. The slow activity of the pantothenic acid concentrate and the apparent stability of the spectacled eye factor to alkali eliminates this vitamin. In our earlier work the spectacled eye condition was cured by filtrates having very little factor W potency,[1] which at least indicates that factor W is not concerned. The inability of corn oil to prevent nutritional achromotrichia (greying of the hair) is indirect evidence that these 2 factors are not identical. Whether this factor is identical with the so-called "accessory factor" in the filtrate, required in addition to vitamin B_6 for the complete healing of acrodynia

TABLE II.
"Spectacled eye" Preventing Potency of Supplements.

No. of rats	Ration	Supplement	Avg healing time, wk
5	J_{29} + B_6 + 1% liver extract	Corn oil	3
4	J_{29} + B_6 +1% liver extract	Alpha tocopherol	Inactive
6	J_{29} + B_6 + 1% liver extract	Fuller's earth filtrate	4-6
2	J_{29} + B_6 + 1% liver extract	Pantothenic acid concentrate	6-8
2	J_{29} + B_6 + 1% liver extract	Alkali inactivated pantothenic acid concentrate	6-8

[3] Oleson, J. J., Woolley, D. W., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 151.

[4] Snell, E. E., Strong, F. M., and Peterson, W. H., *Biochem. J.*, 1937, **31**, 1789.

[5] Elvehjem, C. A., Koehn, C. J., and Oleson, J. J., *J. Biol. Chem.*, 1936, **115**, 107.

[6] Oleson, J. J., Elvehjem, C. A., and Hart, E. B., PROC. SOC. EXP. BIOL. AND MED., 1939, **42**, 283.

is not certain as yet, but the action of corn oil suggests a possible relationship.[7, 8, 9] It is to be emphasized, however, that we observe these eye lesions in the complete absence of the symptoms of acrodynia. The existence of eye erosions distinct from acrodynia has been recently reported by György and Eckardt,[10] but whether their "type III" dermatitis is the same as the spectacled eye condition remains to be shown. The solution of the acrodynia problem may give the solution to the "spectacled eye" problem.

The fact that the scaly tail condition also appears in the rats which show the "spectacled eye" condition and the fact that corn oil cures both conditions suggests a possible relationship between these two conditions. However, it should be pointed out that liver extract and liver extract fractions cure the spectacle eye condition without producing any effect on the scaly tail condition. It should also be pointed out that McKibbin, *et al.*,[2] have produced the scaly tail condition on diets containing 4% liver extract which supplies ample amounts of the "spectacled eye" factor.

Summary. The curative action of corn oil in healing the "spectacled eye" condition has been demonstrated. The identity of the factor in liver which prevents these symptoms with the other members of the vitamin B complex has been discussed. Direct and indirect evidence is offered that, besides the crystalline vitamins contained in the ration, the factor concerned is not identical with pantothenic acid, factor W and the factor preventing nutritional achromotrichia. A possible relationship of this factor to the so-called "accessory factor" preventing acrodynia and of this condition to the "type III" dermatitis of György and Eckardt is pointed out. Further, the relation of this condition to the deficiency of the essential fatty acids is discussed.

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7 Birch, T. W., *J. Biol. Chem.*, 1938, **124**, 775.

8 György, P., *J. Am. Chem. Soc.*, 1938, **60**, 983.

9 Kuhn, R., and Wendt, G., *Ber. Deutsch. Chem. Gesell.*, 1938, **71**, 780.

10 György, P., and Eckardt, R., *Nature*, 1939, **144**, 512.