

JUNE, 1942

RESEARCH BULLETIN 343

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UNIVERSITY OF MISSOURI COLLEGE OF AGRICULTURE  
AGRICULTURAL EXPERIMENT STATION

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# The Relation of Biotin to Perosis in Chicks

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(Publication Authorized June 3, 1942)



COLUMBIA, MISSOURI

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# The Relation of Biotin to Perosis in Chicks

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## ABSTRACT

Perosis in chicks was observed on two different basal rations. One contained alcohol-extracted casein, the other contained raw egg-white. Both were supplemented with all the vitamins, except biotin, that are required by the chick. Every chick on both rations developed a typical dermatitis but some on the casein diet did not develop perosis. One gamma of biotin per chick per day prevented both the perosis and the dermatitis in the chicks which received casein. Intramuscular or intraperitoneal injection of 2 to 5 gamma of biotin per chick per day prevented the dermatitis, but it did not completely prevent perosis in the chicks that received raw egg-white. When the egg-white was heated oral administration of 1.0 gamma of biotin prevented dermatitis, but it did not prevent perosis. These facts indicate that at least one factor in addition to biotin is concerned with this type of perosis.

Several investigators, Eakin, McKinley and Williams ('40), Hegsted, Oleson, Mills, Elvehjem, and Hart ('40), and Ansbacher ('41), have shown that chicks develop dermatitis and grow at a sub-normal rate if their diets do not contain biotin. A recent report by Hegsted, Mills, Briggs, Elvehjem and Hart ('42), states that crystalline biotin is as effective as the biotin concentrate they had used previously. During some of our earlier attempts to determine the amount of biotin required for growth, the chicks which did not receive biotin developed, in addition to the typical dermatitis, a severe perosis. Our observations on the perosis caused by a deficiency of biotin are described in this report.

## EXPERIMENTAL

The chicks were day-old single-comb White Leghorns. The biotin was supplied as a concentrate (S. M. A. No. 200).<sup>1</sup> Since the crude biotin concentrate did not prevent the perosis which developed on certain types of rations, it seemed unnecessary to use the more expensive crystalline product. The unrecognized vitamins required by the chick were supplied as a fuller's earth eluate of liver or of yeast

<sup>1</sup>S. M. A. Corporation, Cleveland, Ohio.

extract and was prepared as follows. Fresh beef liver, or dried yeast, was extracted with boiling water. The water extract was concentrated to a convenient volume and a large portion of the inactive material was precipitated by adding enough 95 o/o alcohol to give a concentration of 50 per cent. After removing the precipitate the filtrate was concentrated until it contained approximately 10 per cent dry matter, and was then treated with fuller's earth at a pH of 1.0. It was adsorbed 4 times in all, with 20 grams of fuller's earth at each adsorption for each 100 grams of dry matter. The combined adsorbates were eluted 3 times with 600 cc. portions of 0.2 N ammonia for each 100 grams of adsorbate. The eluates prepared from yeast and liver extracts had about the same activity. The percentage composition of the basal rations is given in Table 1.

TABLE 1. COMPOSITION OF BASAL RATIONS

Ration	Corn Starch	Sucrose	Casein, acid-washed	Casein, acid-washed and alcohol extracted	Raw Egg-white	Heated Egg-White	Gelatin	Lard	Salt Mixture <sup>1</sup>	Cellu-flour	Fuller's earth eluate of liver or of yeast extract	Each ration contained the following vitamins	
												Per 100 grams of ration	
I	30		35									Vitamin A <sup>2</sup>	6000 I.U.
II		42		30			10	17	4	3	1	Vitamin D <sup>2</sup>	850 "
III		42			30		10	10	4	3	1	Thiamin Chloride <sup>3</sup>	0.8 Mg.
IV-A	42			30			10	10	4	3	1	Riboflavin <sup>3</sup>	1.6 "
IV-B	37			15	20		10	10	4	3	1	Pyridoxine <sup>3</sup>	1.2 "
IV-C	37			25	10		10	10	4	3	1	Calcium Pantothenate <sup>3</sup>	2.0 "
V		42				30	10	10	4	3	1	Choline Chloride <sup>3</sup>	200.0 "
												Inositol <sup>3</sup>	100.0 "
												Nicotinic Acid <sup>3</sup>	1.0 "
												p-Aminobenzoic Acid	30.0 "
												Alpha-tocopherol <sup>3</sup>	0.8 "
												2-Methyl-1, 4-naphthoquinone <sup>3</sup>	1.0 "

<sup>1</sup>Hubbell, Mendel, and Wakeman '37 salt mixture No. 351 with MnSO<sub>4</sub> added to make 0.025 Mn in the ration

<sup>2</sup>A-D Concentrate generously supplied by Mead Johnson and Co., Evansville, Indiana

<sup>3</sup>Generously supplied by Merck and Co., Rahway, New Jersey

### Perosis on Casein Rations

The chicks in the first experiments received Ration I, which contained acid-washed casein and corn starch. Some of the chicks, Groups 1, 2 and 3, received only the basal ration while others, Groups 4, 5 and 6, received biotin in addition. These data are summarized in Table 2, Series A. Every chick in Group 1, which did not receive biotin, developed severe perosis in 4 to 5 weeks. Some of the chicks

in Groups 2 and 3, under observation at a different time, did not develop perosis but all of them developed dermatitis. Every chick in Groups 4, 5 and 6, which received biotin, was normal. One gamma of biotin per chick per day was sufficient to prevent both the perosis and the dermatitis, but the rate of growth was more rapid when the biotin was supplied at a higher level.

It seemed certain from these observations that biotin is required to prevent one type of perosis, but the fact that some chicks in Groups 2 and 3 failed to develop the disease even though they were not supplied with biotin, was disconcerting. As a possible suggestion it should be mentioned that the casein supplied to these chicks was a new preparation, and it may have contained more biotin than the one formerly employed. The casein was therefore purified further by extracting with boiling alcohol for 3 days in a Soxhlet extractor, and substituted for the acid washed casein. Likewise, sucrose was substituted for corn starch in Ration II, and these changes definitely increased the incidence of perosis, Table 2, Series B. Seventy-five per cent of the cockerels and 66.6 per cent of the pullets developed a severe perosis in Group 7, while the chicks which received the same basal ration and 1.0 gamma of biotin per chick per day in Group 8, were normal.

Although the constituents of the basal ration under some conditions apparently carried enough biotin, or some unidentified substance or both to prevent perosis, the amount was never sufficient to prevent dermatitis. Furthermore, the chicks which had perosis nearly always had more severe dermatitis than those which did not have perosis. These observations indicate that larger amounts of biotin are required to prevent dermatitis than are required to prevent perosis, but in view of data to be described later this interpretation may be incorrect.

### **Perosis on Egg-white Rations**

The occurrence in chicks of dermatitis caused by raw egg-white was first described by Lease and Parsons ('34), and their report has been amply confirmed. This was a very puzzling observation but it has been explained recently by Eakin, Snell, and Williams ('40, '41), and Gyorgy, Rose, Eakin, Snell, and Williams ('41), who obtained a fraction from raw egg-white in concentrated form which is capable of inactivating biotin in vitro. The active agent is called avidin or avidalbumin. A diet which protected rats from egg-white injury was no longer protective when this fraction was supplied with it.

TABLE 2. RELATION OF PEROSIS TO COMPOSITION OF RATION

Group	Biotin gamma/ chick/ day	No. of chicks	Final Wt. gms.	Age wks.	Mortal- ity o/o	Perosis	
						Incidence o/o	Index <sup>1</sup>
Series A. Basal ration contained corn starch and acid washed casein							
1	None	1M	355	6	---	100	33.3
		4F	339	6	---	100	43.0
2	None	2M	379	6	---	---	---
		4F	332	6	---	25	7.0
3	None	2M	275	5	---	---	---
		3F	229	5	---	---	---
4	1.0 orally	1M	412	6	---	---	---
		5F	378	6	---	---	---
5	2.0 orally	3M	466	6	---	---	---
		2F	350	6	---	---	---
6	3.0 orally	3M	542	6	---	---	---
		3F	440	6	---	---	---
Series B. Basal ration contained sucrose and acid washed and alcohol extracted casein							
7	None	4M	303	6	---	75	45.8
		3F	266	6	---	66.6	13.0
8	1.0 orally	3M	341	6	---	---	---
		2F	315	6	---	---	---
Series C. Basal ration contained sucrose and 30 o/o raw egg-white							
9	None	7	128	5	29	100	36.6
10(a)	2 and 3 injected	6	183	6	50	100	10.1
11(b)	2.5 and 5.0 injected	6	205	5	00	66.6	6.4
Series D. Basal ration contained corn starch and raw egg-white or raw egg-white and casein							
12(c)	None	4	90	6	75	100	27.7
13(d)	None	5	112	6	40	100	25.5
14(e)	None	5	125	6	80	100	15.5
Series E. Basal ration contained sucrose and 30 o/o of heated egg white							
15(f)	1.0 orally	4	73	2	--	100	severe perosis (discarded)
16(g)	II	5	147	4	20	100	33.3
17(h)	II	4	128	3	--	100	15.4

<sup>1</sup> Calculated by the method of Wilgus, Norris and Heuser '37.

- Intramuscular and intraperitoneal injection of 2.0 gamma per chick per day 1st week and 3.0 gamma per day afterwards.
- Injection of 2.5 gamma per chick per day 1st week and 5.0 gamma per day afterwards.
- 30 per cent raw egg-white.
- 20 per cent raw egg-white and 15 per cent casein.
- 10 per cent raw egg-white and 25 per cent casein.
- Egg-white heated 20 minutes in boiling water.
- Egg-white heated moist for 3 hours at 90 degrees.
- Egg-white autoclaved 6 hours at 15 pounds pressure.

Since raw egg-white inactivates biotin, a ration which contains this protein should supply the minimum quantity of available biotin. In order to test this hypothesis a basal ration which contains 30 per cent of raw egg-white, and sucrose as a source of carbohydrate, Ration III, was supplied to 3 groups of chicks, Table 2, Series C. Every chick, Group 9, which received this ration developed the first symptoms of perosis in 8 to 10 days. The chicks were unable to stand for any length of time and their gait indicated that walking was painful. As the disease progressed the hocks became enlarged and turned in, while the feet turned out. In severe cases the tendon of Achilles slipped out of the condyle. Dermatitis did not develop until about the 4th week.

The chicks in Group 10 received 2.0 gamma of biotin per chick per day during the first week and 3.0 gamma per day during the remainder of the experimental period. The biotin was injected either into the peritoneal cavity or into the breast muscle, but neither method of injection completely prevented perosis. A mild deformity developed in about 10 days, but after that time it seemed to become less severe. Some of the chicks were almost normal at the end of the experimental period, but others had enlarged hocks and perceptible bowing of the legs. The most characteristic symptom was an abnormal gait. The chicks in Group 11 received 2.5 gamma of biotin per day during the first week and 5 gamma daily thereafter, and they developed less marked symptoms of perosis than those in Group 10. Dermatitis did not develop in any of the chicks that received biotin.

Jukes and Bird ('42) reported recently that 50 to 70 per cent of the chicks which received a grain ration that contained 30 cc. of fresh raw egg-white per 100 grams of the ration developed perosis in 3 to 5 weeks. The disease was prevented by heating the egg-white or by intramuscular or intraperitoneal injection of 0.34 gamma of crystalline biotin per chick per day. Ansbacher ('40), also observed a fairly high incidence of perosis in chicks which received a ration that contained 0.1 gamma of biotin per gram of diet. In another series of experiments corn starch was substituted for sucrose and various amounts of casein were substituted for equal quantities of raw egg-white. These data are summarized in Table 2, Series D. The chicks in Group 12 received 30 per cent of raw egg-white, Ration IV-A, but no casein. Those in Group 13 received 20 per cent of raw egg-white and 15 per cent of casein, Ration IV-B. Those in Group 14 received 10 per cent of raw egg-white and 25 per cent of casein, Ration IV-C. Every chick in all three groups, that survived long enough, developed both perosis and dermatitis. Those that received the smaller amounts

of egg-white developed perosis later than when the ration contained sucrose and no protein other than egg-white, either raw or heated. The time required for the dermatitis to developed was about 28 days regardless of the nature of the carbohydrate or protein.

### **Perosis on Heated Egg-white Rations**

It has been known for some time that egg-white after exposure to moist heat does not induce dermatitis, and it seemed desirable therefore to determine whether it still induces perosis. The egg-white, used in Ration V, was dissolved in water and heated under the following conditions. (1) Boiled for 20 minutes by passing live steam into the solution. (2) Heated in an oven for 3 hours at 90 degrees. (3) Autoclaved 6 hours at 15 pounds pressure. These data are summarized in Table 2, Series E. Regardless of how the egg-white was heated, and in spite of the fact that each chick received orally 1.0 gamma of biotin daily, every individual in Groups 15, 16 and 17, developed perosis in about the same time as those that received the raw egg-white. None of them developed dermatitis.

### **THE ANTIPEROSIS COMPLEX**

The complex nature of the dietary factors concerned in perosis in chicks is now apparent, and at least four factors are involved. These are manganese, choline, biotin, and an unidentified organic nutrient which is present in an eluate of a fuller's earth adsorbate of a water extract of beef liver (Richardson and Hogan, '41). The knowledge now available does not preclude the possibility that a fifth factor is involved.

### **DISCUSSION**

The results with rations which contain casein and those which contain egg-white are not in complete agreement. It is very difficult to produce this type of perosis consistently when the rations contain casein and corn starch, but there is no difficulty in producing dermatitis. It was assumed at first therefore that some preparations of casein, and possibly of starch, carry enough biotin as an impurity to prevent perosis but not enough to prevent dermatitis. According to this hypothesis larger quantities of biotin are required to prevent dermatitis than are required to prevent perosis. This agrees with the observations reported by Jukes and Bird ('42). This conclusion, however, is not consistent with the data which are obtained with rations that contain sucrose and egg-white. Chicks which receive rations containing egg-white develop perosis in 8 to 14 days, but they do not develop dermatitis until about 28 days. Furthermore, the injection of 2.0 to 5.0 gamma of biotin per chick per day does not

completely protect them from perosis, but it does completely protect them from dermatitis. It might be argued that the egg-white inactivates some of the biotin even though it is injected into the body. In this case, it must be assumed that some of the avidalbumin is absorbed unchanged from the digestive tract, and that larger amounts of biotin are required to prevent perosis than are required to prevent dermatitis. In agreement with other investigators, we find that the biotin-binding property of egg-white is destroyed by heat if dermatitis is taken as an indication of biotin deficiency. However, this property is not destroyed, if the development of perosis is the criterion. Although the chicks that received heated egg-white and biotin were entirely free of dermatitis, they developed perosis in 8 to 10 days. In addition, the perosis was definitely more severe than it was in chicks that received raw egg-white and biotin.

It is concluded from the observations which are described in this paper that biotin is the sole factor concerned in the prevention of dermatitis but there may be an additional factor concerned in preventing the perosis that develops on the diets described. There is also the possibility that egg-white contains a substance that is capable of inactivating the unidentified antiperotic factor which is present in the fuller's earth eluate. If there is such a compound it is not identical with avidalbumin, at least it is not destroyed by heat.

### SUMMARY

A high incidence of perosis occurs in chicks which receive a ration that contains sucrose and acid-washed and alcohol-extracted casein, supplemented with all the vitamins required by the chick except biotin. All the chicks develop the typical dermatitis even though they do not develop perosis. Both abnormalities develop in about 28 days. Both are prevented by 1.0 gamma of biotin daily per chick.

This type of perosis was not produced consistently when corn starch was substituted for the sucrose and when the casein was not extracted with alcohol, but there was no difficulty in producing the dermatitis.

When raw egg-white is used as a source of protein, chicks develop perosis in 8 to 14 days and develop the typical dermatitis in about 28 days. Intramuscular or intraperitoneal injection of 2 to 5 gamma of biotin per chick per day completely protects the chicks from dermatitis. It does not completely prevent perosis, though it does reduce the severity.

Heating the egg-white destroys its biotin-binding property if dermatitis is taken as an indication of biotin deficiency, but all the



chicks which receive the heated egg-white and biotin still develop perosis.

A deficiency of biotin explains the dermatitis, but it does not completely explain the perosis.

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