depletion, and general debility. It is quite possible, therefore, that the depression of the excretion of 17-ketosteroids observed in acute hepatic insufficiency may have resulted from the secondary effects of the disease, rather than to a rise in the level of active circulating estrogens. That reciprocal depression of androgen formation may occur eventually, as a result of the failure of the liver to accomplish the inactivation of estrogenic compounds, is a definite possibility, and may conceivably account for the persistent diminution in the excretion of 17-ketosteroids which has been observed in chronic hepatic insufficiency.

Summary. The levels of excretion of estrogens and 17-ketosteroids in the urine have been determined in 11 young adult males with acute infectious hepatitis, at frequent intervals, from onset of the disease to convalescence. In 9 moderately severe cases there was a significant elevation in the level of excretion of biologically active estrogens in the urine which persisted at a slowly diminishing elevation until convalescence was considerably advanced. There was no significant increase in the concentration of the urinary estrogens in 2 cases of hepatitis which presented only mild clinical symptoms.

Marked to moderate depression occurred in the level of excretion of urinary 17-ketosteroids early in the course of acute hepatitis, with a return to normal values considerably in advance of the period of diminished estrogen excretion and of clinical recovery.

15227

Adequacy of the Known Synthetic Vitamins for Normal Feathering and Pigmentation in Chicks.

DOUGLAS V. FROST, F. PEIRCE DANN, AND FLOYD C. MCINTIRE.
(Introduced by Carl Nielsen.)
From the Abbott Laboratories, North Chicago, Ill.

The existence of a factor, or factors, in liver or yeast other than pantothenic acid, *p*-aminobenzoic acid, biotin, or inositol necessary for normal pigmentation was indicated from earlier work in this laboratory with rats[1,2] and dogs.[3] McGinnis, Norris, and Heuser[4] likewise indicated the presence of an unidentified factor other than pantothenic acid in brewers' yeast which was necessary for normal feathering and pigmentation in growing Rhode Island Red chicks. Cure or prevention of the achromotrichia developed in rats fed sulfaguanidine or succinylsulfathiazole by administration of folic acid concentrates was reported by Martin[5] and by Wright and Welch.[6] Similar results have been obtained in this laboratory using liver concentrates. It appeared, therefore, that the active factor might be identified with the *L. casei* factor[7] and vitamin B_c.[8]

Attempts have been made to develop techniques capable of assay for the unknown pigmentation factor. Chicks and dogs on highly purified diet and rats on purified diet with added succinylsulfathiazole have been used. Most consistent results have been obtained with growing chicks. In studying the role of the *L. casei* factor in hair and feather pigmentation, concentrates of the vitamin prepared by one of us (F.C.M.) and finally the

[1] Frost, D. V., Moore, R., and Dann, F. P., PROC. SOC. EXP. BIOL. AND MED., 1941, **46**, 507.

[2] Dann, F. P., Moore, R., and Frost, D. V., *Fed. Proc. Soc. Exp. Biol. and Med.*, 1942, **1**, 107.

[3] Frost, D. V., and Dann, F. P., *J. Nutrition*, 1944, **27**, 355.

[4] McGinnis, J., Norris, L. C., and Heuser, G. F., *J. Biol. Chem.*, 1942, **145**, 341.

[5] Martin, G., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 353.

[6] Wright, L. D., and Welch, A. D., *J. Nutrition*, 1944, **27**, 55.

[7] Pfiffner, J. J., *et al., Science*, 1943, **97**, 404.

[8] Angier, R. B., *et al., Science*, 1945, **102**, 227.

TABLE I
Composition of Basal Chick Diet.

	g/100 g		mg/100 g
Sucrose	58.0	Thiamine	0.30
Casein S.M.A. (vitamin-free)	18.0	Riboflavin	0.40
Gelatin	10.0	Pyridoxine	0.40
Salts (O & M)	5.0	d(+) Ca pantothenate	3.30
$CaHPO_4 \cdot 2H_2O$	1.0	Nicotinic acid	10.00
$MnSO_4 \cdot 2H_2O$	0.04	Mixed tocopherols	1.25
Corn oil	5.0	2-Methyl-1,4-naphthoquinone	0.44
Haliver oil	0.03	Biotin	0.036
Cystine	0.3	$ZnCl_2$	1.25
Choline	0.15	$CuSO_4 \cdot 5H_2O$	1.50
Inositol	0.1		

pure synthetic substance were used.* Liver concentrates used in these experiments were first standardized against a liver fraction of known vitamin B_c content.† The standardized liver fraction was later assayed against the synthetic *L. casei* factor. Standardizations against pure vitamin B_c and synthetic *L. casei* factor gave the same values with both *L. casei* and *S. faecalis R.* The active material in our concentrates is hereafter referred to as *L. casei* factor.

The basal diet was designed to supply in adequate amount all dietary factors which had previously been associated with chromotrichial activity in various species, excepting only *p*-aminobenzoic acid. Particular regard was paid to the adequacy of copper,[9,10,11] zinc,[12] pantothenic acid,[13,14,15] biotin,[16] and cystine.[17] The chromotrichial activity originally reported for *p*-aminobenzoic[18] acid in rats has not been verified.[19,15,2] For this reason it was decided not to include it in the diet at the present stage of investigation.

Experimental. Day-old Black Leghorn chicks were fed a basal diet as shown in Table I. In preliminary experiments, concentrates of the *L. casei* factor from liver were used to determine the levels needed for growth. Groups of 10 chicks were used and observations were recorded weekly. It was determined that a level of 400 μg per 100 g of diet gave good growth, feathering, and pigmentation over a period of 12 weeks. Growth was about 10% greater for control chicks receiving 10% liver fraction B (Wilson), but there was no observed difference in feathering and pigmentation. Chicks which received the *L. casei* factor concentrate for only the first 2 weeks grew at a decreasing rate and finally began to lose weight after 7 weeks; however, no failure in pigmentation or feathering was observed in this group even after 10 weeks.

Preliminary experiments were also conducted in which *L. casei* factor concentrates from liver were given 5 times weekly by injection in amounts calculated to supply 1, 2.5, 5, 10, and 20 μg of the factor per day. In this run the fullness of feathering was proportional to the level of *L. casei* factor up to the 10 μg

* We wish to thank Dr. E. L. R. Stokstad of Lederle Laboratories for supplying us the synthetic *L. casei* factor used in these studies.

† This liver fraction was standardized against crystalline vitamin B_c by Prof. W. H. Peterson at the University of Wisconsin.

9 Gorter, F. J., *Nature*, 1935, **136**, 185.

10 Keil, H. L., and Nelson, V. E., *J. Biol. Chem.*, 1931, **93**, 49.

11 Free, A. H., PROC. SOC. EXP. BIOL. AND MED., 1940, **44**, 371.

12 Stirn, E. E., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1935, **109**, 347.

13 György, P., and Poling, C. E., *Science*, 1940, **92**, 202.

14 Unna, K., Richards, G. V., and Sampson, W. L., *J. Nutrition*, 1941, **22**, 553.

15 Henderson, L. M., McIntire, J. M., Waisman, H. A., and Elvehjem, C. A., *J. Nutrition*, 1942, **23**, 47.

16 György, P., and Poling, C. E., PROC. SOC. EXP. BIOL. AND MED., 1940, **45**, 773.

17 Payeek, P., and Baum, H. M., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 271.

18 Ansbacher, S., *Science*, 1941, **93**, 164.

19 Emerson, G., PROC. SOC. EXP. BIOL. AND MED., 1941, **47**, 448.

TABLE II.

Growth, Feathering, and Pigmentation of Chicks Receiving Various Levels of Synthetic *L. casei* Factor by Injection. Comparison with *L. casei* Factor Concentrate from Liver and with 10% Brewer's Yeast Supplement.

	Synthetic *L. casei* factor 1 μg			Synthetic *L. casei* factor 2.5 μg			Synthetic *L. casei* factor 5 μg		
Weeks	Wt, g	Feathering	Pigmentation %	Wt, g	Feathering	Pigmentation %	Wt, g	Feathering	Pigmentation %
1	44			46			46		
2	75			80			84		
3	106			127			146		
4	119			164			211		
5	132	Very poor	0	193	Poor	10	269	Subnormal	25
6	153	" "	0	234	"	10	310	"	40
7	157	" "	0	242	"	40	369	"	40
8	176	" "	0	291	"	40	436	"	60
9	172*	" "	10	308†	"	50	459	"	60

	Synthetic *L. casei* factor 10 μg			*L. casei* factor concentrate 10 μg			10% brewer's yeast		
Weeks	Wt, g	Feathering	Pigmentation %	Wt, g	Feathering	Pigmentation %	Wt, g	Feathering	Pigmentation %
1	44			45			45		
2	78			80			81		
3	130			135			147		
4	195			207			230		
5	264	Normal	100	275	Normal	90	300	Normal	100
6	346	"	100	348	"	90	358	"	100
7	374	"	100	409	"	90	458	"	100
8	477	"	100	495	"	90	594	"	100
9	548	"	100	544	"	90	666	"	100

* Only 3 chicks survived.
† Only 4 chicks survived.

level where it was normal. Depigmentation was marked at the 5 and 10 μg levels. At the lower levels, the feathers were poorly developed and the chicks had a ragged and scrawny appearance. Growth was in proportion to the level of *L. casei* factor given.

When synthetic *L. casei* factor was made available to us for research purposes, the above experiment was repeated at injection levels calculated to supply 1, 2.5, 5, and 10 μg daily of the crystalline material. A control group with 10% brewers' yeast supplement added to the basal diet was run for comparison. The results as to growth, feathering, and pigmentation were essentially similar to those above except that the 10 μg level of synthetic *L. casei* factor appeared adequate for normal pigmentation, whereas the concentrate at this level had not proved adequate.

The results with synthetic *L. casei* factor are shown in Table II. Photographs of representative chicks from the various groups are shown in Fig. 1.

At the 1 and 2.5 μg levels, feathering was so poor that true depigmentation was difficult to discern, although the chicks gave the general appearance of grayness. At the 5 μg level, chicks showed the most definite depigmentation. The plumage of this group was somewhat sparse, but was full enough so that depigmentation was readily apparent. Both feathering and pigmentation were about equal for the group receiving 10 μg *L. casei* factor by injection and the control group fed yeast supplement.

Depigmentation became most pronounced in the proximal portions after the feathers were fairly well developed. The wing and tail feathers were most affected, but nearly all of the feathers were somewhat affected. Depigmentation was not discernible as quickly in the lower level groups as it was at the 5 μg level, probably because feather growth was

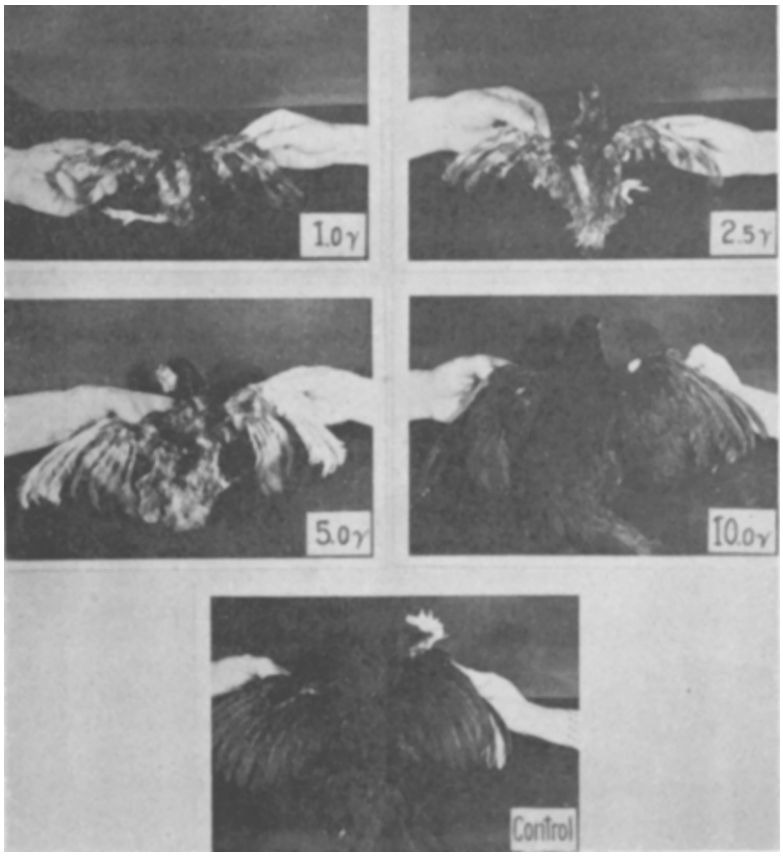

FIG. 1.
Chicks at nine weeks of age representing the groups which received 1, 2.5, 5, or 10 μg daily of synthetic *L. casei* factor by injection or 10% brewer's yeast added to the basal diet.

slower and less complete at the lower levels.

Discussion. A primary need for the *L. casei* factor in the chick would appear to be for feather development. When the need for feather formation is just satisfied, the needs for pigmentation are still not met. Depigmentation then becomes most apparent in the rapidly growing feathers. A still higher level is needed to satisfy both needs completely.

Campbell, Brown, and Emmett[20] have indicated that a level of 20 μg daily of crystalline vitamin B_c isolated from yeast, given either orally or parenterally to growing chicks, produces normal growth, feathering, and blood values. The present work indicates that a level of about 10 μg of synthetic *L. casei* factor is adequate for normal feathering and gives good growth. It appears likely that a somewhat higher level would have given growth comparable to that obtained with 10% brewers' yeast supplement. Evidence for the existence of separate factors required for feathering and growth has been presented by Briggs, Luckey, Elvehjem, and Hart.[21] The multiplicity of forms in which the *L. casei* factor apparently occurs in nature requires clarification with regard to this possibility.

In separate experiments we have used concentrates of a fermentation product highly active for *S. faecalis R.*, but only slightly active for *L. casei*. These concentrates gave very poor growth and feathering when used

[20] Campbell, C. J., Brown, R. A., and Emmett, A. D., *J. Biol. Chem.*, 1944, **154**, 721.

[21] Briggs, G. M., Luckey, T. D., Elvehjem, C. A., and Hart, E. B., *J. Biol. Chem.*, 1944, **153**, 423.

at a level which proved adequate in the case of liver concentrates, based on *S. faecalis R.* assay. Thus it may prove true that the *L. casei* assay is a better indication of activity for higher animals.

The importance of pantothenic acid in normal pigmentation has been adequately emphasized. The interesting observation has been further made[6] that the storage and utilization of pantothenic acid in succinylsulfathiazole-fed rats is favorably influenced by the simultaneous administration of biotin and folic acid-rich concentrates. Early work in this laboratory[1] indicated that low levels, *i.e.*, 40-60 μg of pantothenic acid as it occurred in natural products, were much more effective in prevention of graying in growing rats than levels up to 200 μg per day of d(+)calcium pantothenate. The inference was that other factors were acting synergistically with pantothenic acid. This idea seems to have support in much of the work to date, and it appears probable that the metabolic functions of the *L. casei* factor and pantothenic acid are closely interdependent.

In line with the above reasoning it may now become possible to reconcile the discrepancies between different laboratories regarding the adequacy of pantothenate alone to cure or prevent achromotrichia in rats. The adequacy of given levels of pantothenate for growth and pigmentation may to some extent be governed by the level of *L. casei* factor in the diet, or the rate of intestinal synthesis of this factor. Unna and Richards[22] observed that the requirement of pantothenate for rats decreases from 100 μg per day at 3 weeks of age to 25 μg per day at 10 weeks of age. Furthermore, rats started on 25-50 μg per day of pantothenate became gray but showed gradual restoration of the black color of the fur as they approached maturity. These observations were confirmed by Henderson *et al.*[15] On the other hand, Williams[23] reported the inefficacy of even high levels of pantothenate to cure graying uniformly, as was also observed in this laboratory.[1] Further experiments are indicated in which the *L. casei* factor is supplied together with pantothenate.

The needs of the chick for the *L. casei* factor are apparently not complicated by intestinal synthesis to the same extent as in the rat. For the latter reason, chicks may be considered preferable to other species in studies dealing with the effect of diet on normal pigmentation processes.

It is significant to note that Willier[24] has elaborated on the common role of melanophores in bringing about the pigmentation of hair or feathers in different species and has further postulated that a genetic lethal factor in the melanophore may be the primary cause of graying in man.

Summary. Black Leghorn chicks were raised on a basal diet designed to contain the previously recognized chromotrichial factors, except the *L. casei* factor. Synthetic *L. casei* factor injected 5 times weekly at a level calculated to supply 10 μg per day showed feathering and pigmentation comparable to that of chicks receiving 10% brewers' yeast supplement. Growth was somewhat better on the latter. At a level of 5 μg, feathering was fairly good and depigmentation was marked. At 1 and 2.5 μg levels, feathering was very poor. Growth and feathering were in proportion to the level of *L. casei* factor used.

Acknowledgment is made to Mr. Albin Junnila for technical care of the animals.

[22] Unna, K., and Richards, G. V., *J. Nutrition*, 1942, **23**, 545.

[23] Williams, R. R., *Science*, 1940, **92**, 561.

[24] Willier, B. H., *Ann. Surgery*, 1942, **116**, 598.