Studies on Unidentified Chick Growth Factors Apparently Organic in Nature

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Morrison and associates ('55, '56), Dannenburg and associates ('55), and Camp, Reid and Couch ('55) reported that, when a mixture of crude sources of unidentified growth factors was fed to chicks, the observed growth increases appeared to be due to the presence in these materials of both unidentified organic and inorganic constituents. The evidence for the existence of growth-stimulating substances organic in nature was based upon the markedly greater growth which occurred in chicks when the diet contained the intact supplements in comparison with that obtained with an equivalent amount of mineral matter produced by ashing at temperatures of 525 to 700°C. The procedure of including in the diet a quantity of the ash of unidentified factor supplements equivalent to that present in the amount used to stimulate chick growth has been followed in further work on unidentified chick growth factors apparently organic in nature. The results of this work are presented in this report.

EXPERIMENTAL

Purebred White Plymouth Rock chicks or crossbred chicks of White Plymouth Rock hens mated to Vantress males were used in this investigation. With the exception of one experiment the chicks were hatched from the eggs of hens maintained at the Cornell University poultry plant. The hens were fed a simplified diet composed largely of corn and soybean meal. No sources of unidentified chick growth factors were included in the diet. The hens were housed in pens with raised wire-mesh floors in order to prevent coprophagy.

Duplicate lots of 13 to 20 chicks per lot were subjected to each treatment. A uniform number of chicks per lot was used in each experiment. In 7 of the 12 experiments reported in this paper, each lot contained an equal number of male and female chicks. In the remaining experiments, only male chicks were used. The chicks were placed on experiment at approximately one day of age. The duration of all experiments was 4 weeks.

The chicks were housed in galvanized metal batteries with raised wire-mesh floors, equipped with automatically-controlled electrical heating units. The chicks of each lot were weighed as a group at the start of the experiments and individually every week thereafter. Each chick was identified with a numbered wingband. Feed and water were supplied ad libitum. A weekly record of feed consumption was made at the time the chicks were weighed.

Two basal diets designated diets A and B were used in all experiments except experiment 1 in which diet AA was fed. This diet was identical with diet A except that it contained 5% less soybean protein and 5% more glucose. The composition of diets A and B is given in table 1. The metabolizable energy content of diet A was 3065 Cal./kg and that of diet B, 3495. The protein content (nitrogen × 6.25) of these diets was 27.1 and 30 1%, respectively. Both diets contained an excess amount of protein in relation to energy content. Because of higher energy content, the methionine, glycine, mineral and vitamin additions to diet B were increased so as to make the intake of these materials per chick per day approximately the same as those of the chicks fed diet A. An antioxidant was included in diet B in order to prevent the corn oil from becoming rancid during the course of the experiments.

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TABLE 1

Composition of basal diets

Ingredient	Amount	
	Basal A	Basal B
	%	%
Glucose monohydrate[1]	55.55	45.21
Soybean protein[2]	30.00	33.38
Hydrogenated oil[3]	3.00	—
Corn oil[4]	—	10.00
Cellulose[5]	3.00	3.23
DL-Methionine	0.70	0.75
Glycine	0.30	0.32
Mineral mixture[6]	5.43	5.84
Ash unidentified growth factors (UGF)	1.30	—
Vitamin mixture	0.72[7]	1.25[8]
2,6-Ditertiary butyl-4-methyl phenol (BHT)	—	0.022

[1] Cerelose.
[2] Drackett Assay C-1.
[3] Hydora.
[4] Mazola.
[5] Solka Floc.
[6] The grams per 1000 gm of Analytical Reagent Grade chemicals in the mineral mixture were as follows: 396.1 $CaHPO_4$, 274.8 $CaCO_3$, 159.7 KH_2PO_4, 110.5 NaCl, 46.0 $MgSO_4$, 6.1 $FeSO_4 \cdot 7H_2O$, 6.1 $MnSO_4 \cdot H_2O$, 0.31 $CuSO_4 \cdot 5H_2O$, 0.18 $ZnCl_2$, 0.15 $Na_2MoO_4 \cdot 2H_2O$, 0.048 KI, 0.031 $CoCl_2 \cdot 6H_2O$.
[7] The grams and units per 1000 gm of vitamins and other substances in the vitamin mixture were as follows: 208.3 choline chloride, 34.7 inositol, 6.9 niacin, 2.8 *d*-calcium pantothenate, 2.8 α-tocopheryl acetate, 1.4 thiamine·HCl, 1.4 riboflavin, 0.63 pyridoxine·HCl, 0.56 folic acid, 0.069 menadione, 0.028 biotin, 0.0028 vitamin B_{12}, 6,940 IU vitamin A, 530 ICU vitamin D_3, 740.3 vitamins A, D_3 and E diluents and glucose.
[8] The grams and units per 1000 gm of vitamins and other substances in the vitamin mixture were as follows: 129.8 choline chloride, 21.6 inositol, 5.7 α-tocopheryl acetate, 4.3 niacin, 3.5 *d*-calcium pantothenate, 0.87 thiamine·HCl, 0.87 riboflavin, 0.39 pyridoxine·HCl, 0.35 folic acid, 0.083 menadione sodium bisulfite, 0.017 biotin, 0.0035 vitamin B_{12}, 4,330 IU vitamin A, 865 ICU vitamin D_3, 1.9 diphenyl-*p*-phenylenediamine (DPPD), 830.6 vitamin A, D and E diluents and glucose.

A mixture of crude materials, hereafter designated UGF, and a liver extract were used as sources of unidentified chick growth factors. The former was composed of 50% distillers' dried solubles, 25% fish solubles, and 25% dried whey product. The ash included in diets A and AA was obtained by burning the UGF at 525°C until a light gray product was obtained. The percentage of ash given in diet A, table 1, is the average quantity equivalent to 12% UGF.

The UGF and liver extract[1] used in the experiments were obtained from the same original supplies. When these unidentified factor supplements were included in the basal diet, appropriate adjustments were made so as to maintain the protein content of the diets constant.

The UGF contained 69.4 mg of zinc per kg, and the liver fraction 29.4 mg per kg. The quantities of potassium in the UGF and the liver fraction were 2.1 and 2.93% respectively, and the quantities of sodium were 0.91 and 0.88% respectively.

All values for zinc content presented in this report were determined according to the colorimetric procedure of the A.O.A.C. ('55). The values for potassium and sodium were determined by the procedure described by Mathis ('56).

The results of the experiments were subjected to analysis of variance according to Snedecor ('56). Afterwards, Duncan's multiple range test described by Federer ('55) was applied to the results where necessary in order to locate the sites of significant growth differences.

RESULTS

The first group of experiments was conducted to determine if the intact UGF pro-

[1] The liver extract was Liver Fraction No. 1 of Wilson, Laboratories, Wilson and Company, Chicago, Illinois.

TABLE 2

Effect of crude sources of unidentified factors (UGF) on chick growth

Experiment	Av. weight at 4 weeks		Gain over basal	Gain/feed	
	Basal A	UGF		Basal	UGF
	gm	*gm*	%		
1	337(36)[1]	431(35)	31.7	0.52	0.58
2	339(36)	380(39)	13.7	0.56	0.60
3	360(39)	432(40)	22.4	0.55	0.63
4	344(35)	411(36)	22.0	0.63	0.58
5	322(35)	382(38)	21.4	0.46	0.56
Average	340(210)	407(217)	22.3	0.55	0.59

[1] Survivors; 40 chicks per treatment at start.

moted greater growth in chicks than that observed when the basal diet, containing an equivalent amount of ash from the mixture, was fed. The results of the experimental work are presented in table 2. The intact UGF was found to promote markedly greater growth than that promoted by the basal diet containing the ash. The average increase in gain was 22.3% and the range in gain in the experiments varied from 13.7% to 31.7%. The growth increases were found to be highly significant ($P < 0.01$). The unit gain per unit of feed consumed was also improved to the extent of 7.3%.

O'Dell, Newberne and Savage ('58) pointed out that the basal diets used in the experiments of Morrison and associates ('55, '56) and Dannenburg and associates ('55) were probably deficient in zinc and perhaps potassium. However, by including the ash of an equivalent amount of the intact UGF in the diet the potassium content was increased from 0.25 to 0.52%. This quantity has been found by Leach and associates ('59) to be more than adequate for maximum growth of chicks under the experimental conditions. The amount of zinc added to the basal diet by means of the ash was 8.3 mg/kg. Since the basal diets contained 11.4 ± 3.1 mg of zinc per kg, the ash addition increased the total zinc content of the diets to approximately 19.7 mg/kg. This amount has been found by Zeigler, Leach and Norris ('58) to be sufficient for maximum growth of chicks maintained in a zinc-free environment and fed a diet containing casein but inadequate when the diet contained soybean protein. The increased growth obtained with the intact UGF, however, was not due to zinc functioning independently, since the quantity of zinc supplied by it was no greater than that supplied by the ash.

The possibility that the improved growth was caused by the presence of minerals in the intact UGF, which were sublimed by ashing at a temperature of 525°C, appears ruled out except for selenium. The basal diet was found to contain an adequate quantity of selenium.[2] No evidence exists for a requirement for other mineral elements which sublime at this temperature. The results of the experiments showed, therefore, that the UGF contains an unidentified organic factor or factors required directly for chick growth or which promotes chick growth indirectly by rendering the zinc in the mixture in some unexplained manner more available to the chick.

On completion of these experiments studies were initiated on the unidentified growth factor reported to be present in liver and liver extracts by Edwards and associates ('55) and others. Further work was also conducted on the unidentified growth factors in the UGF. Basal diet B was fed in these studies. This diet was subsequently shown by Leach and associates ('59) to be deficient in potassium, and by Zeigler, Leach and Norris ('58) to be deficient in zinc when the chicks were reared in a zinc-free environment.

The results of two experiments are summarized in the first section of table 3 and show that the addition of 1.5% of liver extract to basal diet B promoted a striking increase in growth. The growth increase was found to be highly significant ($P < 0.01$).

[2] Unpublished results, Leach, R. M., Jr., and L. C. Norris, 1959.

TABLE 3

Interrelationship of unidentified liver-extract factor and zinc

Treatment	Av. weight at 4 weeks	Gain/feed
	gm	
Basal diet B		
Basal (6, 7)[1]	371(59)[2]	0.63
+ 1.5% liver extract (LE)(6, 7)	469(62)	0.66
+ ash = 1.5% LE (7)	380(30)	0.63
+ ash UGF (7)	457(32)	0.65
+ ash UGF + 1.5% LE (7)	532(31)	0.68
+ UGF (6, 7)	524(63)	0.68
Basal diet B with adequate zinc		
Basal (8, 9)	522(53)[3]	0.71
+ 2% liver extract (LE) (8, 9)	576(55)	0.71
+ 0.23% K (9, 10, 11, 12)	571(115)	0.74
+ 0.23% K + 2% LE (9, 10, 11, 12)	588(114)	0.73
+ 0.23% K + UGF (12)	647(32)	0.76

[1] Experiment.
[2] Survivors; 64, 64, 32, 32, 32 and 64 chicks per treatment, respectively, at start.
[3] Survivors; 56, 56, 118, 118 and 32 chicks per treatment, respectively, at start.

In contrast an amount of liver ash equivalent to that in the intact extract failed to stimulate a significant growth increase. When the ash of the UGF was included in the basal diet in an amount equivalent to the quantity of the intact material supplied in the previous group of experiments, a significant growth increase ($P < 0.01$) occurred which was approximately equal to that obtained with the intact liver extract. A still further growth increase was obtained by additions to the basal diet of the intact UGF or a combination of UGF ash and liver extract. This growth improvement was also found to be significant ($P < 0.05$). The ash of the liver extract raised the potassium content of basal diet B from 0.27 to 0.31% and made the diet almost adequate in potassium, while the UGF ash increased the potassium content to 0.52%.

The liver extract ash, however, appeared to lack a substance present in the intact extract and in the UGF ash. The liver extract increased the zinc content of the basal diet 0.44 mg/kg, whereas the UGF ash increased it 6.9 mg/kg. The former quantity of zinc, according to Zeigler and Norris[3] would have no measurable effect on chick growth. On the other hand, the latter quantity would have a marked effect but would not entirely satisfy the zinc requirement of chicks fed a purified diet containing soybean protein. The results suggested, therefore, that the UGF ash stimulated chick growth by increasing the dietary zinc and that the liver extract and the UGF stimulated chick growth because of the presence of unidentified organic substances.

This finding was confirmed by the results of the work with liver extract presented in the second section of table 3. Basal diet B, supplemented with 50 mg zinc per kg, an amount more than sufficient to meet the chick's requirement for this mineral, according to Zeigler, Leach and Norris ('58), was used in these studies. When this diet was supplemented with liver extract an increase in growth was obtained which was significant ($P < 0.05$). Adding 0.23% potassium to the diet promoted a growth improvement approximately equal to that obtained with the liver extract. When both potassium and liver extract were fed, the increase in growth was little greater than that obtained when either one of these materials was fed alone. These results indicated that the growth-promoting effect of liver extract in these experiments was caused by its potassium content. The inclusion of zinc in the basal diet, therefore, appeared to replace the unidentified growth-promoting substance in the intact liver extract. This suggests the possibility that the un-

[3] Unpublished results, 1959.

TABLE 4

Need for unidentified factors by chicks supplied adequate quantities of zinc and potassium in the basal diet

Experiment	Av. weight at 4 weeks		Gain over basal	Gain/feed	
	Basal B	UGF[1]		Basal	UGF
	gm	*gm*	%		
9	586(24)[2]	610(26)	4.38	0.74	0.76
10	571(32)	647(32)	14.28	0.76	0.75
11	564(30)	596(30)	6.12	0.74	0.72
13	579(30)	635(30)	10.37	0.80	0.80
Average	575(116)	622(118)	8.77	0.76	0.76

[1] Distillers' dried solubles used as UGF in experiment 13.
[2] Survivors; 26, 32, 30 and 30 chicks per treatment, respectively, at start.

known organic substance in liver extract acted as a chelating agent, and thereby made the dietary zinc more available. Whether or not the UGF also contained the substance could not be determined from the evidence. Kratzer[4] has observed that zinc functions as a partial substitute for the unidentified, growth-promoting properties of a soybean meal extract. In addition Kratzer and associates ('59) have obtained evidence that EDTA (ethylenediaminetetraacetic acid),[5] a chelating agent, appears to make the zinc in a purified diet containing soybean protein more available.

Although excellent growth was promoted in the chicks by supplying a diet adequate in both zinc and potassium, a still further growth increase was obtained in repeated experiments by supplementing the diet with sources of unidentified chick growth factors. This growth increase was found to be highly significant ($P < 0.01$). The UGF previously described, and distillers' dried solubles[6] were used to provide unidentified growth factors in the experiments.

The results of the experiments are presented in table 4. The average gain in weight promoted by the sources of unidentified growth factors was 8.55% and the range in gain varied from 4.38 to 14.28. The cause of this variation is not clear at present, but may be related to variation in the composition of some of the ingredients used in the basal diet. In work on the growth-stimulating properties of vegetable fats by Dam and associates ('59), evidence of an interaction between the level of corn oil in the basal diet and the UGF was observed, indicating that at times corn oil exerted a sparing effect on the unidentified factors. In spite of the variability in results evidence of the existence of an organic, chick growth-promoting substance in sources of unidentified growth factors not identical with the one in liver extract has been obtained.

SUMMARY

Further evidence has been obtained of the presence, in crude materials, of unidentified substances, apparently organic in nature, which stimulate growth of chicks fed a purified diet containing soybean protein and adequate quantities of all known vitamins. One of these, found in liver extract, appeared to replace zinc to a great extent or to render it more available. Another factor(s), present in a mixture of distillers' dried solubles, dried whey and fish solubles, promoted growth of chicks fed a purified diet adequate in zinc.

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[4] Kratzer, F. W., University of California, Davis, 1959, personal communication to the authors.
[5] Versene.
[6] Supplied by J. A. Wakelam, The Distillers Company, Ltd., London, England.

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