SOME PROPERTIES OF AN UNIDENTIFIED CHICK GROWTH FACTOR FOUND IN CONDENSED FISH SOLUBLES*

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(Received for publication, December 4, 1947)

In previous papers (1, 2) evidence was presented to show that condensed fish solubles produced a marked increase in the growth rate of chicks on diets containing an adequate level of vitamins and essential amino acids. Berry, Carrick, Roberts, and Hauge (3) found that a water extract of fish solubles was also active in stimulating the growth of chicks. Very little other information on the properties of the unidentified growth factor in fish solubles is available. The present investigation was undertaken to study some of the properties of the factor.

EXPERIMENTAL

The chicks used were day-old straight run (New Hampshire ♂♂ X single comb white Leghorn ♀♀) cross-bred chicks which were the progeny of hens on Diet B-1 described previously (2). The experimental groups were housed in electrically heated batteries with raised screen floors. The chicks were individually wing-banded and were weighed at the beginning of the experiment and at weekly intervals thereafter. Feed and water were supplied *ad libitum*. The experiments were terminated at the end of 4 weeks. The basal ration was the same as that reported in previous studies (1, 2) and consisted of ground yellow corn 35, wheat bran 10, wheat middlings 10, dehydrated alfalfa leaf meal 5, soy bean oil meal 28, vitamin test casein 7.5, limestone grit 2.0, steamed bone meal 1.5, iodized salt 0.5, fish oil (2000 vitamin A, 400 vitamin D units) 0.5, and manganese sulfate 0.025 gm., thiamine 0.3, riboflavin 0.6, niacin 5.0, calcium pantothenate 2.0, pyridoxine HCl 0.4, inositol 100.0, choline 150.0, *p*-aminobenzoic acid 10, biotin 0.02, folic acid 0.05, menadione 0.05, and α-tocopherol 0.3 mg.

* Published with the approval of the Director of the Wisconsin Agricultural Experiment Station. Supported in part from funds supplied by the Borden Company, New York, and by the Commercial Solvents Corporation, Terre Haute, Indiana. We are indebted to Merck and Company, Inc., Rahway, New Jersey, for crystalline vitamins, to Dr. B. L. Hutchings of the Lederle Laboratories Division, American Cyanamid Company, Pearl River, New York, for synthetic folic acid, and to the Borden Company, New York, for the supply of condensed fish solubles.

Solubility of the factor was tested in water, 70 per cent MeOH, absolute MeOH, 70 per cent EtOH, 95 per cent EtOH, acetone, and ether. The water, alcohol, and acetone extracts were prepared in the following way. A weighed quantity of condensed fish solubles was mixed with sufficient solvent to result in the desired concentration allowing for the volume of water present in the fish solubles (50 per cent solids). The mixture was stirred mechanically for 1 hour at room temperature and filtered with suction through a Whatman No. 42 filter paper. The residue was reextracted twice with solvent of the desired concentration. The combined filtrates were concentrated and the organic solvents removed by distillation under reduced pressure. The residue remaining in the distillation flask was taken up in water.

The ether extraction was carried out for 24 hours with a liquid-liquid extractor which is a modification of the one described by Hossfeld (4).

Dialysis of fish solubles against distilled water was continued for 48 hours. Synthetic sausage casing membrane was used.

The extracts and the insoluble residues were mixed directly into the basal ration at a level equivalent to either 3 per cent or 6 per cent of the original condensed fish solubles.

The results are presented in Table I. In each trial the unsupplemented basal group and the group fed the basal ration supplemented with 3 per cent condensed fish solubles serve as negative and positive controls. The average weight of the chicks in each lot at 4 weeks of age is reported. A direct comparison of lots in different trials can be made by considering the per cent response which is calculated by considering the difference between the negative and positive controls as 100 per cent.

In confirmation of the observation of Berry *et al.* (3) the active principle was found in the water extract (Lots 3 and 12). Some growth-promoting activity remained in the residue (Lots 4 and 13). The growth factor was extracted by 70 per cent MeOH (Lots 18 and 32) and by 70 per cent EtOH (Lot 14). With these solvents the degree of extraction was comparable to that obtained with water. Filtration and distillation were facilitated by the alcohols. The absolute methanol extract (Lot 20) caused some promotion of growth but a greater part of the activity was present in the residue (Lot 21) in contrast to a lower concentration of this solvent.

The precipitate formed in 95 per cent EtOH at $-4°$ (Lot 28) contained full growth-promoting activity, and only very slight solubility of the factor was evident under these conditions (Lot 27). In acetone the factor was completely insoluble (Lot 25) and full activity was present in the residue (Lot 26).

Dialysates of condensed fish solubles (Lots 22, 35, and 39) gave a full response, indicating that the factor is readily dialyzable through synthetic sausage casing membrane. The growth stimulation obtained was of the

same order as that with water, 70 per cent MeOH, and 70 per cent EtOH extracts.

TABLE I

Average Weight and Comparative Response of Chicks to Various Fractions of Condensed Fish Solubles

Trial No.	Lot No.	Supplement to basal ration		Average weight, 4 wks.	Response
			per cent		*per cent*
1	1	None		173	
	2	3% fish solubles		233	100
	3	H_2O extract, fish solubles	=6	247	123
	4	Residue, Lot 3	=6	226	88
	5	Ether extract, fish solubles	=6	163	−17
	6	Residue, Lot 5	=6	246	+122
	7	As Lot 2, pH 1, autoclaved 20 mins., 15 lbs.	=3	171	−3
	8	As Lot 2, pH 7, autoclaved 20 mins., 15 lbs.		198	42
	9	As Lot 2, pH 12, autoclaved 20 mins., 15 lbs.		168	−8
2	10	None		232	
	11	3% fish solubles		307	100
	12	H_2O extract	=3	287	73
	13	Residue, Lot 12	=3	250	24
	14	70% ethanol extract	=3	285	71
	15	Residue, Lot 14		253	28
3	16	None		197	
	17	3% fish solubles		261	100
	18	70% methanol extract	=6	278	126
	19	Residue, Lot 18	=6	231	53
	20	Absolute methanol extract	=6	266	108
	21	Residue, Lot 20	=6	274	120
	22	Dialysate, fish solubles	=3	264	105
4	23	None		209	
	24	3% fish solubles		298	100
	25	100% acetone extract	=3	217	9
	26	Residue, Lot 25	=3	306	108
	27	95% ethanol extract, −4°	=3	241	35
	28	Residue, Lot 27, −4°	=3	312	115
	29	Enzyme digest	=3	306	108
5	30	None		192	
	31	3% fish solubles		300	100
	32	70% methanol extract	=3	303	103
6	33	None		243	
	34	3% fish solubles		288	100
	35	Dialysate, fish solubles	=3	284	91
	36	Residue, Lot 35		274	69

TABLE I—*Concluded*

Trial No.	Lot No.	Supplement to basal ration		Average weight, 4 wks.	Response
			per cent		*per cent*
7	37	None		176	
	38	3% fish solubles		277	100
	39	Dialysate, fish solubles	≡3	286	109
	40	As Lot 39, heated at 100°, 2 hrs., pH 3		284	107
	41	" " 39, " " 100°, 2 " " 6		296	119
	42	" " 39, " " 100°, 2 " " 9		263	86
8	43	None		218	
	44	3% fish solubles		307	100
	45	Dialysate	≡3	283	73
	46	As Lot 45, norit A filtrate	≡6	228	11
	47	" " 45, " " eluate	≡6	205	−15
	48	" " 45, Lloyd's reagent filtrate	≡6	255	42
	49	" " 45, " " eluate	≡6	226	9

Adsorption of the growth factor from a dialysate of fish solubles was attempted with norit A (solution at pH 3.0) and Lloyd's reagent (solution at pH 1.0). The low activity of the norit filtrate (Lot 46) indicates that the factor was adsorbed. Elution from norit was repeated three times with a mixture of 70 per cent MeOH and 5 per cent NH_4OH at 70°. Removal of the factor from the charcoal was not successful, as was indicated by the eluate (Lot 47). The filtrate from Lloyd's reagent (Lot 48) gave a response of 42 per cent, indicating only partial adsorption. Elution of Lloyd's reagent was repeated three times with 0.5 N NaOH, but no activity was apparent in the eluate (Lot 49).

The heat stability of the growth factor was studied under different conditions. Autoclaving condensed fish solubles (15 pounds pressure for 20 minutes) in 0.1 N HCl and 0.1 N NaOH resulted in complete loss of activity (Lots 7 and 9). In neutral solution over 50 per cent of the activity was destroyed under these conditions (Lot 8). Aliquots of a dialysate of condensed fish solubles at pH 3, 6, and 9 were heated for 2 hours at 100°. This treatment did not cause any loss in activity at pH 3 or 6 (Lots 40 and 41) and only slight loss in activity occurred at pH 9 (Lot 42).

Because of the loss of activity due to autoclaving with acid and alkali, it seemed possible that the factor concerned could be hydrolyzed. To find out whether the activity could be destroyed by enzymatic hydrolysis, a 300 gm. sample of fish solubles was made up to 4 liters in 1 per cent Na_2CO_3 and the pH adjusted to 8.1, 20 gm. of pancreatin and 10 gm. of crude intestinal mucosa were added to the solution, and the mixture was placed in an automatic shaker and incubated for 72 hours at 37°. The enzyme digest

was concentrated by distillation under reduced pressure to a volume of 300 cc. and was mixed into the basal ration at a level of 3 per cent. No destruction of activity resulted from this treatment, since a full growth response was obtained (Lot 29).

DISCUSSION

The growth factor present in condensed fish solubles was found to be soluble in water, 70 per cent methanol, and 70 per cent ethanol. The degree of solubility was decreased in absolute methanol and very slight solubility was evident in 95 per cent ethanol at −4°. The factor was insoluble in ether and acetone. Dialysates of condensed fish solubles contained nearly all of the growth activity present in the original material.

Studies on stability indicate a fair degree of stability to heat in the intermediate pH range. However, autoclaving temperatures at 0.1 N acid or alkali destroyed the growth activity. Enzymatic digestion as carried out did not cause any loss of activity. The factor was apparently strongly adsorbed on norit A and to a less extent by Lloyd's reagent. The elution procedures employed were not successful in removing the active material.

Some relationship seems to exist between the growth factor in fish solubles and other materials with growth-promoting activity for the chick and rat. Jaffé and Elvehjem (5), in studies on the fractionation of a growth factor for rats present in liver, found that the factor was soluble in 60 per cent ethanol. The study also indicated that the factor concerned was strongly adsorbed and could be removed only by exhaustive elution. They noted that condensed fish solubles caused a stimulation in the growth rate of rats equivalent to that obtained with liver fractions. Nichol *et al.* (1) in a study of sources of growth-promoting materials for chicks obtained an increase in the growth rate with whole liver powder and certain other liver preparations comparable to that obtained with fish solubles.

The growth factor in cow manure, described by Rubin and Bird (6) and which was found to be active in previous tests (1) with the same basal ration employed in this study, was found to differ in certain properties from those obtained with fish solubles. The cow manure factor was reported to be non-dialyzable, while the factor in fish solubles can be dialyzed. A difference was also observed in solubility in 95 per cent ethanol. The possibility that more than one factor is involved would seem to have some basis. However, the differences with respect to the properties mentioned may be merely a difference in the manner in which the same factor exists in different source materials.

SUMMARY

The unidentified factor present in condensed fish solubles was found to have the following properties.

1. Soluble in water, 70 per cent methanol, 70 per cent ethanol.
2. Somewhat soluble in absolute methanol and very slightly soluble in 95 per cent ethanol.
3. Insoluble in ether and acetone.
4. Dialyzable through a cellophane membrane.
5. Heat-stable (100° for 2 hours) over a range of pH 3 to 9; no loss in activity was caused by enzymatic digestion.

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