Adenosine as Growth Factor for Chicks Fed Purified Diet.* (22482)

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Barnett and Bird(1) found that chicks responded consistently to sources of unknown growth factors if fed a purified basal diet purposely contaminated with feces of older chickens.

A concentrate prepared from penicillin fermentation residue was furnished by E. R. Squibb and Sons, and stated to be highly active by chick assay and by microbiological assay. Upon fractionation it yielded, at one stage, a considerable quantity of a crystalline material. Ultimate analyses and comparative infrared spectra revealed that this material was adenosine(2). Adenosine proved to be inactive in the microbiological assay, but active in the chick assay.

Methods. The microbiological assay procedure followed was that of Schaefer and McCormack(3). Day old New Hampshire X Silver Cornish chicks from depleted dams(1) reared in electrically heated batteries with raised wire floors were used in all chick assays. Ten chicks were started in each experimental lot. The purified ration in Table I, and the method of fecal contamination(1) were used except in Exp. 2 where the feces were omitted. All supplements were added at the expense of sucrose. During Exp. 2, a deficiency of vit D developed, and the level of this ingredient was increased from 600 ICU to 1200 ICU per kg of ration. Even at this level mild symptoms of rickets appeared and the practice of administering 2 drops of haliver oil twice weekly was followed subsequently. This eliminated the vit. D deficiency. Some cases of ataxia and some deaths were observed in groups not receiving fish solubles. Inasmuch as maximum responses were obtained, in many cases, only when 2 widely differing sources of unidentified growth factors were included in the diet, the initial test of adenosine was made with dried whey or fish solubles. Subsequent tests have always included one group in which adenosine was the only supplement. The adenosine was a commercial sample prepared from yeast, and stated by the supplier† to be chromatographically pure. The levels of adenosine were chosen arbitrarily, but were known to be considerably higher than the level furnished by the above-mentioned concentrate. Adenosine was fed at 150 mg/kg of diet in the first 2 experiments and at 300 mg/kg in the last 3 trials.

Results. In the first experiment adenosine elicited a pronounced growth response when added to the diet which contained 2% dried whey. In the second experiment there is some question about the significance of response to adenosine in the presence of dried whey because only one male and 4 females remained in the control group at 4 weeks of age. In subsequent experiments no response to adenosine occurred in the presence of whey. Adenosine gave a definite response in only one of the 5 experiments when fed in addition to 3% of fish solubles. In 2 other experiments there was a suggestion of a response in the presence of fish solubles (Table II).

In all 4 of the experiments in which adenosine was fed alone a response was obtained; however, in Exp. 3 the response occurred only in the males. Table III shows the average of all males from this series of experiments which received the basal diet and the average of all males which received only adenosine supplementation. The same data are presented for the females and the average of the male and female averages. A statistical analysis by the method of Titus and Hammond(4) showed that the males which received adenosine were significantly larger than the males which received the basal diet. Also the unweighted mean of the sexes in the adenosine group was significantly greater

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† Nutritional Biochemicals Corp.

TABLE I. Basal Ration Used in Chick Assay for Unidentified Growth Factors.

	g/kg
Sucrose	628
Isolated soybean protein (Drackett C-1)	250
*Salts premix	60
Soybean oil	45
DL Methionine	6
Glycine	3
Choline chloride	2
Inositol	1
†Vit premix in sucrose	5
	1000

Plus the following (per kg):

Alpha tocopherol acetate	10 mg
Vit D	600 ICU
" A	15,000 USP

* Salts premix

$CaCO_3$	3803.2
K_2HPO_4	2580
$CaHPO_4 \cdot 2H_2O$	1190
NaCl	1340
$MgSO_4 \cdot 7H_2O$	816
Fe Citrate $6H_2O$	220
$MnSO_4 \cdot H_2O$	40
KI	6.4
$CuSO_4 \cdot 5H_2O$	2.4
$ZnCl_2$	2.0
	10,000.0

† Vit premix

Vit B_{12}	.02 mg
Biotin	.2
Menadione	.5
Pyridoxine	4
Pteroylglutamic acid	4
Riboflavin	6
Ca Pantothenate	20
Thiamin	10
Niacin	50
p-Aminobenzoic acid	100
Sucrose to total	5 g

than in the basal group. However, the females which received adenosine were not significantly larger than the controls.

In each of the 4 experiments in which a comparison is possible, 3% of fish solubles gave a more pronounced growth response than adenosine. In 2 of 4 experiments whey improved growth in the presence of fish solubles.

TABLE III. Effect of Adenosine Supplementation—Exp. 2-5. Mean chick wt at 4 wk in g.

Treatment	♂	♀	Avg of ♂ & ♀ means
Basal	176 (22)	173 (18)	174 (40)
Adenosine*	222 (11)	187 (13)	204 (24)
Difference	46†	14‡	30†

* Fed at 150 or 300 mg/kg of diet.
† Significant at 5% level(4).
‡ Not significant.
Figures in parentheses represent No. of birds.

Discussion. The response to adenosine, the greater response to fish solubles, and the fact that in most cases adenosine failed to improve the diet containing fish solubles suggest that adenosine might be one component of the growth factor complex of fish solubles. The existence of such a complex is indicated by the reports of Morrison *et al.*(5) and Couch *et al.* (6) that the ash accounts for part, but not all of the growth promoting effect of fish solubles. Other compounds similar to adenosine including adenine, kinetin(7), 5'-adenylic acid, and adenosine triphosphate have been studied for their growth promoting effects. Preliminary results indicate that none is superior to adenosine.

In these experiments and in many not reported here maximum responses were always obtained with the combination of fish solubles and whey. In some experiments whey did not give an additional response when added to the diet containing fish solubles. In some previous experiments the converse was true, *i.e.*,

TABLE II. Response from Adenosine Supplementation. Wt in g at 4 weeks.*

Additions to basal diet	Exp. 1		Exp. 2		Exp. 3				Exp. 4		Exp. 5	
	Adenosine level, mg/kg of diet											
	0	150	0	150	0	300	0	300	0	300	0	300
							♂ only					
None	152	—	192	224	169	167	175	197	152	196	180 216‡†	215 293‡†
2% dried whey	153	192	163†	190	178	158	165	171	149†	147	191 278‡	278‡†
3% fish solubles	244	227	253	271	224	268	253	303	251	270	239 311‡	296‡
2% dried whey + 3% fish solubles	242		296	274	280		309		244			

* All figures are avg of male and female averages except column designated "males only."
† Only 1 remaining male or female with 2 to 5 of opposite sex in these groups.
‡ Protein (Drackett C-1) increased to 40%.

fish solubles sometimes failed to give an additional response in diets containing whey. One supplement alone sometimes failed to elicit a response, as in the experiments reported here.

These results are not interpreted as contradicting reports from other laboratories that fish solubles and whey contain different unknown factors. They seem to indicate that the dietary requirements for the different unknowns vary from time to time for obscure reasons.

Other species have been observed previously to respond favorably to dietary purines and purine derivatives under certain conditions. Frost and Elvehjem(8) noted a growth response when adenine nucleotides were fed to rats on a low niacin diet. Huff and Bosshardt(9) found that mice which were fed 2% succinylsulfathiazole in a low fat diet gave a growth response to purines and purine derivatives.

Summary. In 5 experiments growth of chicks was increased by feeding adenosine at 150 or 300 mg kg of purified diet. Fish solubles at the rate of 3% of the diet gave a more pronounced response than did adenosine. Adenosine did not consistently elicit a response in the presence of either fish solubles or dried whey.

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Influence of Season on EEE Infection in English Sparrows. (22483)

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Clinical eastern equine encephalomyelitis (EEE) is not observed in man or horses in the United States during the winter months. Antibody surveys and attempts to isolate virus from wild birds and mosquitoes indicate that the virus is not active or is at a very low level of activity during the winter in the southern United States(1). Cessation of mosquito activity may explain this pattern in more northern areas but some species are active in considerable numbers throughout the winter in lower southern areas. It has been shown that lower temperatures such as occur in southern areas during the winter do not adversely influence the ability of mosquitoes to transmit the virus(2). Neither can the lack of virus activity be correlated with the absence of a susceptible bird population since susceptible birds migrate into the southern areas during the winter months. It has been shown that changes in day length and an internal reproductive rhythm influence the physiology, reproduction, and migratory behavior of birds(3,4). It is possible, therefore, that the cessation of virus activity may be due to changes in response to the virus in birds brought about by seasonal changes.

The present study was conducted to determine whether the response of birds to EEE infection was altered by seasonal influences.

Materials and methods. English sparrows (*Passer domesticus domesticus*) were trapped