TABLE II.
Effects of PGA and 4-Amino PGA on Growth and Survival of Mice in Experiment 2. Five mice were used per group.

Group	Supplement in mg per kg of diet		Body wt and (in parentheses) No. of survivors					Avg survival time in days*
	PGA	4-amino PGA	0	1	3	5	8 wk	
15	0.1	0	10	14	21	24	22 (5)	
16	1.0	0	10	13	19	19	19 (5)	
17	10	0	10	15	20	22	24 (5)	
18	100	0	11	13	20	22	24 (5)	
19	0.1	0.1	10	15	20	24	24 (5)	
20	1.0	0.1	10	16	21	26	28 (5)	
21	10	0.1	10	16	21	23	24 (5)	
22	100	0.1	11	16	23	26	30 (5)	
23	0.1	0.3	10	(0)				4
24	1.0	0.3	10	13 (4)	20	23	26 (4)	
25	10	0.3	10	13	18	20	21 (5)	
26	100	0.3	9	14	18 (4)	21	24 (4)	
27	0.1	1.0	10	9 (1)	(0)			5
28	1.0	1.0	10	13 (1)	(0)			8
29	10	1.0	10	9 (1)	(0)			7
30	100	1.0	10	(0)				5
31	0.1	3.0	10	(0)				6
32	1.0	3.0	11	(0)				4
33	10	3.0	10	(0)				4
34	100	3.0	10	(0)				4

* In the case of groups in which all the animals died during the experimental period.

of pteroylglutamic acid. This finding may be contrasted with the inhibitory effect of the 4-amino compound on *S. fecalis* R which is reversed by pteroylglutamic acid.

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Relation Between Induced Hyperthyroidism and an Unidentified Chick Growth Factor.*

A. R. ROBBLEE, C. A. NICHOL, W. W. CRAVENS, C. A. ELVEHJEM, AND J. G. HALPIN.
From the Departments of Biochemistry and Poultry Husbandry, University of Wisconsin, Madison.

The use of biological assays for study of unidentified growth factors is often restricted by the extent to which they can measure low concentration of a factor in a given material. This may be due to the small response of the animal to a given supplement. A series of studies have shown that the chick requires an unidentified growth factor found in condensed fish solubles,[1] whole liver powder, and certain other liver fractions.[2,3] The increased growth rate of the chick as a result of adding supplements carrying the factor has been used as an assay in determining some of the prop-

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1 Robblee, A. R., Nichol, C. A., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G., *Poultry Science,* 1948, in press.

2 Nichol, C. A., Robblee, A. R., Cravens, W. W., and Elvehjem, C. A., *Poultry Science,* 1948, in press.

3 Nichol, C. A., Robblee, A. R., Cravens, W. W., and Elvehjem, C. A., *J. Biol. Chem.,* 1947, **170**, 419.

TABLE I.
Effect of Supplements on the Growth Rate of Chicks.

Trial No.	Lot No.	Supplements to basal ration	Avg wt at 4 wks	Mortality %
1	1	None	120	25
	2	3% condensed fish solubles	220	0
	3	.25% desiccated thyroid U.S.P.	129	42
	4	.25% " " + 3% condensed fish solubles	203	25
	5	.25% " " + .05 cc Reticulogen (Lilly)/bird/day*	171	25
2	6	None	178	0
	7	3% condensed fish solubles	249	0
	8	.125% desiccated thyroid	137	0
	9	.125% " " + 3% condensed fish solubles	279	0
3	10	None	141	17
	11	3% condensed fish solubles	205	0
	12	.03% iodinated casein	96	33
	13	.03% " " + 3% condensed fish solubles	238	8
4	14	None	129	15
	15	3% condensed fish solubles	191	15
	16	.125% desiccated thyroid	134	40
	17	.125% " " + 3% condensed fish solubles	221	0
	18	.02% iodinated casein	117	40
	19	.02% " " + 3% condensed fish solubles	232	5
5	20	None	159	0
	21	3% condensed fish solubles	259	0
	22	.05 cc Reticulogen/bird/day*	240	0
	23	.125% desiccated thyroid	156	17
	24	.125% " " + 3% condensed fish solubles	249	17
	25	.125% " " + .05 cc Reticulogen/ bird/day*	233	0
	26	.02% iodinated casein	122	8
	27	.02% " " + 3% condensed fish solubles	273	0
	28	.02% " " + .05 cc Reticulogen/ bird/day*	251	0

* Injected.

erties of the factor.[4] However, in more recent work (unpublished) with low concentrations of the factor the response obtained is sometimes such that it is questionable whether it may be attributed to the presence of the factor, or merely to normal variation between the experimental groups of chicks.

The recent work of Betheil, Wiebelhaus, and Lardy[5] and of Ershoff *et al.*[6] offers a method which may be helpful in improving certain biological assays for unidentified growth factors. They found that the administration of desiccated thyroid to rats resulted in poor growth and a shortened survival period. It was suggested that feeding desiccated thyroid increases the requirement of the growing animal for some unknown factor(s), and that this increased requirement is fulfilled by feeding materials containing the unidentified antithyrotoxic factor. Liver and yeast were found to be sources of the antithyrotoxic material. It has been long recognized that hyperthyroidism increases the animals' requirement for various nutrients. The relationship of experimentally induced hyperthyroidism to increased requirements of many of the known vitamins has been reviewed.[5]

The observations of Betheil *et al.*[5] suggested that production of a condition of hyperthyroidism in chicks might be useful in

4 Robblee, A. R., Nichol, C. A., Cravens, W. W., Elvehjem, C. A., and Halpin, J. G., *J. Biol. Chem.*, 1948, **173**, 117.

5 Betheil, J. J., Wiebelhaus, V. D., and Lardy, H. A., *J. Nutrition*, 1947, **34**, 431.

6 Ershoff, B. H., PROC. SOC. EXP. BIOL. AND MED., 1947, **64**, 500.

increasing the effective assay range, and sensitivity of chicks to the unidentified chick growth factor found in condensed fish solubles and certain liver fractions. The present investigations were initiated to study the effect of thyroid active materials on the degree of response of chicks to supplements containing the unidentified factor or factors.

Experimental. Day-old (New Hampshire ♂♂ x Single Comb White Leghorn ♀♀) chicks were used in all the studies. The chicks were progeny of hens fed diet B1, described in a previous report.[1] The experimental groups were housed in electrically heated batteries with raised screen floors. The chicks were wing-banded and 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 four weeks. The basal diet used was the same as that used in previous trials.[1] It consisted of the following ingredients: ground yellow corn 35, wheat bran 10, wheat middlings 10, dehydrated alfalfa meal 5, soybean oil meal 28, vitamin test casein 7.5, limestone grit 2.0, steamed bonemeal 1.5, iodized salt 0.5, fish oil (2000A-400D) 0.5, $MnSO_4H_2O$ 0.025 g; thiamine 0.3, riboflavin 0.6, niacin 5.0, calcium pantothenate 2.0, pyridoxine HCl 0.4, inositol 100, choline chloride 150, *p*-aminobenzoic acid 10, biotin 0.02, folic acid 0.05, menadione 0.05, α-tocopherol 0.3 mg. Supplements to the basal ration were made at the expense of the corn.

Results. The results obtained are presented in Table I. In trial 1 the addition of 0.25% desiccated thyroid to the basal diet resulted in slow growth and high mortality (lot 3). The growth rate in this case was not significantly different from that of the basal group (lot 1). The addition of either 3% condensed fish solubles to the diet containing desiccated thyroid; or the daily injection of 1 U.S.P. unit of reticulogen (Lilly), a concentrated liver extract used in the treatment of pernicious anemia, gave a significant increase in growth rate. In both cases, however, the average weight attained at 4 weeks of age was somewhat below that of the positive control group in which condensed fish solubles alone was added to the basal ration (lot 2).

In the second trial a lower level of desiccated thyroid was used. In this case, a level of .125% desiccated thyroid in the diet (lot 8) resulted in a significantly lower growth rate than was obtained with the basal group (lot 6). The addition of 3% condensed fish solubles and .125% desiccated thyroid to the basal diet (lot 9) resulted in a significant increase in growth above that of the positive control (lot 7) which was supplemented with fish solubles alone.

Another source of thyroidal activity was tested in trial 3. Iodinated casein (3.07% thyroxin) was added to the basal ration at a level of .03%. A decrease in growth rate and an increase in mortality resulted (lot 12). The effect obtained when 0.03% iodinated casein and 3% condensed fish solubles were added was similar to that observed in the previous trial using desiccated thyroid. The average weight at 4 weeks of age (lot 13) was significantly above that of the positive control (lot 11).

The results in trial 4 are essentially in agreement with those of the previous 2 trials. The addition of desiccated thyroid to the basal ration (lot 16) did not result in a lower rate of growth than was obtained with the basal ration alone, but was accompanied with a marked increase in mortality. Iodinated casein (lot 18) caused both a decreased growth rate and high mortality. When the basal ration was supplemented with either of these materials along with 3% condensed fish solubles (lots 17, 19) a growth response greater than that which was obtained with condensed fish solubles alone (lot 15) resulted.

Trial 5 was carried out to determine whether or not reticulogen injections would fully substitute for condensed fish solubles in rations containing iodinated casein or .125% desiccated thyroid. The results show that reticulogen was effective in both cases (lots 25, 28) in overcoming the thyrotoxic effect of these materials. The administration of desiccated thyroid did not cause a decreased growth rate (lot 23). The growth rate was depressed in the group fed 0.2% iodinated casein (lot 26). In both cases mortality was higher than in the control groups. The addition of con-

densed fish solubles and iodinated casein gave an added growth response, but this effect was not observed with desiccated thyroid and fish solubles.

Discussion. The data presented here show that either condensed fish solubles or reticulogen (Lilly) serve to counteract the thyrotoxic effect of desiccated thyroid in the chick. This result parallels the observations of Ershoff *et al.*[6] and of Betheil *et al.*[5] who noted the anti-thyrotoxic effect of liver and yeast when desiccated thyroid was fed to rats.

A similar protective action was also obtained when iodinated casein was used in place of desiccated thyroid. This would indicate that the thyrotoxic effect is attributable to an increased thyroid activity. Since the protective action is exerted by both condensed fish solubles and reticulogen (Lilly) it would appear that the anti-thyrotoxic factor is identical to the unidentified growth factor described previously.[1,3]

In addition to the protective action of condensed fish solubles a further effect was noted in these experiments. An increased growth rate was obtained when either 0.125% desiccated thyroid or .02-.03% iodinated casein was added to the ration containing condensed fish solubles. The effect is similar to that observed by Irwin, Reinecke and Turner[7] who reported a stimulation in growth of chicks when iodinated casein was added to the ration. However, in the experiments reported herein the addition of either desiccated thyroid or iodinated casein to the basal ration without fish solubles caused a decreased growth rate and high mortality and it was only in the presence of an adequate amount of the factor supplied by condensed fish solubles that a growth stimulation was obtained.

The levels of iodinated casein that were found to be effective were considerably lower than those employed by Irwin *et al.*[7] They reported that 36 g of iodo-casein (3.1% thyroxin) per cwt. of feed was the optimum dosage level and that variations from this level were not effective. In the experiments reported herein the effective levels ranged from 9.08 to 13.62 g iodinated casein (3.07% thyroxin) per 100 lb. of feed. Higher levels were not tested. In a recent report Wheeler, Hoffman, and Graham[8] found that a diet containing 10 g of thyroprotein (3.0% thyroxin) per cwt. of feed resulted in a significant increase in uniformity and mean body weight of male birds at 12 weeks of age. The level of iodinated casein used was within the range we found to be effective.

The growth stimulation obtained may be due to an increase in the basal metabolic rate of the chick. Evidence that the administration of thyro-active materials to animals causes an increase in the requirement of many nutrients has been adequately reviewed by Betheil *et al.*[5] It appears that in the presence of sufficient amounts of all the nutrients, the increased basal metabolic rate results in a more rapid growth rate than is exhibited by the normal or untreated individual rather than a thyro-toxicity. It is quite evident, however, that a deficiency in the ration of the unidentified factor(s) found in condensed fish solubles results in a thyro-toxicity when desiccated thyroid or iodinated casein is administered.

The results that have been obtained by various investigators would seem to be dependent on 2 factors: the completeness of the ration and the level of thyro-active material fed. The ration used by Wheeler *et al.*[8] containing both fish meal and meat meal as protein supplements, would likely meet both of these requirements; however the basal ration used by Turner, Irwin, and Reinecke[9] and by Irwin *et al.*[7] might be submarginal with regard to the unidentified factor of condensed fish solubles.

The results of the study indicate that an experimentally induced hyperthyroidism may be an extremely useful tool in biological assay for unidentified factor(s) required by the chick for optimum growth. A level in the diet of .125% desiccated thyroid or levels of .02 to .03% iodinated casein resulted in an

7 Irwin, M. R., Reineke, E. P., and Turner, C. W., *Poultry Science,* 1943, **22**, 374.

8 Wheeler, R. S., Hoffman, E., and Graham, C. L., *Poultry Science,* 1948, **27**, 103.

9 Turner, C. A., Irwin, M. R., and Reinecke, E. P., *Poultry Science,* 1944, **23**, 242.

increase in the assay range for the unidentified factor.

The increase in growth rate which was obtained when iodinated casein or desiccated thyroid was added to the ration containing condensed fish solubles has not been studied beyond 4 weeks of age. Work is now in progress to determine the possible value of a combination of fish solubles and iodinated casein in commercial feeding.

Summary. A thyrotoxic condition in the chick, induced by feeding desiccated thyroid or iodinated casein was effectively counteracted either by supplementing the diet with condensed fish solubles or by the injection of reticulogen.

An increased growth response was obtained upon the addition of either desiccated thyroid or iodinated casein to a ration containing adequate amounts of the known and unidentified chick growth factors.

The addition of either 0.125% desiccated thyroid or 0.02 to .03% iodinated casein to the basal ration resulted in an improved assay range for the unidentified chick growth factor(s) in condensed fish solubles and reticulogen.

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