tion with the observation of Kaplan[6] who found that irradiation of a lymphosarcoma *in vivo* with 400 r significantly increased the number of metastases over the number occurring in unirradiated controls.

Summary. Evidence is presented that irradiation with small doses of X-rays decreases the time—latent period—required for a migrating cell to appear at the margin of a fresh explant of normal adult chicken heart. The maximum shortening of the latent period occurs with a dose of about 30 r. With larger doses of X-rays the latent period increases again, although a dose of the order of 1,000 r is necessary before the latent period is as long as that of the controls.

[6] Personal communication.

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Activity of Vitamin B_{12} in the Growth of Chicks.*

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The requirement of the growing animal for certain dietary essentials can be increased by inducing a hyperthyroid condition. Ershoff,[1] and Betheil *et al.*[2] observed that liver effectively counteracted a thyrotoxicity in rats which were fed desiccated thyroid. Robblee *et al.*[3] found that the addition to the basal ration of either desiccated thyroid or iodinated casein improved the effective assay range for an unidentified chick growth factor. Work has been in progress[4] for some time on the properties of the growth stimulating components of condensed fish solubles. Fractionation procedures indicated similarities between the chick factor and the material in liver which is active in the treatment of pernicious anemia. Injectable liver extracts were found to be active in promoting the growth of chicks fed rations which were complete in the known growth essentials.[5]

Rickes *et al.*[6] reported the isolation from liver of a red crystalline compound termed vitamin B_{12} which was active in microgram quantities for the remission of pernicious anemia in relapse. More recently Ott *et al.*[7] showed that crystalline vitamin B_{12} had "animal protein factor" activity for the chick. Our data confirm this observation and indicate that vitamin B_{12} is highly active in stimulating the growth of the hyperthyroid chick.

Method. The chicks (New Hampshire ♂♂ x Single Comb White Leghorn ♀♀) were the progeny of hens fed diet B_1 described previously[8] in which protein was provided by

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TABLE I.
Response of Chicks to Supplementation with Vitamin B_{12}.

Group No.	Supplement	No. of chicks surviving	G gain during 14-day test period	Hemoglobin, g
1	None	7	105	8.33
2	3% condensed fish solubles	10	190	8.52
3	Vit. B_{12} 0.75 γ/100 g ration	10	152	8.42
4	" " 1.5 γ/100 g "	9	193	8.98
5	Liver extr. (Lilly, reticulogen 20 U.S.P. units/cc), 0.5 U.S.P. unit bird/day*	10	191	8.52
6	Vit. B_{12}, 0.01/γ/bird/day*	10	126	8.84
7	" " 0.1 γ/ " "	10	177	8.81
8	" " 0.5 γ/ " "	10	184	8.60

* Injected intramuscularly.

yellow corn, wheat, soybean oil meal, fish meal and meat scrap. The experimental groups were housed in electrically heated batteries with raised screen floors. Feed and water were supplied *ad libitum*. The basal ration contained ground yellow corn 35, soybean oil meal 28, wheat bran 10, wheat middlings 10, dehydrated alfalfa meal 5, vitamin test casein 7.5, limestone grit 2.0, steamed bonemeal 1.5, iodized salt 0.5, fish oil (2000A-400D) 0.5, $MnSO_4 \cdot H_2O$ 0.025, iodinated casein (Protamone, 3.07% thyroxine) 0.05 g; thiamine 0.3, riboflavin 0.6, niacin 5.0, calcium pantothenate 2.0, pyridoxine 0.4, p-aminobenzoic acid 10, choline chloride 150, inositol 100, folic acid 0.05, biotin 0.02, menadione 0.05, and α-tocopherol 0.3 mg.

The chicks were wing-banded and weighed when one day old and placed on the basal ration. Individual weights were recorded at weekly intervals. Selection of the birds to be used for the experiment was made at the end of a 2 week depletion period. Mortality during the first 2 weeks was 9%. In order to limit individual variation, two criteria for selection were used: (1) weight (chicks below 60 or above 105 were discarded) and (2) rate of gain (the % gain during the second week was calculated for each bird and those within the range of 33 to 59% were kept). Of the chicks surviving the 2 week depletion period 17% were discarded on the basis of weight and 16% were discarded on the basis of % gain. The selected chicks were distributed (10 per group) in such a way that the average weights of the groups fell between 85 and 88 g and the average % gain ranged from 47 to 48%.

Supplements were added on the 15th day and continued for a 14 day period. Supplementation of the basal ration with 3% condensed fish solubles was made at the expense of corn. The vitamin B_{12} solution (25γ/ml) supplied by Dr. D. F. Green of Merck and Company, Inc., Rahway, N. J., was diluted with distilled water and thoroughly mixed into the basal ration by a mechanical mixer to provide 0.75 γ and 1.5 γ of vitamin B_{12} per 100 g. Injections of vitamin B_{12} and liver extract were made into the pectoral muscle using a 1 cc tuberculin hypodermic syringe calibrated in 0.01 cc. Dilutions of vitamin B_{12} with distilled water were such that 0.2 cc injected every other day provided the dosages listed in Table I. Hemoglobin determinations were made on all chicks on the final day of the test period.

Results. The results are presented in Table I. The positive control for groups given vitamin B_{12} in the basal ration was a group receiving a supplement of 3% condensed fish solubles. Groups receiving vitamin B_{12} by injection were compared with a group injected with a liver extract (Lilly, reticulogen, 20 U.S.P. units per cc), at a dosage of 0.5 U.S.P. units per bird per day. The two positive controls showed average weight differences from the negative control of 85 g (condensed fish solubles) and 86 g (reticulogen).

Pure vitamin B_{12} at a level of 0.75 γ per 100 g of ration caused an average increase in weight of 47 g over the unsupplemented group. At a level of 1.5 γ of vitamin B_{12} per 100 g of ration the average weight in-

crease was 88 g more than the negative control.

Vitamin B_{12} was injected at 3 dosage levels: 0.01 γ, 0.1 γ and 0.5 γ per bird per day. The growth response was 21 g at the 0.01 γ level, 72 g at the 0.1 γ level and 79 g at the 0.5 γ level.

No significant difference was noted in the average hemoglobin values of the experimental groups.

Discussion. It is apparent that pure vitamin B_{12} is able to replace the growth stimulating properties of condensed fish solubles or injectable liver extract under the experimental conditions described. It is a reasonable assumption that the properties of these complex materials which have been under study by means of fractionation procedures and chick growth assay, are due to the occurrence of vitamin B_{12}.

The thyrotoxic condition in chicks induced by feeding a ration containing 0.05% iodinated casein is characterized by the hyperexcitability of the birds and depressed rate of gain in weight. The growth response to supplementation is not dependent upon a hyperthyroid condition but the use of a basal ration containing iodinated casein increases the magnitude of the growth response.[3] The role of vitamin B_{12} in counteracting a thyrotoxic condition will require further detailed investigation.

When vitamin B_{12} was fed at a level of 0.75 γ per 100 g of ration the growth response was highly significant and the rate of gain was approximately half maximal under the conditions of this assay. At a level of 1.5 γ vitamin B_{12} per 100 g of ration the growth response compared closely with that of a group receiving 3% condensed fish solubles. This material is recognized as a potent source of the animal protein factor and the rate of gain induced by a 3% level of condensed fish solubles was observed in previous studies to be close to maximum.

Injection of vitamin B_{12} at a level of 0.01 γ per bird per day resulted in a marginal growth response. At a dosage of 0.5 γ per bird per day the response compared closely with that of a group injected with liver extract (Lilly, reticulogen) at a level of 0.5 U.S.P. unit per bird per day. This liver extract was previously observed to be highly effective in stimulating the growth of the hyperthyroid chick.[5] The response observed at a level of 0.1 γ vitamin B_{12} per bird per day was not appreciably lower that that at the 0.5 γ level.

Summary. Vitamin B_{12} administered orally or parenterally completely counteracted a thyrotoxic condition in chicks produced by feeding a basal ration containing 0.05% iodinated casein. A level of 0.75 γ vitamin B_{12} per 100 g of ration resulted in a half maximal growth response. At a level of 1.5 γ vitamin B_{12} per 100 g of ration the growth response after a two week test period was 88 g compared to 86 g for a group supplemented with 3% condensed fish solubles. A response of 72 g resulted from the intramuscular injection of 0.1 γ vitamin B_{12} per bird per day.

Pure vitamin B_{12} can replace the animal protein factor activity of condensed fish solubles and injectable liver preparations.

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