hand, since vit. B_{12} increased the total thiouracil intake, it would appear logical to expect a greater thyroid inhibition than in the rats which received thiouracil without the vitamin. This would account for the lower concentration of I^{131} in the thyroids of the former as compared to the latter rats. On the whole, it seems reasonable to assume that thyroid uptake of a tracer dose of I^{131} is a better index of thyroid activity than thyroid weight. The former is believed to reflect direct thyroid secretory ability, whereas the latter reflects an indirect action on the thyroid by pituitary thyrotrophic hormone and does not definitely indicate either increased or decreased thyroid function.

It is interesting to consider whether vit. B_{12} can induce normal body growth in hypothyroid rats, as suggested by this study. Thus, vit. B_{12} completely counteracted the growth-retarding action of thiouracil, while the latter was still able to depress thyroid activity. It is possible that vit. B_{12} does not require the mediation of the thyroid to exert its anabolic effects on growth.

Summary. The effects of crystalline vit. B_{12} on thiouracil action was determined in immature female rats for a 30-day period. The vitamin completely counteracted the growth-inhibiting action of thiouracil, and this was accompanied by a considerable increase in food consumption. Although vit. B_{12} decreased the thyroid hypertrophy induced by thiouracil, the uptake of radioactive iodine (I^{131}) by the thyroids was even less than in the rats which received thiouracil only. It is suggested that vit. B_{12} may be able to induce normal growth in hypothyroid rats.

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Effects of Vitamin B_{12} on Normal Thyroid Function in Rats.*† (18143)

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Vitamin B_{12} can counteract the retardation of growth in young rats which results from administering toxic amounts of thyroid powder or thyroprotein(1-4). The mechanism by which vitamin B_{12} achieves this effect has not been explained, although Monroe and Turner(5) presented data indicating that the vitamin increases the catabolism of administered thyroxine in chicks. If this is so, then vitamin B_{12} may also favor the catabolism of endogenous thyroid hormone, in which case pituitary thyrotrophic hormone secretion would be stimulated with a subsequent increase in thyroid secretion rate. It was considered of interest therefore, to determine whether or not the administration of vitamin B_{12} would alter normal thyroid function in immature rats.

Procedure. Young male and female rats of the Carworth strain were placed on experiment for 20 and 30 days respectively. With the exception of 2 groups, all rats were fed the following basal ration: yellow corn meal, 35%; ground wheat, 25%; linseed oil meal, 10%; whole milk powder, 20%; alfalfa leaf meal, 6%; brewers yeast, 3%; and table salt,

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TABLE I. Effects of Vitamin B_{12} on Normal Thyroid Function in Rats.

Group	Treatment	Avg body wt		Avg thyroid wt		Radioactivity—avg counts per sec.		
		Orig., g	Final, g	Actual, mg	Per 100 g body wt, mg	Per thyroid	Per mg thyroid	Per 100 g body wt
				Female rats—30 days trial				
1	Controls	60.3	135.2	12.5	9.3 ± 1.4*	25.2 ± 3.8*	2.1 ± .6*	18.9 ± 3.5*
2	40 μg B_{12}	60.4	147.7	12.2	8.3 ± 1.7	26.8 ± 6.7	2.3 ± .7	18.2 ± 5.0
3	80 μg B_{12}	60.7	153.8	14.5	9.4 ± 1.3	28.4 ± 7.8	2.0 ± .5	18.7 ± 5.8
				Male rats—20 days trial				
4	Controls	57.2	117.2	10.9	9.4 ± 1.3	9.0 ± 1.5	0.7 ± .1	6.9 ± 1.2
5	80 μg B_{12}	56.0	139.8	11.8	8.3 ± 1.8	10.0 ± 4.5	1.0 ± .4	7.1 ± 2.2
6	Controls†	56.0	136.1	12.7	9.4 ± 2.1	16.6 ± 9.6	1.3 ± .5	12.4 ± 7.4
7	80 μg B_{12}†	56.1	142.1	12.6	8.7 ± 1.9	19.1 ± 6.0	1.6 ± .5	13.7 ± 4.5
				Male rats—20 days trial				
8	Controls	55.6	122.5	9.2	8.5 ± 0.6	26.0 ± 2.5	2.8 ± .2	21.6 ± 2.2
9	40 μg B_{12}	56.7	128.8	11.2	8.8 ± 0.7	21.9 ± 2.0	2.0 ± .1	17.0 ± 1.4
10	80 μg B_{12}	56.3	140.2	11.0	8.0 ± 0.5	23.4 ± 2.1	2.1 ± .2	16.7 ± 1.4
11	.16% thyroprotein	56.5	97.7	6.4	6.5 ± 0.7	0.06 ± 0.05	0.01	0.06 ± 0.04
12	.16% thyroprotein + 40 μg B_{12}	56.2	126.0	6.2	4.8 ± 0.4	0.13 ± 0.08	0.02	0.10 ± 0.06

* Standard error of mean $= \sqrt{\frac{\Sigma d^2}{n(n-1)}}$

† Received modified diet in which casein, corn oil and cerelose were substituted for whole milk powder.

1%. Vit. B_{12}§ was mixed into the ration in amounts of either 40 or 80 μg per kg of feed. For 2 groups of male rats, 0.16% thyroprotein¶ was incorporated in the ration. Four hours prior to sacrifice, each rat was injected intraperitoneally with a tracer dose of I^{131} estimated to contain 0.2 μc of radioactivity. The thyroids were carefully removed, weighed and placed in small copper discs. After allowing the thyroids to dry overnight, the radioactivity of each was counted separately under a thin mica-end window counter. All rats were housed in a constant temperature animal room at 75°F.

Results. The data are summarized in Table I. The first experiment was performed on 3 groups of female rats. It can be seen that the addition of either 40 (group 2) or 80 μg of vit. B_{12} (group 3) to the ration increased the final body weights of the rats above that of the controls (group 1). Apparently the basal ration did not contain an amount of B_{12} sufficient for optimal growth. Vit. B_{12} did not alter the thyroid weight of these rats when calculated on a body weight basis. The uptake of I^{131} by the thyroids of all three groups was the same whether calculated on a thyroid, per mg of thyroid or on a body weight basis.

The results of the next experiment on 4 groups of male rats are essentially in agreement with the data on female rats. The inclusion of 80 μg of vit. B_{12} per kg of ration did not alter thyroid weight or uptake of I^{131}. It is notable that the controls (group 6) which received the modified diet gained more body weight than the controls (group 4) fed the basal ration.

Vit. B_{12} did not alter thyroid function in the next experiment, as can be seen by comparing the data in groups 9 and 10 with group 8. The small differences in concentration of I^{131} between the thyroids of the three groups are not statistically significant. The administration of thyroprotein (group 11) significantly reduced the final average body and thyroid weights when compared with the

§ Crystalline vit. B_{12} in the form of a triturate of NaCl was made available through the courtesy of Dr. D. F. Green of the Veterinary Division, Merck and Co.

¶ Thyroprotein (Protamone) was made available through the courtesy of Cerophyl Laboratories, Kansas City, Mo.

controls (group 8). The thyroids of these rats were apparently nonfunctional, as indicated by the virtual absence of I^{131} in the glands. In fact, the radioactivity in these thyroids did not differ statistically from that present in the atmosphere (background). Although the administration of 40 μg of vit. B_{12} (group 12) completely overcame the inhibitory effects of the thyroprotein on growth, it did not counteract the effects of the latter on thyroid activity.

Discussion. These experiments indicate that the administration of vit. B_{12} does not alter normal thyroid function in young rats, even when given in amounts sufficient to increase body growth. Apparently, vit. B_{12} does not affect the metabolism or excretion of endogenous thyroid hormone, since such changes would have altered thyroid activity and uptake of I^{131}. It seems reasonable to conclude that vit. B_{12} can increase body growth in rats independently of the thyroid.

On the whole, these data also do not support the view that vit. B_{12} increases the turnover within the body of administered thyroid materials, since the vitamin did not at all overcome the thyroid-inhibiting action of thyroprotein. However, it is possible that the dose of thyroprotein required to completely inhibit thyroid activity was lower than the amount given, and hence its increased metabolism by vit. B_{12} was not reflected by the thyroid.

Summary. Crystalline vit. B_{12} was fed to immature rats of both sexes in order to determine whether the vitamin could alter thyroid function in normal or thyroprotein-treated rats. The growth rate of the rats supplemented with the vitamin was increased above that of the normal or thyroprotein-treated controls, but there was no significant effect on thyroid weight or uptake of I^{131}. It is concluded that vit. B_{12} does not alter normal thyroid activity in rats.

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Studies on the Destruction of Red Blood Cells. VIII. Molecular Orientation in Sickle Cell Hemoglobin Solutions.* (18144)

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Sherman(1) reported "without interpretation" the observation that "under the polarizing microscope characteristic sickle cells exhibit a definite birefringence". Since that time, several writers have used this finding as evidence that the hemoglobin molecules assume an orderly arrangement in sickled erythrocytes(2,3). When hemoglobin was removed from the red cells, Ponder(2) demonstrated that the "ghosts" would not become sickle shaped on exposure to low oxygen tensions. Recent studies by Pauling *et al.* (3,4) have shown that certain physical and chemical properties which differentiate sickle cell hemoglobin from normal hemoglobin result from an alteration in the structure of the globin portion of the molecule. Human deoxygenated hemoglobin is said to be less soluble than the oxygenated form, suggesting that the phenomenon of sickling might be related to incipient crystallization of the

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