experiments reported here, has also been noted by Mitchell *et al.*(9) in studies of amylase, adenosine deaminase and "adenylic acid phosphatase" in Taka-diastase on their filter paper chromatopile. In an initial run with a $(NH_4)_2SO_4$ gradient at pH 6.5, the enzymes were separated, the "phosphatase" being least adsorbed(9). In second runs with samples from activity peaks, however, the enzyme failed to move. Presumably a strongly adsorbing component of the Taka-diastase was acting as a displacing agent in the first run. The enzymes could be made to move in the second runs by raising the pH to higher levels. Runs at a pH of 6.5 and constant $(NH_4)_2SO_4$ concentrations gave a wide spread of the enzyme activity, with a frontal peak in most cases. Such results would follow from the present observations on two types of adsorption, that firmly held and that which can be eluted, the latter perhaps being held on the former.

The adsorption of the milk phosphatase on various Celites is roughly paralleled by the adsorption of certain viruses on Celites, suggesting that with viruses also the clay in the Celites might be the active adsorbent. The pneumonia virus of mice, for example, is strongly adsorbed at pH 6.8 from 0.15 M NaCl on Filter-Cel, Celite no. 505, and Super-Cel, less strongly on Hyflo Super-Cel and Celite no. 503(4). Here also elutions were successful only at high pH values, pH 9 and above.

Summary. The adsorption of a preparation of bovine alkaline phosphatase on Filter-Cel was investigated in respect to amount of adsorbent, concentration and kind of salt, pH and temperature. Calcined Celites were poor adsorbents for the phosphatase.

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Effects of Vitamin B_{12} on Diethylstilbestrol and Thyroprotein Action in Male Rats.*† (18434)

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Recently vit. B_{12} has been demonstrated to counteract the inhibition of growth induced by feeding large doses of thyroid-active substances(1-4) or thiouracil(5) to young rats. It was considered possible that vit. B_{12} might likewise counteract the inhibition of growth which has been observed in young rats treated with diethylstilbestrol(6). It was also of interest to determine whether the effectiveness of vit. B_{12} in these respects could be explained by an increase in food consumption and/or an increase in the catabolism or rate of excretion of the hormones administered.

Methods. Immature male albino rats (Carworth strain) were divided into 10 uniform groups of 10 each, and were fed the following basal ration for 20 days: 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, 1%. Diethylstilbestrol‡ or thyroprotein‡ were mixed into the ration in amounts of 0.10 and 0.16 per cent respectively. Vit.

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TABLE I. Effects of Vitamin B_{12} on Diethylstilbestrol and Thyroprotein Action in Male Rats.

Group	Treatment	Avg body wt		Avg total gain in body wt, g	Avg daily food intake, g	Avg testis wt		Avg sem. ves. wt	
		Orig., g	Final, g			Actual, mg	Per 100 g body wt, mg	Actual, mg	Per 100 g body wt, mg
1	Control	55.0	115.6	60.6	9.6	1207.6	1016.3 ± 94.7*	48.2	41.5 ±4.1*
2	.2 μg B_{12}	58.6	120.8	62.2	9.5	1011.7	834.1 ± 99.5		
3	.1% stilb.†	55.0	80.9	25.9	6.0	272.4	331.3 ± 43.6	63.8	79.0 ± 5.1
4	.1% stilb. .2 μg B_{12}	55.1	91.1	36.0	6.8	268.5	293.8 ± 26.3	67.8	73.8 ± 7.4
5	.1% stilb. .4 μg B_{12}	58.0	98.4	40.4	7.6	333.4	340.8 ± 37.7		
6	.16% thyro.‡	58.4	87.8	29.4	9.3	196.1	225.8 ± 14.6		
7	.16% thyro. .2 μg B_{12}	57.2	98.7	41.5	10.8	213.4	217.9 ± 22.1		
8	.1% stilb. .16% thyro.	55.5	67.5	12.0	7.0	176.8	258.7 ± 17.0	55.5	83.6 ± 6.8
9	.1% stilb. .16% thyro. .2 μg B_{12}	55.5	80.7	25.2	8.5	189.1	234.3 ± 16.8	61.9	76.9 ± 5.3
10	.1% stilb. .16% thyro. .4 μg B_{12}	57.8	88.0	30.2	8.9	229.7	266.0 ± 31.6		

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

† Diethylstilbestrol.
‡ Thyroprotein.

B_{12}‡ was injected subcutaneously in doses of 0.2 or 0.4 μg daily. All rats were housed in a temperature-controlled animal room at 75°F. Body weight and food intake data were collected every second day. On the 20th day the animals were killed and weighed. The testes were removed from all and the seminal vesicles from half of the rats, and weighed on a Roller-Smith balance.

Results. Body growth and food intake. The data are summarized in Table I. The average final body weights and daily food consumption of the controls (group 1) were about the same as the rats supplemented with vit. B_{12} (group 2). The diethylstilbestrol-treated rats (group 3) gained less than half the weight and consumed less than ⅔ as much food as the controls. Injections of 0.2 (group 4) or 0.4 μg of vit. B_{12} (group 5) partially counteracted the growth and appetite-depressing actions of the estrogen, with the larger dose of the vitamin showing the greater effect.

Thyroprotein (group 6) reduced growth considerably but had no appreciable effect on food intake. When vit. B_{12} was added (group 7) it partially overcame the inhibition of growth and increased the total food consumption above that of the controls. The most drastic retardation of growth occurred when diethylstilbestrol and thyroprotein were fed together (group 8). Total food consumption was intermediate between that consumed by the groups given diethylstilbestrol or thyroprotein only. Vit. B_{12} (groups 9 and 10) partially overcame these inhibitory effects of the two hormones on growth and appetite.

Testes and seminal vesicles. There were no significant differences between the testes weights of the controls (group 1) and rats injected with vit. B_{12} (group 2). Diethylstilbestrol (group 3) reduced the weight of the testes to about ⅓ of that in the controls, presumably through inhibiting FSH secretion by the anterior pituitary. The weight of the seminal vesicles was doubled on the basis of body weight, probably as the result of a

‡ Diethylstilbestrol and crystalline vit. B_{12} (Cobione) were kindly supplied by Dr. D. F. Green of the Veterinary Division, Merck and Co., Rahway, N. J., and thyroprotein (Protamone) by the Cerophyl Laboratories, Inc., Kansas City, Mo.

direct stimulation of the seminal vesicles. Vit. B_{12} (groups 4 and 5) did not modify these actions of diethylstilbestrol on the testes or seminal vesicles.

Thyroprotein (group 6) decreased the weight of the testes even to a greater degree than diethylstilbestrol. The addition of vit. B_{12} (group 7) did not alter this result. The effects of diethylstilbestrol and thyroprotein together (group 8) on the testes and seminal vesicles were also not changed by the addition of vit. B_{12} (groups 9 and 10).

Discussion. These data indicate that vit. B_{12} can at least partially overcome the growth-retarding action of diethylstilbestrol in young male rats. It is possible that a greater dose of the vitamin than used in these experiments would have completely overcome these inhibitory effects of diethylstilbestrol. The greater food consumption by the vitamin-supplemented rats was at least partially responsible for the increased growth.

Vit. B_{12} did not alter the effects of diethylstilbestrol or thyroprotein on the testes or seminal vesicles. This is in contrast to the observation of Ershoff *et al.*(7,8) that desiccated whole liver or yeast can overcome the inhibition of ovarian development in immature rats fed massive doses of alpha estradiol. It is possible that these food substances contain a factor or factors other than vit. B_{12} which are effective in this respect. Differences between the actions of natural estrogens, such as estradiol, and diethylstilbestrol(6) must also be considered.

Unfortunately, the data presented here cannot be considered as evidence for or against the view that vit. B_{12} favors the disappearance of thyroprotein or diethylstilbestrol from the body. The large amounts of diethylstilbestrol or thyroprotein fed the rats may have been more than sufficient to induce maximal responses by the testes and seminal vesicles, and hence any relatively small change in the catabolism or excretion of these hormones would not be reflected in the response of the two organs.

Monroe and Turner(9) recently reported that vitamin B_{12} may be able to increase the catabolism of administered thyroxine in baby chicks, but no effect of vit. B_{12} could be demonstrated by the senior author on normal thyroid function in rats(4) or chicks(10). Although the ability of vit. B_{12} to counteract the growth-retarding effects of large doses of diethylstilbestrol or thyroprotein in rats may be attributed in part to its stimulation of greater food intake, other channels for its effectiveness are also indicated.

Summary. The effects of crystalline vit. B_{12} on several actions of diethylstilbestrol and thyroprotein were determined in 100 young male albino rats. Diethylstilbestrol and thyroprotein were incorporated into the ration in amounts of 0.10 and 0.16% respectively, and vit. B_{12} was injected subcutaneously in doses of 0.2 or 0.4 μg daily. Vit. B_{12} partially counteracted the growth-retarding action of these two hormones when given individually or together, and also increased food consumption. The vitamins did not alter the ability of diethylstilbestrol or thyroprotein to depress testes weight or the ability of the former to increase the weight of the seminal vesicles.

Addendum. Further data obtained since this manuscript was submitted indicate that vit. B_{12} probably does not alter the turnover of thyroprotein in the rat. The increase in the basal metabolism of rats given thyroprotein was found to be unaffected by vit. B_{12} administration.

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