the validity of assessing the toxicity or nontoxicity of a test compound on findings based on the use of a single diet.

Summary. Immature rats were fed graded levels of DBH in conjunction with either a highly purified or natural food stock ration. A significant difference in response to DBH administration was observed between rats fed the 2 diets. On the stock ration DBH when fed at levels of 0.1%, 0.15% or 0.2% of the diet had little, if any, adverse effect on weight increment and none on gross appearance. When fed at comparable levels in the purified ration, however, DBH caused a significant retardation in growth, an unhealthy appearance, varying degrees of alopecia, and at the 0.2% level, death. The protective factor or factors in the stock ration was distinct from any of the known nutrients.

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Dinitrophenol Inhibition of Pituitary Adenoma Formation in Mice Fed Propylthiouracil. (28046)

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Surgical thyroidectomy, injection of large doses of NaI^{131}, or prolonged administration of 6-propyl-2-thiouracil (PTU) or related drugs are known to induce pituitary adenomas in mice(1-5). Treatment with thyroxine will prevent the appearance of the tumors (2,6), perhaps by direct action of the hormone upon the pituitary(7,8,9). 2,4-Dinitrophenol (DNP) administered systemically will inhibit release of thyrotropin(10) but, in contrast to thyroxine, DNP injected directly into the pituitary or into the hypothalamus will not inhibit thyrotropin release(8). Its effect appears to be indirect. The present study was undertaken to determine whether DNP would affect induction of pituitary tumors in mice during prolonged feeding of PTU.

Materials and methods. Six groups, each containing 60 four- to five-week-old C57Bl mice, were fed Purina Fox Chow meal supplemented with PTU and/or DNP as indicated in Table I. The mice were maintained on these diets for 17 months, after which animals still alive were sacrificed. The pituitaries were weighed and prepared for histological examination. The objective criterion for adenoma formation was a pituitary weighing more than 4 mg. No control pituitaries weighed more than 1.5 mg.

Results. Adenomas were produced by both 10 and 12 g of PTU per kg of diet (Table I). These tumorous pituitaries were characterized by multiple adenomas consisting of large cells with relatively clear cytoplasm. In some instances, the predominant cells were deep staining, arranged in cords, and in general resembled a slow-growing malignant tumor. However, no evidence of metastases or vascular invasion was seen and mitotic figures were infrequent.

Incorporation of 0.5 g of DNP per kg of diet reduced the incidence of pituitary tumors caused by either dose of PTU by at least 75%. "Tumors" which did appear in mice fed both PTU and DNP were small and were characterized histologically by ill defined areas of swollen cells which had some cytoplasmic granules but there were no circumscribed adenomas.

No pituitary tumors were observed in control animals fed Fox Chow meal alone or in

TABLE I. Incidence of Pituitary Tumors in C57B1 Mice Fed Propylthiouracil (PTU) and Dinitrophenol (DNP).

Group	A	B	C	D	E	F
DNP level in diet (g/kg)	0	.5	0	0	.5	.5
PTU level in diet (g/kg)	0	0	10	12	10	12
# of mice living 17 mo	28	38	24	29	20	29
# of mice with pituitary "tumors"*	0	0	15	21	3	3
% of mice with pituitary "tumors"*	0	0	62	72	15	10
Median wt of pit. "tumors" (mg)*			7	10	5	9
Avg wt of pit. "tumors" (mg)*			10	18	5	9

* Avg weight of control pituitaries was .83 ± .03 mg. Pituitaries weighing more than 4.0 mg are tabulated as "tumors".

animals fed a diet containing only DNP. Pituitaries of these 2 groups had normal histological appearance. Thyroid hyperplasia was grossly apparent in mice fed PTU alone, but not in mice fed PTU plus DNP.

Discussion. The reversal of propylthiouracil pituitary carcinogenesis by incorporation of DNP in the diet is additional evidence that the effect of PTU in tumor formation depends upon its disturbance of thyroid function. Gorbman(11) suggested that carcinogenic effects of PTU feeding might depend upon non-specific toxicity of the diet coupled with specific antithyroid activity. Although the present experiment does not test this hypothesis, the results demonstrate that general toxicity alone is not sufficient to produce pituitary adenomas.

The present experiment does not indicate clearly the site of DNP action and its mode of action. Greer *et al.*(8) were unable to suppress TSH release by direct injection of DNP into either pituitary or hypothalamus, even though systemic administration of DNP gives this effect(10). Although both thyroxine and DNP will uncouple oxidative phosphorylation, they appear to act by different mechanisms(12). The similarity of DNP and thyroxine in preventing PTU induced pituitary tumors may likewise be achieved by different mechanisms. However, the fact that the DNP also reversed PTU induced thyroid hyperplasia is strong evidence that the ultimate effect was reduction of pituitary activity. It appears probable that both thyroxine and DNP inhibit pituitary tumorigenesis by this means.

One might predict that DNP would cause cessation of growth of hormone dependent thyroid(13) and pituitary(14) tumors which do not grow in the presence of active thyroid tissue. This is being investigated.

Summary. Incorporation of 0.5 g of 2,4-dinitrophenol per kg of diet reduced the incidence of pituitary adenomas caused by prolonged feeding of 6-n-propyl-2-thiouracil.

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