major reactions: (a) twitchings of the thigh and leg muscles, (b) marked vasoconstriction.[4] Within 30 to 45 minutes after the onset of the perfusion with dibenamine (1:250,000 to 1:25,000) when the action of large amounts (50-1000 γ) of epinephrine is completely blocked, nicotine still induces notable vasoconstriction and visible twitchings of the muscles.

Discussion. Dibenamine hydrochloride used in concentrations of 1:1,000,000 to 1:25,000 prevents epinephrine-induced vasoconstriction as tested in the Laewen-Trendelenburg preparation. The protective effect afforded by this drug appears to be very potent, for it can block the action of very large amounts of epinephrine (1000 γ). This is practically a total adrenolytic effect. A comparative study with benzyl-imidazoline hydrochloride (priscol),‖ another synthetic autonomic blocking agent,[5] used under the same experimental conditions, exhibits a similar protective action but only for smaller amounts of epinephrine. This is in agreement with the results obtained by other investigators experimenting in intact animals.[2,6] The inhibitory effect of dibenamine on epinephrine does not seem to be due to a direct chemical interaction between the 2 molecules. The *in vitro* experiments reported above seem to support this view. In addition to being adrenolytic, dibenamine exhibits also a marked sympathicolytic action as shown by studies in the intact animal.[1,2] Since dibenamine is an adrenolytic and sympathicolytic agent, its site of action is probably at the neuroeffector cells or between the postganglionic nerve endings and the latter.

Within 30 to 50 minutes after the onset of the perfusion with dibenamine, when the action of large amounts of epinephrine is completely inhibited, small amounts of both hypertensin and nicotine still exhibit a marked vasoconstrictor activity. These facts seem to indicate that dibenamine is either specifically adrenolytic or that these various vasoconstrictor substances do not have the same site of action.

Summary. 1. Dibenzyl - β - chloroethyl amine hydrochloride (dibenamine) inhibits the vasoconstrictor action of epinephrine, as tested in the Laewen-Trendelenburg preparation in the frog. Its adrenolytic action is very potent, for it blocks even large amounts of epinephrine (50-1000 γ). 2. When the action of large amounts of epinephrine is completely inhibited by dibenamine, both hypertensin and nicotine still exhibit a marked vasoconstrictor activity. 3. The peripheral locus of action of dibenamine seems to be at the neuroeffector cells or between the postganglionic nerve endings and the latter.

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Effect of Thiouracil and Estrogen on Lactogenic Hormone and Weight of Pituitaries of Rats.*

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The weight of the hypophysis increases subsequent to thyroidectomy,[1-4] and this may

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be due to an augmentation of the basophil cells[4,5] which are presumed to secrete thyrotropin.[6-8] The acidophils, on the other hand, become markedly decreased following thyroidectomy.[9] The administration of goitrogenic substances, like thyroidectomy, can produce similar effects on the hypophysis. Thus, they have been reported to increase the weight of the pituitary,[10] increase and hypertrophy the basophil cells, and reduce the number and size of the eosinophils.[11-13]

The acidophils of the hypophysis are the source of the lactogenic hormone,[6,7,14] and hence it would be expected that this hormone would be reduced following thyroidectomy. However, the only 2 reports available on this subject are not in agreement. McQueen-Williams reported that thyroparathyroidectomy in male rats reduced the lactogen content of the pituitary,[15] whereas Reineke, Bergman and Turner found no apparent change in the pituitary content of this hormone following thyroidectomy in young goats.[16]

It was thought that since thiouracil is similar in its effects to thyroidectomy, it would offer a considerable advantage in a restudy of the relation of thyroid deficiency to the secretion of hypophyseal lactogenic hormone. In the rat it would make unnecessary the surgical removal of the thyroids, with the inevitable concomitant removal of the parathyroids which creates an additional deficiency in the animal. It was also considered of interest to determine the effects of estrogen in combination with thiouracil on the lactogen content of the pituitary. Estrogen has been shown to be a powerful stimulator of lactogenic secretion, greatly increasing its content in the pituitary,[17-19] in the blood[20] and initiating milk secretion in several species.[18,21,22] Would thiouracil inhibit the dynamic effect of estrogen on pituitary lactogenic secretion?

Experimental. The first experiment ran for 24 days, and was performed on young female albino rats of a standard strain we have been using for years.† The control rats (Group 1) received no special treatment while Group 2 was fed a ration containing 0.1% thiouracil‡ during the 24-day period. Group 3 was given 100 I.U. estrone‡ daily during the last 10 days of the 24-day period, while Group 4 received 0.1% thiouracil for the first 14 days, and thiouracil plus 100 I.U. estrone daily during the last 10 days of the experiment.

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† We are indebted to Dr. A. G. Hogan, Chairman of the Department of Agricultural Chemistry, University of Missouri, for a generous supply of these rats.

‡ We wish to thank Dr. Mark Welsh of the Lederle Lab., Pearl River, N.Y., for the thiouracil; Dr. D. W. MacCorquodale of the Abbott Research Labs., North Chicago, Ill., for the estrone; and Dr. D. F. Green of Merck and Co., Rahway, N.J., for the diethylstilbestrol used in this study.

TABLE I.

Experiment 1. Normal Female Rats.

Group	No.	Sex	Treatment	Avg body wt		Avg No. lactogen units*			Avg pit. wt, mg	Avg pit. wt†/ 100 g B.W.	Avg thyroid wt, mg	Avg thyroid† wt/ 100 g B.W., mg
				1st day	24th day	per pit.	per 100 g body wt	per mg pit. tissue				
1	10	F	[1]Controls	108	130	8.5	6.0	1.25	6.76	5.18 ± .22	12.5	9.6 ± .61
2	9	F	[1]0.1% Thiouracil	109	129	5.5	4.2	0.61	8.93	6.90 ± .22	57.6	44.5 ± 2.85
3	10	F	[2]100 I.U. estrone daily from 14th	110	128	12.0	9.3	1.49	8.05	6.29 ± .17		
4	10	F	[2]0.1% Thiouracil + 100 I.U. estrone, 14th through 24th days	116	126	5.5	4.3	0.60	9.04	7.15 ± .20	46.0	36.5 ± 3.51

Experiment 2. Castrate Male Rats.

				1st day	21st day							
1	7	M	Controls	132	180	1.7	0.9	.18	9.17	5.09‡	10.6	6.5 ± .03
2	8	M	[3]10 γ Diethylstilbestrol daily	129	130	3.2	2.5	.41	7.81	6.02	12.8	9.5 ± .95
3	6	M	[3]10 γ Diethylstilbestrol + 0.1% Thiouracil daily	128	134	3.0	2.2	.25	11.60	8.69	53.1	38.8 ± 3.18

[1,2,3] Groups assayed and compared with each other in same 10 pigeons.
* Reece-Turner units.[17]
† Standard error of the mean.
‡ Individual weights were not available for determining S.E.M.

The second experiment§ ran for 21 days and included 3 groups of young male rats which had been castrated about 10 days earlier. Group 1 served as controls and received subcutaneous injections of .05 cc of corn oil daily. Group 2 was given injections of 10 μg of diethylstilbestrol‡ in corn oil daily while Group 3 similarly received 10 μg of diethylstilbestrol by injection plus 0.1% thiouracil in the feed daily.

All the rats were killed by etherization, and the pituitaries and thyroids were removed and weighed to the nearest .01 mg. Each group of pituitaries was macerated with a small mortar and pestle, suspended in an exact volume of distilled water, and the equivalent of 2 female or 6 male pituitaries was injected intradermally over one side of the crop glands of 10 pigeons. Two different groups of pituitaries were assayed and compared with each other in the same 10 pigeons by injecting over each side of the crop gland. The crop glands were rated for lactogen units by the Reece-Turner method.[17]

Results. Experiment 1. The lactogenic hormone in the pituitaries of the thiouracil-treated rats (Group 2) was reduced below that in the controls (Group 1), averaging 4.2 units compared to 6.0 units per 100 g body weight. The rats which received estrone alone (Group 3) showed a considerable increase in pituitary lactogen over the controls, averaging 9.3 units per 100 g body weight. Group 4, which received the same amount of estrone but got thiouracil in addition, not only showed no increase in lactogenic hormone over the control rats but had as little (4.3 units per 100 g body weight) as in the animals treated with thiouracil alone.

The pituitaries of the thiouracil, and thiouracil plus estrone-treated groups were definitely heavier than in the control rats, both increasing about the same amount per 100 g of body weight. The thyroids of both these groups weighed much more than in the controls, but the increase in thyroid weight was less in the group which received thiouracil plus estrone.

Experiment 2. It will be seen that in this experiment the lactogen content of the pituitaries of the thiouracil plus stilbestrol-treated rats (Group 3) rose just as much above the controls (Group 1) as the animals which received stilbestrol alone (Group 2). It must be pointed out that the Group 3 rats in this experiment do not correspond to the thiouracil plus estrone-treated group in the first experiment. The former received thiouracil and estrogen together during the entire 21-day period, while the latter got only thiouracil for the first 14 days and thiouracil with estrogen during the last 10 days of the 24-day period.

The pituitaries of the rats which were given thiouracil plus stilbestrol (Group 3) weighed considerably more than the animals treated with stilbestrol alone (Group 2) or the controls (Group 1). The average thyroid weight of the rats in Group 3 was increased approximately 5-fold over the controls.

Discussion. The reduction in the lactogen content of the pituitary following thiouracil treatment confirms the results reported by McQueen-Williams in thyroparathyroidectomized rats. This is believed to support the contention that the eosinophils, which are decreased following thyroidectomy or goitrogen treatment, are the source of lactogenic hormone. It is interesting that whereas the combination of thiouracil and estrogen in the first experiment did not evoke an increase in pituitary lactogen, it did do so in the second experiment. It is believed this difference can be accounted for in the somewhat different procedure followed with the 2 groups. Thus, the former received the estrogen only after a preliminary treatment with thiouracil for 2 weeks whereas the latter group received thiouracil and estrogen together during the entire length of the experiment. It would seem that in the first experiment the administration of thiouracil alone during a 2-week preliminary period reduced the eosinophils in the hypophysis to such a low level that they were rendered incapable of responding

§ This was part of another experiment by V. Hurst, J. J. Trentin and the authors to determine the effects of thiouracil plus estrogen on mammary growth.

to subsequent estrogen stimulation and hence evoked no lactogen increase. In the second experiment on the other hand, the simultaneous administration of estrogen with thiouracil checked the depressing effect of the latter on the eosinophils and enabled the estrogen to evoke its normal increase of pituitary lactogen.

The increase in the pituitary weight following thiouracil treatment corroborates a similar report on thiourea-treated rats,[10] and is in line with the findings in thyroidectomized animals. The recognized ability of estrogen to increase pituitary size was evident both in the estrone and diethylstilbestrol-injected rats. However, the greatest augmentation of pituitary weight occurred in the rats which received the above estrogens in combination with thiouracil.

Summary. The administration of 0.1% thiouracil in the feed for 24 days to young female rats reduced the lactogenic hormone content of the pituitary below that in normal rats. The proven ability of estrogen to increase the lactogen content of the pituitary remained unimpaired in rats which were given the estrogen together with thiouracil for 21 days. However, when rats received thiouracil alone for a 2-week preliminary period, the subsequent administration of estrogen plus thiouracil for 10 days failed to maintain even the normal level of lactogenic hormone.

The pituitary and thyroid weights of the thiouracil as well as thiouracil plus estrogen-treated rats were increased above the normal controls. The thyroid hypertrophy in the rats which received estrogen and thiouracil for 10 days following a preliminary treatment of thiouracil alone for 2 weeks was less than in the rats which received only thiouracil for the same period.

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A Brief Insulin Tolerance Test.*

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Meduna *et al.*[1-3] by determining reducing substance (which is predominantly glucose) in venous blood at fasting and at 5, 10, 15, 20, 25, 30, 35, 45, 60, 90 and 120 minutes following the injection of 0.1 unit insulin per kg body weight, had found that the nadir was shallower and later in oneirophrenics than in normals, and had observed, in individual, normal records, a bump—*i.e.* a failure to fall or actual rise—before the nadir at which sympathetic counter-regulation initiates a rapid rise in blood-sugar peaking about the 45th minute. He had also found that throughout all of these changes the amount of reducing substances other than glucose was constant. The present work was begun in 1943 to increase the certainty of his separation and, by samples at the 12th and 17th minutes, to define the bump on the descending limb and finally, to construct a brief test for clinical use.

On the assumption that the effect of insulin in lowering the blood-sugar was proportional to its initial value, Meduna had expressed the observed decrease in percent of fasting value, and subsequent experience indicates that this is probably the best expression. He used colorimetric determination of alkaline ferricyanide reduced by tungstic acid filtrates from 0.1 ml of venous blood and this, by chance, gives mean fasting values of approximately 100 mg %, so that the mean

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