TABLE 1

NUMBER OF CORMELS WITH LOW (0–2) AND HIGH (3–5) TETRAZOLIUM RATINGS COMPARED WITH PERCENTAGE GERMINATION 40 DAYS AFTER PLANTING

Variety	Treatment	Tetrazolium ratings		Percentage germination
		0–2	3–5	
Elizabeth the Queen	Room temperature	39	1	8
	Cold storage	2	38	68
Margaret Beaton	Room temperature	36	4	26
	Cold storage	5	35	77
Leading Lady	Room temperature	40	0	3
	Cold storage	25	15	41
Valeria	Room temperature	40	0	1
	Cold storage	4	36	28

tissues that is not associated with germinability. The color pattern is centered mainly on the parenchymatous tissue and the perimeter of the cormel. As the sheathing husk dries and hardens, the color reaction decreases until the depth of dormancy is reached and hardly any reddening occurs. From that time on, intensity of the reaction to tetrazolium and capacity for germination rise in parallel. The rise may be rapid or slow, depending on the variety and the conditions of storage or treatment.

A description of experiments which led to the application of this method to gladiolus cormels will be published elsewhere by the senior author.

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Mechanism of Suppression of Nontransmissible Pneumonia in Mice Induced by Newcastle Disease Virus[1]

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In 1942 Burnet (*1*) reported that two successive intranasal inoculations of Newcastle disease virus (NDV) produced extensive influenza-like pneumonia in the mouse and that neither the infection nor the lesion could be transferred by serial passage in mice. This unusual host-virus relationship was later studied extensively by Ginsberg (*2*) and by Davenport (*3*), independently. The data obtained by these investigators were in substantial agreement and indicated that the production of pulmonary lesions by a large amount of NDV paralleled many known host-virus interactions that occur in a true viral infection except that demonstrable viral multiplication[2] did not occur (*2, 3*).

In this laboratory (*4*) the microbial product APM was found to exert a definite but transient suppressive effect on the development of pneumonia in mice following infection with influenza A virus. The fact that APM failed to exert any appreciable antiviral effect *in vitro* or *in vivo* prompted studies which led to the demonstration of its capacity to suppress the nontransmissible pneumonia in mice induced by NDV. Extensive studies with pneumonia virus of mice (*5*) and influenza A virus (*6*) as well as with NDV (*2, 3*) have indicated that factors other than viral multiplication per se are involved in the production of lesions. It would appear that in the case of NDV and of influenza A virus, APM might selectively affect such a factor.

The California strain of NDV was used throughout these studies and was propagated in the allantoic sac of embryonated eggs in the usual manner. Infectivity titrations on pooled allantoic fluid were carried out in embryonated eggs as previously described (*7*). The infective titer of mouse lung tissue was determined in a similar manner except that the diluent for homogenized lung tissue contained 500 units each of penicillin and streptomycin/ml to insure bacterial sterility and that all eggs were chilled overnight after 48 hr of incubation before collection of allantoic fluid. Hemagglutination tests were carried out at 4° C (*8, 9*).

Inoculation and injection of mice. Large numbers of albino mice weighing 18–20 g each were inoculated intranasally under light ether anesthesia with 0.1 ml of undiluted allantoic fluid containing 10^9 ID_{50} of NDV. After inoculation of NDV the mice were distributed at random into identical cages, 6–8 mice per cage. The cages were previously marked as control mice or as mice to be injected with APM and with the scheduled date of sacrifice and examination. Injections of APM were made subcutaneously under the loose skin on the backs of the mice. Groups of 10–15 mice each were sacrificed at appropriate daily intervals and the lesion score (*10*) and average weight of the lungs (weight of Petri dish plus 10 lungs less weight of dish after removal of lungs, divided by 10) were recorded (Table 1). The microbial product APM was prepared as originally described (*4*) from culture filtrates of *Achromobacter* sp. 134.

Suppression of pneumonia was readily effected by daily injections of APM. Large numbers of mice were inoculated intranasally with NDV. One half of these received daily subcutaneous injections of 1.0 mg APM

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[2] The term viral multiplication as used in this communication is restricted to the formation of new infectious particles. Ginsberg (*2*) has noted that although multiplication of incomplete virus was not detected, the possibility of its presence could not be definitely eliminated.

TABLE 1

FAILURE OF APM TO AFFECT RESOLUTION OF PNEUMONIA IN MICE

Days after inoculation of NDV*	Control I/T	Control lesion score L/M	Control lesion score %	APM† I/T	APM† lesion score L/M	APM† lesion score %
3	19/19	63/95	66			
7	22/24	56/120	47	20/22	52/110	47

I/T = Number infected/total.
L/M = Total lesion score/total maximum lesion score.
% = L ÷ M × 100.
Lesion score : ½ = 5% lung tissue consolidated ; 1 = 5–25% ; 2 = 26–50% ; 3 = 51–75% ;4 = 76–100% ; 5 = dead mouse with lungs consolidated.
* 10^9 ID_{50} intranasally.
† 1 mg APM injected subcutaneously daily beginning on the 3rd day after inoculation of NDV.

beginning 1 hr after inoculation of NDV. The remaining mice served as controls. Twelve to 15 mice from each group were sacrificed daily for 6 days and on the 9th day after inoculation. The lesion score, expressed as percentage pneumonia, and average weight of the lungs were determined as described. The results are presented graphically in Fig. 1. As expected (*2, 3*), the maximum of pneumonia was

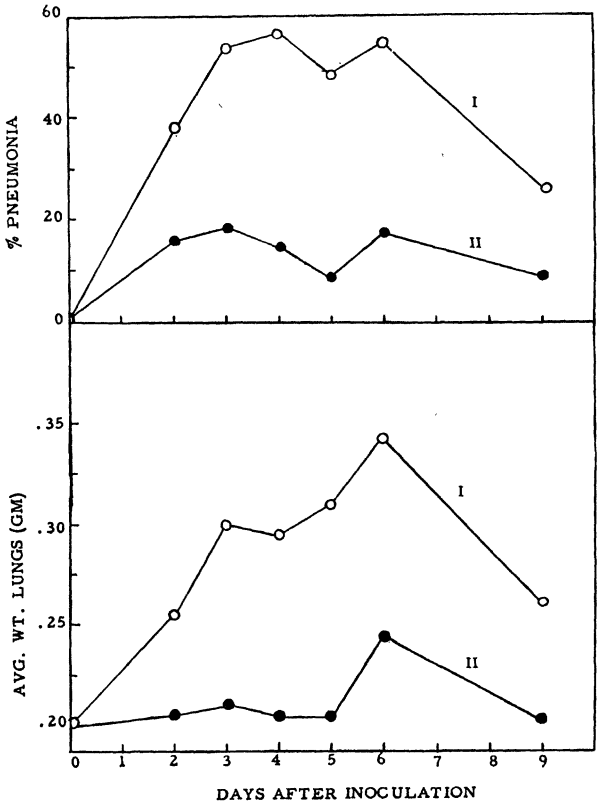

FIG. 1. Effect of daily injections of APM on development of pneumonia. I, control; II, seven daily injections of APM beginning 1 hr after instillation of NDV.

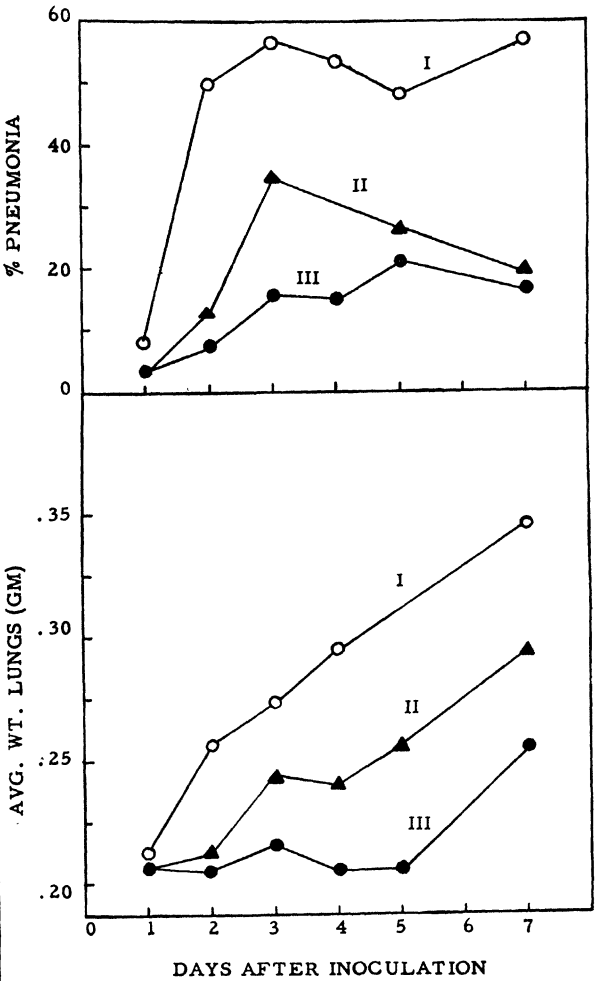

FIG. 2. Effect of early treatment with APM on development of pneumonia. I, control; II, two daily injections of APM beginning 1 hr after instillation of NDV; III, three daily injections of APM.

reached in the control group (curve I) on the 3rd day after inoculation of NDV and severe pneumonia continued for 3 additional days. By the 9th day after inoculation, however, resolution of the pneumonia was well under way. It is evident (curve II) that the pneumonia was considerably reduced in those mice which received 7 daily injections of APM.

When daily injections of APM were discontinued after the second injection, pneumonia continued to develop. In this experiment 17 groups of 12 mice each were inoculated with NDV. Six of these groups were set aside and served as controls. The remaining mice received 2 daily injections of 1.0 mg APM beginning 1 hr after inoculation of NDV. Of these, 5 groups of 12 mice each received one additional injection of APM on the following day (i.e., the 2nd day after inoculation of NDV). Appropriate groups of 12 mice each were sacrificed at daily intervals and the lesion score and average weight of the lungs were recorded. The data (Fig. 2) show that if daily injections of APM were discontinued during the period of rapid

extension of the lesion, pneumonia continued to develop.

When injections of APM were delayed until 48 hr after inoculation of NDV the development of pneumonia was arrested. Large numbers of mice were inoculated with NDV and then separated into 13 groups of 12 mice each. Five these groups were set aside as controls and an equal number received 1.0 mg daily injections for 5 days beginning 1 hr after inoculation. In the 3 remaining groups, daily injections of APM were not begun until 48 hr after instillation of NDV. Appropriate groups of mice were sacrificed daily and the lesion score and average weight of the lungs were noted. The data (Fig. 3) show that daily injections of APM could be delayed for as long as 48 hr after inoculation of NDV and still arrest the development of pneumonia.

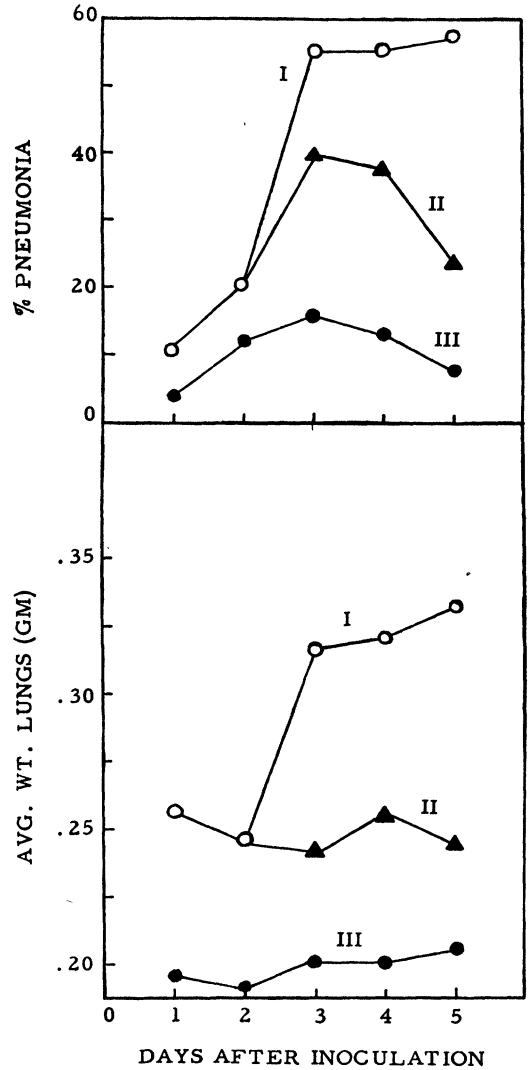

FIG. 3. Effect of delayed administration of APM on development of pneumonia. I, control; II, daily injections of APM begun 48 hr after instillation of NDV; III, daily injections begun 1 hr after instillation of NDV.

However, when daily injections of APM were delayed until the time of maximal pneumonia, i.e., 72 hr after inoculation of NDV, APM was without effect. In this experiment, as before, large numbers of mice were inoculated intranasally with NDV. One group of 19 mice was sacrificed on the 3rd day after inoculation and the lesion score and average weight of the lungs were recorded. A 2nd group of 24 mice received daily injections of 1.0 mg APM beginning on the 3rd day after inoculation. A 3rd group of 22 mice served as controls. On the 7th day after inoculation of NDV mice in the 2nd and 3rd groups were sacrificed and examined (Table 1). It will be seen that APM was without effect when daily injections were delayed for 72 hr and that it did not affect resolution of pneumonia. Thus, it would appear that APM is effective only during the period of rapid extension of the lesion.

Daily injections of APM failed to alter appreciably the infective titer of lung tissue. Eighty mice were inoculated intranasally with 10^9 ID_{50} of NDV. One hour after inoculation 40 mice were injected subcutaneously with 1.0 mg APM and daily thereafter for 2 days. An equal number were set aside as controls. Ten mice from each group were sacrificed 2, 24, 48, and 72 hr, respectively, after inoculation. Lungs from the respective groups were pooled and stored at $-70°$ C. Five to 8 days later the various pools of lung tissue were thawed, 10% suspensions of lung tissue were prepared by grinding lungs from each subgroup in a mortar with sterile sand, and infectivity titrations were carried out in embryonated eggs as previously described (Fig. 4). As expected

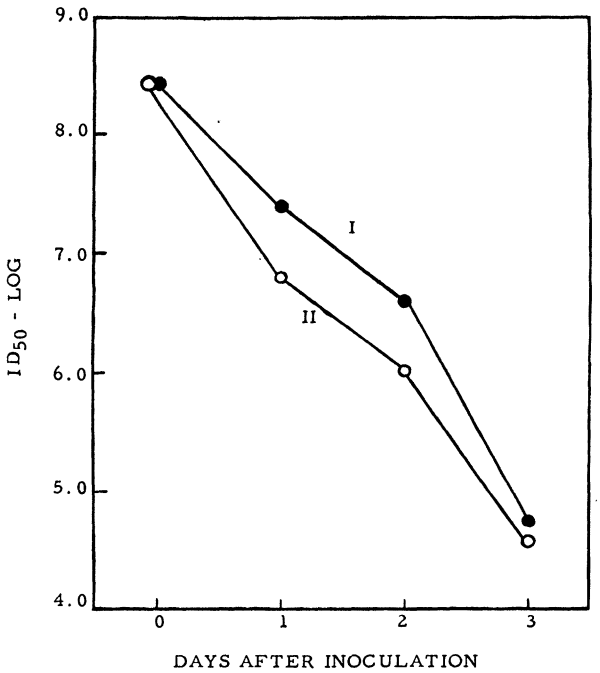

FIG. 4. Effect of daily injections of APM on infective titer of lung tissue. I, daily injections of APM beginning 1 hr after instillation of NDV; II, control.

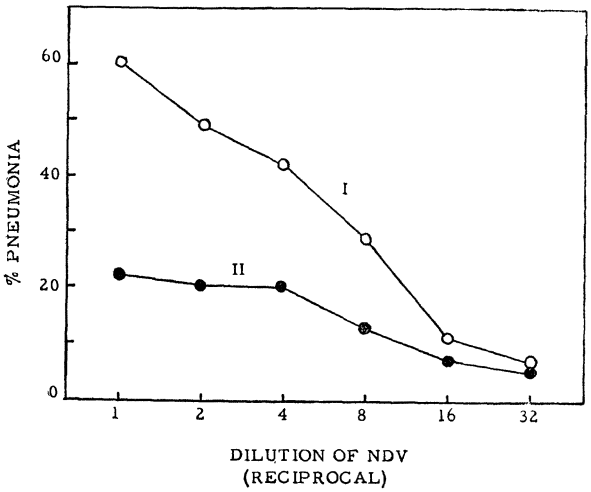

FIG. 5. Effect of dilution of NDV on suppressive action of APM. I, control; II, daily injections of APM beginning 1 hr after instillation of NDV.

(*2, 3*), the infective titer of lung tissue from the control group (curve II) decreased from $10^{-8.4}$ when removed 2 hr after inoculation to $10^{-4.6}$ on the 3rd day after inoculation. Daily injections of APM which definitely suppressed the development of pneumonia failed to affect appreciably the infective titer of lung tissue (curve I).

Curiously, dilution of the inoculum of NDV did not increase the effectiveness of APM in suppressing development of pneumonia. In fact, the data indicate a reduction in effectiveness of APM against limiting dilutions of NDV in the experiment described below. Serial 2-fold dilutions of NDV were inoculated intranasally into 6 groups of 40 mice each. One hour later half of the mice in each group were separated and these mice were injected with 1.0 mg APM at that time and daily thereafter for 2 days. The remaining mice in each group served as controls. On the 3rd day after inoculation of NDV, when the maximum of pneumonia was attained with all dilutions of NDV (*2*), the mice were sacrificed and the lesion scores were recorded (Fig. 5). It will be seen, (a) that the effectiveness of APM was not increased when the amount of NDV inoculated was decreased, and (b) that APM had little or no effect on pneumonia induced by limiting dilutions of NDV.

It will be recalled (*4*) that APM did not inactivate infective NDV *in vitro* when NDV was titrated in eggs in the presence and absence of APM. However, the injurious effect of a large amount of NDV could, conceivably, be neutralized by direct contact with APM. To test this possibility serial 2-fold dilutions of both APM and NDV, respectively, were mixed *in vitro* with one another and with saline. After 90 min at room temperature, 0.1-ml amounts of the various mixtures were instilled intranasally into groups of 5 mice each. All mice were sacrificed on the 3rd day after inoculation and the lesion scores were recorded. Unfortunately, inoculation of concentrations of APM alone of 2.0 mg/ml or higher caused pulmonary consolidation grossly indistinguishable from that caused by NDV. However, maximum tolerated amounts of APM (1.0 mg/ml) failed to inactivate *in vitro* the injurious effect of NDV diluted one-eighth.

The data presented here indicate that the nontransmissible pneumonia in mice induced by the intranasal instillation of a large amount of NDV was definitely modified by subcutaneous injections of APM only during the period of rapid extension of the lesion. However, APM failed to exhibit antiviral (*4*) or antitoxic properties *in vitro,* nor was its effectiveness increased when the inoculum of NDV was decreased. The postulated incomplete virus (*11–14*) has been purposely omitted from consideration in this report in the absence of evidence of its existence in this particular host-virus relationship (*2, 3*). That its presence could not be definitely eliminated has been noted (*2*). However, its relationship, if any, to the nontransmissible pneumonia induced by NDV remains obscure.

Current knowledge (*2, 3*) of host-virus interactions relative to the production of this nontransmissible pneumonia together with the absence of demonstrable antiviral properties of APM (*4*) make it difficult not to conclude that APM selectively inhibits the development of the lesion. Considerable evidence has been presented that the production of lesions may not be related to viral multiplication per se in the case of pneumonia virus of mice (*5*), influenza A virus (*6*), as well as NDV (*2, 3*). Recently, Davenport (*3*) has proposed the hypothesis that cell injury may result from the synthesis or introduction of sufficient infective virus to exceed the level tolerated by the host cell; and that the virus, or its degradation products, may combine firmly with an essential cellular constituent thus creating cellular imbalance leading to hypertrophy, hyperplasia, or necrosis of the cell. If this is true, the suppressive effect of APM on pneumonia induced by NDV could be the result of competition between APM and virus for such a cellular constituent. Thus, in the absence of viral synthesis and in the presence of sufficient APM for effective competition, the cell could gradually rid itself of virus without cellular imbalance or injury and subsequent production of lesions (Figs. 1 and 4). On the other hand, suppression of pneumonia following infection with influenza A virus by APM (*4*) would be expected to be transient in the presence of continuing viral synthesis.

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The Effect of Phenothiazine, N. F. (Green) on the Uptake of I^{131} by the Rat Thyroid[1]

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The drug phenothiazine (thiodiphenylamine) is an anthelmintic in common use by veterinarians in the care of hogs, sheep, and cattle and is frequently used in human treatment. As a regular constituent of the diet of livestock it is often used as a preventive. The use of this drug as an anthelmintic has been reviewed by Davey and Innes (*1*). Collier, in a series of papers, has studied various biochemical effects. The latest of these (*2*) dealt with the inhibitory effect of the drug on succinoxidase and cytochrome oxidase in a beef heart preparation.

Our interest in phenothiazine was aroused by the accidental inclusion of the drug in one lot of our stock rat food; thus without our knowledge, our entire rat colony was fed the drug daily for a period of about 2 weeks. Some of these rats were used in a routine class experiment to demonstrate the uptake of iodine by the thyroid. The failure of the gland to take up iodine (I^{131}) and the concurrent discovery of the inclusion of phenothiazine in the diet led to the following preliminary investigations in the influence of the drug on the uptake of I^{131} by the thyroid.

A group of Sprague-Dawley rats weighing between 150 and 200 g were placed on a Remington iodine-deficient diet (*3*). For the times indicated in Tables 1 and 2, commercial phenothiazine, N. F. (Green) was mixed with the diet. The phenothiazine was added to the salt mixture as recommended by the U. S. Department of Agriculture on a basis of 1 g of the drug to 10 g of salt mixture. In the Remington diet the concentration of drug amounted to 0.1% by weight of the total diet. The average daily consumption of the drug by each rat was calculated to be approximately 100 mg/kg of body weight. This figure appears to be between 50 and 75% higher than that recommended for prophylactic feeding in sheep, but it is only a small fraction of the treatment dose used in livestock.

In the first experiment (Table 1), the drug was added to the food after the animals had been maintained on the Remington diet for 3 days; in the second, more complete experiment (Table 2), the animals were fed the Remington diet for 10 days before the addition of phenothiazine. The I^{131} was administered intraperitoneally 24 hr before the animals were killed. For the animals on phenothiazine for only 24 hr, the I^{131} was administered 2 hr after the drugged food was offered, and, since this was done late in the afternoon, it is assumed that very little food had been eaten by the time the radioactive isotope was administered.

It is obvious from these preliminary experiments that phenothiazine, as it is normally administered to stock animals, has a marked effect on the rat thyroid uptake of I^{131}. Although the data recorded in Table 1 are incomplete, they indicate that the drug produces its effect almost immediately; for, when the drug and the iodine isotope were administered within 2 hr of each other, the thyroids, were found to be suppressed to a degree equivalent to those of animals fed phenothiazine for longer periods of time. This immediate effect was further demonstrated by the lack of significant difference in the uptake of I^{131} by the thyroids of animals fed the drug for 2, 3, or 4 days (Table 2). The difference in the uptake between

[1] This study was aided in part by a grant from the Atomic Energy Commission.

TABLE 1

I^{131} UPTAKE BY RAT THYROID—REMINGTON DIET 6 DAYS

Groups	No. of animals	Thyroid wt., mg	% Uptake, total gland[1]	% Uptake,[2] mg	Thyroid/blood ratio[3]
Controls	3	17	9.7	0.56	13,500
Phenothiazine 3 days	2	18	0.9	0.05	137
Phenothiazine 2 days	2	19	1.9	0.10	313
Phenothiazine 1 day	2	15	1.3	0.09	860

[1] Radioactivity in total gland ÷ total radioactivity injected.
[2] Radioactivity/mg of thyroid ÷ total radioactivity injected.
[3] Radioactivity/mg of thyroid ÷ radioactivity/ml of blood.

TABLE 2

I^{131} UPTAKE BY RAT THYROID—REMINGTON DIET 14 DAYS
(All figures represent the average of 4 animals ± S.E.)

Groups	Thyroid wt., mg	% Uptake, total gland[1]	% Uptake,[2] mg	Thyroid/blood ratio[3]
Controls	16.9 ± 1.6	28.5 ± 5.1	1.85 ± 0.33	26,450 ± 1,291
Phenothiazine 4 days	13.6 ± 1.1	1.6 ± 0.03	0.12 ± 0.04	697 ± 120
Phenothiazine 3 days	14.5 ± 1.6	1.2 ± 0.16	0.09 ± 0.16	598 ± 181
Phenothiazine 2 days	12.4 ± 0.6	1.7 ± 0.15	0.14 ± 0.04	574 ± 212

[1] Radioactivity in total gland ÷ total radioactivity injected.
[2] Radioactivity/mg of thyroid ÷ total radioactivity injected.
[3] Radioactivity/mg of thyroid ÷ radioactivity/ml of blood.