Discussion.—Survival of RNase activity of fresh tissues during fixation and embedding is not surprising with respect to the rather rough handling of the tissues involved in isolation of crystalline RNase[7]. The methods used include extraction of crude RNase with acids, therefore the effect of the Carnoy fixative on fresh tissues might be explained accordingly[2]. Since RNase generally can be characterized as a very thermostable enzyme[8], which fact also formed the basis for preparation of the original digestion fluid for histochemical purposes[9], the stability towards embedding in paraffin seems rather easily understood. Further, as recently reviewed[10], the active centre of the RNase molecule seems well protected against 'denaturing' agents, since enzymatic activity is fully retained despite severe changes in the organized structure of the native molecule'. The inhibitory effect of formaldehyde has been utilized in recent investigations[11].

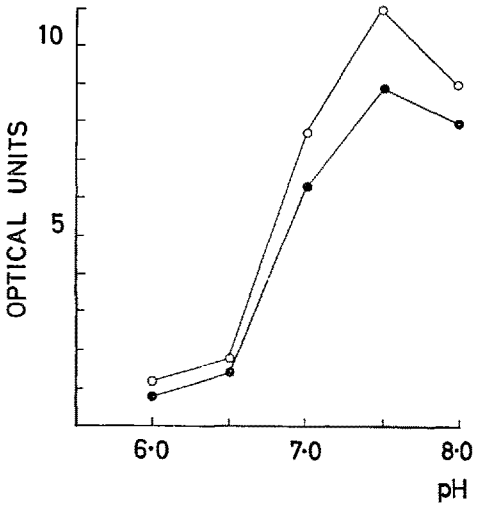

Fig. 2.

From the histochemical point of view, these results raise the question of the prerequisites for localization of RNase activity in sections, to allow for studies on the controlling action of the enzyme in RNA and protein metabolism[12] on the microscopical level. However, in the opinion of the authors, such attempts seem premature, until the demonstration has been made that the activity observed is responsible for the release of RNA from the sections[1]. Such studies are in progress in this institute.

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Zusammenfassung

In gefriergetrockneten, nach Carnoy fixierten Paraffinschnitten des Rattenpankreas kann die gesamte Ribonukleaseaktivität des frischen Gewebes nachgewiesen werden. Bei Fixierung ohne vorhergehende Gefriertrocknung verschwanden etwa 15–30% der ursprünglichen Aktivität. Nach Fixierung in Formaldehyd war die Ribonukleaseaktivität erloschen.

Effect of Reserpine (Serpasil®[1]) on Increased Oxygen Consumption Induced by Triiodothyronine[2]

Recent clinical reports suggest that reserpine may be of value in reducing the basal metabolic rate[3], combating tachycardia[4], and alleviating symptoms[5] associated with hyperthyroidism. In view of the observations that reserpine antagonizes the increase in oxygen consumption produced by dessicated thyroid in guinea pigs[6] and thyroxine in rats[7], it was thought worthwhile to determine whether the same would hold true for triiodothyronine, a purified principle of recent interest.

Materials and Methods. 16 male guinea pigs, weighing from 460 to 525 g, were divided, according to Fisher and Yates' tables of random[8], into two groups, *A* and *B*, containing 4 and 12 animals respectively. Each animal occupied a separate cage in a room in which the temperature was thermostatically maintained at 21°C. Ground Purina Rabbit Pellets, tap water, and cabbage were provided *ad libitum*. Group *B* was rendered hyperthyroid with intraperitoneal injections of 125 μg of DL-triiodothyronine[9] per kilogram body weight daily for 4 days. Group *A* served as placebo treated[10] controls.

After significant degrees of hyperthyroidism were produced, one half of the animals in Group *B* received reserpine (serpasil®) intraperitoneally, 0·5 mg/kg on the first day, followed by 0·35 mg/kg of body weight on the second and third days. Control animals in Group *B* received corresponding amounts of placebo therapy in the same manner.

A modification of Richards and Collison's closed chamber technique[11] was used to measure oxygen consumption; the apparatus, methods, and conditions were described in a previous paper[6]. All readings were made at 25·0 ± 0·5°C. Oxygen consumption was

[1] Reserpine (Serpasil®) used in these experiments was generously supplied by Ciba Pharmaceutical Products, Inc., Summit, New Jersey.

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Table I

Effect of reserpine on oxygen consumption

Day	Liters oxygen/(kg body weight) $^{2/3} \times 0{\cdot}1$/h			% difference of Means *	P of difference**
	Placebo-treated euthyroid animals Group A(4)	Hyperthyroid animals Group B (12)			
		Reserpine-treated (6)	Solvent-treated (6)		
0	7·6 ± 0·30	11·0 ± 1·04	11·2 ± 0·68	+ 1·8	> 0·3
1	7·9 ± 0·36	9·8 ± 0·57	11·0 ± 0·83	+10·8	> 0·01
2	7·5 ± 0·59	7·6 ± 0·77	8·9 ± 0·65	+14·6	< 0·01
3	7·7 ± 0·40	6·3 ± 0·03	8·7 ± 0·50	+16·1	< 0·01
5	7·5 ± 0·48	5·5 ± 1·08	7·8 ± 0·54	+30·8	< 0·01
7	7·2 ± 0·88	5·7 ± 0·80	7·3 ± 0·38	+21·9	< 0·01
9	7·3 ± 0·89	6·0 ± 0·67	7·2 ± 0·60	+16·7	< 0·01
11	7·5 ± 0·65	6·7 ± 0·85	7·3 ± 0·75	+ 8·2	> 0·3

( ) = number of animals per group.

* % difference of means =

$$\frac{\text{(solvent-treated)} - \text{(reserpine-treated)}}{\text{solvent-treated}} \times 100.$$

** *P* of difference = probability of difference between means of reserpine treated and solvent-treated animals.

Figures represent means ± standard deviations.

determined before starting reserpine therapy, 24 h after each of the first three injections, and on days 5, 7, 9, and 11. Values for oxygen consumption were corrected to standard temperature and pressure, and were expressed as liters of oxygen per (kilogram body weight)$^{2/3} \times 0{\cdot}1$ per hour[12]. Metabolic changes were analyzed statistically for the significance of the difference between group means by student's 't' test[8].

All animals were autopsied at the conclusion of the experiments and examined for changes in organ weights and evidence of infection.

Results.—The data related to oxygen consumption have been summarized and their significance computed (Table I).

Euthyroid animals.—A relatively steady state of oxygen consumption was observed throughout the experiments in this group (Table I).

Hyperthyroid animals.—The animals treated with solvent vehicle showed a gradual return of oxygen consumption to euthyroid levels within 5 days following cessation of triiodothyronine treatment. Reserpine treatment produced a significant decrease in oxygen consumption after one day; maximum depression occurring at 5 days. At this time, these animals were considered to be hypothyroid since there was significantly less oxygen consumed by this group than in euthyroid control animals. After reserpine treatment was terminated, oxygen consumption gradually rose to euthyroid values at the conclusion of experiments.

Discussion.—Reserpine reduced the oxygen consumption of 'thyroid-intact' guinea pigs, rendered hyperthyroid with triiodothyronine, to hypothyroid levels. This suggests that the effect of endogenous thyroid hormone as well as the effect of exogenous triiodothyronine on oxygen consumption were antagonized. These results are in general accord with previous observations in this laboratory in guinea pigs[6]. In these experiments oxygen consumption was also reduced to hypothyroid levels in animals rendered hyperthyroid with dessicated thyroid and in euthyroid animals. No significant effect was observed after reserpine treatment in animals rendered hypothyroid with thiouracil. Furthermore, KUSCHKE and GRUNER have reported that reserpine antagonizes the increase in oxygen consumption produced by thyroxine but not that produced by 2,4-dinitrophenol[7]. These observations, along with those of the present paper, suggest that a certain amount of circulating thyroid hormone is necessary before reserpine can exert its effect, and that a peripheral antagonism for triiodothyronine and thyroxine appears to be the major mechanism of reduction of oxygen consumption. Direct thyroid[13] or central hypothalamo-hypophyseal effects seem less likely but may also be involved as contributing factors.

Table II

Changes in body weights

	Group A (euthyroid controls)	Group B (hyperthyroid animals)		Solvent minus reserpine-treated animals
		Reserpine-treated	Solvent-treated	
% change in body weight*	– 5·0	– 22·5	+ 1·5	+24·0
Probability of difference .	> 0·3	< 0·05	> 0·3	< 0·05

* Average percent change in body weight at 4 days from pre-reserpine or pre-solvent treatment control values.

Changes in body weights which occurred in reserpine-treated animals were probably due to decreased food and water intake during drug therapy[14] (Table II). At autopsy there was no evidence for infection nor was there any consistent change in weight of the liver, spleen, kidney, adrenal, lung, and thyroid between the reserpine and solvent treated animals.

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Résumé

Chez des cobayes rendus hypothyroïdiens au moyen de la DL-triiodothyrosine, la Réserpine diminue la consommation d'oxygène. Les résultats présentés suggèrent que la Réserpine agit comme un antagoniste de la DL-triiodothyrosine. L'action de l'hormone thyroïdienne (exogène ou endogène) sur la consommation d'oxygène est empêchée probablement par un mécanisme localisé à la périphérie.

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