Fraktion	Anzahl	positiv		Grösse der Induktion			Art der Induktion				
		Zahl	%	gross	mittel	klein	archencephal	deuterencephal	Rümpfe	Schwänze	unbestimmt
E 87 I – II . .	38	30	79	20	8	2	–	20	8	6	8
E 94 I	17	17	100	10	7	–	1	15	2	2	2
E 97 II	22	19	86	8	8	3	–	3	9	15	4

Der deuterencephale Induktionsstoff ist in NaCl- und KCl-Lösungen der Ionenstärke $\mu = 0{,}6$–$1{,}0$ löslich und fällt beim Verdünnen auf $\mu = 0{,}3$ zum Teil wieder aus. Die Tabelle enthält als Beispiel Fraktion E 94 I.

Wie die Abbauversuche mit Trypsin zeigten, ist der Proteinanteil dieser Fraktion für die Induktionsfähigkeit sicher erforderlich. Dagegen ist noch nicht geklärt, ob auch die in der Fraktion enthaltene Desoxyribonukleinsäure hierfür notwendig ist.

Um zu prüfen, ob spinokaudale und deuterencephale Induktionsstoffe in der Zelle in verschiedenen Formbestandteilen enthalten sind, wurde nach dem Verfahren von DOUNCE[3] eine Zellkernfraktion hergestellt. Sie induzierte vorwiegend deuterencephal. Dagegen induzierte die Fraktion, welche nach dem Abtrennen der Zellkerne hochtourig abzentrifugiert wurde, vor allem spinokaudal, daneben aber auch deuterenzephal. Diese Faktoren konnten ebenfalls mit Pyrophosphatpuffer (s. oben) extrahiert werden (Tabelle E 97 II).

Aus dem Überstand des hochtourig zentrifugierten Embryonalextraktes (s. oben) konnte nach der Streptomycinsulfatmethode[4] ein Ribonucleoproteid gewonnen werden, das ebenfalls deuterencephal induzierte. Die Wirksamkeit war jedoch schwächer als die von Fraktion E 94 I (Tabelle). Nach 3 min langem Erhitzen im siedenden Wasserbad induzierte das Ribonucleoproteid auch archencephal.

Die Untersuchungen werden fortgesetzt.

Der Deutschen Forschungsgemeinschaft danken wir für die Unterstützung der Arbeit.

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Heiligenberg-Institut, Heiligenberg (Bodensee), den 25. März 1957.

Summary

From chick embryos, fractions were prepared which, after implantation into new gastrulae, preponderantly give deuterencephalic or spinocaudal inductions. The behaviour of these fractions after incubation with thioglycolic acid or hydrolysis with trypsin is described.

[3] A. L. DOUNCE in *The Nucleic Acids II* (Academic Press, New York 1900), p. 105.

[4] S. S. COHEN, J. biol. Chem. *168*, 511 (1947).

Demonstration of Ribonuclease Activity in Sections from Carnoy Fixed Rat Pancreas

In an earlier communication[1], persisting ribonuclease (RNase) activity in sections from Carnoy-fixed rat pancreas was considered to be an explanation for the complete removal of ribonucleic acid (RNA) through incubation in McIlvaine's buffer without addition of RNase. On the other hand, the only partial release of the RNA of formaldehyde-fixed tissue should be due to chemical degradation. The present communication demonstrates RNase activity in Carnoy-fixed material, and the inhibitory effect of the formaldehyde fixation procedure.

Experimental.—Small, fresh or frozen-dried[2] pieces of pancreas from decapitated rats were mechanically shaken in 100 ml of Carnoy's fluid (ethanol:chloroform:acetic acid = 6:3:1) for 1·5 h at room temperature, and then washed twice with absolute ethanol for 10 min. Other fresh pieces from the same glands were fixed in LILLIE's[3] buffered formaldehyde solution (pH 7·0) for 24 h at room temperature, washed with running distilled water for 36 h, and dehydrated with alcohol (50% for 2 h, 96% for twice 2 h, absolute ethanol for three times 2 h). All dehydrated tissues were left in ether for three times 2 h, evacuated, weighed, and immersed in molten paraffin (m.p. 58°C) *in vacuo* at 59° ± 0·1°C for 30 min. Embedding followed at ice-water temperature. The paraffin blocks were sectioned at 7 μ setting of the microtome, and distributed equally between 50 ml centrifuge tubes[2]. After deparaffination with petroleum ether and washing with ether, each tube contained about 50 mg of evacuated material. 5 ml of distilled water were added to the tubes, and their content homogenized with a Janke-Kunkel stainless-steel homogenizer. Further homogenizations with additional amounts of water were used for determination of the suitable tissue concentration in relation to substrate concentration, as demonstrated below. RNase activity was measured with the slightly modified ANFINSEN *et al.*[4] method. 0·1 ml aliquots of the homogenates were added to McIlvaine's buffer, which contained yeast nucleic acid ('Hefenukleinsäure', Merck, Germany, purified according to VISCHER and CHARGAFF[5]), at room temperature, and mechanically shaken in glass-stoppered centrifuge tubes at 37° ± 0·01°C for 60 min. Total volume: 4 ml. The reaction was stopped through addition of 4 ml of ice-cold 10% perchloric acid containing 0·25% uranyl acetate, and the samples left at + 2°C for approximatively 60 min. After the first 20 min, no more acid-soluble material was released within the experimental period. Following centrifugation, the absorption of the supernatants was measured at 260 mμ in a Beckman Model DU quartz spectrophotometer with photomultiplier, using 10 mm cells. The readings were used in calculating the amounts of acid-soluble material released from RNA due to RNase action, with correction for substrate and tissue blanks. Thus, the activity is given in optical units at 260 mμ. All deter-

[1] S. LAGERSTEDT, Exper. *12*, 425 (1956).

[2] S. LAGERSTEDT, Z. Zellforsch. *45*, 472 (1957).

[3] R. D. LILLIE, *Histopathologic Technic* (Blakiston, Philadelphia 1948).

[4] C. B. ANFINSEN, R. R. REDFIELD, W. L. CHOATE, J. PAGE, and W. R. CARROL, J. biol. Chem. *207*, 201 (1954).

[5] E. VISCHER and E. CHARGAFF, J. biol. Chem. *176*, 715 (1948).

minations, also those of the blanks, were made at least in duplicate. The points in the curves are the means of such determinations. The pH of the digests was measured (with the glass electrode in a Beckman pH-Meter) in parallell experiments both before and after incubation. Some special procedures will be described below.

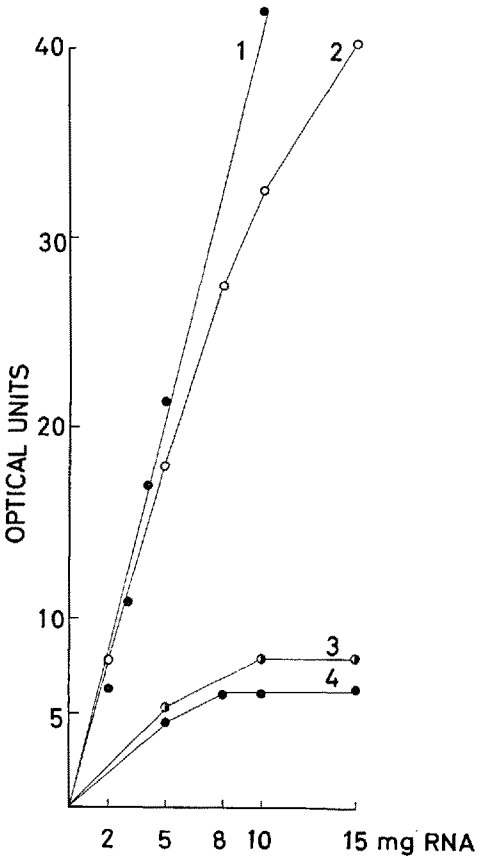

Fig. 1.

Influence of Tissue and Substrate Concentrations.—Preliminary experiments showed that no more than 15 mg of RNA per digest were tolerable in maintaining a constant pH throughout the digestions. Thus, a tissue concentration had to be used, permitting constant absorption values within a safe range of substrate concentrations less than 15 mg RNA per 4 ml volume. A typical dilution experiment, using the technique described above, and with sections from fresh Carnoy-fixed material, is shown in Figure 1. Curve 1 derives from a digest containing 10 mg tissue; Curve 2: 1 mg; Curve 3: 0·05 mg; and Curve 4: 0·02 mg. Obviously, in this example, about 0·02 mg will allow for 10 mg RNA per digest. In all experiments reported, the tissue concentration has been adjusted to fit 10 mg of the substrate.

Comparison of RNase Activity in Sections from Carnoy- and Formaldehyde-Fixed Tissue.—As demonstrated in Table I, formaldehyde-fixed tissues (CH_2O) did not show measurable RNase activity, in contrast to Carnoy-fixed material from the same pancreas (CARNOY), although about 500 times higher tissue concentrations were used in testing the sections after formaldehyde fixation. A lot of the Carnoy-fixed sections were secondarily fixed in the formaldehyde solution for 24 h, and, after repeated washings with distilled water, dialysed against distilled water for 36 h in the cold room. After dehydration and drying, they also failed to show measurable RNase activity (Carnoy + CH_2O). The same results were obtained using frozen-dried material. The formaldehyde-fixed material was found to be inactive also at pH values between 5·0 and 8·0 and when using lower tissue concentrations.

Table I

Effect of Formaldehyde fixation on RNase activity at pH 7·5

Fixation	Approximate amounts of tissue μg	Absorption readings at 260 mμ			Calculated activity
		Tissue + RNA	Tissue	RNA	
CARNOY. . .	20	0·639	0·091	0·200	
		0·641	0·089	0·219	0·34
CARNOY	10×10^3	0·270	0·060	0·269	
+ CH_2O. . .		0·309	0·055	0·210	−0·01
		0·371	0·059	0·309	
CH_2O. . . .	10×10^3	0·368	0·060	0·291	0·01

The pH-Dependability of the RNase Activity in Sections from Carnoy Fixed Tissue.—The pH of the buffer was varied between 5·0 and 8·0. Optimal activity was found around pH 7·5 (Fig. 2). The curve with open circles derives from fresh Carnoy-fixed sections, the other one from frozen-dried Carnoy-fixed material. The curves are not quantitatively comparable.

Influence of Fixation and Embedding on the RNase Activity.—Fresh tissues were homogenized in distilled water. Approximatively 0·1 ml aliquots were delivered with a braking pipette[6] into small, open gelatin capsules (Park, Davis & Co., No. 3). These were, either directly or after freeze-drying, fixed in Carnoy's solution, and embedded in paraffin, closely following the scheme given above for pieces of tissue. The RNase activity at pH 7·5 was compared to that of the fresh homogenate. As shown in Table II, no decrease in activity could be observed in the frozen-dried material. Carnoy-fixation of fresh homogenates caused a loss of about 15 to 30% of the original activity. The paraffin embedding procedure did not exert any measurable influence on the results.

[6] H. HOLTER, C. r. trav. lab. Carlsberg, Sér. chim. *24*, 399 (1943).

Table II

Influence of fixation and embedding on the RNase activity calculated from the absorption readings at 260 mμ. The figures in brackets give the number of homogenates tested. The standard error is that for the sum of test homogenate and blanks.

Experiment No.	Fresh homogenate	Fresh CARNOY fixation	Fresh CARNOY fixation and embedding	Freeze-drying, CARNOY fixation and embedding
1	0·535 ± 0·019 (4)	0·456 ± 0·008 (2)	–	0·538 ± 0·011 (2)
2	0·488 ± 0·004 (6)	–	0·342 ± 0·011 (4)	0·501 ± 0·009 (4)
3	0·405 ± 0·005 (4)	0·318 ± 0·008 (4)	0·315 ± 0·007 (4)	–

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].

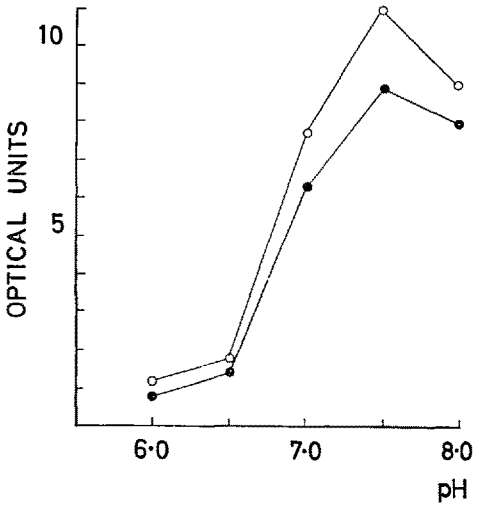

Fig. 2.

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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Department of Histology, University of Lund, Sweden, February 27, 1957.

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[11] S. Bradbury, Quart. J. micr. Sci. *97*, 323 (1956).

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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.

[2] This work was supported by a grant from Ciba Pharmaceutical Products, Inc.

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[9] DL-triiodothyronine (trionine®) used in these experiments was generously supplied by Hoffmann-La Roche, Inc., Nutley, N. J.

[10] Isotonic saline was used for placebo therapy.

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