merely misleading in reference to imprinting.

It seems likely that James failed to obtain an effect with his control animals simply because the animals did not frequent the area in which the imprinting object was placed (*3*).

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Ribonuclease of Euglena gracilis

Abstract. An enzyme in extracts of *Euglena gracilis* splits both purine and pyrimidine internucleotide bonds of ribonucleic acid. Its *p*H optimum is at 4.5; it is very heat-labile and is rather insensitive to inhibition by metal ions or sulfhydryl group reagents. A partial purification of the enzyme is described.

Recently, several enzymes of the protistan *Euglena gracilis* were examined in connection with other metabolic studies. Because of the special taxonomic position of this type of organism, it seemed of interest to compare the properties of some of these enzymes with those of similar enzymes from plant and animal sources. This report deals with the isolation, partial purification, and properties of a ribonuclease from *E. gracilis*. Ribonucleases from a wide variety of tissues and organisms have been described in the literature (*1, 2*).

Mass culture and harvesting of *Euglena,* as well as the extraction of the enzyme, were carried out as previously described (*3*). The ribonuclease assay was a modification of a published spectrophotometric procedure in which the ultraviolet-absorbing material remaining in solution, when the undegraded and partially degraded ribonucleic acid is precipitated from the reaction mixture by uranium acetate at *p*H 3.0, is determined at 260 mμ (*4*). The incubation was carried out at a *p*H of 4.5 in the presence of 0.05*M* acetate buffer for a period of 2 hours with 0.25 ml of the enzyme extracts or corresponding amounts of the purified fractions. The symbol Δ^{260}, which is used to express the enzymatic activity in Fig. 1 and throughout the text, represents the difference between the absorption in the experimental tubes and controls at 260 mμ.

The time course of the degradation of ribonucleic acid by the *Euglena* enzyme is shown in Fig. 1*A*. As shown in Fig. 1*B*, the enzyme has a very sharp optimum of activity at *p*H 4.5. This value is at the lower end of the range of *p*H optima (4.5 to 5.5) reported for a number of plant ribonucleases, while the enzymes of animal origin tend to have a higher optimal *p*H (*2, 5*). Unlike a number of other ribonucleases, the *Euglena* enzyme was found to be extremely heat-labile. When the enzyme extract was heated to 68°C at a *p*H of 4.6 in the presence of 0.05*M* acetate buffer, all activity was lost within 2 minutes. As in the case of pancreatic ribonuclease (*6*), the activity of the *Euglena* enzyme increased with increasing ionic strength, with an optimum at approximately 0.5*M* sodium chloride (Fig. 1*C*). Metal ions, which have been shown to inhibit pancreatic ribonuclease (*7*), were found to affect the enzyme from *Euglena* at rather high concentrations only (Table 1). The sulfhydryl group reagents *o*-iodosobenzoate and *p*-chloromercuribenzoate, which were tested at concentrations from $10^{-5}M$ $10^{-3}M$, were without effect, except for *p*-chloromercuribenzoate, which had a somewhat inhibiting effect at the highest concentration.

An approximately 15-fold purification of the enzyme was achieved by two ammonium sulfate fractionations; in the first, at *p*H 8.5, the protein precipitating between 38 and 53 percent of saturation in ammonium sulfate was collected; in the second, protein precipitating at *p*H 7.0, between 39 and 51 percent of saturation. These fractionations were followed by the removal of inactive protein by adsorption on calcium phosphate gel (*8*) and a final ammonium sulfate precipitation, at *p*H 7.0, between 39 to 51 percent of saturation, to concentrate the enzyme solution. While the over-all yield in enzymatic activity by this procedure was only of the order of 20 percent, an essentially phosphomonoesterase-free ribonuclease was obtained.

Table 1. Inhibition of *Euglena* ribonuclease by various metal ions at four concentrations. Assay as in text.

Cation	Inhibition (%)			
	$10^{-5}M$	$10^{-4}M$	$10^{-3}M$	$10^{-2}M$
Fe^{+++}	16	20	65	100
Cu^{++}	6	11	40	76
Zn^{++}	19	18	23	96
Pb^{++}	17	43	48	81

The specificity of this enzyme preparation was examined by two methods. In the first, its activity against ribonucleic acid was compared to its activity against the so-called ribonucleic acid "core." This "core" is obtained by exhaustive digestion of ribonucleic acid by pancreatic ribonuclease, and it is resistant to further degradation by this enzyme (*9*). It has been shown to consist of a mixture of oligonucleotides containing mainly purine bases (*9*). When *Euglena* ribonuclease was allowed to act on this "core" and on undegraded ribonucleic acid at *p*H 4.5, values of 0.720 and 2.040, respectively, were ob-

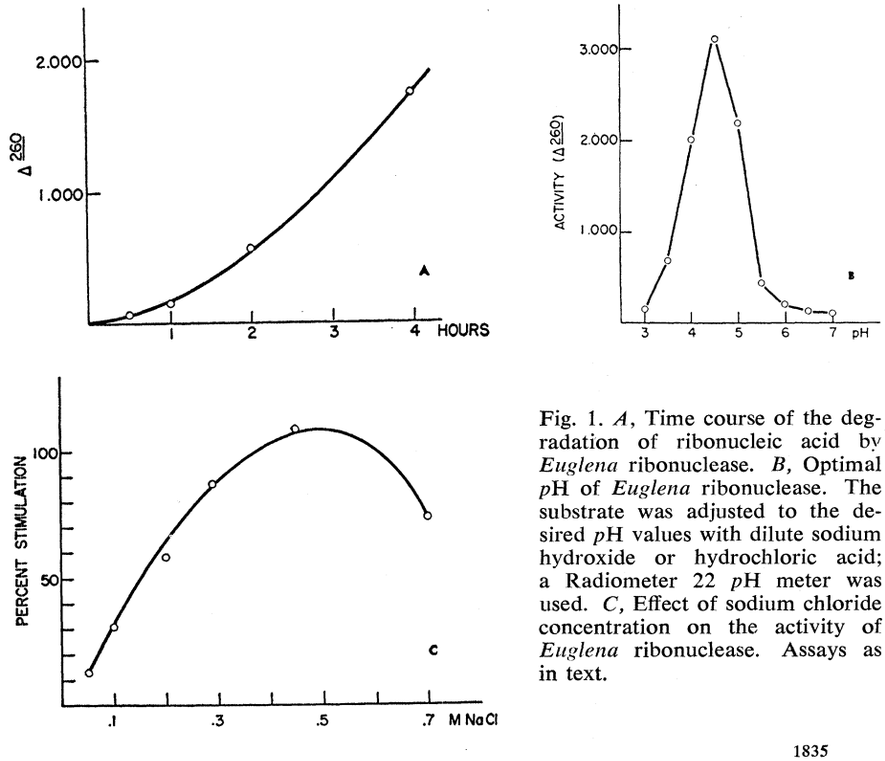

Fig. 1. *A*, Time course of the degradation of ribonucleic acid by *Euglena* ribonuclease. *B*, Optimal *p*H of *Euglena* ribonuclease. The substrate was adjusted to the desired *p*H values with dilute sodium hydroxide or hydrochloric acid; a Radiometer 22 *p*H meter was used. *C*, Effect of sodium chloride concentration on the activity of *Euglena* ribonuclease. Assays as in text.

tained for Δ^{260}, while pancreatic ribonuclease (*10*) at *p*H 7.3 gave a Δ^{260} of 0.930 with ribonucleic acid and of 0.000 with the "core." Unlike the pancreatic enzyme, the *Euglena* ribonuclease therefore was capable of further degrading the "core"—an indication that it does not share the specificity of pancreatic ribonuclease for pyrimidine internucleotide bonds. This was confirmed by experiments in which the enzymes were allowed to act on polyadenylic and polyuridylic acid (*11*) and in which the reaction products were analyzed by paper chromatography. Both polymers were degraded by the enzyme from *Euglena,* while pancreatic ribonuclease attacked only the polyuridylic acid (*12*).

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Effect of Reserpine on Ventricular Escape

Abstract. Catecholamine depletion by reserpine diminishes the tendency of the ventricle to escape from vagal suppression. Neither spinal section nor adrenalectomy enhances the reserpine effect. Norepinephrine restores the characteristic occurrence of ventricle escape during vagal stimulation.

Krayer and his co-workers (*1*) have described the importance of catecholamines to the rhythmicity of the sinus pacemaker, and Roberts and Modell (*2*) have shown that catecholamine activity is even more important to the rhythmicity of the ventricular pacemaker. It has been recently indicated that an adrenergic mechanism may play a role in the phenomenon of vagal escape (*3*). If catecholamines are important to the intrinsic rhythmicity of the ventricle, then ventricular escape from vagal control should be influenced by catecholamine depletion. This report (*4*) describes experiments designed to explore this relationship. Reserpine was used to deplete the amines.

In cats with the spinal cord sectioned at C1 or in cats anesthetized with Dial urethane (0.6 to 0.7 ml/kg), the right vagus nerve was stimulated. With appropriate vagal stimulation, the sinus rate may be slowed sufficiently to permit ventricular escape—that is, there is an unmasking of ventricular rhythmicity through the removal of sinus dominance. In each experiment the minimal intensity of vagal stimulation which would permit ventricular escape was used. Therefore, while the stimulus parameters varied, animals were compared at equivalent stimulus responses. Pulses of 6 to 14 volts, frequency of 10 to 40 cy/sec, and pulse duration of 0.1 msec applied for at least 20 seconds (*5*) were usually sufficient to produce ventricular escape. Reserpine (0.5 to 10.0 mg/kg) was injected intravenously 24 to 50 hours before the experiment. Since there was no significant difference in the effects of reserpine in the dose range employed, the results at all dose levels were pooled and averaged. The data are summarized in Table 1.

Table 1. The effect of reserpine on ventricular escape during vagal stimulation. S.E., standard error.

Procedure	No. of animals	Av. slowest sinus rate (induced by vagal stimulation) (beats/min. ± S.E.)	Av. ventricular escape rate* beat/min ± S.E.	Incidence of asystole†	Av. duration of asystole (sec ± S.E.)
		Anesthetized animals			
Control	15	49 ± 8	64 ± 8	2/15	7
Reserpine-treated	14	14 ± 5	28 ± 7‡	11/14	17 ± 2
		Animals with spinal-cord section			
Control	14	60 ± 6	66 ± 5	0/14	0
Reserpine-treated	4	7 ± 2	20 ± 11	4/4	14 ± 4

* See text for explanation. † Periods longer than 5 seconds. ‡ Based on 11 animals, since three developed only sinus escape.

In 15 untreated, anesthetized animals, when the sinus rate decreased during vagal stimulation to an average of 49 beats per minute, the rate of ventricular escape averaged 64 beats per minute and stabilized at an average of 75 beats per minute. Untreated animals with spinal-cord section responded in a similar manner, indicating that ventricular escape is not primarily related to sympathetic tone. In all cases, a sinus rhythm reappeared promptly after the cessation of vagal stimulation.

In animals treated with reserpine, ventricular escape still occurred, but there was a striking difference in the sinus rate at which this escape from vagal control appeared. Furthermore, ventricular rate after escape from vagal control also differed greatly from the comparable ventricular rates in the untreated animals. In 14 anesthetized animals, when the sinus rate was slowed by vagal stimulation to an average of 14 beats per minute, the independent ventricular rate averaged 28 beats per minute. These rates are significantly lower than those of the controls ($p < .01$). There was a similar response in four reserpine-treated animals with spinal-cord section. During vagal stimulation, asystolic periods of more than 5 seconds duration developed in only two of 29 untreated animals; such periods occurred in 15 of 18 reserpine-treated animals. In addition, the duration of asystole was considerably greater in the reserpine-treated animals (Table 1). The lower independent ventricular rate and the longer period of asystole after reserpine treatment indicate that the effect of reserpine on the ventricle is a consequence of an altered ventricular rhythmicity.

The appearance of ventricular escape during asystole suggests other factors not influenced by reserpine. In all reserpine-treated animals with spinal-cord section and in two reserpine-treated anesthetized animals, ventricular escape during asystole developed with the onset of convulsions. Thus, it seemed possible that such concomitants of anoxia as the liberation of potassium or the mobilization of cardiac catecholamine stores not depleted by reserpine (*6*) provoked ventricular escape. Other sources of catecholamines probably did not play a role, since bilateral adrenalectomy after reserpine treatment (in one anesthetized animal and in two animals with spinal-cord section) did not further diminish the ability of the ventricle to escape from vagal suppression.

In 12 animals treated with reserpine the administration of norepinephrine prevented, during vagal stimulation, the