which increases the activity of isoniazid on *M. tuberculosis in vitro*[2]. The mechanism by which OABA is metabolized in mycobacteria remains entirely unknown. We may state only that in tested concentrations OABA itself does not interfere with the growth of either strain used. It might be suggested that it is somehow connected with the metabolism of nicotinic acid. The latter is now supposed to be the point that is attacked by isoniazid, as suggested by KRÜGER-THIEMER[5]. However an explanation of the facts presented cannot be given before we know the precise mechanism of isoniazid activity against *M. tuberculosis*.

Z. ŠIMÁNĚ and R. URBANČÍK

Tuberculosis Research Institute, Prague-Bulovka (Czechoslovakia), September 4, 1959.

Zusammenfassung

Anthranilsäure antagonisiert die Isoniazid-Aktivität gegen *M. tuberculosis in vitro*. Dieser Antagonismus beruht auf keinem physikalisch-chemischen Einfluss der Anthranilsäure auf die Geschwindigkeit der Isoniazid-Spaltung im flüssigen Nährboden.

Inhibition of Sea Urchin Egg Cleavage by Ribonuclease[1]

I. *Lytechinus variegatus - Arbacia punctulata*

In view of the demonstrated inhibitory action of ribonuclease (RNase) on cell division in amphibian eggs[2,3] and certain other cells[4], it seemed of interest to examine for similar action of ribonuclease on cleavage of sea urchin eggs. This material is of special interest because of the demonstration by MAZIA of ribonucleic acid in the isolated mitotic apparatus of sea urchin eggs[5] and the report of ribonucleic acid in the cell surface of *Arbacia* eggs by LANSING and ROSENTHAL[6]. Because of the differences in biological activity found in different ribonuclease preparations[7], several crystalline enzyme samples were used. They were obtained from the following commercial sources: Armour, Sigma, Worthington, and Nutritional Biochemical Corporation. These enzymes were tested for action on fertilized eggs of two species of sea urchins, *Lytechinus variegatus* and *Arbacia punctulata*. Eggs were added to sea water solutions of the enzyme preparations at selected intervals following fertilization and before second cleavage. They were subsequently examined for cleavage without removal from the RNase solutions. Appropriate concentration of all four enzyme preparations (M. W. = 14,000) inhibited cleavage of both species. However, as the Table shows, the several preparations are not equally effective. Similar results were obtained in eight other experiments. This variation in activity may be due to differing proportions of acid and alkaline ribonuclease in the several preparations[8]. Several other enzymes did not affect cleavage of *Arbacia* eggs. These include trypsin (see also [9]), lysozyme, and deoxyribonuclease. Aside from an inhibiting action on cleavage, the enzyme preparations had other effects. These include a collapse and partial lysis of the fully formed *Lytechinus* fertilization membrane (this effect was not pronounced in *Arbacia*) and an egg jelly precipitating action by all four enzyme preparations. However, these effects are not related to the inhibition of cleavage by RNase because eggs from which fertilization membranes had been removed were also inhibited by RNase. Such demembranated *Lytechinus* eggs increased (without lysis) 25% in diameter upon standing in the same ribonuclease. This swelling suggests action on the cellular membrane. The almost complete inhibition of the cleavage of sea urchin eggs reported here has been obtained with eight different batches of eggs. In five other experiments performed independently by one of us (C. B. M.) an inhibition was obtained (with Armour and Worthington RNase at the concentration level of $4 \cdot 10^{-4}$ *M*) but not such a pronounced one. In this case, merely a retardation of the cleavage was obtained (RNase-treated are two to three divisions behind controls) followed by abnormal development sometimes resulting in death of the embryo. This could indicate that the permeability to RNase might be very dependent on the actual condition of the cellular membrane in the case of this material. On the other hand, preliminary observations on *Lytechinus* suggest an increased ribonuclease sensitive period, 20 to 40 min before cell division. Such a critical stage, also observed by RUSTAD for U.V. sensitivity[5], may be found to be correlated with a specific action of ribonuclease on some part of the mitotic apparatus.

Source of Ribonuclease	3×10^{-4}	Concentration	
		$1{\cdot}5 \times 10^{-4}$ M	7×10^{-5} M
Nutritional Biochemical	1	1–2	2–4
Armour	1	2–4	2–8
Sigma	1	2–8	Young morula
Worthington	1	Young morula	Young morula

Cell stage at which *Arbacia* egg cleavage was blocked by three concentrations of the preparations, in conditions where complete inhibition was achieved (controls are at the stage young morulas)

L. LEDOUX* and C. B. METZ

Oceanographic Institute, Florida State University, Tallahassee (Florida), October 28, 1959.

Résumé

La ribonucléase exerce une action inhibitrice sur le clivage des œufs d'oursins. La source commerciale de l'enzyme, la concentration utilisée et l'état des œufs déterminent les effets obtenus. Ceux-ci varient entre une inhibition totale immédiate et un ralentissement des divisions cellulaires, suivi d'un développement anormal éventuellement abortif.

En outre, la ribonucléase a une action complexe sur la membrane de fertilisation, la couche hyaline et la membrane cellulaire. Cette dernière action pourrait être liée aux effets antimitotiques observés.

* Permanent address: Radiobiology Laboratory, CEN, Mol, Belgium.

[1] Contribution No.126 from the Oceanographic Institute, Florida State University, Tallahassee (Florida). Aided by a grant from the National Science Foundation.

[2] L. LEDOUX, J. LECLERC, and F. VANDERHAEGHE, Nature *174*, 793 (1954).

[3] J. BRACHET and L. LEDOUX, Exp. Cell Res., Suppl. *3*, 27 (1955).

[4] J. BRACHET, *Biochemical Cytology* (Academic Press, New York 1957), p. 157.

[5] D. MAZIA, Adv. biol. med. Phys. IV (1956). – R. C. RUSTAD, Exp. Cell Res. *16*, 575 (1959) and in press.

[6] A. I. LANSING and T. B. ROSENTHAL, Biol. Bull. *97*, 263 (1949).

[7] L. LEDOUX and J. BRACHET, Biochem. biophys. Acta *16*, 290 (1955).

[8] L. LEDOUX, unpublished.

[9] A. TYLER and C. B. METZ, Pubbl. Staz. zool. Napoli *27*, 128 (1955).