Decrease of Bacteriotropic Activity of Isoniazid on *M. tuberculosis* in the Presence of Anthranilic Acid

As shown by BÖNICKE[1], *o*-aminobenzoic acid (anthranilic acid, OABA) has no influence on the activity of isoniazid on *M. phlei*. We have, in our own experiments[2], reached the same conclusion with the saprophytic Mycobacterium ATCC 607. It was now of interest to find out whether this is true also in the case of virulent mycobacteria.

We attempted at first to exclude the possibility of physicochemical interactions between isoniazid and OABA, which could have resulted in the influence of OABA on the speed of isoniazid decomposition, even in the absence of mycobacteria in the medium. The decrease of isoniazid in concentration 20·0 µg/ml in Youmans' liquid medium, with or without 1% human albumine, and in the presence or absence of 50·0 µg/ml OABA, at 37°C, was followed by means of the method for isoniazid determination of WOLLENBERG[3]. Results are summarized in Table I.

It may be seen that OABA in the tested concentrations has no influence on the speed of isoniazid decomposition in Youmans' medium, either with or without human albumine. Therefore, further results might be attributed mainly to the interference of OABA with the mechanism of isoniazid action on *M. tuberculosis*.

The possible synergistic or antagonistic influence of OABA on the isoniazid activity was traced in the liquid Youmans' medium without albumine. It was distributed in 50 ml amounts in 250 ml Erlenmeyer flasks and seeded with one loopfull of a 15-days' old Youmans' surface culture of $H_{37}Ra$ strain of *M. tuberculosis*[4]. After 21 days incubation at 37°C all flasks were autoclaved, the bacterial mass separated by filtration on Schleicher-Schüll paper discs No. 589[2] and transferred from filter discs into Kjeldahl flasks. Then total nitrogen of the bacterial mass was determined by the usual Kjeldahl method. Isoniazid (Nicetal-Wander) and OABA (Lachema-Czechoslovakia) concentrations, as well as results of this experiment, may be found in Table II.

Later another experiment was started on Youmans' liquid medium with 1% human albumine. The medium was distributed in 5·0 ml amounts in tubes, which were then seeded with 0·05 ml of a 10 days old Dubos culture of the $H_{37}Rv$ strain of *M. tuberculosis*[4]. Results were read after 14 days' incubation at 37°C. The content of tubes was transferred into Kjeldahl flasks and total nitrogen determination of the grown bacterial mass was performed as mentioned above. Isonizid and OABA concentrations, as well as results of this experiment, are to be seen in Table III.

Results of both experiments were analysed statistically with use of the F-test. Thus it was found that with the $H_{37}Ra$ strain OABA in concentration of 10·0 µg/ml antagonised significantly the bacteriotropic activity of isoniazid in 99·5% confidence limits. With the $H_{37}Rv$ strain and the same OABA concentration, differences between the activity of isoniazid alone and isoniazid with OABA were within the 95·0% confidence limit and this result is therefore not significant. However, by increasing the OABA concentration from 10·0 to 25·0 or 50·0 µg/ml, significant results were obtained in the 97·5 or 99·5% confidence limits respectively.

The decrease of bacteriotropic potencies of isoniazid in the presence of OABA is very interesting in view of the exactly opposite activity of *p*-aminobenzoic acid,

Table I

Medium	Days	Isoniazid*	Isoniazid +OABA*
Youmans	0	100	100
	1	81	84
	3	52	55
	5	36	35
	9	23	22
Youmans	0	100	100
1% albumine	1	58	50
	3	32	29
	5	24	19
	9	16	13

* Isoniazid concentration in % terms; 100% equals to 20·0µg/ml; OABA concentrations 50 µg/ml

Table II

Drug	mg of N	Statistical significance
Isoniazid	0·80	
0·05 µg/ml	0·80	
	0·59	91·54 31·33
		31·33 = $F_{1,4}$ (0·005)*
Isoniazid	2·24	
0·05 µg/ml	2·38	
OABA		
10·0 µg/ml	2·80	

* 0·5% critical value of $F_{1,4}$-distribution

Table III

Drug	mg of N	*F*-Test	Statistical significance for comparison with iconiazid alone
Isoniazid . . .	4·35		
0·1 µg/ml	4·88		
	4·08		
Isoniazid . . .	4·35		
0·1 µg/ml	4·88	6·26 7·71	not found
OABA 10·0 µg/ml	4·61	7·71 = $F_{1,4}$ (0·05)	
Isoniazid . . .	5·02	19·69 12·22	2·5%
0·1 µg/ml	4·62	12·22 = $F_{1,4}$	significance level
OABA 25·0 µg/ml	5·02	(0·025)	
Isoniazid . . .	4·88	38·53 34·12	1%
0·1 µg/ml	5·02	34·12 = $F_{1,3}$	significance level
OABA 50·0 µg/ml		(0·01)	

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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*.

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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)

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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.

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