THE BREAKDOWN OF ATROPINE BY BACTERIA*

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744 strains of micro-organisms have been examined for their ability to break down atropine. Many strains of the genera *Corynebacterium* and *Pseudomonas* may occur as contaminants of atropine and eye preparations containing atropine.

Sterility tests on atropine eye drops (Polish Pharmacopoeia III) often demonstrated the presence of Gram-negative motile rods which could survive and even multiply in the eye drops. There are many reports of soil bacteria which are able to break down tropine alkaloids (Lypacewicz, 1930; Küster, 1952; Mozejko, 1955). We have now examined the organisms isolated from contaminated atropine preparations and from other related environments for their ability to break down atropine.

Experimental

Materials and methods. Altogether 744 strains of micro-organisms belonging to 15 genera were examined. The strains were isolated from atropine eye drops, from solid atropine, from the air of the Pharmacy Division rooms at the Clinic Hospital No. 1 in Gdansk, and the Department of Microbiology, from clinical material, from soil and from a departmental stock culture collection. The micro-organisms were isolated from air by the plate sedimentation method, from soil by Lypacewicz's 1930 method, and from drugs and preparations by Kedzia and Barteczko's method (1959). Media used were Lypacewicz's (1930) and a modification of this, and also 1 per cent atropine eye drops.

The number of viable cells was determined by Chabbert's method 1955) and a surface plate method, the quantity of alkaloids in the media and the drugs by *p*-dimethylamidobenzaldehyde (Wasicky, 1915), or a colorimetric method (Vitali, 1881) and a modification of this method (Allport and Wilson, 1939; Allport and Jones, 1942).

Results

Table I shows the occurrence of atropine breakdown by micro-organisms of 15 genera; 54 strains out of the 744 were able to break down the alkaloid; 38 of these belonged to the genus *Pseudomonas*, 10 to the genus *Corynebacterium*, 3 strains to *Bacillus*, one to *Aspergillacae* and one to *Saccharomyces*.

The ability to break down atropine was not a stable property. The storage of strains in meat broth agar led to a loss of this ability in less than 45 days. Frequent subculturing on meat broth agar shortened

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this time. Strains which had lost their ability to break down atropine could again acquire this character after making 7–15 subcultures on meat broth agar containing atropine. Fig. 1 shows the time course of atropine breakdown in a modified Lypacewicz's medium caused by two

TABLE I

ABILITY OF THE ISOLATED STRAINS TO BREAKDOWN ATROPINE

	Total	Number of positive strains
1. *Micrococcus*	88	1
2. *Sarcina*	291	—
3. *Gaffkya*	11	—
4. *Neisseria*	18	—
5. *Staphylococcus*	4	—
6. *Escherichia*	48	—
7. *Serratia*	19	—
8. *Proteus*	17	—
9. *Achromobacter*	12	—
10. *Pseudomonas*	41	38
11. *Corynebacterium*	11	10
12. *Erwinia*	2	—
13. *Bacillus*	66	3
14. *Aspergillaceae*	70	1
15. *Saccharomyces*	1	1
16. *Non-identified*	45	—
	744	54

of the most active strains belonging to the genera *Corynebacterium* and *Pseudomonas*, and by filtrates of 48-hr. cultures of these strains. Active strains of *Corynebacterium* and *Pseudomonas* immediately after isolation destroyed almost 100 per cent of the alkaloids present in the medium in 36–48 hr. of incubation at 30°. Active strains belonging to other genera destroyed atropine at a slower rate and to a lesser extent.

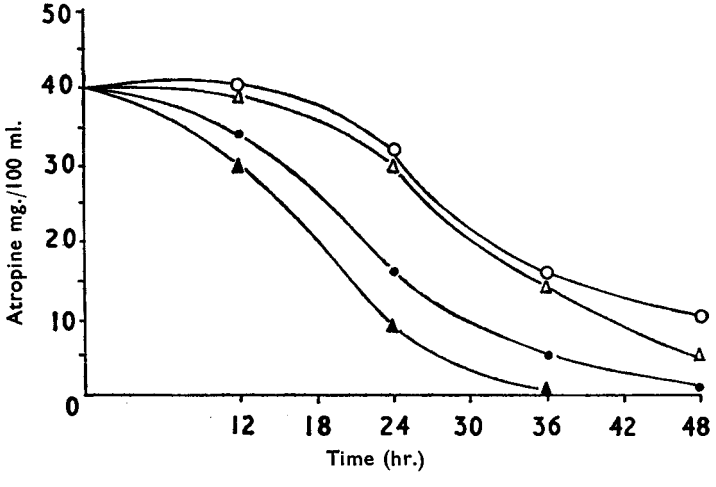

FIG. 1. The time: concentration course of atropine breakdown caused by cultures and filtrates of the *Corynebacterium* (No. 31) and *Pseudomonas* (No. 32) strains on the modified Lypacewicz's medium.

—●—●— No. 32, culture. —○—○— No. 31, culture. —▲—▲— No. 32, filtrate of 48-hr. culture. —△—△— No. 31, filtrate of 48-hr. culture.

Sterile filtrates of 48-hr. cultures of *Pseudomonas* and *Corynebacterium* strains caused atropine breakdown indicating the presence of alkaloid inactivating enzymes in the filtrates. Atropine-destroying strains were also able to break down hyoscine (scopolamine). The maximum atropine concentration was 0·5 per cent of strains belonging to the genus *Corynebacterium*, and about 3·4 per cent for strains belonging to genus *Pseudomonas*.

Table II shows the ability to cause atropine breakdown by strains isolated from the air of the hospital dispensary and from atropine eye drops contaminated while in use, and from solid atropine. Of the 555

TABLE II

ABILITY TO BREAKDOWN ATROPINE OF STRAINS ISOLATED FROM AIR OF THE HOSPITAL PHARMACY, FROM ATROPINE EYE DROPS CONTAMINATED IN THE COURSE OF THEIR USE AND FROM SOLID ATROPINE

Strains isolated from	Number of isolated strains	Number of isolated strains breaking-down atropine	
		Corynebacterium	*Pseudomonas*
Air	303	1	–
Eye drops	202	–	8
Solid atropine	50	–	2
Total	555	1	10

strains 11 caused atropine breakdown. Eight of these were isolated from atropine eye drops, 2 from solid atropine and 1 from the dispensary air. All the atropine-destroying strains isolated from eye drops and solid atropine belonged to the genus *Pseudomonas*. The strain isolated from air belonged to *Corynebacterium*. A loss of about 20 per cent was found in 2 of the 8 samples of atropine eye preparation from which alkaloid-destroying strains had been isolated.

DISCUSSION

The ability of bacteria to break down atropine can be frequently observed in micro-organisms of the genera *Corynebacterium* and *Pseudomonas*. Micro-organisms breaking down atropine are common contaminants of eye preparations, and the air of the rooms where drugs are prepared may be the source of contamination. Species of *Corynebacterium* are of little importance as atropine inactivators, since the amount of atropine present is bacteriostatic for these organisms, but *Pseudomonas* species are able to degrade atropine in the concentration commonly used.

REFERENCES

Allport, N. L. and Wilson, E. S. (1939). *Quart. J. Pharm. Pharmacol.*, **12**, 399–408.
Allport, N. L. and Jones, N. R. (1942). *Ibid.*, **15**, 238–250.
Chabbert, Y. (1955). *Ann. Biol. Clin.* **13**, 37–40.
Kedzia, W. and Barteczko, I. (1959). *Farmacja Polska*, **15**, 85–89.
Küster, E. (1952). *Zbl. Bakter. Parasit.*, **158**, 350–357.
Lypacewicz, J. (1930). *Acta Soc. Bot. Polon.*, **7**, 553–581.
Mozejko, M. (1955). Ref. XIII. Zjazdu Midrob. Pol. Poznan.
Vitali, D. (1881). *Zbl. Anal. Chem.*, **20**, 563–574.
Wasicky, R. (1915). *Ibid.*, **54**, 393–397.