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Non-tuberculosis Mycobacteria in Africa

3. Formamidase Activity—its Evaluation and Practical Application *

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The third study in a series on the prevalence of non-tuberculosis mycobacteria in Africa is devoted to the investigation of the formamidase activity of 288 cultures of mycobacteria, already typed by a battery of standard tests as pathogenic or atypical (184 strains) and saprophytic (104 strains). Of the latter, 96 (92.3%) were formamidase-positive, as compared with only 6 (3.3%) of the former. A close correlation was observed between the speed of growth on Löwenstein-Jensen medium and formamidase activity, 98 (96.1%) of the positive strains showing visible growth within 1-3 days. The relation between formamidase activity and growth on nutrient media was less clear-cut, however, and it was concluded that for the routine differentiation of saprophytic from other mycobacteria the formamidase test should be combined with simple tests such as speed of growth on L-J medium and ability to grow on nutrient media. Russel's method and Nessler's reagent for the detection of ammonia in the formamidase test were compared; the authors consider the former to be preferable, since the reaction is easier to read.

The need for a simple procedure suitable for adoption in routine diagnostic work in tuberculosis laboratories for the identification of mycobacteria, in particular for the differentiation of saprophytes from other types, has often been felt. A number of typing methods have been investigated, but all turned out to be rather too laborious and complicated for routine practice (Engback 1954; Meissner, 1958; Šula & Šulová, 1959; Marks & Trollope, 1960; Schmiedel, 1960; Szabo & Vandra, 1961; Smith & Steenken, 1961; Tarshis, 1962; Jones & Kubica, 1963; Murohashi & Yoshida, 1963; Takeya et al., 1963; Wilbur & Kubica, 1963; Baturo, 1964).

In 1961, Nagayama, Konno & Oka reported the occurrence in certain mycobacteria of an enzyme that catalyses the formation of ammonia from formamide. These workers tested various types of mycobacteria and found that this enzyme—formamidase—showed activity only in saprophytic mycobacteria. The results of their work were confirmed by Hauduroy & Muftic (1963), who found the formamidase test useful for differentiating saprophytic from other mycobacteria. Different modifications of the original technique of Nagayama et al. have been suggested (Muftic, 1964; Dyhno et al., 1964).

In some areas of Africa, the frequency of isolation of atypical and saprophytic mycobacteria is very high. Examination of 161 cultures of non-tuberculosis mycobacteria isolated in Nigeria showed that 44.7% of them appeared to be saprophytes. The routine procedures used at present in tuberculosis diagnostic laboratories permit the identification of saprophytes only after a battery of tests (including the time-consuming determination of drug-sensitivity) has been carried out. In this connexion we thought it would be of interest to find out whether the formamidase test alone would be sufficient to identify saprophytes soon after a culture has been isolated or whether it would have to be used in combination with other simple tests.

The present study was undertaken to confirm the effectiveness of the formamidase test on mycobacteria already typed by the whole complex of standard tests.

MATERIAL AND METHOD

For our formamidase tests we chose the modification of Dyhno et al. (1964). The technique was as follows. All mycobacteria were grown on Löwenstein-Jensen medium (without starch) either for 1

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week (rapid growers and saprophytes) or for 3 weeks (all the remaining cultures); 2 loopfuls of culture in 0.5 ml of phosphate buffer (pH 7.2) were mixed with 0.5 ml of formamide solution (0.1 ml of formamide in 250 ml of distilled water). The tubes were incubated at 37°C for 4 hours. The liberation of ammonia was detected by Russel's method (1944) and/or by means of Nessler's reagent. Of the 288 strains of mycobacteria tested, 242 were received from different parts of Africa—in particular from Nigeria (161) and Kenya (64), and further from Basutoland —now Lesotho—(2); Zanzibar—now in Tanzania— (2); Southern Rhodesia (7); Malawi (2); and the Seychelles Islands (4)—and the remaining 46 from different laboratories in Europe, Asia, Australia and America. The assignment of the atypical mycobacteria isolated in Africa to the various groups of Runyon's classification was based on the following

TABLE 1

FORMAMIDASE ACTIVITY OF MYCOBACTERIA ISOLATED IN DIFFERENT PARTS OF THE WORLD

Group of mycobacteria	Europe, Asia, America and Australia			Africa			Total
	Type or place of isolation	Formamidase		Type or place of isolation	Formamidase		
		absent	present		absent	present	
Tuberculosis	human	1		human	14		26
	bovine	1					
	avian	5					
	drug-sensitive (freshly isolated in Prague)	5					
Photochromogens	Faktor	1		Nigeria	1		5
	Svizensky	1		Southern Rhodesia	1		
				Seychelles Islands	1		
Scotochromogens	Turkey	2		Zanzibar	1		23
				Southern Rhodesia	2		
				Basutoland	2		
				Kenya	4		
				Nigeria	12		
Unpigmented	Battey	3		Southern Rhodesia	1		92
	Australia	1		Malawi	1		
				Seychelles Islands	1		
				Kenya	19		
				Nigeria	66		
Rapid growers	Fortuitum		6	Southern Rhodesia	1		38
				Malawi	1		
				Zanzibar	1		
				Seychelles Islands	2		
				Nigeria	10		
				Kenya	17		
Saprophytic	Rubrum		1	Southern Rhodesia		2	104
	Smegmatis	1	5	Kenya		10	
	N 17		1	Nigeria	3	69	
	Unidentified species	2	2				
	Rabinowitch		1				
	Pellegrini	1					
	Battaglini	1					
Total		46			242		288

TABLE 2
CORRELATION BETWEEN THE RESULTS OF THE FORMAMIDASE TEST AND THE SPEED OF GROWTH ON LÖWENSTEIN-JENSEN MEDIUM

Result of formamidase test	Speed of growth (days)				Total
	1-3	4-6	7-14	15-21	
			Saprophytic mycobacteria		
+++	36	1			37
++	38	2			40
+	18		1		19
−	6	1	1		8
Total	98	4	2	—	104
			Atypical or pathogenic mycobacteria		
+++					—
++	6				6
+					—
−	8	44	68	58	178
Total	14	44	68	58	184

tests: growth at room temperature, catalase activity, niacin test, speed of growth on Löwenstein-Jensen medium, growth on nutrient broth and agar at 37°C, cellular morphology, pigmentation in darkness and after exposure to light, and sensitivity to 7 mycobacteriophages. Detailed reports on the information collected from all these tests have been presented by Zykov, Roulet & Gaya (1967) [1] and Zykov, Donec & Godovannyi (1967).[2]

RESULTS

Among the 288 strains tested, formamidase activity was detected mainly in saprophytic mycobacteria. Table 1 shows the results for all cultures. It may be seen that among the 184 pathogenic or atypical strains (tuberculosis, photochromogens, scotochromogens, unpigmented and rapid growers) there were only 6 (3.3%) cultures (*Mycobacterium fortuitum*) that showed formamidase activity. On the other hand, of the 104 saprophytic strains, 96 (92.3%) were formamidase-positive.

All 288 strains were subcultured on Löwenstein-Jensen medium (without starch) and the speed of growth was measured (Table 2). Of the 102 formamidase-positive strains, 98 (96.1%) showed visible growth within 1-3 days. There was thus a close correlation between speed of growth and formamidase activity. As to the relation between formamidase activity and growth on nutrient media (broth and agar), the results were less clear-cut, as can be seen from Table 3. Of the 249 cultures tested, 89 were formamidase-positive and 160 were formamidase-negative. But it was observed that, while 86 (96.6%) of the formamidase-positive cultures grew on both broth and agar media, 19 (11.9%) of the formamidase-negative cultures did so also; moreover, as many as 21 (13.1%) of the formamidase-negative strains grew on one or other of the media. It is worth mentioning here that most of the formamidase-negative mycobacteria that grew on broth and/or agar media were differentiated from the saprophytic mycobacteria on the basis of their slow speed of growth.

In view of the anomalous results mentioned above, we believe that for the routine differentiation of saprophytic from other mycobacteria the formamidase test should be combined with other simple tests, such as growth on Löwenstein-Jensen medium and growth on nutrient media after incubation at 37°C for 1 week. As for the differentiation of saprophytes

[1] See the article on page 927 of this issue.
[2] See the article on page 939 of this issue.

TABLE 3

CORRELATION BETWEEN THE RESULTS OF THE FORMAMIDASE TEST AND GROWTH ON NUTRIENT MEDIA

Formamidase activity	No. of cultures [a]	Growth on agar (A) and broth (B)			
		AB+	AB−	A+	B+
		Saprophytic mycobacteria			
Positive	84	82	1	1	0
Negative	3	3	0	0	0
		Atypical or pathogenic mycobacteria			
Positive	5	4	1	0	0
Negative	157	16	120	3	18

[a] There were 19 saprophytic strains and 20 atypical or pathogenic strains (including 14 of *Myco. tuberculosis*) that were not tested for growth on simple media.

from the *Myco. fortuitum* group, which were also formamidase-positive rapid growers, it would be necessary to apply some other test (possibly the phenolphthalein-sulfatase test).

With regard to the formamidase-test technique, we attempted to find out if a longer time of incubation would bring forth better results. However, no difference was found in the results read after 5, 6 or 7 hours of incubation. For 33 strains of mycobacteria the presence of ammonia was detected by both Russel's method (1944), and Nessler's reagent. The results were as follows: 22 strains were negative by both methods, 7 were positive by both methods, and 4 were positive by Russel's method but negative with Nessler's reagent. This difference will need some further investigation, but we are inclined to think that Russel's method is preferable since the colour change indicating a positive reaction (colourless→blue) is easier to detect than that indicating a positive Nessler reaction (orange→brown). With the technique we used no formamidase activity was observed in any of the groups of atypical mycobacteria except *Myco. fortuitum*. The possibility suggested by Muftic (1964), that the formamidase test might be used to distinguish between *Myco. fortuitum* and *Myco. tuberculosis* needs further careful investigation. We cannot draw any final conclusions from the formamidase-positive results we obtained with *Myco. fortuitum*, since the number of cultures examined was too small

RÉSUMÉ

De nombreux travaux ont été consacrés à la recherche d'une méthode simple permettant d'identifier les mycobactéries et, plus spécialement, de différencier les souches saprophytes. Les avantages de l'épreuve de la formidase, dont l'intérêt est apparu lors d'études antérieures, sont évalués dans le présent article.

On a recherché l'activité formamidasique de 288 cultures déjà classées par les méthodes usuelles en mycobactéries pathogènes ou atypiques (184 souches) ou en mycobactéries saprophytes (104 souches). Parmi ces dernières, 96 (93,2%) renfermaient de la formamidase, alors que parmi les premières, 6 (3,3%) seulement faisaient preuve d'une activité enzymatique similaire. On notait une corrélation nette entre l'activité et la vitesse de croissance sur milieu de Löwenstein-Jensen, 98 (96,1%) des souches positives donnant une culture visible en 1 à 3 jours. La relation entre l'activité enzymatique et la nature du milieu de culture était moins nette.

Selon les auteurs, on peut le plus aisément différencier les souches saprophytes des autres mycobactéries en associant la recherche de l'activité formamidasique et des tests simples comme la mesure de la vitesse de croissance sur milieu de Löwenstein-Jensen et l'appréciation de l'aptitude à la croissance sur divers milieux nutritifs. Pour déceler l'ammoniac produit au cours de l'épreuve, il semble préférable d'employer la méthode de Russel où la positivité est indiquée par un virage du milieu de l'incolore au bleu plutôt que la méthode de Nessler dont les résultats sont de lecture plus difficile (virage de l'orange au brun).

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