A STRAIN OF MYCOBACTERIUM ISOLATED FROM SKIN LESIONS OF A COLD-BLOODED ANIMAL, *XENOPUS LAEVIS*, AND ITS RELATION TO ATYPICAL ACID-FAST BACILLI OCCURRING IN MAN

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(With Plates 3–4)

INTRODUCTION

The first reference in the literature to acid-fast bacilli in cold-blooded animals is that of Sibley (1889), who found these organisms in subcutaneous nodules of an Italian snake, *Tropidonotus natrix*, which had died in captivity. In 1897, Bataillon, Dubard & Terre described a swelling, the size of a pigeon's egg, they found on the ventral surface of the body wall of a carp; the lesion was non-caseous and contained acid-fast bacilli which grew at room temperature and also at 37° C. Animals injected with cultures of the carp strain showed no evidence of immunity. Friedmann (1903) performing a necropsy on a tortoise, which had been kept in a tank of sea water, isolated an acid-fast bacillus from lesions found in the lungs and he recorded that this organism conferred a degree of immunity in guinea-pigs and cattle. Küster (1905) quoted that Weber & Tante had noted naturally occurring 'tuberculosis' in frogs and that the water and moss in the amphibians' tanks contained a cold-blooded type of tubercle bacillus. Küster, himself, examined a total of 500 frogs and 50 other reptiles and isolated acid-fast bacilli from the livers of three frogs. The strains grew readily at 28° C. but not at 37° C.; they produced liver and lung lesions when injected into cold-blooded animals but none when injected into rabbits, rats or mice. He does not record whether any of his test animals showed skin lesions. Baerthlein & Toyoda (1913) isolated acid-fast bacilli from the livers of frogs maintained in the laboratory; these strains were smooth on first isolation but rough variants grew out after 4 months. On subculture, and on passage through frogs, the rough variant failed to revert to the smooth parent form, but rabbit immune sera were found to contain agglutinins and complement-fixing antibodies to both rough and smooth strains. Neither of the strains, however, reacted with sera from rabbits immunized with human, bovine or avian type of *Mycobacterium tuberculosis* or with anti-lepra sera.

In man there are several records of infections of the skin associated with the presence of acid-fast bacilli which are not *Myco. tuberculosis*. Gellerstedt (1944) collected seven cases in which the patients developed a nodule in the skin which subsequently ulcerated. Although acid-fast bacilli were seen in large numbers in the lesions they could not be cultivated on artificial media. Specimens from the ulcers of two of the patients were inoculated into guinea-pigs, rabbits, rats and

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mice but apparently failed to take; when the animals were sacrified 3 months later no evidence of any lesion could be found.

MacCallum, Tolhurst, Buckle & Sissons (1948) reported the association of an acid-fast bacillus with superficial skin ulceration on the exposed surface of the extremities of a number of patients living in farming districts of Victoria in Australia. The authors were unable to trace the source of the infection, but as the lesion in each case was limited to the exposed surface of the arm or leg, it appeared likely that a pre-existing abrasion determined the site of the ulcer. The ulcers were intractible to treatment; after the slough separated there was a gelatinous mass which could be readily wiped off with gauze. Direct smears from the ulcer showed an abundance of acid-fast bacilli which could be cultivated at 33° C. but not at 37° C. The organisms isolated were fully investigated and clearly they could be neither *Myco. tuberculosis* or *Myco. leprae,* but they had pathogenic properties and were designated *Myco. ulcerans.*

Linell & Norden (1954) isolated an acid-fast bacillus from skin ulcers in thirty-one patients, all of whom were frequent users of one particular swimming bath. The walls of the bath were finished in rough concrete and acid-fast bacilli with the same characters as those recovered from the patients were isolated from the submerged surface of the concrete walls and from the water itself. The ulcers were probably the result of infected abrasions from the rough sides of the bath for the authors produced identical lesions in their own skin by scratching with a needle which had been rinsed in a suspension of the bacilli. One week after the inoculation there was reddening and infiltration at the site of the scratch. A few days later an epithelial crust developed which subsequently separated to expose a small ulcer containing acid-fast bacilli. Biopsy of the ulcer showed a histological picture similar to that seen in the patients: the corium contained tuberculoid cell foci with giant cells of the Langhans' type and round cell infiltration around the tuberculoid structures. One of the volunteers infected himself a second time and the lesion that developed was in no way different from that seen on the first occasion. The bacterium of the organism was fully investigated and named *Myco. balnei.*

PRESENT INVESTIGATION AND RESULTS

An adult female *Xenopus laevis,* maintained in the laboratory, was noticed to have a brown nodular lesion on its back. When first observed the patch was about 3 mm. in diameter with an irregular surface and edge. The dorsal surface of each thigh had two similar lesions 2 mm. in diameter. Three days later twelve small blisters appeared on the back and sides of the animal (Pl. 3, fig. 1).

The animal fed normally on cubed liver and was kept isolated in a glass aquarium for 3 months when it was sacrificed by anaesthesia. At necropsy the viscera were found to be normal; the histological examination of the skin showed the lesions to be situated mainly in the superficial portion of the dermis, but the dense connective tissue of the deep dermis was also involved. The overlying pigment layer was intact. The lesions consisted of closely packed masses of endothelioid cells, macrophages, lymphcytes and polymorphs. Very little collagen fibre was

present. Superficially there was a zone of necrosis; there was no evidence that the capillaries were not patent. Numerous clumps of acid-fast bacilli were to be seen (Pl. 3, fig. 2*a*, *b*).

Fluid from the blisters stained by Ziehl–Neelsen revealed slightly curved acid-fast bacilli $2 \times 1\mu$ in size. The fluid was inoculated on to two sets of the following culture media: 2·6% blood agar, MacConkey agar, Dorset egg and Lowenstein–Jensen media, respectively; one set was incubated at 24° C. and the other at 37° C. One month later the only culture showing any growth was the one on Lowenstein medium which had been incubated at 37° C.; this was a moist smooth growth resembling that of avian tubercle bacilli (Pl. 4, fig. 3). The organisms were acid-fast, showing beading and palisade arrangement, and the individual bacterial cell size varied considerably; a few were about 4μ long but the longer forms—8–15μ—predominated. The primary growth was subcultured to Lowenstein medium with glycerin and to glycerin-free serum agar for incubation at 24 and 37° C. The addition of glycerine significantly improved the growth of the culture incubated at 37° C.; no growth occurred at 24° C., even after 3 months incubation.

Surface cultures on 5% glycerin broth took 9 months to become established (Pl. 3, fig. 4). The filtrate from this growth was concentrated by evaporating to one-tenth of the original volume and the resulting fluid was termed 'acid-fastin'; it was used in the manner of Old Tuberculin (OT) in testing for sensitivity and for coating sheep's RBC in the Middlebrook haemolysin test which will be described later.

The organism, to which I have given the name *Mycobacterium xenopei*, was sensitive to both streptomycin and PAS but of doubtful resistance to INAH, growing at a concentration of 0·2 μmg./ml., but not at 1·0 μmg./ml. It was urease positive, catalase positive, peroxidase negative, nitrate was reduced and there was no colour change in the cyto-chemical test of Dubos–Middlebrook (1948), see Table 5.

Biological reactions resulting from inoculation with blister fluid and cultures

A. *Blister fluid inoculation.* Two mice were inoculated intraperitoneally and subcutaneously, two guinea-pigs and one rabbit intramuscularly. Two *Xenopus laevis* were inoculated—one intracutaneously and one into the dorsal sac—and a further one was kept in contact with the naturally infected sick animal for 12 weeks. All these animals were carefully examined at 7 or 12 weeks after inoculation and none of them showed any pathological lesion.

B. *Inoculation with bacterial cultures.* A 14-day-old culture from Lowenstein medium was suspended in distilled water. Large clumps were allowed to sediment and the supernatant suspension was standardized against 'opacity-tubes' representing *Bacterium coli* suspensions. Six mice were given an estimated dose of 0·2 mg. intravenously and four mice 0·8 mg. intraperitoneally.

Mice sacrificed at 4 or 12 weeks had enlarged spleens and isolated tubercles in the lungs. Direct smears of the liver, spleen and kidney showed massive clusters of acid-fast bacilli reminiscent of the Yersin type of tuberculosis. Cultures of heart-

blood, peritoneum and spleen were positive. Stained sections of the spleens from these animals showed an unusual number of giant cells; there were numerous clumps of acid-fast bacilli but they were not identifiable with any specific histological lesion (Pl. 4, fig. 5). None of the mice exhibited the peritonitis or scrotal lesions found by MacCallum *et al.* (1948) in their mice inoculated with *Myco. ulcerans*; a subculture of this organism obtained from the National Collection of Type Cultures failed to induce these changes in the mice used in this laboratory, but they did not belong to the Dobwolskaja–Sawadskaja strain.

Two guinea-pigs and one rabbit were given intramuscularly 4 mg. of a culture of *Myco. xenopei* and sacrificed at 6 and 12 weeks, respectively. A caseous abscess, containing the infective organism, was found in the muscle at the site of inoculation.

A 3-month-old hen was inoculated with 4 mg. of the culture into the right chest muscle. The animal broke its leg 6 weeks later and had to be killed; no local lesion was found and there was no evidence of any visceral infection. Heart-blood cultures after 10 weeks incubation remained sterile.

Two *Xenopus* were given intraperitoneal injections of 1000×10^6 bacilli of a 4-week-old culture of *Myco. xenopei*. They were examined at 4 weeks and 7 months, respectively. Cultures from the dorsal sac of the first animal gave a mixed growth of *Streptococcus pyogenes*, Lancefield Group A, and acid-fast bacilli identical to those inoculated. No organism was recovered from the second animal, but histological examination of the kidneys revealed one focus between the tubules consisting of a collection of polymorphs and lymphocytes; acid-fast bacilli were present in this little granuloma.

A third xenopus received 8000×10^6 organisms intraperitoneally and was sacrificed after 3 months. Externally no abrasions or granulomata were seen, but beneath the surface of the skin covering the dorsal, ventral and femoral sacs numerous small granulomata were to be found, their diameter varying from 1 to 3 mm. There was one caseous nodule 5 mm. across. Connective strands of fibrous tissue between the dorsal and ventral surfaces of the dorsal sac also contained small granulomata. Macroscopic lesions were not seen in any of the viscera. Smears from the granulomata revealed acid-fast bacilli and the ground-up tissue inoculated on to glycerinated Lowenstein media yielded cultures of the infecting organism after 6 weeks incubation at 37° C. Histological examination of the deep surface of the skin and of the connective fibrous tissue showed focal collections of lymphocytes and some neutrophils with necrotic areas containing numerous acid-fast bacilli. There were also a few endothelioid and giant cells. The lung showed one necrotic focus with surrounding lymphocytes and a few endothelioid type cells and the ovary contained a little nodule with a necrotic centre. Lesions were not seen in the spleen or liver.

A fourth xenopus was given 8000×10^6 organisms into the dorsal sac. This animal died of an intussusception 7 months later. An area 4×2 mm. on the dorsal surface of the right thigh appeared to be ulcerous and histological examination of the skin (Pl. 4, fig. 6) revealed granulomata with superficial ulceration and numerous acid-fast bacilli within the many macrophages. The majority of the liver cells and the nodular collections of macrophages in the splenic pulp contained many

Table 1. *Biological reactions resulting from inoculation of mice, guinea-pigs, rabbits, hens and toads with blister fluid or* Mycobacterium xenopei *culture*

	Blister fluid		Culture	
Mice	1 × IP (7)	1 × SC (7)	6 × IV 0·2 mg. (12)	4 × IP 0·8 mg. (12)
Guinea-pigs	2 × IM (7)		2 × IM 4 mg. (6)	
Rabbits	1 × IM (12)		1 × M 4 mg. (12)	
Hens	Nil		1 × IM (6)	
Xenopus	1 × IC (12)	1 × DS (12)	1 × IP 1 mg. (4)	1 × IP 1 mg. (28)
	1 × contact (12)		1 × IP 8 mg. (12)	1 × DS 8 mg. (28)

Figures in brackets = time in weeks after infection when necropsy was performed.
IP = intraperitoneal; IM = intramuscular; SC = subcutaneous; IC = intracutaneous; DS = into dorsal sac.

acid-fast bacilli. Cultures of the dorsal sac fluid yielded discrete colonies of *Myco. xenopei* (Pl. 4, fig. 7).

The experiments outlined above show the fulfilment of Koch's 'postulates' in that the organism isolated from the naturally occurring lesion in the *Xenopus laevis*, after cultivation for several generations on artificial laboratory media, was able to reproduce the original disease in the susceptible animal. The infecting dose was large, however, and more than 3 months elapsed before the skin lesions developed.

Development of skin sensitization after infection

Two guinea-pigs and one rabbit were inoculated with a dose of 4 mg. of a culture of *Myco. xenopei* and 4 weeks later the animals were skin-tested in parallel with Old Tuberculin (OT) and the *Myco. xenopei* acid-fastin; a tuberculous guinea-pig was included as a control.

Table 2. *Allergy production at 4 weeks*

		Positive reaction in dilution of	
Animal	Infected with	OT	Acid-fastin
1 × Guinea-pig	*Myco. tuberculosis*	1/100	Undiluted
2 × Guinea-pigs	*Myco. xenopei*	Undiluted	Undiluted
1 × Rabbit	*Myco. xenopei*	Undiluted	Undiluted

Table 2 shows that while the tuberculous guinea-pig reacted to OT diluted 1/100 and to undiluted acid-fastin the animals infected with *Myco. xenopei* reacted to OT and acid-fastin only in the undiluted form; intradermal injection of either reagent in any dilution failed to produce a skin reaction.

The positive reaction in the tuberculous control guinea-pig to diluted OT was a 2 cm. area of redness with induration; undiluted acid-fastin in this animal resulted in a reaction with a diameter of 0·8 cm. The reactions in the test animals were between 1·5 and 2·0 cm. in diameter; the reactions in the rabbit to both OT and acid-fastin became indurated and were palpable as nodules up to the end of a week.

The strain of *Myco. xenopei* under test therefore had little power to induce allergy in guinea-pigs or rabbits and failed to cause more than a local abscess in these animals when injected intramuscularly.

Serological tests

The haemagglutination test for antibodies against tubercle bacilli antigens, introduced by Middlebrook & Dubos (1948), was later modified by Middlebrook (1950) by the addition of complement to the reacting system. It thus became a haemolytic test which, however, did not necessarily measure the same antibody as the original test. Nevertheless, it was decided to apply this haemolytic test to the study of *Myco. xenopei* in the hope that it might reveal information of value about the antigenic structure of this organism with regard to its relationship with the tubercle bacilli.

Table 3. *Middlebrook–Dubos haemolysin test*

Immune sera		Haemolysin titre with sheep cells sensitized with: Old Tuberculin	Acid-fastin
Rabbit	Anti *Myco. xenopei*	640	2560
	Anti *Myco. tuberculosis*		
	Human	312	160
	Bovine	1280	160
	Avian	1280	1280
Mouse	Anti *Myco. xenopei*	20	40
Toad	Anti *Myco. xenopei*	20	<20
Guinea-pig	Anti *Myco. xenopei*	20	80
Controls from normal rabbit, toad and guinea-pig		<20	<20

Immune rabbit sera were prepared from killed suspensions of *Myco. xenopei*, human, bovine and avian types of *Myco. tuberculosis*. Employing 'Technique C' of Middlebrook (1950) these four immune sera were tested against two lots of sensitized sheep's RBC's; one lot was coated with Lederle's special sensitive Old Tuberculin and the other with the *Myco. xenopei* acid-fastin referred to above. Included in the tests were also sera from mice, toads and guinea-pigs which had been injected with living *Myco. xenopei*. Table 3 shows the haemolysin titres obtained with the various antisera in the parallel tests and it is of interest to note that high titres of the same order were recorded for *Myco. xenopei* and avian tubercle bacilli anti-sera in the test with sheep's cells coated with acid-fastin. This is not surprising because of the many cultural characteristics shared by these two strains of acid-fast organisms. The antibody titres in the mouse, toad and guinea-pig were not significant.

It seemed possible that sera from patients infected with atypical acid-fast bacilli tested against the two suspensions of sensitized red cells might yield results of diagnostic value; some of these sera might, at any rate, have a higher titre against the acid-fastin-coated red cells than those treated with OT. Sera from eighteen patients from whom atypical acid-fast bacilli had been cultivated were examined by the method indicated above but the results were disappointing and offered no such evidence.

For the differentiation of this type of chromogen from *Myco. tuberculosis* it has

been necessary to confine observations to the atypical bacterium itself by preparing a special 'acid-fastin' and a specific rabbit immune serum from each atypical strain. These products are then tested against the 'Standard' antibody and antigen of the human strain of *Myco. tuberculosis* (H 37 RV) and the standard atypical strain ('Goss'; Nassau, Schwabacher & Hamilton, 1958). Table 4 shows the cross-reactions between the raw standard sera and the final highly specific sera after reciprocal absorption.

Table 4. *Middlebrook–Dubos haemolysin test*

	Sheep cells coated with antigen	
Specific serum	H 37 RV	Goss
H 37 RV	12,800	1,600
Atypical acid-fast bacilli (Goss)	40	12,800
Reciprocal absorption		
H 37 RV serum absorbed Goss culture	6,400	< 100
Goss serum absorbed H 37 RV culture	< 100	8,000

DISCUSSION

The present author (Schwabacher, 1933) redirected attention to the widespread distribution in dust and water of saprophytic acid-fast bacilli, and to the inability to differentiate, on purely microscopical evidence, these forms from tubercle bacilli. Saprophytic acid-fast bacilli can be demonstrated in large numbers in the slime adherent to the mouths of most cold-water taps; in hot dry atmospheres this tends to dry and flake off as dust particles containing the acid-fast, still viable, organisms which can thus be disseminated widely and, falling on moist surfaces, can commence to colonize.

Animals from whom cold-blooded strains of acid-fast bacilli have been isolated, have in all instances been in contact with stagnant water. The skin lesions described by Gellerstedt (1944), MacCallum *et al.* (1948) and Linell & Norden (1954), would appear to have resulted from the contamination of the abrasions with organisms of this type since they were situated on those parts of the arms and legs uncovered by clothing.

In considering the taxonomy of the acid-fast bacilli I have been guided by the standards established by Griffiths (1907, 1911), and in Table 5 the essential features of the recognized types of *Myco. tuberculosis* have been set out for comparison with those of the acid-fast bacilli isolated from water and skin lesions referred to in this communication.

In recent years there have been reports of atypical mycobacteria having been isolated from stuta and resected lungs (*Lancet*, 1957) and it would seem that Tarshis & Frisch (1952) are not alone in their belief that there is a group of acid-fast bacilli midway between the saprophytic bacilli and the human tubercle bacilli which can be distinguished by biochemical tests. In guinea-pigs these chromogens tend to produce areas of central necrosis with abscess formation surrounded by granulation

Table 5. *Comparative bacteriology of acid-fast bacilli isolated from water, skin lesions, and mycobacteria of known types*

						Myco. tuberculosis		
		Saprophytic	*Myco. ulcerans*	*Myco. balnei*	*Myco. xenopei*	Human	Bovine	Avian
Pigment formation	On Lowenstein–Jensen	Yellow to orange	Mustard to yellow	Whitish yellow	Yellow to orange	Only on serum of young cow	Nil	Nil
Growth	At 22° C.	+	Slight	+	Nil	Nil	Nil	Nil
	At 37° C.	+	Slight best at 31° C.	Nil best at 31° C.	+	+	+	+
	At 45° C.	Slight	Nil	.	Nil	Nil	Nil	+
	At 55° C.	Slight	Nil	.	Nil	.	.	±
	On nutrient agar	+	Nil	.	Nil	Nil	Nil	+
	On glycerin nutrient agar	+ +	Nil	.	Nil	Nil	Nil	+ +
	On serum agar	+	+	.	+	+	+	+
	On glycerin serum agar	+ +	+ +	.	+ +	+ +	+	+ +
	On coagulated serum	+	+	.	+	+ +	+	+ +
	On horse-blood agar	+	+	.	+	.	.	.
	On glycerine broth	+	.	.	+	Thick pellicle	Thin film	Thin film or granular *deposit*
	Producing gelatin liquifaction	Nil	Nil	Nil	Nil	.	.	.
	Nitrate reduction	+	+	Negative	+	.	.	.
	Thermolabile point	30 min. at 60° C.	30 min. at 60° C.	10 min. at 56° C.	30 min. at 56° C.	15 min. at 60° C.	15 min. at 60° C.	15 min. at 60° C.
	Urease	+	+	.	+	.	.	.
	Catalase	+	+	.	+	+	+	+
Cytochemical test	Ref: Dubos & Middlebrook (1948)	No colour change	No colour change	No colour change	No colour change	Colour change	Colour change	No colour change

Table 5 (*cont.*)

		Saprophytic	*Myco. ulcerans*	*Myco. balnei*	*Myco. xenopei*	*Myco. tuberculosis*		
						Human	Bovine	Avian
Sensitivity	To Streptomycin	S	S	S	S	.	.	.
	To P.A.S.	S	S	R	S	.	.	.
	To I.N.A.H.	Resistant	Resistant	Resistant	Doubtful resistance. Growth on 0·2. Not at 1·0 μg.	.	.	.
Pathogenicity	Guinea-pigs, 1 mg. i.m.	Nil	Nil	Nil	Nil	D	D	Y
	Rabbits, 1 mg. i.v.	Nil	Epididimitis	Epididimitis	Nil	±	D	Y
	Hen, 1 mg. i.v.	Nil	Nil	Nil	Nil	Nil	Nil	++
	Rat inoculated with pus from patient or peritoneal fluid from infected rat	Nil	Ascites, oedema of hind legs, ulceration of scrotal skin, lesions of the epididymis		.	.	.	.
	Mice, 0·2 mg. intraperitoneally or 1·0 mg. intraperitoneally	Nil			Y	D	D	±
	Mice, 0·2 mg. i.v.	Nil			Y	Y	Y	Y
	Lizards	.	Nil	.	.	.	.	.
	Frogs, 0·8 mg. s.c.	Skin ulceration, positive cultures from lesion and dorsal sac fluid	.	Liver cultures positive	Skin ulceration, positive cultures from lesion and dorsal sac fluid	Nil	Nil	Nil
References		Schwabacher (1933)	MacCallum *et al.* (1948)	Linell & Norden (1954)	.		Wilson & Miles (1946)	

D = disseminated tuberculosis. Y = Yersin type tuberculosis.
± = variable slight pathogenicity.

tissue infiltrated by polymorphs. Cross-immunization studies indicate that the chromogenic strains share antigenic properties with human tubercle bacilli but that the two groups have specific component characteristics of their own. I believe those acid-fast bacilli which are photochromogenic to be of the same order as those isolated from water. The mechanism of microbial pathogenicity or the factors governing individual host parasite relationship cannot be explained. It is known that saprophytes, introduced in sufficient numbers, will, on occasions (as shown by Linell & Norden, 1954), become established as primary pathogens and it is not surprising, therefore, that these saprophytes exhibit slightly different behaviour *in vitro* as a result of their adaption to a different environment (see Table 5.)

This power of adaptation is illustrated in the case of *Myco. xenopei* which, isolated from a cold-blooded animal, failed to grow *in vitro* at 24° C., but grew well at 37° C. (only slightly at 31° C.), yet an inoculation of a massive dose of the culture into another cold-blooded animal of the same species eventually reproduced the original disease.*

The poorly or non-pigmented mutant acid-fast bacillus, emerging in culture as a result of chemotherapy, is of equal importance to the chest physician. Its power of infectivity in contacts has not yet been assessed and it remains the responsibility of the bacteriologist to investigate all mycobacteria as fully as possible with a view to elucidating the incidence of cross-infection of these mutant strains. Investigations on these lines are continuing.

Castelnuovo, Gaudiano, Morelli & Sciarrone-Polizzi (1958) claim to be able to type strains of mycobacteria by their specific phages. It seems reasonable to believe that transduction of a genetic factor relating to virulence could change a saprophytic mycobacterium into a mycobacterium of greater virulence. Alteration of Brucella virulence has been observed by Braun (1954) who, with the aid of DNA transformed a rough nonvirulent strain into a smooth virulent type.

SUMMARY

1. A brief review of the literature on acid-fast bacilli found in skin lesions is given.

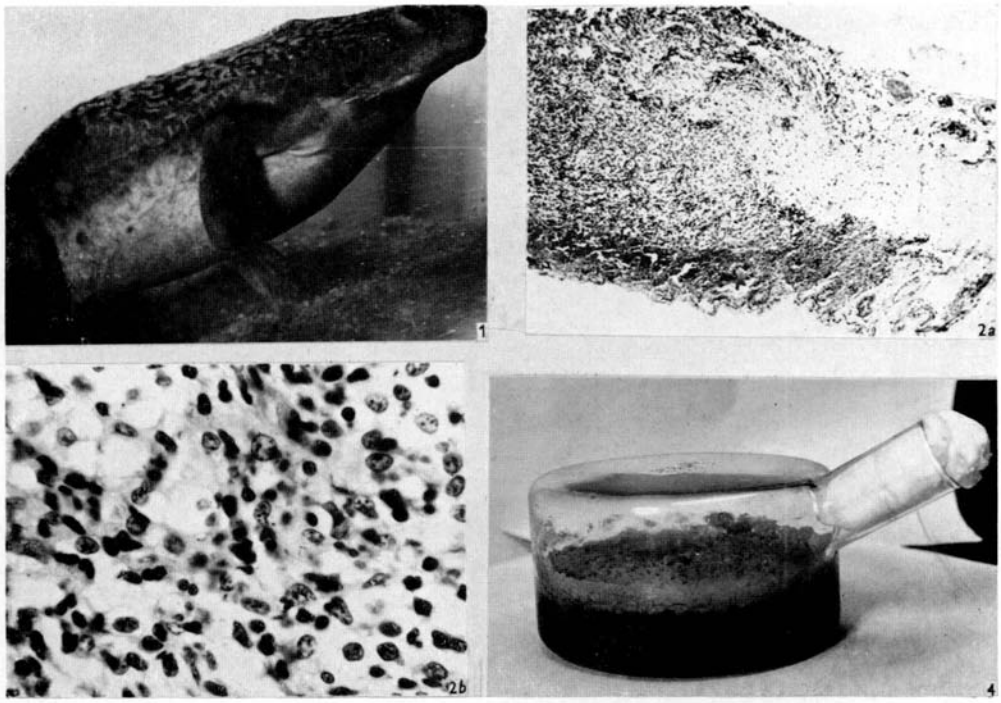
2. The characteristics are described of an acid-fast bacillus isolated from a skin lesion found in the South African *Xenopus laevis*.

3. Reasons are given to support the view that chromogenic acid-fast bacilli cultured from skin lesions belong to the group of 'Saprophytic acid-fast bacilli'.

4. The possibility of using the Middlebrook–Dubos haemolysin test as a tool to differentiate atypical acid-fast bacilli is discussed.

Thanks are due to Dr Charles Pike for all the histological reports, to Mr T. R. West for the microphotographs, to Dr Piersma (Messrs Lederle) for the gift of special sensitive Old Tuberculin for the Middlebrook test, to Dr Nassau for patients' sera and the 'Goss' antigen and finally to Mr W. J. Fincham for the titrations of the haemolysin titres.

* One of three toads injected intraperitoneally with 0·8 mg. of a paprophytic mycobacterium from a tuberculosis sputum died after 15 months with multiple skin lesions, similar to those seen in natural Xenopus infections. Acid-fast bacilli were present in the lesions and were also cultured from the dorsal sac.



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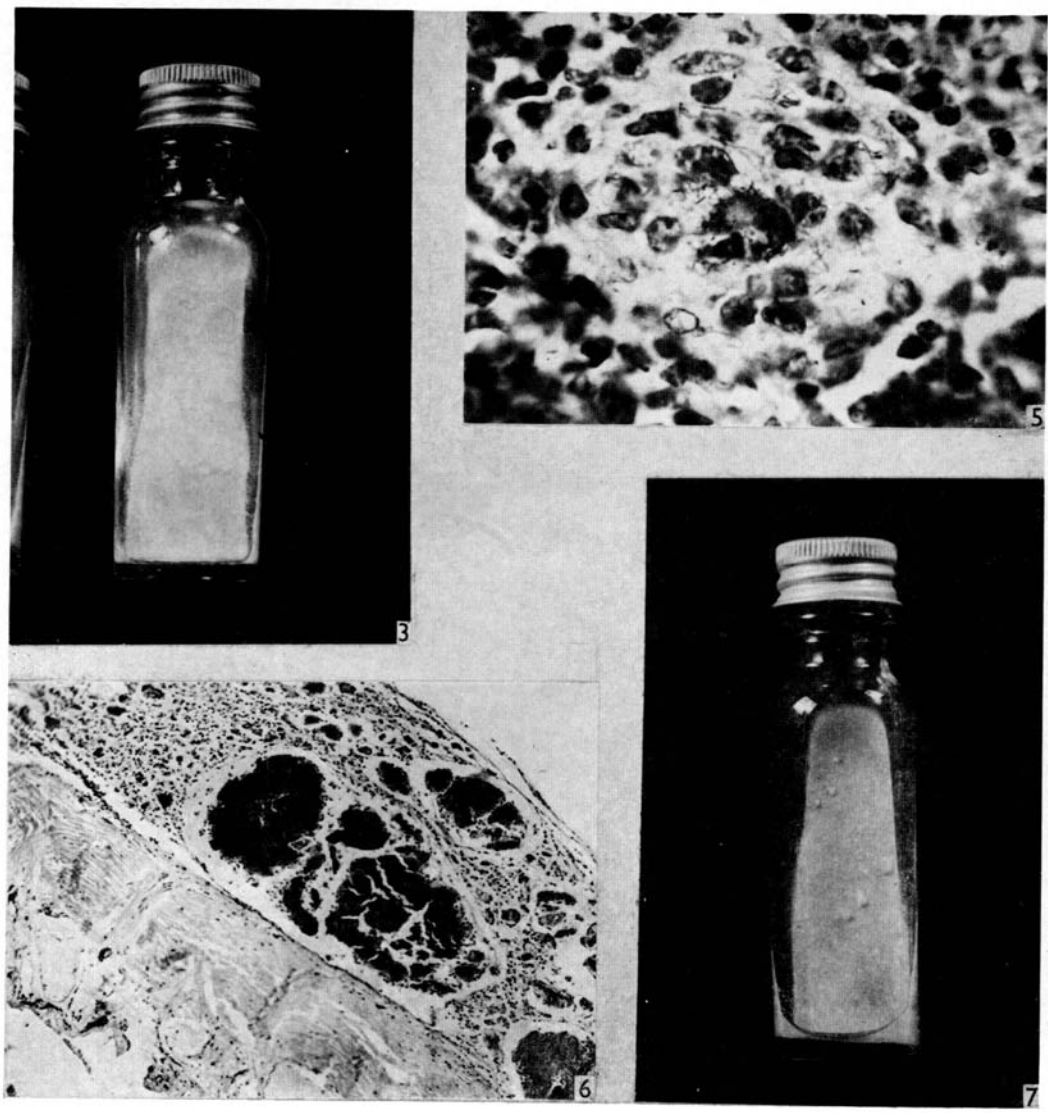

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EXPLANATION OF PLATES

Plate 3

Fig. 1. Blisters on skin of *Xenopus laevis*.

Fig. 2. Histology of naturally occurring disease, description page 58. *a*. Granuloma, magnification × 100. *b*. Acid-fast bacilli, magnification × 800.

Fig. 4. Surface growth on 5% glycerin broth. The concentrate of the broth was termed 'acid-fastin'.

Plate 4

Fig. 3. Smooth, pigmented growth of acid-fast bacilli from blister fluid, primary isolation.

Fig. 5. Splenic lesion in a mouse inoculated by the intravenous route, description page 60.

Fig. 6. Skin lesion of an artificially infected *Xenopus laevis*. Granuloma with large clumps of acid-fast bacilli.

Fig. 7. Cultures of dorsal sac fluid from an inoculated *Xenopus laevis*.

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