New Results in the Chemotherapy of Mycobacterioses

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Since Rifampicin (RAMP) has become available for the chemotherapy of mycobacterioses, new schemes for successful first treatment of tuberculosis have become possible. Now the same good results can be obtained in the chemotherapy of chronic tuberculosis, resistant to many antituberculous drugs. This is why we should also anew investigate the practicability of RAMP in the treatment of so-called atypical mycobacterioses. As the evaluation of such data on a clinical basis is not possible because of the rarity of cases, and the bacteriologic determination of resistance does not give a satisfying answer as to the therapeutic effect of drugs used alone or in combination, we wish to offer, in the following pages, ways for the assessment of therapeutic effects in animals or in man. One of the experimental methods is screening the therapy in infected animals.

Mice infected with *M. kansasii* will show readily reproducible courses of infection, which, as worked out by Yoshida [3] (Fig. 1), give a good therapeutic response to combination therapy including Rifampicin. These strains are often as sensitive as *M. tuberculosis* $H_{37}Rv$ *in vitro* to Rifampicin. The large number of mycobacteria in these slowly developing infections makes necessary somewhat higher dosages in comparison with those used for *M. tuberculosis*. Rifampicin in triple or quadruple combination with streptomycin (SM), Isoxyl, certain sulfonamides, ethionamide, isoniazid (INH) or ethambutol (EMB), offers some promising aspects for therapy. Generally speaking, it is best to recommend a quadruple combination.

Beside the therapy of swimmingpool-infections, the determination of an effective treatment of infections with *M. marinum* is very important because of its analogy to *M. leprae*. Infections with *M. marinum* in mice are virulent enough to serve also as an experimental model for the evaluation of antimycobacterial drugs. Intravenous injection at first causes skin lesions in the tail, feet, mouth and ears (in male animals, in addition, the testicles) with large numbers of mycobacteria (Fig. 2). Later on we find progressive mycobacterioses of the lung (Fig. 3). In survival-time tests (under extremely hard conditions), as well as in the treatment of these serious peripheral processes, Rifampicin at a dosage of 20 mgm. and 50 mgm./kgm. was again very effective (Fig. 4). Similar results (as with the effective dose of 10 mgm./kgm. of Rifampicin) were obtained in spite of the clinical course of the infection with 50 mgm./kgm. of B.663, 100 mgm./kgm. of sulfamethoxypyrazine, 50 mgm./kgm. of ethionamide and 50 mgm./kgm. of DDS according to the survival time of the animals (Fig. 5). Ten mgm./kgm. of INH and 100 mgm./kgm. of Ledermycin were shown to be less effective, and 100 mgm./kgm. of Isoxyl caused the lowest effect. In these experiments only Rifampicin could significantly control the peripheral processes. A combination of Rifampicin with one of the above mentioned tuberculostatics showed some therapeutic improvement compared with the activity of the partner, but the effectivity of this combination was not better than that of Rifampicin alone. The combination with Ledermycin resulted in a decrease of activity. In most experiments, however, the course of generalizing infections was effectively influenced by treatment with twin combinations (Fig. 6); the most active are Rifampicin and B.663 (Fig. 7), and Rifampicin and ethionamide (Fig. 8). Not so good results were obtained by combinations of Rifampicin and Kelfizine, DDS (Fig. 9) or Isoxyl. The combination of Rifampicin and INH showed lower activity than Rifampicin alone. The individual effects of Rifampicin and Ledermycin are nearly nullified when these two drugs are combined—similarly to their activity against the peripheral lesions (Fig. 10). No decrease of activity was to be observed by adding 10 mgm./kgm. of INH to 20 mgm./kgm. of Rifampicin and 100

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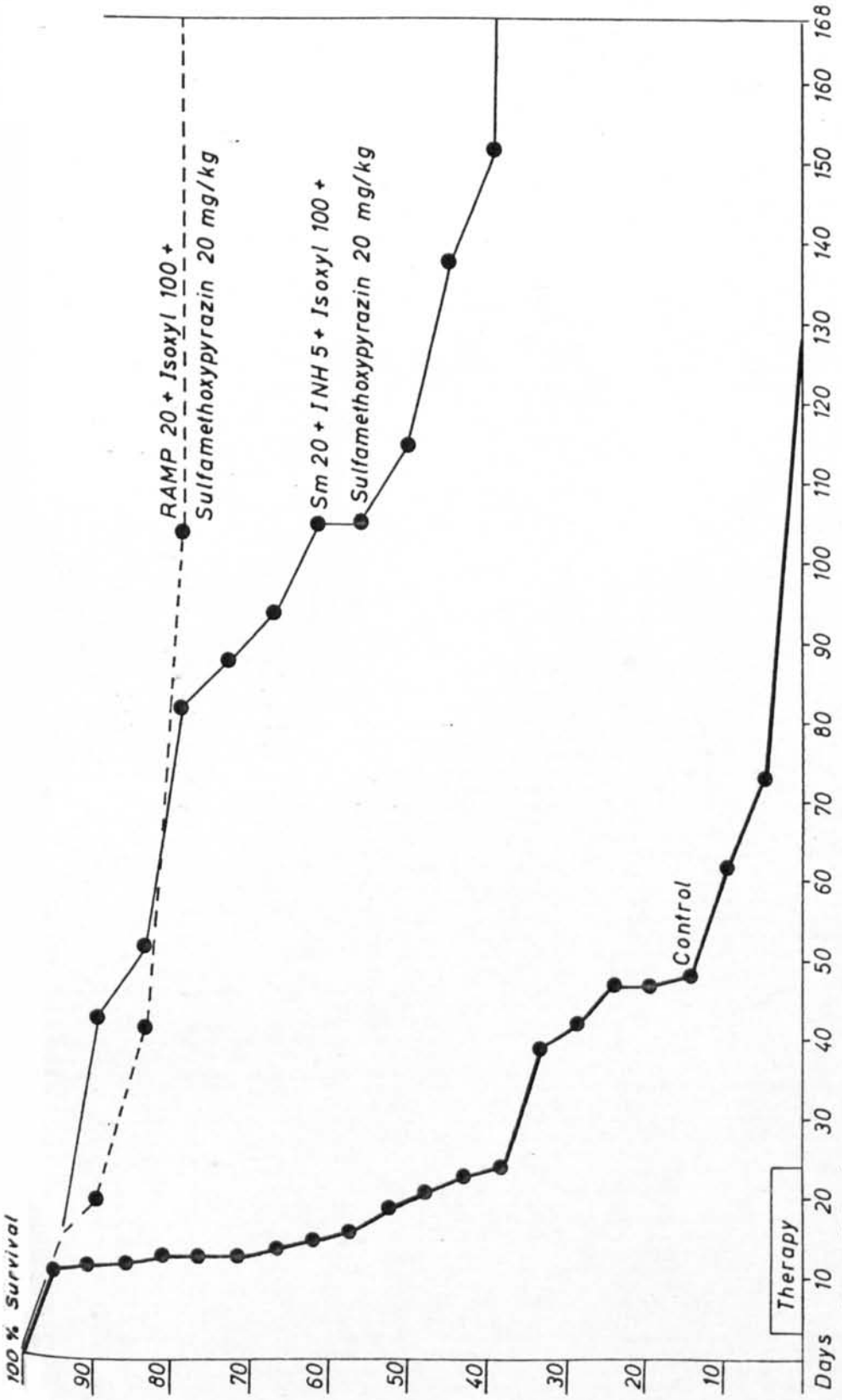

FIG. 1. Combination therapy with Rifampicin in mice infected with *M. kansasii* 11813 (this strain was isolated from a patient). Dose of infection: 5 mgm./ml., 0.025 ml./gm. mouse, administered intravenously. • = one animal died of infection.

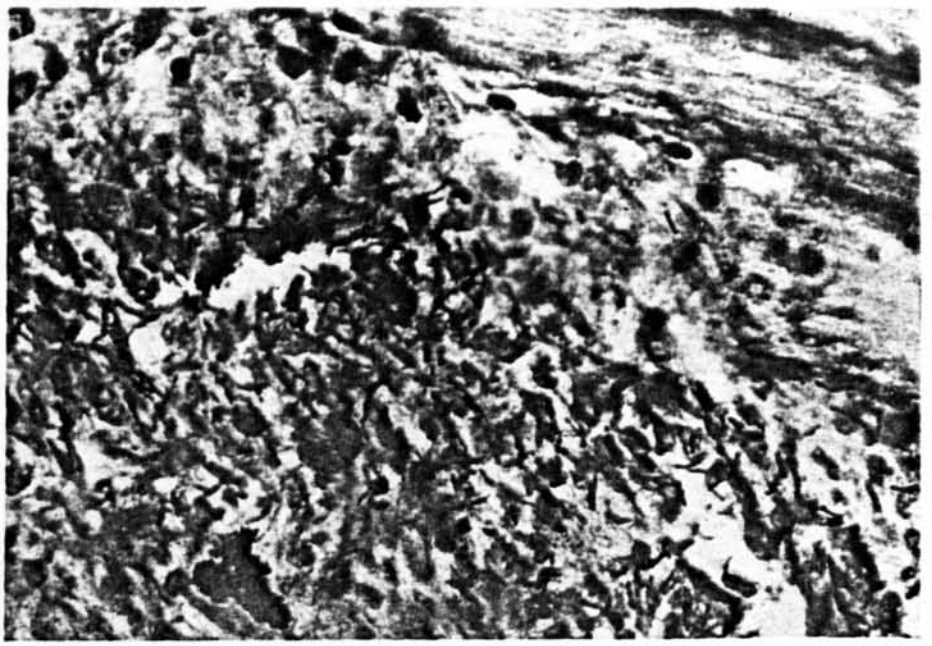

FIG. 2. Infection with *M. marinum* in mice. Skin lesions on tail. Ziehl-Neelsen staining of mycobacteria.

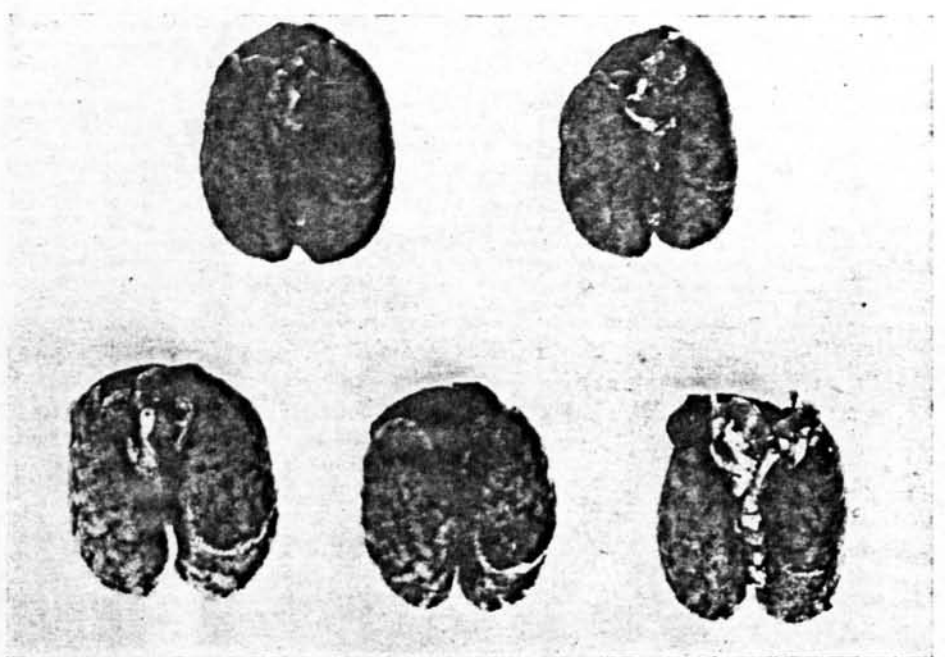

FIG. 3. Final lung processes after infection with *M. marinum*. Laboratory animal, mouse.

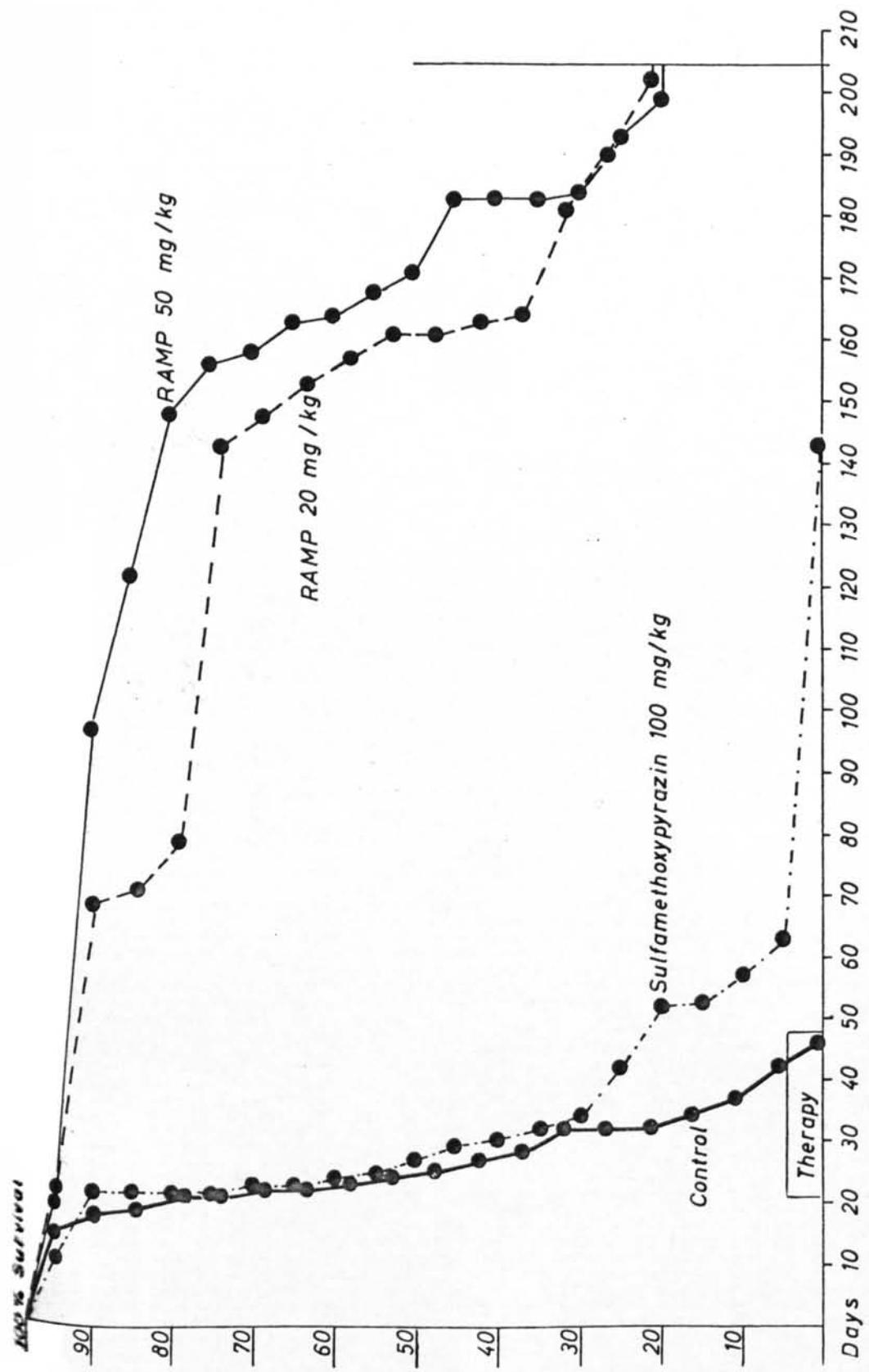

FIG. 4. Experimental infection with *M. marinum* 1254 isolated from human skin lesions. Dose of infection 2 mgm./ml., 0.025 ml./4gm. mouse. • = one animal died of infection.

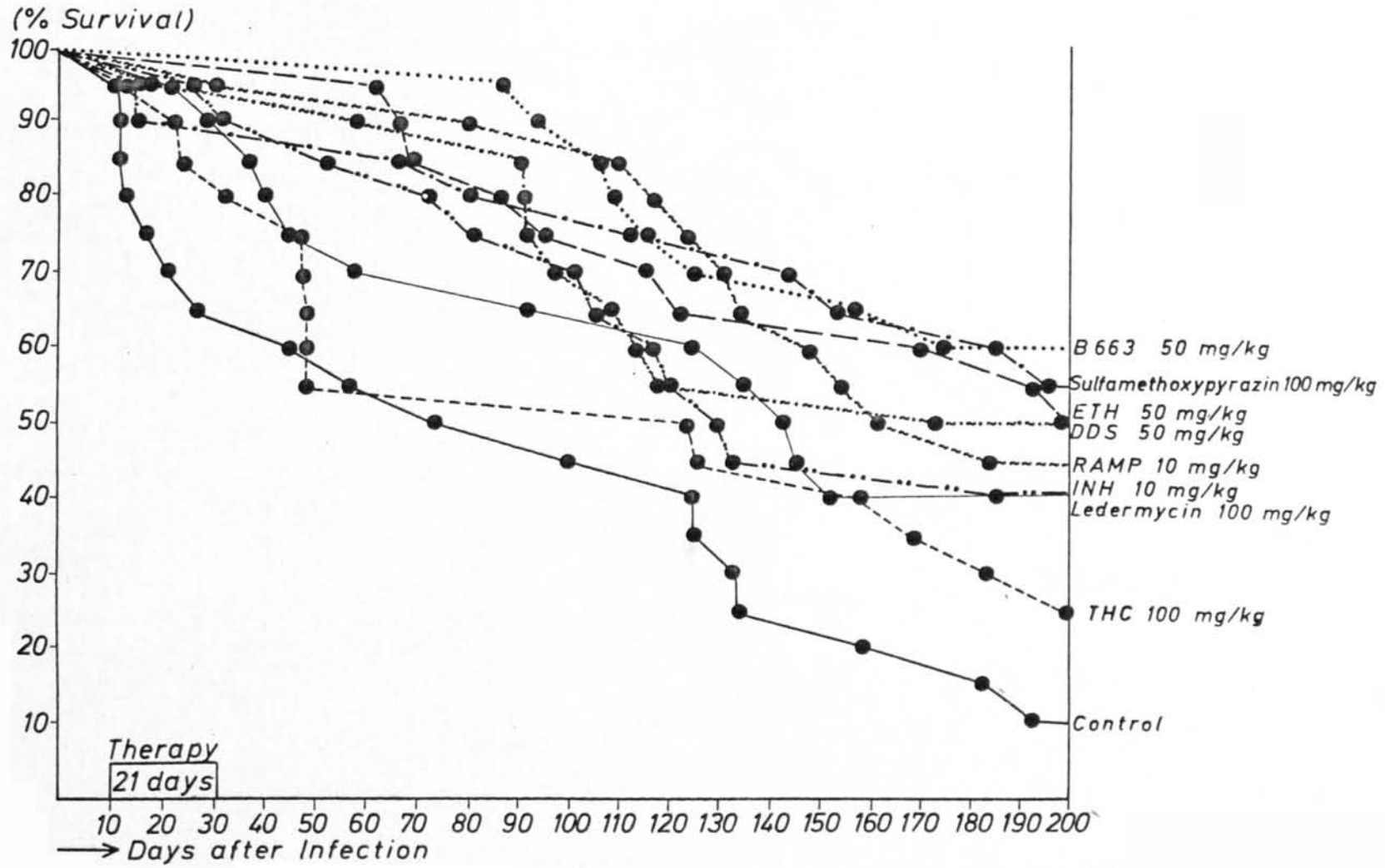

FIG. 5. Test conditions as in Fig. 4. Chronic course of disease. Several forms of monotherapy.

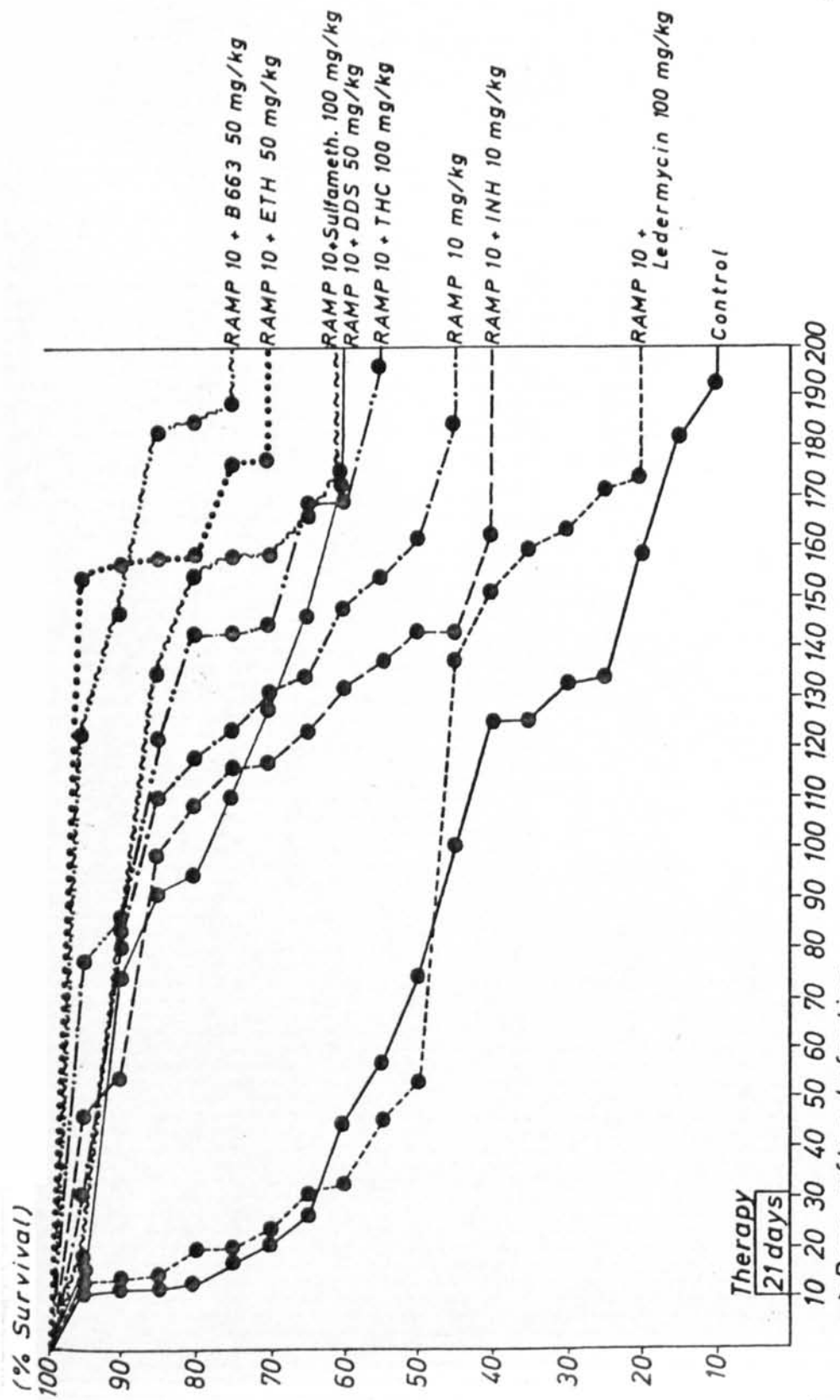

FIG. 6. Test conditions as in Fig. 5. Comparison of double combinations.

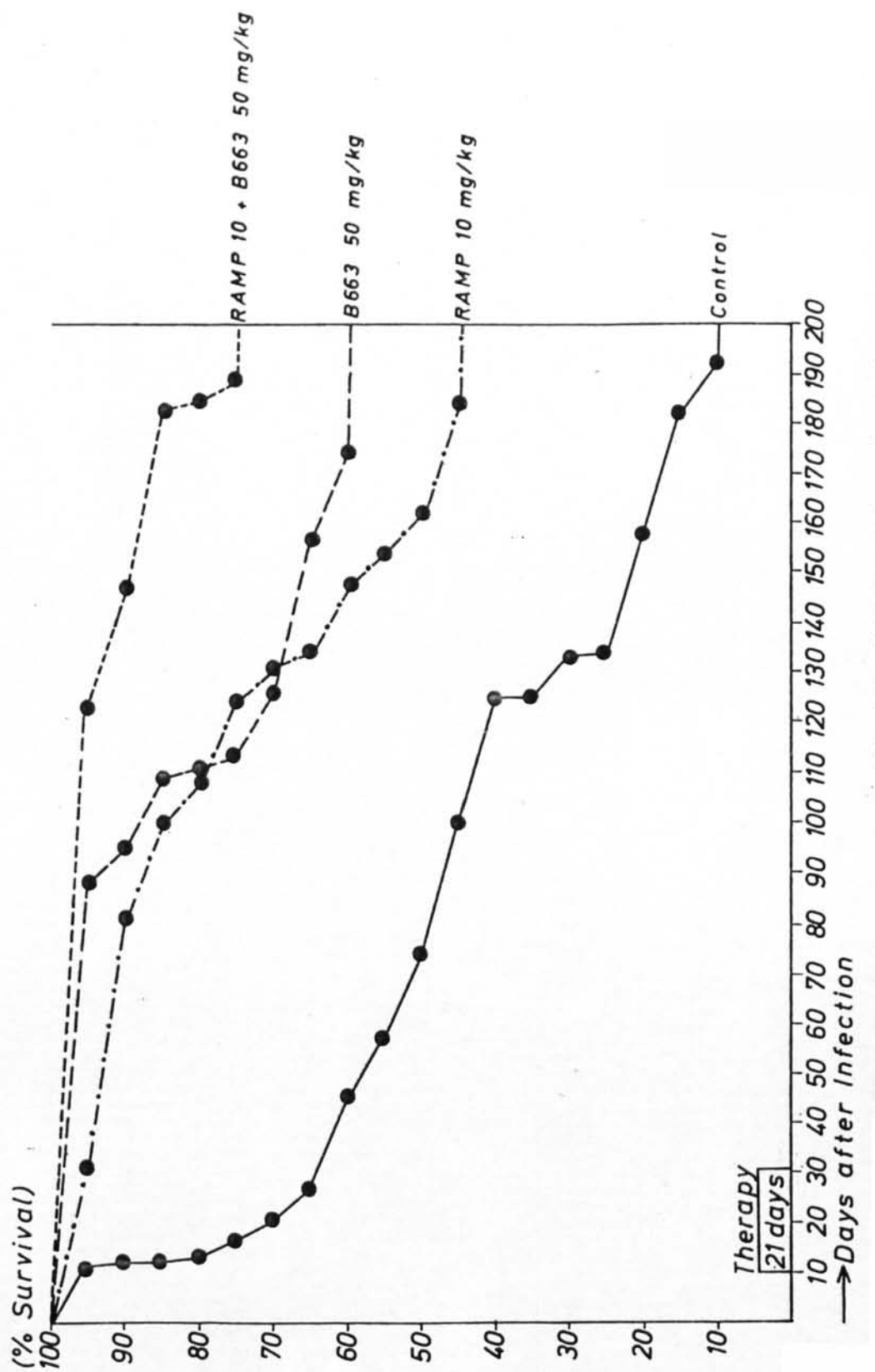

FIG. 7. Test conditions as in Fig. 5. Rifampicin and B.663 used alone and in combination.

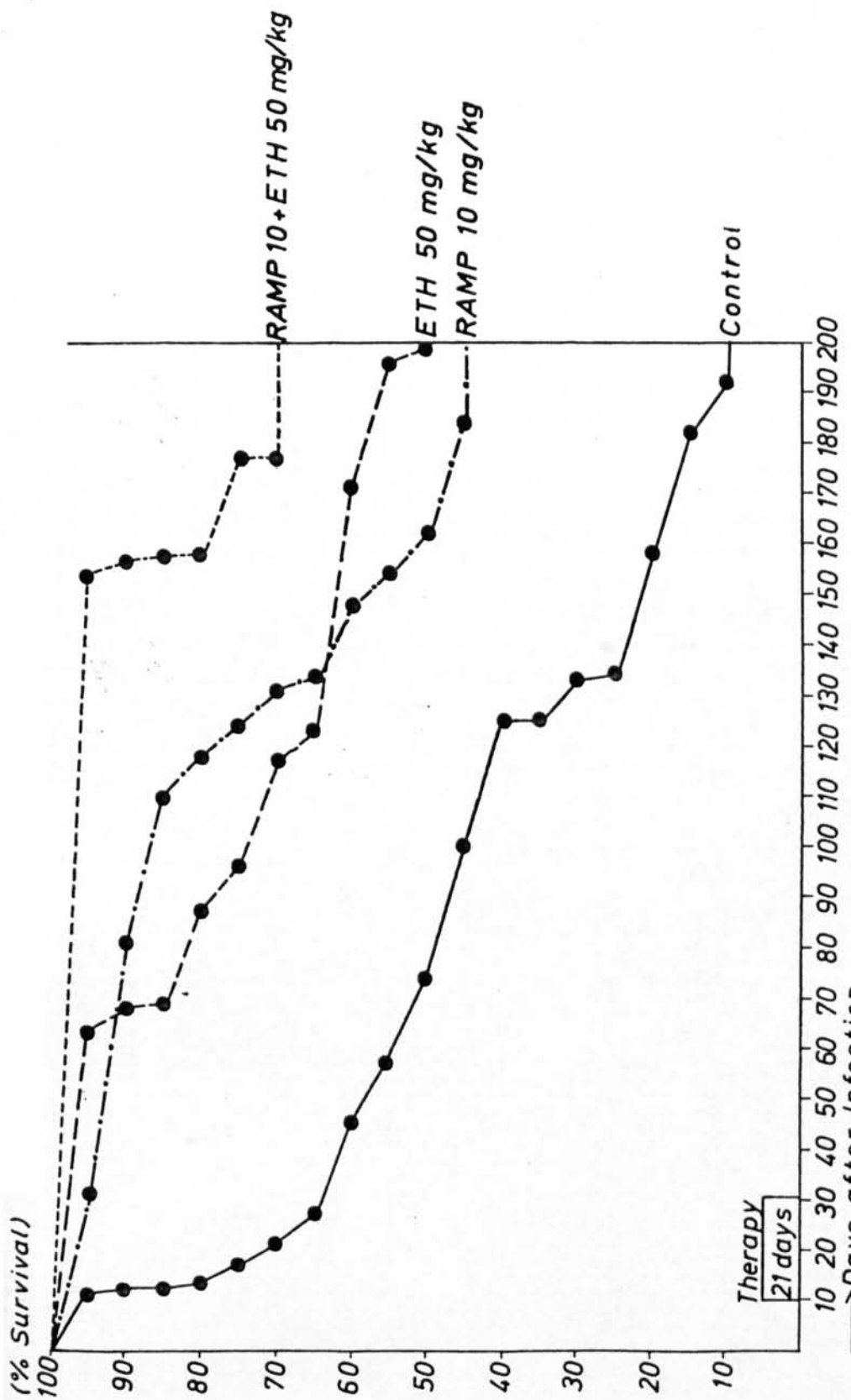

FIG. 8. Test conditions as in Fig. 5, Rifampicin and ethionamide used alone and in combination.

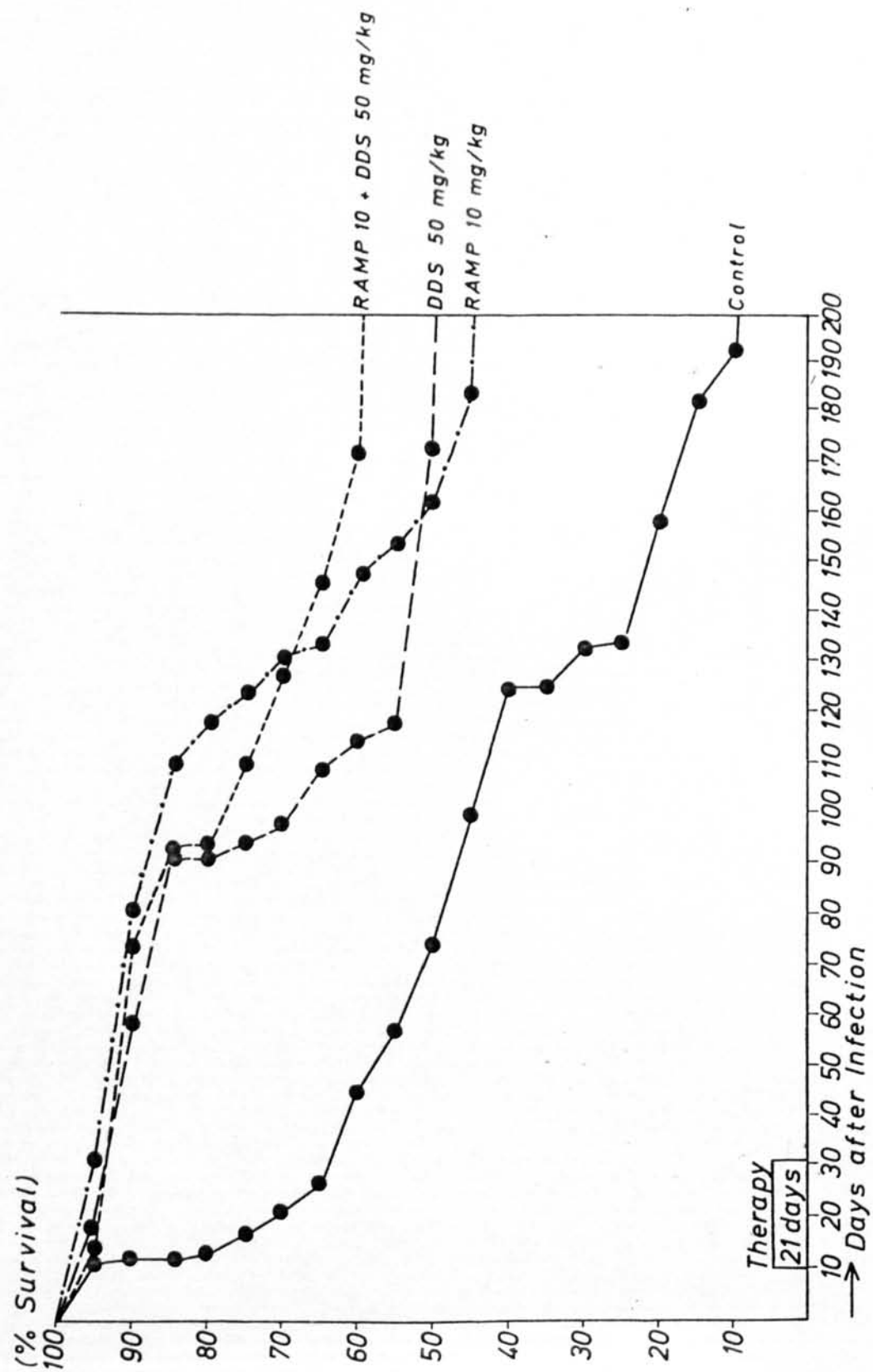

FIG. 9. Test conditions as in Fig. 5. Rifampicin and DDS used alone and in combination.

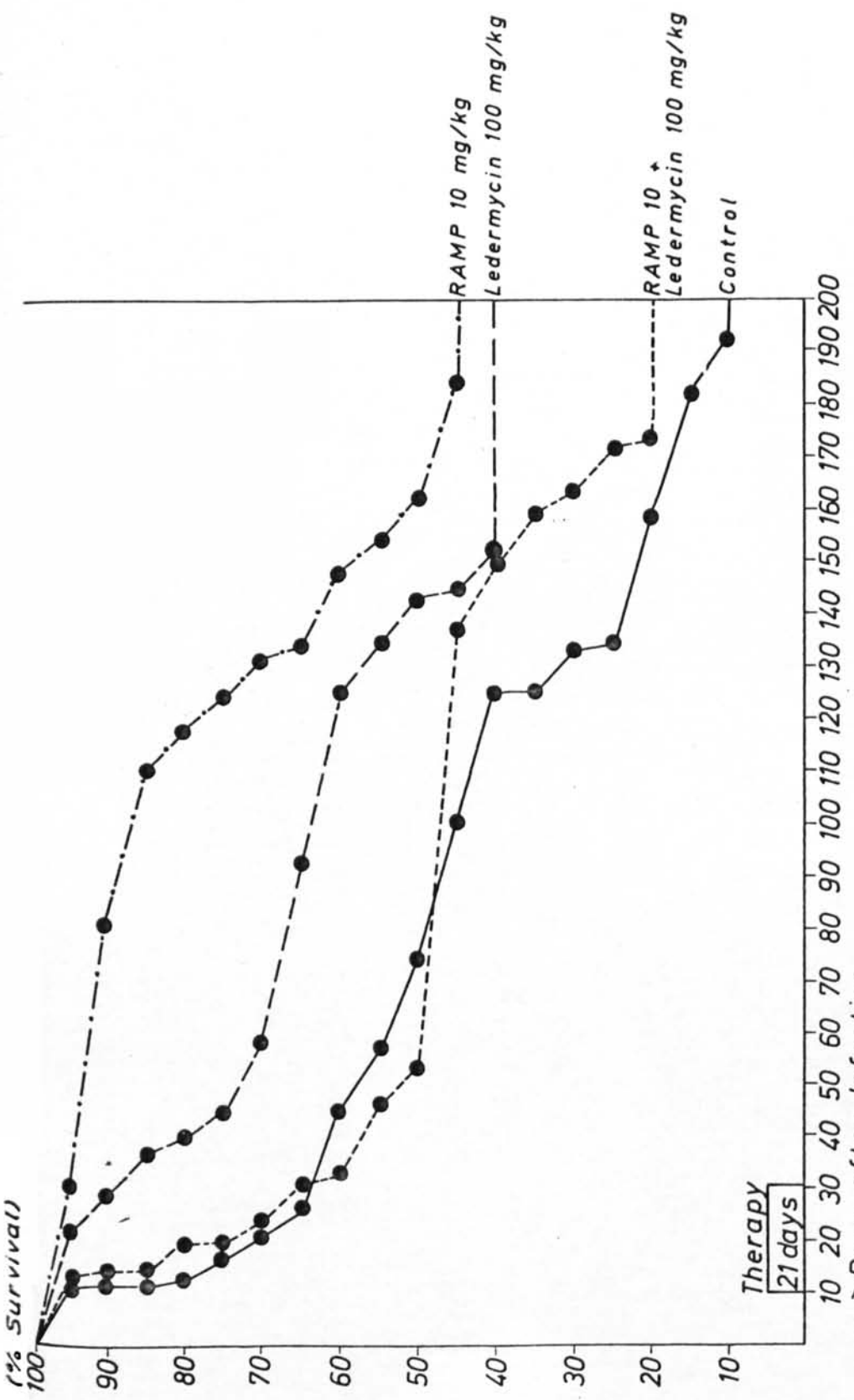

FIG. 10. Test conditions as in Fig. 5. Rifampicin and Ledermycin used alone and in combination.

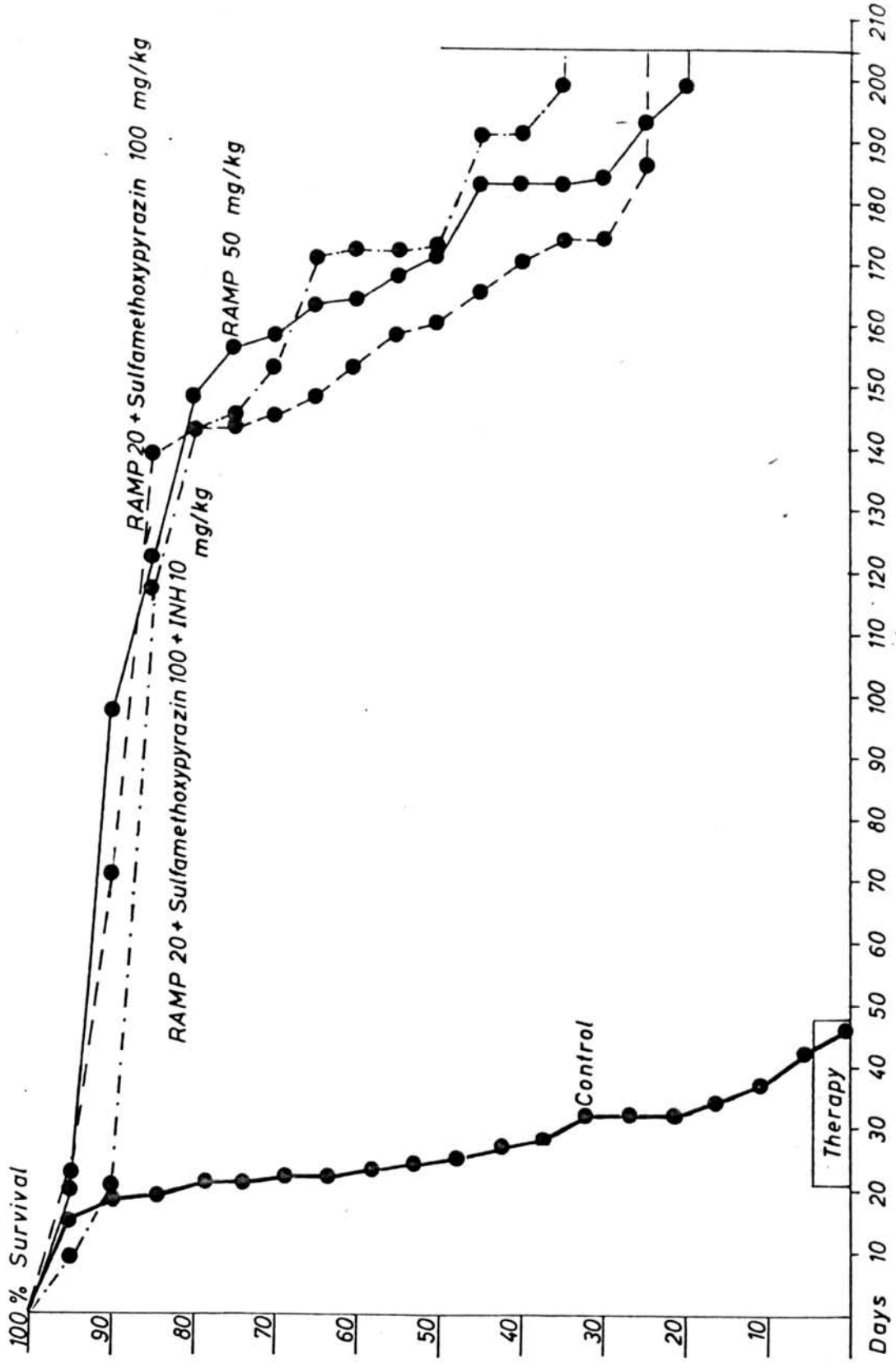

FIG. 11. Test conditions as in Fig. 4. Improvement of the twin-combination by addition of INH in comparison with high doses of Rifampicin (50 mgm./kgm.)

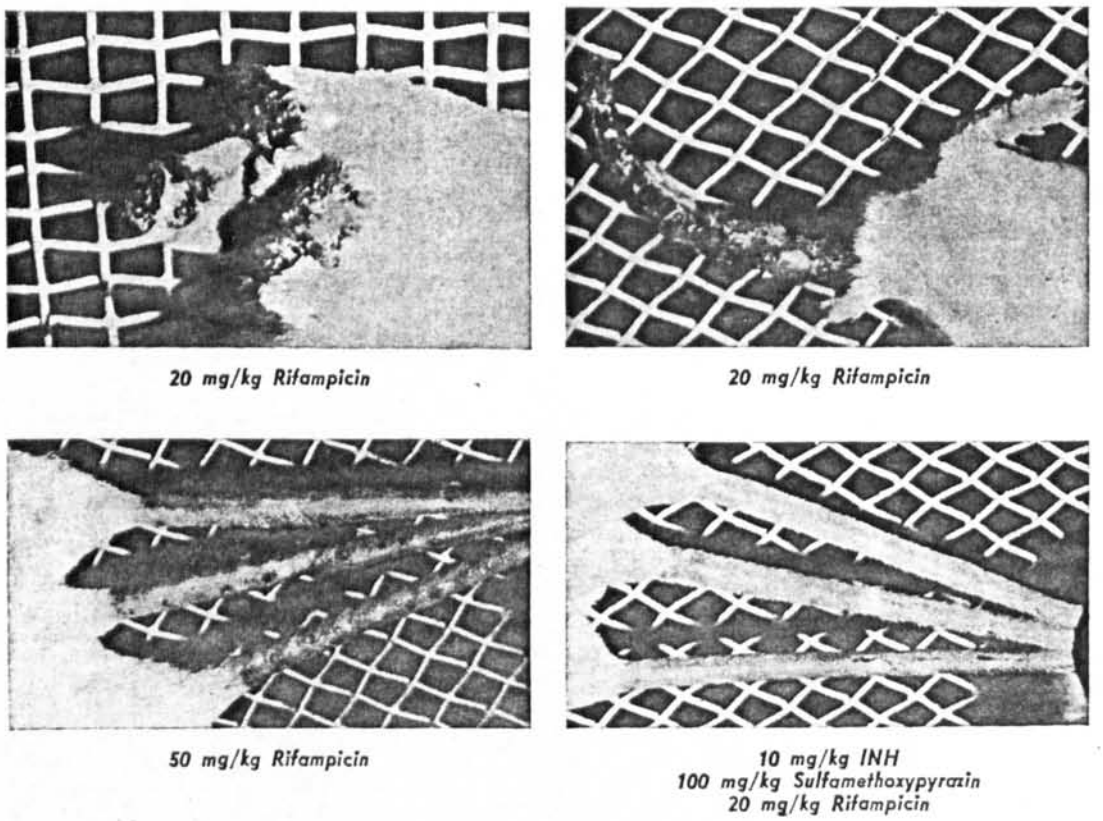

FIG. 12. Example showing effectivity of therapy on serious processes eight days after statement of therapy.

mgm./kgm. of sulfamethoxypyrazine; in this case the effectivity of this drug was again to be seen (Fig. 11). The combination of these three drugs is effective in both cases, i.e., the generalizing and the peripheral infection. Similar results were obtained by 50 mgm./kgm. of Rifampicin as monotherapy. The treatment of the serious peripheral processes by 20 mgm./kgm. of Rifampicin alone showed some failures, whereas the triple combination of Rifampicin, sulfamethoxypyrazine and INH, as well as the monotherapy by 50 mgm./kgm. of Rifampicin, produced a complete healing of the ulcerations in all animals (Fig. 12). The results gathered from monotherapy and twin combinations offer the possibility of several other and perhaps better triple combinations.

The fact that the drugs used for the treatment of leprosy, viz., DDS, B.663 and sulfonamides, are also effective against *M. marinum* infections and show promising effects in combination with Rifampicin, may be an indication for the use of such infections as analog models for leprosy itself.

As "atypical" mycobacteria are often less virulent, the building of experimental models with laboratory animals encounters many difficulties. Large numbers of mycobacteria are necessary as infection doses. This accelerates the course of the infections on the one hand and on the other brings about obstacles in analyzing the data. For this reason we developed a special kind of serum activity determination which enables us to compare the activity of several chemotherapeutic agents against different mycobacterial strains and species. This method has the advantage of being independent of the virulence of the mycobacteria for laboratory animals.

For this kind of experiment we show the serum activity (Fig. 13) after oral applica-

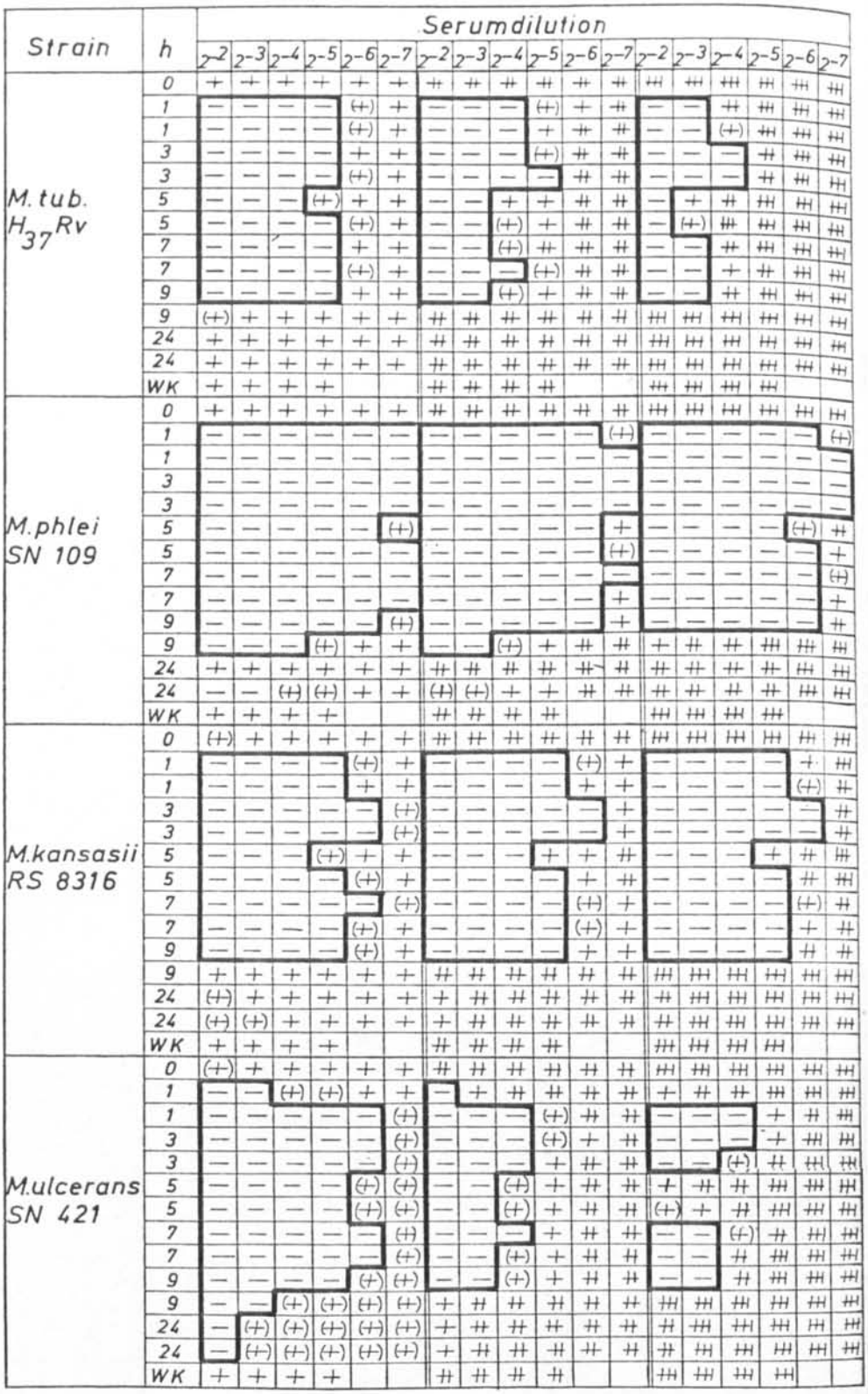

FIG. 13. Determination of serum activity in rabbits after oral application of 50 mgm./kgm. Rifampicin against mycobacterial

Strain	h	Serumdilution																	
		2^{-2}	2^{-3}	2^{-4}	2^{-5}	2^{-6}	2^{-7}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	2^{-6}	2^{-7}	2^{-2}	2^{-3}	2^{-4}	2^{-5}	2^{-6}	2^{-7}
M.marinum SN 1254	0	+	+	+	+	+	+	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++
	1	−	(+)	+	+	+	+	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++
	1	(+)	+	+	+	+	+	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++
	3	−	(+)	+	+	+	+	−	++	++	++	++	++	+++	+++	+++	+++	+++	+++
	3	−	−	+	+	+	+	−	+	++	++	++	++	+++	+++	+++	+++	+++	+++
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	9	−	(+)	+	+	+	+	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++
	24	+	+	+	+	+	+	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++
	24	(+)	+	+	+	+	+	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++
	WK	+	+	+				++	++	++				+++	+++	+++			
M.smegmatis SN 7	0	+	+	+	+	+	+	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++
	1	−	−	(+)	+	+	+	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++
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	24	−	(+)	(+)	+	+	+	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++
	WK	+	+	+	+			++	++	++	++			+++	+++	+++	+++		
M.avium 13839	0	+	+	+	+	+	+	++	++	++	++	++	++						
	1	(+)	(+)	(+)	(+)	(+)	(+)	++	++	++	++	++	++						
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	9	(+)	+	+	+	+	+	++	++	++	++	++	++						
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	24	+	+	+	+	+	+	++	++	++	++	++	++						
	WK	+	+	+	+			++	++	++	++								
M.fortuitum SN 203	0	+	+	+	+	+	+	+	++	++	++	++	++	+++	+++	+++	+++	+++	+++
	1	+	+	+	+	+	+	+	++	++	++	++	++	+++	+++	+++	+++	+++	+++
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	9	+	+	+	+	+	+	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++
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	24	+	+	+	+	+	+	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++
	24	+	+	+	+	+	+	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++
	WK	+	+	+	+			++	++	++	++			+++	+++	+++	+++		

strains. Medium: Lockemann. Serum dilution 2^{-6} and 2^{-7} with an addition of 4% serum. Inoculation: 6×10^{-4} mgm.

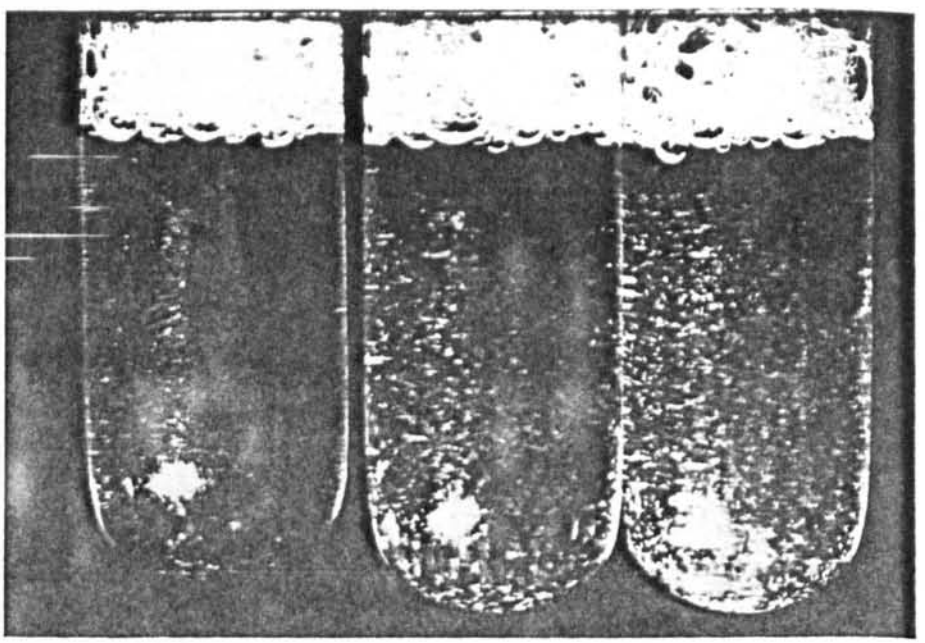

FIG. 14. Example of the growth control +, ++, +++.

tion of 50 mgm./kgm. of Rifampicin against inoculations with different mycobacteria. Blood samples were taken 1, 3, 5, 7, 9 and 24 hours after application of Rifampicin at every given point of time from two rabbits. A mixture of 1 ml. of the serum with 3 ml./culture-broth (Lockemann) was prepared, and by 1:2 steps diluted up to 1:128, i.e., 36 readings over one day. The test tubes were inoculated with 6×10^{-4} mgm. mycobacteria. The resulting growth of the mycobacteria was labelled as (+), +, ++ and +++; '—' means that no bacterial growth could be observed. These results were compared with the zero values of bacterial growth and control runs without drugs. The results of total inhibition are pointed out by a dark line. The inhibition may be unchanged after three readings, e.g., with *M. phlei*, or may be decreased when mycobacteria grow in numbers in the control (+, ++ and +++) tubes (Fig. 14), e.g., with *M. ulcerans*. The differences in the inhibition of several mycobacterial strains with varying sensitivity at the same serum concentrations are significant.

In order to check the usefulness of this method and to demonstrate the inhibition effect of different schemes Fig. 15 has been prepared. It shows the serum activity destination with rabbit serum according to the model in Fig. 13. Fifteen different strains of 10 species were tested under 10 therapeutic regimens. Lack of bacterial growth ('—') was analyzed and illustrated only by the height of the column, which is a direct representation of the inhibition grade. The time and the grade of serum activity are determined in the same manner. An arrow-shaped column represents activity going beyond the measured concentrations. The horizontal readings show the therapeutic effect of a regimen; vertical readings show the inhibitory effectivity of one medication against the strains tested. These data give us the following important results:

Rifampicin is effective at a dosage of 50 mgm./kgm. according to our test conditions (1:2 dilution, inoculation with 6×10^{-4} mgm. mycobacteria) against *M. tuberculosis, M. bovis, M. kansasii, M. ulcerans, M. intracellulare, M. phlei* SN 109, *M. marinum* and one strain of *M smegmatis* There are some extremely sensitive mycobacteria, as *M. bovis Vallée, M. phlei* SN 109 and, as earlier described (2), but not shown in this figure, some strains of *M. tuberculosis* and

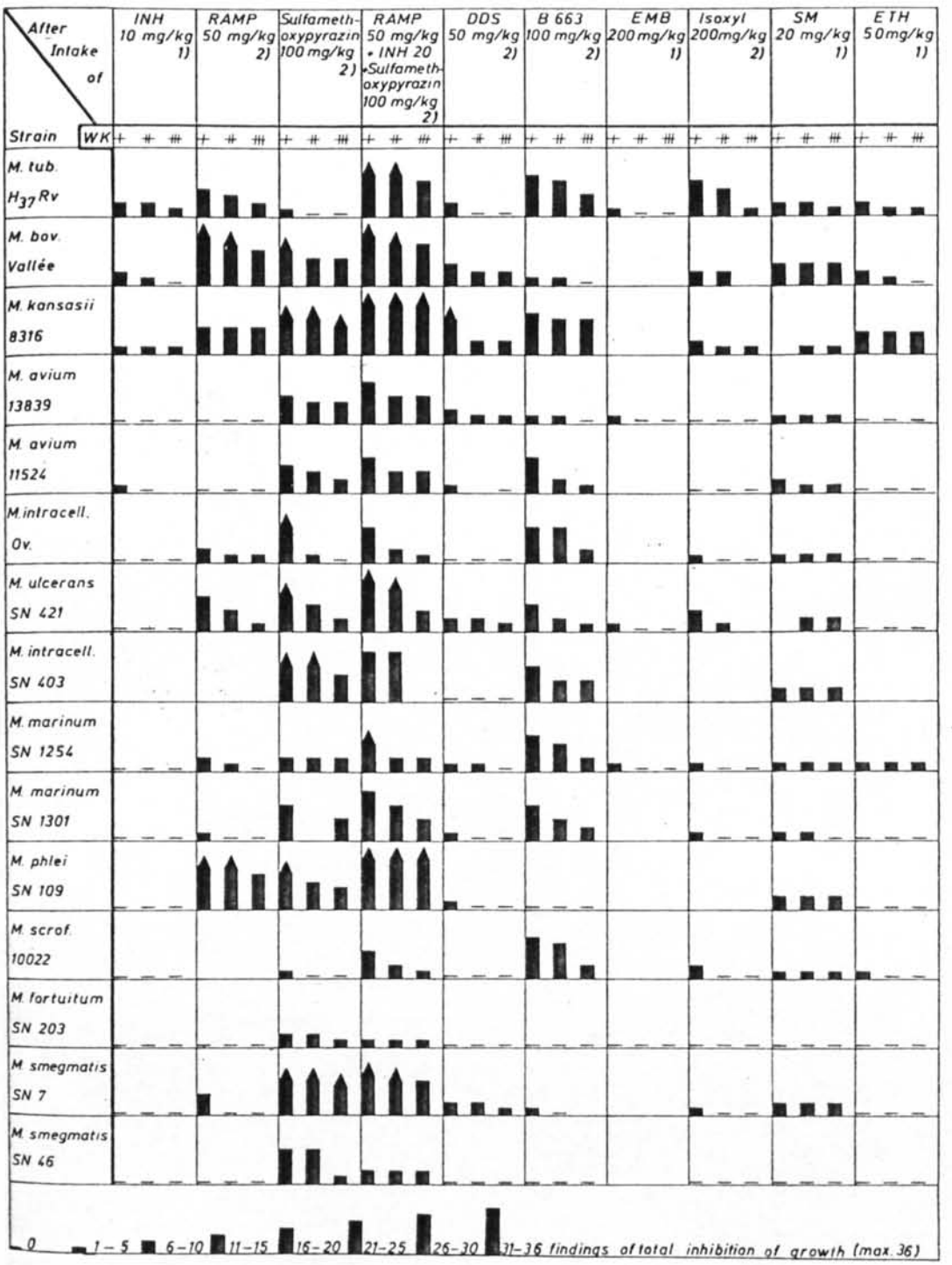

FIG. 15. Survey of serum activity determination in rabbits. Height and duration of the total inhibition labelled as column. 1) After single application. 2) After eight days' pretreatment.

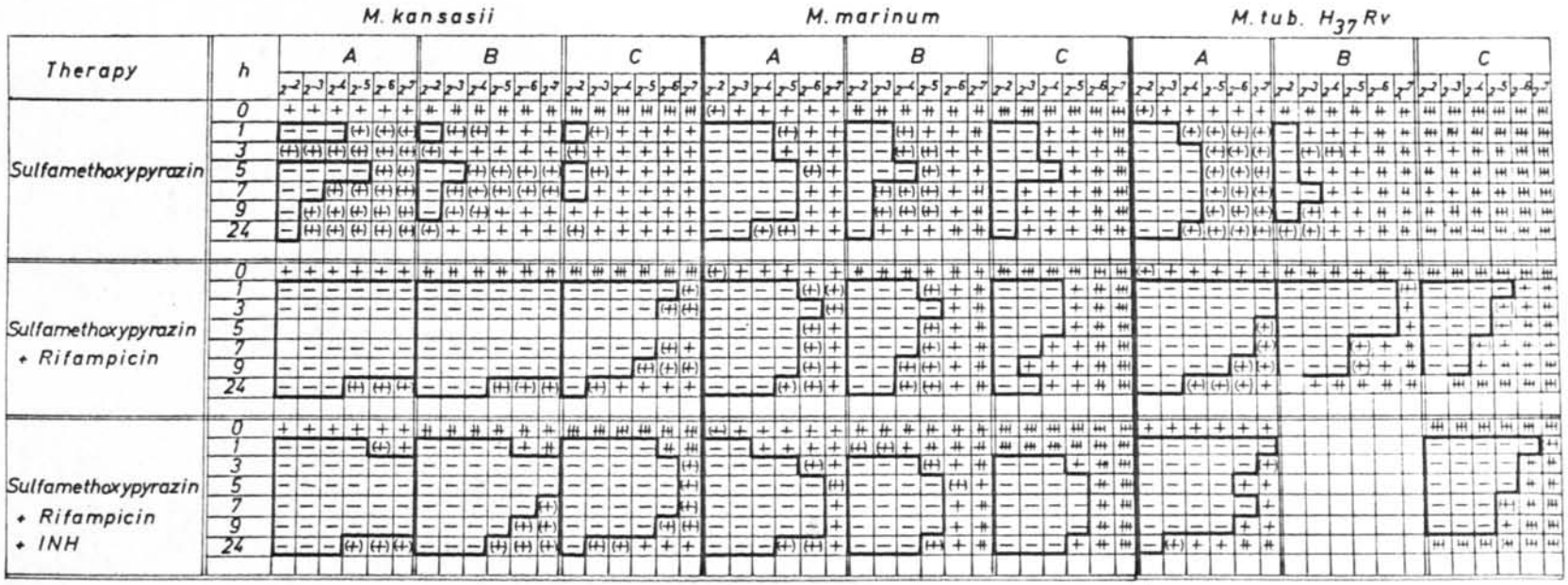

FIG. 16. Serum activity determination in healthy test persons.

Test person Schr. Sulfamethoxypyrazine 20 mgm/kgm. 1X
Test person K. Sulfamethoxypyrazine 20, Rifampicin 10 10 mgm/kgm. 1X
Test person R. Sulfamethoxypyrazine 20, Rifampicin 10, INH 5 mgm/kgm. 1X

Medium: Lockemann
Inoculation: 1 Tr. of 10^{-2}
Serum dilution: 2^{-6} and 2^{-7} with an addition of 4% serum

A = Reading WK +
B = Reading WK ++
C = Reading WK+++

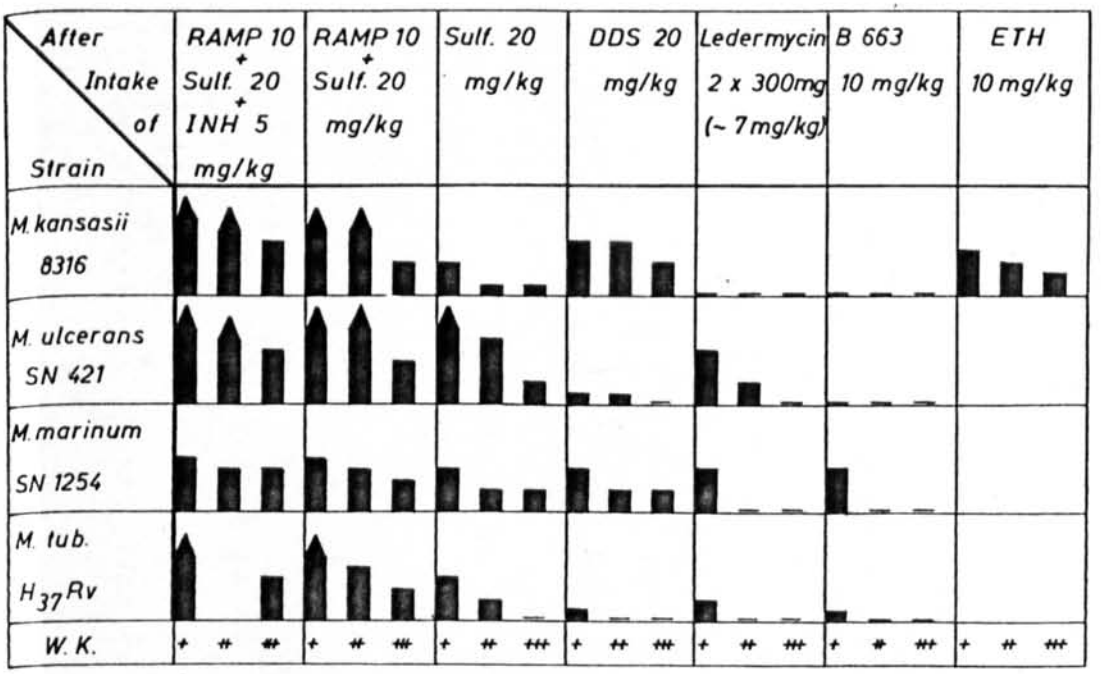

FIG. 17. Survey of serum activity determination in healthy test persons. Assessment as in Fig. 15.

M. intracellulare. These data are always in agreement with the sensitivity tests and are extremely valuable as a basis for the evaluation of therapeutic dosages. *M. tuberculosis* $H_{37}Rv$ and *M. kansasii* 8316 are very similar in their sensitivity. *M. marinum* is less sensitive than *M. tuberculosis* $H_{37}Rv$.

Sulfonamides might be a very important factor in the therapy of "atypical" mycobacterioses; e.g., sulfamethoxypyrazine had the broadest spectrum of activity among the here tested substances and even inhibits rapidly growing mycobacteria which up to now have been a serious problem for chemotherapy. Sulfamethoxypyrazine is more effective against several strains and remarkably better tolerated than DDS. Experiments for comparison of several sulfonamides will be continued.

Most significant is the increasing inhibitory effect by combining Rifampicin, sulfamethoxypyrazine and INH in a triple combination, the activity of which exceeds most frequently the mentioned measure range. Even if there are small or no inhibition effects after monotherapy we can observe an increase in therapeutic effectivity after combination treatment, e.g., against *M. avium* infections.

Streptomycin also has an inhibitory effect on most of the mycobacteria tested. It has important therapeutic value even in relatively low concentrations against infections with *M. kansasii* and *M. avium*, as shown earlier (1).

Isoniazid and ethionamide show only a small spectrum of activity. The effectivity of ethionamide is already evident in monotherapy against *M. kansasii* and *M. marinum* infections in mice. Our experimental data show that it is a good drug for combination therapy, too. INH can be used as third or fourth drug in several combinations.

Another drug, B.663, causes extremely high serum levels after application of a single dose of 100 mgm./kgm., and shows a very broad antibiotic spectrum. The high dose is not indicated for long term therapy in rabbits.

Isoxyl in a dose of 200 mgm./kgm. is active against strains of *M. tuberculosis*, *M. bovis*, *M. kansasii* and *M. ulcerans*, and, according to a control growth '+', even against *M. intracellulare*, *M. marinum*, *M. scrofulaceum* and some *M. smegmatis* strains. It is suitable, too, for combination therapy against infections with the above mentioned mycobacteria.

The test conditions of ethambutol show extraordinarily unsatisfactory results in rabbits.

The usefulness of these data, found in rabbits, for chemotherapy in man is dependent on whether the tolerated doses will cause a corresponding antibacterial activity. These problems can easily be solved by means of the above described method as being worked out for *M. marinum* and other mycobacteria (Fig. 16). From Fig. 17 we can gather that twin or triple combinations administered in tolerable doses reveal excellent results. It is also shown that the tested sulfonamide has a better or equal activity, as compared with DDS at the same dosage, against *M. marinum* and *M. ulcerans*. A well tolerated dose of sulfamethoxypyrazine is more valuable against *M. kansasii* than DDS, which on the one hand is very active, but in the concentrations used is already too toxic for therapy in man. Already a single dose of 10 mgm./kgm. of B.663 inhibits growth of *M. marinum* and *M. tuberculosis* $H_{37}Rv$ according to control growth '+'. This drug shows significant accumulation in the body so that a blood level control after long-term treatment is necessary; nevertheless, its serum activity in man is irrefutable, too.

As the sensitivity data are extremely variable, even for one kind of "atypical" mycobacteria—more variable than in the species *M. tuberculosis*—it is necessary to work out an individual therapy in man. The dosages and combinations can be easily evaluated by means of the above mentioned method, resulting in a specific treatment of "atypical" mycobacterioses. In the same way it is possible to cure infections caused by *M. avium*, only if a plan of therapy is formulated by the best combination of tolerated drugs under permanent control of the serum level (the same is due to poly-resistant tuberculoses). The preliminary advice of the regimen can easily be screened in rabbit experiments.

SUMMARY

1. By means of the method briefly described it is possible to evaluate drug combinations against almost any kind of mycobacteriosis.

2. By including serum of patients or healthy test persons we can adapt the experimentally gained results to conditions in man.

3. Although a large group of substances when used alone are effective against all mycobacterioses, it is, however, to be recommended that for the purpose of therapy they be employed in combinations of several drugs.

4. The combinations of chemotherapeutic agents found by this experimental screening allow successful therapy at dosages which are well tolerated by men.

5. The combination of Rifampicin, sulfamethoxypyrazine and INH proved to be therapeutically effective against most mycobacterioses. Screening for other combinations of therapeutic value will be continued.

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