In this paper, the third the authors have prepared on N-dodecyl-1,3-propanediamine, the chemical's value as a practical disinfectant against Mycobacterium tuberculosis is described, with tentative comments on toxicity and sensitization.

Tuberculosis Disinfection With Diamine

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THE CHEMICAL N-dodecyl-1,3-propanediamine (hereafter called "diamine") has shown high growth inhibiting properties for tubercle bacillus suspensions (*1*). Additional experiments were performed to test the use of diamine as a practical disinfectant against tuberculous sputum, and to inquire into its toxicity, skin irritating and sensitizing properties.

Methods

The solubility of diamine is high in ethyl or isopropyl alcohol but low in water. Triton X-100 (Rohm and Haas, polyethylene glycol alkylaryl ether) permitted concentrated diamine solutions in alcohol to remain clear on further dilution with distilled water, even when sodium hydroxide was present. Solution or suspension methods (diamine, Triton X-100 and sodium hydroxide by weight, isopropyl alcohol by volume) are given for each experiment.

Inclusion of small proportions of sodium hydroxide enhanced the antibacterial potency of diamine, as is clearly shown in this report. Diamine is alkaline but has a low buffer capacity, and sodium hydroxide probably aids its action by maintaining a more nearly optimal alkaline pH.

The concentrations of isopropyl alcohol and Triton X-100 that contacted tubercle bacilli and sputum contaminants were generally rather low, and alcohol was only used once in a final concentration above 10 percent. Concentrations of isopropyl alcohol alone as high as 10 percent and Triton X-100 alone as high as 2 percent, or both together in these respective strengths, showed no inhibition against tubercle bacilli or sputum contaminants in a whole sputum test.

Dried Sputum Film Test

Purulent sputum pools containing large numbers of tubercle bacilli were shaken with beads 3 hours at 37° C. to make fairly fluid and homogeneous mixtures (*2*). Films from 0.05 ml.

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of such sputum, spread over 2 x 3 cm. rectangles on cover slips, were dried in the dark, generally at room temperature. Stained films from different pools showed from approximately 5 to more than 100 single acid-fast rods or small clumps per oil immersion field.

For the test, usually run in duplicate, a film was dropped into 40 ml. of diamine, held for the desired time at 20° C., quickly rinsed in 40 ml. of water to remove excess disinfectant or in a like volume of sodium ricinoleate to further neutralize some disinfectant (*3*), and then dropped into 40 ml. of water or weak sodium hydroxide containing glass beads. Hard shaking broke the cover slip and dispersed the film. This film-containing liquid was cultured on 3 slants, generally Loewenstein-Jensen medium, in 0.1 ml. volumes. Control films contacted water instead of diamine and were finally shaken in weak sodium hydroxide, before culturing, to inhibit the growth of contaminants. A maximum 0.25 percent of a film or 0.000125 ml. of original sputum was inoculated on each slant, which generally produced over 50 colonies in every control culture. Unfortunately diamine often dissolved rather large fractions of the films, so that culture inoculums tended to be below the stated maximum.

Guinea pigs were sometimes inoculated subcutaneously in parallel with cultures using volumes as large as 5 ml. or a maximum 12.5 percent of a film.

Whole Sputum Test

A measured volume of diamine (double the desired final concentration) was added to an equal volume of sputum, thoroughly shaken and held at 20° C. or room temperature for 30 minutes with intermittent agitation. It was then quickly diluted 100 times in water or weak sodium hydroxide and inoculated in 0.1 ml. volumes on culture slants. Guinea pigs were sometimes also given much larger subcutaneous inoculums. The final diluent sometimes contained small amounts of potassium ricinoleate which somewhat checked the action of diamine but had no demonstrable effect on the growth of tubercle bacilli. Control sputum plus water was finally diluted in 1 percent sodium hydroxide to control contaminants.

The advantages of this test were:

1. A known amount of sputum went completely through the test.
2. It was easy to run.
3. Four times as much original sputum (0.0005 ml.) was planted on each slant as the maximum culture inoculum (0.000125 ml.) in the dried sputum film test.

The disadvantage of the whole sputum test, apparently not a major one with diamine (*3*), was that 1 percent of the test dilution of disinfectant went through to the final culture.

Diamine on Concrete or Glass Surfaces

Two techniques were employed on concrete or glass surfaces. In one, 3 x 4 cm. rectangles on concrete or glass were contaminated with sputum which was allowed to dry. They were later covered with varying diamine dilutions. In the other, diamine was applied and allowed to dry and sputum was later placed over the same areas and allowed to dry. Care was always taken to keep the sputum boundaries within the diamine areas. These techniques were performed at room temperature. Sputum only was placed on control areas.

For the test a sterile swab was dipped into water or weak sodium hydroxide, rubbed over a rectangle and rinsed in the original fluid. This was repeated several times and cultures were then inoculated from the rinse fluid. Guinea pigs were also injected in one experiment. Control areas were swabbed with and rinsed in weak sodium hydroxide.

Results

A number of experiments with the dried sputum film technique pointed in the same direction. Two such tests are summarized. In the first test a 1 percent diamine stock solution in 50 percent isopropyl alcohol without sodium hydroxide was diluted in distilled water to 1:2,500 and 1:5,000 diamine concentrations. Films contacted these dilutions for 30 minutes. When films were shaken in 0.5 percent sodium hydroxide as the final diluent before culturing, 1:2,500 diamine caused complete growth inhibition and 1:5,000 produced marked but incomplete inhibition. When water was used as the final diluent, however, the 1:2,500 diamine con-

centration caused only partial inhibition of growth. The approximate mean colony count per tube in the control cultures was 300.

In the second test a stock solution containing 1 percent diamine, 1 percent sodium hydroxide, and 4 percent Triton X-100 in 60 percent isopropyl alcohol was diluted in distilled water to 1:1,000 and 1:5,000 diamine concentrations. Films were immersed for 10 minutes and were finally shaken in water before culturing. Growth inhibition was complete at the 1:1,000 dilution and two guinea pigs were negative for tuberculosis. At the 1:5,000 dilution growth inhibition was marked but incomplete and both guinea pigs were positive for tuberculosis. The approximate mean colony count per tube in the control cultures was 100.

These experiments illustrate the range in which diamine has caused complete inhibition by the dried sputum film technique, when weak sodium hydroxide was present either in the original diamine formulation or in the final diluting fluid. In the first experiment diamine proved definitely less effective when tested in the absence of sodium hydroxide.

Two representative experiments illustrating the whole sputum test technique are summarized. A tuberculous sputum pool, which yielded over 1,000 colonies per control slant, was used. Exposure to diamine was 30 minutes at 20° C.

In the first test diamine was diluted in water or in 0.1 percent sodium hydroxide from a 1 percent diamine, 10 percent isopropyl alcohol stock solution, and mixed with equal volumes of undiluted sputum. The final diluent before inoculating cultures and guinea pigs was water without sodium hydroxide but containing 1:6,000 potassium ricinoleate. Control sputum plus water contacted 1 percent sodium hydroxide in the final diluent to control contaminants before culturing.

A 1:625 final dilution of diamine without sodium hydroxide caused a moderate but not complete inhibition of *Mycobacterium tuberculosis*, and two guinea pigs were positive. However, it prevented all growth of contaminants. A 1:1,250 dilution also completely inhibited the contaminants but had no effect on the tubercle bacilli. A 1:2,500 diamine dilution no longer affected the contaminants.

When diamine was diluted in weak sodium hydroxide, it completely inhibited tubercle bacilli and contaminants at a 1:1,250 final dilution with two negative guinea pigs. At 1:2,500 it markedly but not completely inhibited *M. tuberculosis* and two guinea pigs were positive, but it still completely inhibited contaminants. Contaminants were no longer affected at 1:5,000.

In the second experiment (see table) final dilutions of 1:2, 1:10, and 1:50 of the same highly positive sputum contacted diamine which was diluted from a 2 percent diamine, 95 percent isopropyl alcohol stock solution throughout the test ranges with distilled water only. Before culturing, one portion of each diamine-sputum mixture was diluted 100 times in water containing 0.05 percent potassium ricinoleate. Cultures were inoculated 45 seconds later and duplicate cultures were made after the other portion of each mixture had been diluted in 0.05 percent potassium ricinoleate containing 1 percent sodium hydroxide. This technique in a whole sputum test minimizes the action of sodium hydroxide, save for its effect on contaminants. The sputum-water controls were cultured 45 seconds after dilution in 1 percent sodium hydroxide.

In the table the growth inhibiting capacity of

Whole sputum test with diamine (N-dodecyl-1, 3-propanediamine) using 3 sputum dilutions

Final sputum dilutions in test	Highest final diamine dilutions causing complete growth inhibition of			
	Mycobacterium tuberculosis—diluent before culturing contained		Sputum contaminants—diluent before culturing contained	
	No NaOH	1 percent NaOH	No NaOH	1 percent NaOH
1:2	[1] 1:250	1:500	1:1, 000	[2] 1:2, 000
1:10	1:1, 000	1:1, 000	1:4, 000	[2] 1:32, 000
1:50	1:4, 000	1:4, 000	1:16, 000	[2] 1:64, 000

[1] This final diamine dilution contained just under 20 percent by volume isopropyl alcohol which undoubtedly reenforced the 1:250 diamine action. The alcohol concentration in the other diamine dilutions is considered inconsequential.

[2] Not run at higher dilutions.

diamine against sputum is shown to be a function of the amount of sputum contacted by the germicide. This is a rigorous test considering the high content of organic matter and tubercle bacilli in this sputum. There were over 200 colonies per control tube for sputum diluted 50 times. Under practical conditions contamination with the quantity of tubercle bacilli represented by even the 1:50 dilution would be the exception.

The brief contact of the 1:2 and 1:10 sputum controls with 1 percent sodium hydroxide almost, but not completely, checked the growth of non-acid-fast organisms in cultures from these specimens (not shown in the table). The same contact for all diamine-treated sputums caused complete decontamination, which indicates that even the most dilute diamine employed had some effect on these extraneous organisms. This test again shows that diamine works more effectively against contaminants than against *M. tuberculosis* in sputum.

The effect of diamine on concrete surfaces was evaluated in three experiments. Fairly rough and absorbent concrete stepping stones were used. The pH of concrete, determined by swabbing areas with water and rinsing the swabs in the same water several times, approximated 9.0 in a number of tests, and these surfaces were also shown to have some buffer capacity. This should theoretically make concrete a favorable surface for decontamination by diamine.

The effect of sodium hydroxide was definitely exaggerated in the first two experiments, which is considered permissible because one can well use sodium hydroxide with diamine when disinfecting a concrete floor.

In the first experiment 0.2 ml. volumes of sputum were applied to the rectangles. Considerable sputum soaked into the concrete and drying was rapid. After the sputum had dried, 2 ml. of a turbid aqueous diamine suspension was flooded slowly over each rectangle during a 3-minute period. The diamine was spread evenly with a swab as it was applied. Much of it was absorbed by the concrete and the swab removed most of the excess. After 20 minutes a new swab was dipped into 40 ml. of 0.25 percent sodium hydroxide, the rectangle was swabbed, and the swab rinsed in the sodium hydroxide. This was performed 5 times and the resultant mixture of diamine, sputum, and sodium hydroxide was left at room temperature until all areas had been treated and swabbed, then 6 cultures were inoculated from each mixture. Control areas were flooded with water instead of diamine and likewise swabbed into sodium hydroxide.

The test was run on duplicate concrete surfaces, and the cultures of control areas developed over 300 colonies per tube. The highest dilution of diamine that caused complete growth inhibition was 1:5,000 for one surface and 1:2,500 for the other. In the latter instance 1:5,000 diamine produced almost complete inhibition.

In the second experiment 1:500 and 1:5,000 aqueous diamine dilutions in 1.5 ml. volumes were slowly flooded on concrete rectangles and allowed to dry. Two hours later 0.05 ml. amounts of positive sputum were spread over the treated and control areas and were left overnight at room temperature. The test was completed on the next day as in the first experiment.

Control colonies approximated 50 per tube, but no growth appeared in cultures from the 1:500 and 1:5,000 diamine-treated rectangles. This experiment also contained tests with 1:500 diamine made up in half saturated urea and further diluted to 1:5,000 with water, and 1:500 diamine in 2 percent Clorox which was likewise further diluted. Urea favors diamine solution and, as dried urea is slightly deliquescent, it was thought that this mixture might be more effective than aqueous diamine only. This was not proved although diamine in urea also caused complete inhibition at 1:500 and 1:5,000 concentrations. The diamine-Clorox mixtures were incompatible and separated. Even the preparation of 1:500 diamine and 2 percent Clorox failed to cause complete inhibition of growth.

The last experiment was planned to test the residual action of diamine that had been dried for varying periods on concrete and glass surfaces and to minimize the effect of sodium hydroxide. Diamine was diluted in water, from a clear stock solution of 2 percent diamine in 99 percent isopropyl alcohol and 3 percent Triton X-100 to final concentrations of 1:300, 1:1,000, and 1:3,000. Rectangles on glass and concrete were flooded with 0.25 ml. to 0.4 ml. of these

dilutions. The concrete rapidly absorbed most of the diamine before drying, but diamine concentrated on the glass surfaces by evaporation. The surfaces were stored in the dark at room temperature for 11 days, then like areas were prepared and allowed to dry for 4 hours. At this time sputum containing tremendous numbers of tubercle bacilli was diluted 10 times in water and placed in 0.05 ml. volumes on each treated area and on untreated control rectangles. The surfaces were stored overnight in the dark. Half of the treated surfaces were then swabbed 3 times and rinsed in 10 ml. of water and immediately inoculated on 3 cultures, while the other treated areas went through 0.5 percent sodium hydroxide before culturing. Some guinea pigs were inoculated from the concrete surface mixtures at the time that cultures were made. Half the control areas were treated with 0.5 percent and the remainder with 1 percent sodium hydroxide.

Control cultures from both glass and concrete developed over 200 colonies of *M. tuberculosis* per slant and many of these cultures from concrete also showed non-acid-fast contaminants.

None of the glass surfaces, including control areas, yielded growth of non-acid-fast contaminants. Cultures from glass areas on which sputum had been placed 4 hours after diamine were also all negative for *M. tuberculosis* through the 1:3,000, or highest diamine dilution.

When diamine had remained on glass for 11 days before sputum was added and the areas were swabbed and diluted with sodium hydroxide, growth of tubercle bacilli was completely inhibited by the 1:300 and 1:1,000 and almost completely inhibited by the 1:3,000 diamine dilutions. When water was used for final swabbing and dilution, complete growth inhibition was produced by only the 1:300 concentration of diamine. Dilutions of 1:1,000 and 1:3,000 caused marked and moderate degrees of inhibition, respectively.

Results of this test on concrete were disappointing. Probably insufficient volumes of diamine were applied, which mostly soaked into the concrete and was no longer available to contact the sputum.

Diamine on concrete in 1:300 concentration for 4 hours completely inhibited growth on cultures and 4 guinea pigs were negative for tuberculosis. The 1:1,000 strength caused marked though not complete inhibition when swabbed and rinsed with sodium hydroxide but only moderate inhibition when treated with water.

Diamine on concrete for 11 days gave only a moderate degree of inhibition under both methods of final swabbing and dilution at 1:300, and 2 guinea pigs were positive for tuberculosis. No evidence of activity was apparent for the 1:1,000 concentration.

Toxic and Sensitizing Properties

A few preliminary tests on toxicity, skin irritability, and sensitizing properties of diamine were performed.

Intravenous injection of diamine into mice caused rapid death in a dose of 150 mg./kg., but 30 and 60 mg./kg. were tolerated and the mice appeared healthy 3 days later. Diamine was rather irritating by intraperitoneal injection, in doses as low as 40 mg./kg., into mice and killed all animals tested within 24 hours.

Two guinea pigs received 1 to 1.5 ml. of 1:300 diamine by stomach tube, lost weight for several days, and died. Another pig given a like volume of 1:1,000 diamine survived and gained weight for 2 months.

Diamine was not found to be unduly irritating to the skin in dilutions that might be used for practical disinfection. One volunteer rinsed his hands with 1 to 0.2 percent diamine many times for more than a year and has often allowed it to dry without any effect other than a slight tingling sensation. A 10 percent solution, allowed to dry on the forearm, produced a mild inflammatory reaction followed by slight desquamation.

Diamine is definitely irritating on intracutaneous injection. This was shown in two guinea pigs which, however, failed to develop measurable skin sensitivity to diamine under the following conditions. They first received 0.05 ml. intradermal diamine doses in 1:100, 1:300, 1:1,000, 1:3,000, and 1:10,000 dilutions. The 1:3,000 strength caused a minimal necrosis and this dose was repeated 3 times weekly until 9 such injections had been given. After a 19-day rest another 1:3,000 dose caused reac-

tions indistinguishable from previous responses to this concentration (*4*).

Diamine Compared With Phenolic Disinfectants

Sputum film tests have been run in this laboratory on several phenolic disinfectants (*5*). Exposure of films to disinfectants was 30 minutes, and the final diluent contained 0.25 percent sodium hydroxide. The highest dilutions of three representative phenolic preparations that caused complete growth suppression were:

Liquor Cresolis Saponatus U.S.P. 1:200.
Lysol (Lehn & Fink Products Corp.) 1:200.
Amphyl (Lehn & Fink Products Corp.) 1:400.

As shown in the present report, 1:2,500 diamine produced complete growth inhibition under the same conditions excepting that the final diluent contained 0.5 percent sodium hydroxide.

Conclusions

Under natural conditions of contamination and infection, *Mycobacterium tuberculosis* is always accompanied by exudate. N-dodecyl-1, 3-propanediamine (diamine) is shown to be an efficient disinfectant of tuberculous exudate. This is especially true when diamine is incorporated in weak sodium hydroxide. The evidence was developed by rigorous methods in which cultural results have been supported by animal infection tests.

Diamine has an even greater growth inhibiting power for sputum contaminants and also, from unreported experiments, for heavy concentrations of *Escherichia coli* and *Micrococcus pyogenes* var. *aureus*. Hence, it can be considered a growth inhibiting agent with a wide antibacterial action although its effectiveness against spore formers is not known.

The dried film and moist sputum testing procedures imitated the two varieties of contamination likely to occur. Each method has advantages and disadvantages. That the results using either technique were not far apart makes it possible to obtain the advantages of both. Thus, washing diamine from the film did not reduce its effectiveness, and the somewhat uncertain findings for film disinfection by diamine plus sodium hydroxide were corroborated in the surer moist sputum technique.

The experiments with the use of diamine on contaminated surfaces, limited to concrete and glass, suggest that similar results might be expected for other smooth, inert, or alkaline surfaces.

It seems reasonable to employ the sodium hydroxide-containing preparation at a diamine dilution of 1:1,000 for heavily contaminated articles such as specimen bottles and emesis basins. For less heavily contaminated areas such as floors in the immediate vicinity of patients, bed frames, bedside tables, and laboratory surfaces, a dilution of 1:2,500 is suggested. A dilution of 1:5,000 would seem adequate for surfaces possibly, but not probably, contaminated, such as sanatorium and hospital floors and counters in general. A minimum contact period of 30 minutes is recommended.

If applications to fixed surfaces are not rinsed but allowed to dry in situ, an additional advantage should be gained. A smooth, inert, or alkaline surface mopped twice weekly should maintain a considerable disinfecting potential, especially if soap is not employed.

Sodium hydroxide should be incorporated with diamine unless the resultant alkalinity is contraindicated, in which case strengths from 1:250 to 1:1,000 are recommended.

Diamine is not recommended for the routine disinfection of sputum. The intimate mixing required for a sure effect would be entirely impractical. For extreme accidental contamination, such as the spilled content of a specimen bottle, the area should be flooded with concentrated diamine solution (1:200 approximately) containing sodium hydroxide, the disinfectant and the contaminating material should be mixed well and the entire area covered with newspapers or similar material for at least an hour.

Summary

The disinfectant action of N-dodecyl-1,3-propanediamine (diamine) against highly positive tuberculous sputum was evaluated in several ways. Its activity, particularly in the presence of sodium hydroxide, was high.

Diamine has a broad spectrum antibacterial

action. It inhibits the growth of sputum contaminants more effectively than that of *Mycobacterium tuberculosis*. Its activity against bacterial spores, however, is not known.

Diamine does not appear to be an undue skin irritant. While no sensitizing ability was found, this possibility should be kept in mind. It appears promising as a surface disinfectant for tuberculosis hygiene and probably for more general sanitation also.

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Record Number Rehabilitated

A record number of 66,273 handicapped persons were restored to productive employment through the State-Federal vocational rehabilitation program during the fiscal year ending June 30, 1956.

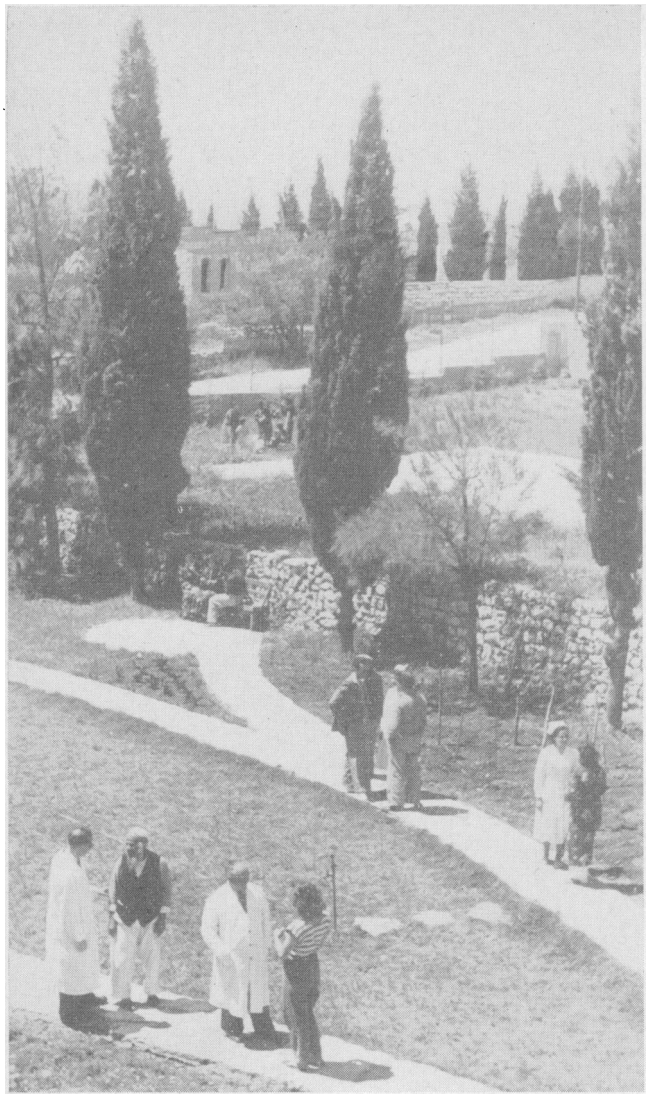
This was the highest total since the start of the public rehabilitation program in 1921 and was about 14 percent above the 57,981 rehabilitated in fiscal 1955.

The record figure includes 65,640 persons restored to employment directly through the State-Federal vocational rehabilitation program. The other 633 persons were placed in jobs through specific projects such as rehabilitation centers and shelter workshops developed jointly by community organizations and State rehabilitation agencies and financed, in part, by Federal grants.

Of the total rehabilitated last year, approximately 3,500 entered such "shortage" professional fields as education, medicine, and engineering. About 8,200 are in skilled trades, and approximately 6,200 work on farms. Most of the others are in managerial, sales, and service jobs or in unskilled work.

Roughly 13,000 of those rehabilitated had been dependent on public assistance immediately prior to or during the rehabilitation process and had been receiving approximately $11.1 million a year in aid payments. The total cost of rehabilitating these people was $9.2 million.

More than 48,000 of the rehabilitants were unemployed at the time they began receiving rehabilitation help, and most of the others were in unsafe, part-time, or otherwise unsuitable jobs or were in danger of losing their jobs because of disability.



Public Health Nurses in Israel

Hadassah medical organization nurses shown in some of their activities. *Above:* A "home call" to a roving Bedouin family is made to arrange for delivery of an expected child at the modern Beersheba hospital. *Left:* Patients at Safad's restored tuberculosis hospital find the garden a pleasant place for nurse consultation. *Below:* At Kiryat Yovel, a nurse joins the queue of families awaiting inoculations at this project for family-centered treatment.

Photographs: Hazel Greenwald