TABLE IV.
Effect of Rate of Growth of *S. aureus* FDA and of Temperature on the Activity of Penicillin (1.5 units per ml) in Rabbit Serum.

| Incubation of cultures at given temp. (in hrs) | Viable organisms per ml in thousands Temperature of Incubation | | | | | |
|---|---|---|---|---|---|---|
| | 37°C | | 39°C | | 42°C | |
| | Control | Penicillin present | Control | Penicillin present | Control | Penicillin present |
| 0 | 120 | 120 | 120 | 120 | 120 | 120 |
| 2 | 850 | 600 | 2700 | 550 | 800 | 120 |
| 4 | 11000 | 30 | 17000 | 10 | 46000 | 5.0 |

the death of *S. aureus* FDA and a strain of *S. agalactiæ*. At temperatures higher than that leading to maximum growth of the organism, penicillin kills *S. aureus* FDA faster than it does at the temperature of maximum growth. At the temperatures slightly higher than optimum, there still exists an interrelation between growth and the killing of bacteria by penicillin. It is believed that only at excessively high concentration, or very high temperature, does penicillin kill organisms while they are not undergoing growth.

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## Use of Beta Beta' Dithiocyano Diethyl Ether (RID-O) to Control Mite Infestations in Mice.*

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When it was noticed that part of a colony of 3,000 mice used for cancer research were losing hair, they were examined for mites. The skin on the abdomen was scraped with a small sharp scalpel on which a drop of glycerine had been placed. The scrapings were then put in a drop of glycerine on a glass slide and examined with a microscope. Glycerine was used because mites remain active in this medium for many hours. The species of Acarina (mites) found was identified as *Myobia musculii*.

Since rotenone and derris root powder were not available, an attempt was made to kill the mites with DDT.† The results were disastrous because the DDT powder was much more toxic for mice than it was for mites. Some papers on the toxicity of DDT for mice, rats, cats, etc., appeared about this time and confirmed the observation that it is toxic for mice.[1,2,3] Animals that lick their fur and thus ingest agents applied externally, cannot be treated with DDT. In addition to the toxicity, DDT was of no value in this case because it did not appear to kill the mites. This lack of lethal effect of DDT on mites may be related to the fact that mites are not insects, but arachnids.

The next insecticide tried was a liquid

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† A small sample of DDT was very kindly supplied by the Research Department of the School of Pharmacy of the University of Maryland.

1 Lillie, R. D., and Smith, M. I., *Public Health Rep.*, Washington, D.C., 1944, **59**, 979.

2 Nelson, A. A., Draize, J. H., Woodard, G., Fitzhugh, O. G., Smith, R. B., Jr., and Calvery, H. O., *Ibid.*, 1944, **59**, 1009.

3 Woodard, G., Nelson, A. A., and Calvery, H. O., *J. Pharm. and Exp. Therap.*, 1944, **82**, 152.

(TOXITE) designed for the treatment of red mites and insects infesting poultry and other animals. The label states that this is a mixture of xylenols, para cresol, meta cresol, mineral oil, and hydroxytoluenes. This was sprayed on the mice, and while it seemed to kill the mites with which it came in contact, it also killed some of the mice. About this time, cubé root, a substitute for derris root, was obtained. This supposedly contained 5.2% rotenone and it was assumed that it would be no more toxic than derris root. This proved to be an erroneous assumption, because when 300 mice were dusted with this material, 50 of them died during the next 3 days.

Finally, after all these unfortunate experiences with rodenticidal insecticides, a material was found that is extremely lethal for mites and relatively non-toxic for mice. The purpose of this paper is to describe its use and discuss its toxicity in the hope that this information will be of value to others who use mice. This may also prove to be applicable to other animals, but it has not been extensively tested. It has been used on rabbits, guinea pigs, and dogs without noticeable toxicity. A dog was effectively cleared of fleas.

The material found to be effective in controlling mites is a recently developed commercial roach, ant, and bedbug powder called RID-O‡ which contains as the active ingredient 7% beta beta' dithiocyano diethyl ether. Before application to the mice, this product was diluted with 1 to 1½ times its weight of powdered talc. This decreased the tendency to form clumps, and, therefore, the powder was more easily blown into the fur. The mice, the wood shavings, and the container in which the mice were kept were all dusted. The blower or dusting device was so designed that it was not necessary to remove the mice from the glass bowls or cages for the treatment. Large numbers of mice could, therefore, be treated in a relatively short time.

The dusting device essential for the successful application of this powder mixture was constructed from a bottle with a two-hole rubber stopper and several pieces of rubber and glass tubing.§ This was attached to an air jet which was adjusted so that the stream of air which carried the powder was just sufficient to ruffle the fur of the mice. If excess amounts of powder accumulated on the mice, this was partially blown off by a stream of compressed air which circumvented the powder bottle. It was desirable and almost necessary to carry this operation out in a chemical hood for the powder, when inhaled, was found to be irritating to the mucous membranes.

This system has many advantages over usual methods of treating mice with powders. When mice were individually dipped in a powder bowl, the eggs, which were not killed, came off into the powder. Mite-free mice subsequently treated, picked up some of these eggs and thus became infested with mites. With the method described in this paper, such spreading of mites or their eggs is not possible.

When mite-infested mice were treated with RID-O as described above, the mites died in less than 2 minutes, or before they could be scraped off and examined under a microscope. A comparative study of the lethal effect of DDT and RID-O on mites was therefore made. Two sets of mites from the same animal were placed on glass slides. One set was treated with RID-O and examined immediately. The mites stopped moving in about one minute after application. The other set of mites was given a heavy treatment of 10% DDT in talc. These DDT-treated mites floundered about in this powder for several hours. DDT may eventually be lethal for mites, but even this is doubtful as living mites can usually be isolated from mice treated with this substance. In contrast to this, living mites have not been isolated from mice treated with RID-O.

The one unsatisfactory feature of the RID-O treatment is that it does not kill the eggs or larvæ. It is, therefore, necessary to repeat the treatment at appropriate intervals if the mites are to be eliminated from the colony. That RID-O is practically non-toxic was

‡ We are indebted to the Leeds Chemical Co., 509 Pratt St., Baltimore 1, for a generous supply of RID-O.

§ Space limitations prevent a more detailed description which will be supplied by the author on request.

TABLE I.
Toxicity of Insecticides Applied to Mice. Twenty Mice in Each Group.

| Group | Treatment | | No. of mice dying during intervals indicated (weeks) 1 | 3 | 5 | 7* | 9 | 11 | % dead | 15 | 19 | 23 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | RID-O | daily | 0 | 0 | 0 | 0 | 0 | 0 | 0 | Discontinued. | | |
| 2 | '' | biweekly | 0 | 0 | 0 | 1 | 0 | 0 | 5 | Mice used for RID-O | | |
| 3 | '' | weekly | 1 | 0 | 0 | 0 | 0 | 1 | 10 | feeding test. | | |
| 4 | '' | monthly | 0 | 0 | 0 | 1 | 0 | 0 | 5 | See Table II. | | |
| 5 | TOXITE | daily | 1 | 2 | 1 | 1 | 0 | 0 | 25 | 0 | 0 | 0 |
| 6 | '' | biweekly | 0 | 0 | 0 | 1 | 4 | 0 | 25 | 1 | 1 | 0 |
| 7 | '' | weekly | 0 | 0 | 0 | 3 | 0 | 0 | 15 | 0 | 0 | 0 |
| 8 | '' | monthly | 0 | 1 | 1 | 0 | 0 | 0 | 10 | 0 | 0 | 0 |
| 9 | DDT | daily | 0 | 1 | 1 | 4 | 2 | 0 | 40 | 0 | 0 | 0 |
| 10 | '' | biweekly | 0 | 1 | 0 | 0 | 2 | 1 | 20 | 1 | 0 | 0 |
| 11 | '' | weekly | 0 | 1 | 1 | 0 | 0 | 0 | 10 | 0 | 2 | 1 |
| 12 | '' | monthly | 0 | 1 | 0 | 0 | 0 | 0 | 5 | 0 | 3 | 2 |

* Treatment stopped at end of 8 weeks.

TABLE II.
Toxicity of RID-O When Mixed with the Diet.

| Group | No. of mice | Fed RID-O | Amt* per 3 g diet | Deaths during intervals indicated—in weeks 1 | 2 | 3 | 4 | 6 |
|---|---|---|---|---|---|---|---|---|
| 1 A | 10 | 3 wks | 1 mg | 1 | 1 | 1 | 0 | 0 |
| B | 10 | 1 day | 1 | 0 | 0 | 0 | 0 | 0 |
| 2 A | 8 | 3 wks | 10 | 1 | 2 | 0 | 0 | 0 |
| B | 10 | 1 day | 10 | 0 | 0 | 0 | 0 | 1 |
| 3 A | 8 | 3 wks | 5 | 0 | 0 | 0 | 0 | 0 |
| B | 10 | 1 day | 5 | 3 | 0 | 0 | 0 | 0 |
| 4 A | 10 | 3 wks | 25 | 1 | 3 | 0 | 0 | 0 |
| B | 9 | 1 day | 25 | 0 | 0 | 0 | 0 | 0 |

* The amounts in this column refer to amounts of active ingredients in the RID-O.

established in several experiments. In one of these, the relative toxicity of DDT, TOXITE, and RID-O was tested by treating one group of 60 mice (20 in each set) with these agents daily except Sundays, for a period of 8 weeks. In 3 other groups, the mice were treated biweekly, weekly, and monthly, respectively.

The results are shown in Table I. In the mice treated daily with RID-O for 8 weeks, none of the 20 mice died. Five (20%) of the mice treated daily with TOXITE died, while 8 (40%) of the mice treated daily with DDT died. In the groups of mice treated less frequently, the deaths due to TOXITE and DDT were not so numerous.

At the end of 11 weeks, the animals that had been dusted with RID-O were fed diets containing various amounts of RID-O. In general, each of the former groups was divided in 2: one was fed RID-O for 3 weeks; the other was fed the RID-O diet for only one day. In Groups 2 and 4, it may be seen in Table II, there was some mortality. The RID-O diets in these cases contained 10 mg and 25 mg of beta beta' dithiocyano diethyl ether per 3 g of diet. These concentrations were so high that the animals refused to eat and some of these deaths were attributed to starvation rather than to the toxicity of the substance. The results of this experiment were very inconsistent as seen by Table II. There is some indication that a tolerance to the drug can be built up and that the natural resistance of the animals varies considerably.

*Summary.* Beta beta' dithiocyano diethyl ether (RID-O) has been found to be very effective in controlling mites and exhibits a relatively low toxicity in mice. This chemical may be found useful as an insecticide or an acaricide on other laboratory and domestic animals.