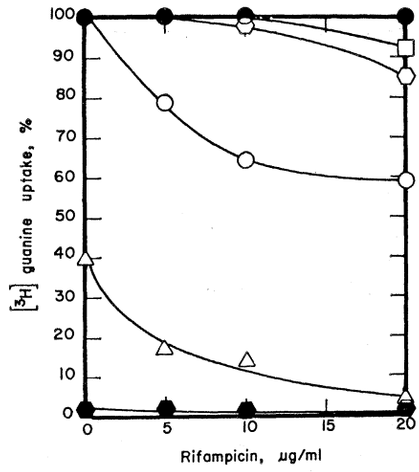

Fig. 2. Dose response to rifampicin of [^{3}H]guanine incorporation in the presence or absence of amphotericin B. Symbols: ●, no amphotericin B; □, 0.005 μg of amphotericin B per milliliter; ○, 0.01 μg of amphotericin B per milliliter; ○, 0.02 μg of amphotericin B per milliliter; △, 0.04 μg of amphotericin B per milliliter; ●, 0.16 μg of amphotericin B per milliliter.

its yeast-like phase. For this reason, our studies on three clinical isolates (*2*) were done on organisms converted to the yeast-like phase and subsequently maintained in this unicellular condition at 37°C on 2 percent glucose, 1 percent yeast extract, 1.5 percent agar (*3*). For these studies a 7-day-old yeast phase culture was transferred to Salvin's broth in sufficient quantity to yield 2×10^5 to 4×10^5 cells per milliliter of stock suspension which was then incubated at 37°C with constant agitation for 24 hours (*4*). After a prolonged lag phase, incorporation of [^{3}H]guanine was linear for 24 to 48 hours, and growth studies revealed the cells to be budding and in logarithmic growth. The effects of drugs on incorporation of [^{3}H]guanine and on viability of cells in colony counts were measured on replicate logarithmically growing cultures. The minimum inhibitory concentration (MIC) for each drug was defined as the lowest concentration that measurably decreased RNA synthesis and viability. Viability studies were performed on samples taken at 0 time and after 24 hours' incubation. All subcultures were incubated at 25°C for at least 2 weeks before they could be read. Because of the filamentous growth, colony counts could only be estimated. Only tenfold differences could be appreciated by comparing growth of the treated cultures with controls at various dilutions. Synergy was defined as a more than additive effect of the drugs in combination on [^{3}H]guanine incorporation into nucleic acid, and a decrease of at least 2 logs in colony counts when compared to the effects of either drug alone.

The MIC determined for amphotericin B alone against all three isolates was 0.04 μg/ml (Fig. 1), and confirmed the values previously reported (*5*). The MIC of rifampicin for the three isolates was 80 μg/ml. At 100 μg of rifampicin per milliliter there was a 76 percent drop in [^{3}H]guanine incorporation into the trichloroacetic acid–insoluble fraction of the cell (data not shown).

Low concentrations of amphotericin B potentiated the antifungal effects of rifampicin against the yeast-like phase of *H. capsulatum* (Figs. 1 and 2) on all three isolates. Our previous studies have shown that rifampicin was also an effective agent against the yeast *Saccharomyces cerevisiae* when it was used with amphotericin B (*6*). This effect was shown to be caused by an alteration of the permeability barrier of the cytoplasmic membrane of these yeasts by amphotericin B and an increased penetration of the second agent into the cell.

The concentrations of amphotericin B and rifampicin at which synergy against *H. capsulatum* was observed concentrations well below the respective MIC of each drug. The effect on [^{3}H]-guanine incorporation of both drugs together was greater than the additive effect of the drugs given separately. A similar effect was observed on viable colony counts.

The combination of both drugs, at concentrations well below the MIC, decreased colony counts by at least 2 logs, whereas each drug alone at these concentrations had no visible effect on viability. The combination containing 0.04 μg of amphotericin B per milliliter and 20 μg of rifampicin per milliliter was fungicidal, whereas a fungicidal effect could not be achieved with each drug alone even at much higher concentrations.

These in vitro studies may have important clinical implications. The combination of amphotericin B and rifampicin was fungicidal at concentrations readily attainable in vivo and this may be important in decreasing the prolonged period of treatment required for cure of histoplasmosis. This observation will have to be further evaluated with in vivo studies.

G. S. KOBAYASHI
G. MEDOFF, D. SCHLESSINGER
C. N. KWAN, W. E. MUSSER
Department of Medicine, Divisions of Dermatology and *Infectious Diseases*, and *Department of Microbiology, Washington University School of Medicine, St. Louis, Missouri 63110*

References and Notes

1. P. Reddy, D. F. Gorelick, C. A. Brasher, H. Larsh, *Amer. J. Med.* **48**, 629 (1970).
2. Subcultures of primary human isolates of *Histoplasma capsulatum*, G217 A and G217 B, were obtained in the yeast phase of growth from Dr. Ram P. Tewari, Rutgers University, New Brunswick, N.J.; the Down's isolate is from the stock culture collection of this laboratory.
3. G. S. Kobayashi and P. Guilacci, *Sabouraudia* **5**, 180 (1967).
4. S. B. Salvin, *J. Bacteriol.* **59**, 312 (1950).
5. G. Hildick-Smith, H. Blank, I. Sarkany, *Fungus Diseases and Their Treatment* (Little, Brown, Boston, 1964).
6. G. Medoff, G. S. Kobayashi, C. N. Kwan, D. Schlessinger, P. Venkov, *Proc. Nat. Acad. Sci. U.S.A.* **69**, 196 (1972).
7. Supported in part by NIH training grant AM 05611 and research grant AI 06213, and American Cancer Society grants NP-38A and P-477. Rifampicin was a gift from Dr. P. Sensi, Gruppo Lepetit, Milan, Italy.

19 June 1972

Chemical Spraying as Reported by Refugees from South Vietnam

Abstract. *Ninety-eight refugees who had been exposed to chemical sprays in South Vietnam were interviewed in Hanoi. Most reported effects on eyes and skin and gastrointestinal upsets. Ninety-two percent suffered fatigue, prolonged or indefinite in 17 percent of cases. Reports of abortions and monstrous births in sprayed humans and animals and of substantial numbers of deaths among fish, fowl, and pigs were also given.*

In the years since the initiation of the extensive use of chemical agents such as defoliants and harassing agents by the United States in Indochina, considerable attention has been devoted to the potential ecological consequence of this massive chemical intrusion (*1*, *2*). However, little attention has been paid, in the evaluation of the ecological and human effects of the defoliants, to their direct consequences for the sprayed population, despite the fact that for several years claims have been appearing that humans and animals exposed

to the chemical sprays sicken or die (*3*, *4*).

During our visit to Hanoi from December 1970 to January 1971 (*5*), we commenced an attempt to study these effects, with the active cooperation of Vietnamese physicians. There is a steady trickle of evacuees from South Vietnam arriving in the North, often for hospitalization or medical care. A proportion of these have come from the sprayed areas. During our period in Hanoi, we interviewed several of these evacuees concerning their experience of the chemical sprays; and on the basis of these interviews we constructed a questionnaire, which was translated into Vietnamese by Dang Vinh Thien of the Department of Scientific and Technical Information and subsequently administered under the supervision of Thon That Tung and Nguyen Xuân Huyên of Hospital A. Because we were anxious to survey only one member from each household, 3 or 4 months of screening of those evacuees who were not too sick to participate were needed to establish 98 respondents who could describe chemical spraying.

Of the 98 evacuees, 80 were men, most aged between 25 and 45. This disproportionately high percentage of men reflects the long and arduous journey that the evacuees must make. The first of the group to arrive had left the South in November 1970, the latest in April 1971. The pilot interviews had revealed the tremendous upheaval experienced by the peasant population. In the face of bombing, fighting, defoliation, and forced population movements, a normally static agricultural people had become nomads. Therefore we decided to focus our questioning on their most recent experience of chemical weapons. Ninety-five percent of the group has been sprayed at least twice, 60 percent had been sprayed three or more times; two said they had been sprayed 21 and 30 times, respectively. While we may have doubts about the precision of these two claims, it is clear that peasants commonly experienced chemical weaponry repeatedly. We tried to allow for the effects of respondents remembering other and worse experiences and merging these with the last spraying by separately questioning for these. We have, however, not reported these other events, and all subsequent questions referred only to the most recent spraying episode that the individual had experienced.

Asked what the spray had touched, 93 percent said trees; 89 percent, crops; 86 percent, people; 84 percent, water; and 70 percent, animals. It has been suggested that leaflets are dropped prior to a crop destruction sortie (*6*); among the surveyed group 93 percent said that no leaflets were dropped or other warnings given before the raid. In 91 percent of the cases the chemical had been sprayed from the air, and the descriptions were compatible with the known properties of Agents Orange, White, and Blue, and of CS (*7*).

Asked the effect of the spray on plants, the respondents gave replies which make familiar reading when set against the classical description of exposure to herbicides. The leaves of large trees yellowed, shriveled, and fell; banana plants died; crops withered, rotted, and became inedible—specifically mentioned were cassava, maize, rice, sweet potato, bananas, and coconut. Ninety-five percent of the group said that trees were damaged or died, and 89 percent reported that crops were destroyed or became inedible. Forty-eight percent of the group stated that fish in ponds and rivers died following the spraying (some singled out fish in still water as suffering the heaviest casualties), and 39 percent said that domestic animals died, particularly the smaller animals, such as fowl and pigs (reported by 22 and 18 percent, respectively). Five percent claimed that cattle had died; others referred to cattle going into convulsions—"struggling"—or getting skin disorders, but surviving. Five percent also spontaneously (the direct question was not asked) referred to abortions or monstrous births occurring among the animals. Only 7 percent specifically stated that their domestic animals were unaffected, the remainder either did not keep animals or did not know the effects. These effects are compatible with earlier claims made both in the Western press and by Vietnamese observers (*3*).

Informants gave a history of having been in fields, houses, shelters, or jungle when the spray planes came over. Nevertheless, most had inhaled the spray and been wetted by it. The initial effect on the eyes was a reddening or inflammation or temporary loss of vision (79 percent); a few (5 percent) reported that their sight was permanently "dimmed" following exposure. Such effects are more compatible with the known effects of CS than the defoliants. Vomiting and nausea (in 58 percent) seemed to pass fairly rapidly for most of the group, and diarrhea was present in only a third. Skin burning or reddening occurred in 56 percent of the group, and 16 percent reported longer-term or persistent effects, including pustules, scabs, and eczema; these were frequently described as difficult to cure. It may be noted that a persistent chloracne has been reported as one consequence of exposure to Dioxin (*8*). All the initial effects were described as beginning within a period of 3 hours from the spraying episode, so must in large measure be due to direct contact. A variety of other effects, ranging from "intoxication" to "nosebleed," were reported by one third of the group. Ninety-two percent of the informants reported fatigue, weariness, or dizziness after the spraying. Of these, 17 percent reported that the fatigue was prolonged; "unconscious for 3 or 4 days," "felt as if I was dismembered," "unendurable fatigue," and "fatigue lasting forever" were among the descriptions.

One of our pilot interviews in Hanoi was with a woman suffering from acute asthenia (fatigue), unable to do much other than to lie in bed; she described this weakness as having begun after the spraying. The doctors treating her were puzzled as to the cause of the asthenia; it was not seen in roughly matched groups evacuated in the same way but from areas that had not been sprayed, nor had psychotropic agents alleviated this patient's symptoms. While fatigue syndrome is the not uncommon consequence of exposure to extreme conditions (*9*), the clinical picture in this case is compatible with the peripheral neuropathy reported in patients exposed to percutaneous absorption of 2,4-D (*7*) used as a herbicide in the United States (*10*); it is also similar to the effects of some of the organophosphorus insecticides.

Finally, respondents were asked to report on the effects of the spraying on others in their village. In general, they reported effects similar to those on themselves, but 11 percent stressed long illnesses among the elderly or children, and 8 percent described deaths from chemicals in their villages. In addition, 4 percent of the respondents spontaneously referred to human abortions as one sequel of the spraying episode.

The sequelae of this chemical intrusion for the human population often appear to be prolonged and painful, and the assessments of the toxicology of individual agents are made largely irrelevant by the number of times they

are sprayed and the complex combinations of agents which may be involved in any one episode. Thus to the ecological havoc wrought by the defoliation campaign (*1*, *2*) and the potential teratogenic (*2*, *3*) and mutagenic (*3*) effects must be added direct effects on the exposed population, including prolonged asthenia and skin disorders.

The possible potentiating effects of eye irritation in an environment where trachoma is endemic must also not be overlooked. Nor to our knowledge has there yet been any substantial study of the potential carcinogenic effects of the chemical agents. It is difficult to obtain an estimate of the potential population thus at risk following the chemical war. Figures provided by the Democratic Republic of Vietnam and the Peoples Republic of China speak of more than 1.5×10^6 people having been poisoned (*11*), and the scale on which the chemicals have been applied (*2*) would certainly make this figure a feasible one.

Although the defoliation campaign has been largely phased out in Vietnam, we cannot be sanguine that we are merely cataloging past damage that is not being repeated elsewhere. The use of chemical defoliants by the Portuguese against guerrillas in Angola is authenticated (*12*), and there have been repeated reports that the chemicals are still in use on a large scale elsewhere in Indochina, notably Laos (*13*), despite the reductions in their use in Vietnam itself. It is urgent that clear information on the effects of chemical sprays on humans and lower animals be obtained, in view of the continued use of these agents in Indochina and elsewhere.

HILARY A. ROSE
Department of Social Administration, London School of Economics and Political Science, London, England
STEVEN P. R. ROSE
Department of Biology, Open University, Walton, Bletchley, Buckinghamshire, England

References and Notes

1. G. H. Orians and E. W. Pfeiffer, *Science* **168**, 544 (1970); A. H. Westing, *BioScience* **21**, 893 (1971); *ibid.*, p. 1225; Committee on Commerce, *Effects of 2,4,5-T on Man and the Environment* (Serial 91-60, Government Printing Office, Washington, D.C., 1970).
2. Herbicide Assessment Commission of the AAAS, preliminary report 1970, in *War Related Civilian Problems in Indochina*, part 1, Vietnam (Senate Judiciary Committee, Washington, D.C., 1971), pp. 113–131; R. T. Cutting, T. H. Phuc, J. H. Ballo, M. W. Benenson, C. H. Evans, *Congenital Malformations in Hydatiform Moles and Stillbirths in the Republic of Vietnam 1960–1969* (Government Printing Office, Washington, D.C., 1970).
3. Ton That Tung, Trinh Kim Anh, Bach Qúoc Tuyên, Dao Xuân Tra, Nguyên Xuân Huyên, *Vietnam. Stud. (Hanoi)* **19**, 53 (1971).
4. M. F. Kahn, in *CBW: Chemical and Biological Warfare*, S. Rose, Ed. (Beacon, Boston, Mass., 1969), p. 87; A. Lacombe, J. P. Farriaux, G. Fontaine, P. M. Muller, *Rev. Pediat.* **3**, 207 (1967).
5. Our visit was at the invitation of the Committee for the Dissemination of Science and Technology of the Democratic Republic of Vietnam, and under the auspices of the World Federation of Scientific Workers. Its details have been reported elsewhere [S. Rose and H. Rose, *New Sci.* **49** (No. 735), 134 (1971).
6. A. W. Galston, in *CBW: Chemical and Biological Warfare*, S. Rose, Ed. (Beacon, Boston, Mass., 1969), p. 62.
7. Herbicide abbreviations are as follows: 2,4-D, 2,4-dichlorophenoxyacetic acid; Agent Orange, a mixture of 2,4-D and 2,4,5-trichlorophenoxyacetic acid; Agent White; a mixture of 2,4-D and picloram; Agent Blue, dimethyl arsenic acid; and CS, *o*-chlorobenzylmalononitrile.
8. H. Bauer, K. H. Schulz, U. Spiegelberg, *Arch. Gewerbepath. Gewerbehyg.* **18**, 538 (1961).
9. R. J. Lifton, *Death in Life: Survivors of Hiroshima* (Random House, New York, 1967); L. Eitinger, *Concentration Camp Survivors in Norway and Israel* (Allen & Unwin, London, 1964).
10. N. P. Goldstein, P. H. Jones, J. R. Brown, *J. Amer. Med. Ass.* **171**, 1306 (1959).
11. Stockholm International Peace Research Institute, *The Problem of Chemical and Biological Warfare* (Humanities, New York, 1971), vol. 1, pp. 176–177.
12. The relevant press reports on this are compiled in A. Humbaraci, *Threats to the Ecology of Southern Africa* (Zambian Information Office, Rome, 1971).
13. K. Boupha, *Report to the International Conference on Medicine and the Indochina War, Paris (1971)*.
14. We thank Ton That Tung, Nguyên Xuân Huyên, and the patients of Hospital A for their cooperation in making this survey; and A. H. Westing for helpful discussions.

19 January 1972; revised 16 June 1972 ■

Development of a Receptor on a Foreign Nerve Fiber in a Pacinian Corpuscle

Abstract. *When the sensory fiber of a Pacinian corpuscle (in cat mesentery) is transected (at the inferior mesenteric nerve) transduction fails within 30 hours: the nerve ending produces no generator potentials in response to mechanical stimulation. Electrically elicited nerve impulse conduction continues for at least another 18 hours. A transducer mechanism develops on a regenerating nerve fiber when this fiber enters the denervated corpuscle. Such transducer development takes place on myelinated fibers from the inferior mesenteric nerve, which normally supplies corpuscles, as well as on myelinated hypogastric nerve fibers, which normally do not go to corpuscles, including fibers larger than the original corpuscle afferents.*

The mechanoreceptor Pacinian corpuscle develops by an interaction between a sensory nerve cell and an epithelial or fibroblastic (lamella) cell: as the nerve cell's axon (myelinated) grows into the embryonic target tissue, lamella cells there begin to proliferate and eventually to envelop the axon terminal forming a laminated capsule around it (*1*) (Fig. 1, I). In the course of this development, a highly sensitive receptor mechanism differentiates on the axon terminal, capable of transducing mechanical into electrical energy (*2*). This transducer mechanism is distinct from the mechanism of nerve impulse conduction: it produces a local, graded electrical potential (generator potential) in response to mechanical stimulation (strain) (*3*), is insensitive to tetrodotoxin (*4*), and has a high temperature coefficient (*5*). The transducer differentiation appears to be restricted to the nonmyelinated terminal portion, where the axon is in intimate contact with the lamella cells. The remainder of the axon inside and outside the capsule is insensitive to mechanical stimuli of the order of magnitude to which the terminal responds (*6*), and the lamella cells do not partake in the transduction (*7*).

Is the differentiation of the transducer mechanism a specific property of the Pacinian corpuscle axon, or are other axon types capable of such differentiation if they interact with the lamella cells?

This question and related ones are asked here for the Pacinian corpuscle of the cat's mesentery. The approach is to cut the nerve supply of the corpuscle in the adult animal and to reinnervate it with a foreign nerve fiber after the original one has degenerated.

The Pacinian corpuscle of the cat's mesentery (mesocolon) is well suited for a study of this kind. These sense organs, about 1 mm long and 0.5 mm thick, are visible to the naked eye; their afferent axons, which often run separate from each other over several centimeters, are easily seen in the translucent mesocolon and dissected with low-power optics (Fig. 2). The capsule survives after many months of denervation if its blood supply is intact.

To denervate the corpuscle, the in-