**DISCUSSION**

*Dr. Weibel:* I have a comment with respect to the covering of dividing cells by type 1 cells. It would be nice to conceive of these cells hiding beneath the type 1 cell lining while they divide, but I don't think you can really make this point. You will often find type 2 cells partially covered by flaps of type 1 cells. Assuming that about one-third of the type 2 cells are covered by type 1 cell flaps, then about 15 percent of the type 2 cell profiles occurring on random sections should appear to be completely covered; this point should be looked at carefully.

*Dr. Evans:* The point we are making results from our interpretation of the data we have collected. These data consist of examples of cells during mitosis and labeled type 2 cells observed in the electronmicroscope at the time intervals of 1, 4, 5, 8, and 12 hours after injection of tritiated thymidine. At one hour, we observed that 13 percent of the labeled type 2 cells were covered by more than half of their free surface by the adjacent type 1 cells. At this time, the labeled cells have not divided, and our value of 13 percent is similar to your reference to 15 percent as the number of type 2 cells covered by the adjacent type 1 cells seen in sections under normal conditions. As cell division progressed, we noted an increase in the percentage of labeled type 2 cells covered by the adjacent type 1 cells. At five hours, the value was 36 percent, and at 12 hours 45 percent were covered. From our observations with the electronmicroscope of 31 type 2 cells during mitosis, we found that all were lying on the basement membrane and that 14 were partially covered by the adjacent type 1 cells. We interpret these data to indicate that as type 2 cells undergo division, they move apart over the basement membrane and beneath the adjacent type 1 cells so that one or both sister cells are partially covered by type 1 cells.

**Toxic Effects of the Herbicide Paraquat**

*Renate D. Kimbrough, M.D.*

Paraquat is a general weed killer of the bipyridyl family of herbicides (1, 1'-dimethyl 4,4' bipyridylum). Paraquat is available either as the dichloride or dimethyl sulfate salt, both compounds are water soluble. In the United States the following formulations are available: Ortho paraquat, a 29.1 percent solution, Ortho dualparaquat, a 42 percent solution, and Ortho Spot, weed and grass killer, a 0.44 percent solution. Paraquat was developed in the 1950's, and came onto the market in Great Britain in 1962. It was registered for use in the United States in 1964. Registration also permits aerial spraying of diluted formulations.

According to a recent editorial in Lancet,1 the total number of human deaths known to the manufacturer from paraquat poisoning is now 124 worldwide. In addition, 60 persons who have recovered from paraquat poisoning are also known. The fatal dose in adults is thought to be about 15 ml of a 20 percent solution, or a "mouthful" as it is described in most case histories. Formulations which contain 5 percent of paraquat if accidentally swallowed are usually not fatal. Fatal poisoning in almost all cases has been due to ingestion of the material. The initial symptoms of poisoning consist of burning of the mouth and throat, followed by nausea and vomiting. After a latent period of up to several days, increasing respiratory distress develops, a transient effect is also observed on kidney, liver and heart function and transient neurologic signs are seen, but death is usually the result of a progressive fibrosis and epithelial proliferation that occurs in the lungs.

Dermal exposure to the paraquat concentrate may result in severe skin irritation. Diluted spray solutions may produce slight to moderate irritation. Paraquat is absorbed through the skin. In toxicity studies conducted in our laboratory we found that the acute dermal LD50 in rats was 80 mg paraquat/kg body weight in males and 90 mg/kg in females, while the acute oral LD50 in male rats was 100 mg/kg and in females 110 mg/kg.2 These findings indicate that there does not seem to be a great difference between the oral and dermal toxicity in rats.

McDonagh and Martin3 reported four cases of paraquat poisoning in children. One of the patients, a three-year-old boy, was admitted to the hospital with superficial burns on his right thigh and hands 24 hours after he had spilled a 20 percent paraquat concentrate (Gramoxone) on his clothes. He had also been seen playing near a pool of the liquid. About six to eight hours after admission an erythematous rash appeared on his thigh and hands. The rash developed into a large first degree burn. No lesions were seen in his mouth. In spite of the fact that the child did not appear to have ingested any paraquat, it was present in his urine. This case illustrates that paraquat is also absorbed through human skin.

As pointed out earlier, paraquat is registered in certain instances for spraying by airplane in the United States. Fortunately, the particle size is large enough so that direct inhalation does not seem to present a problem unless high pressure spray or air blast equipment is used. According to Gage the "no-effect" level for a "respirable" paraquat aerosol in the rat is about 0.1 mg/M3 for repeated six-hour exposures, which would suggest that 0.1 mg/M3 would not be excessive for occupational exposure. The threshold limit value for paraquat in workroom air in the United States set by the American Conference of Government Industrial Hygienists is 0.5 mg/M3 at present.

Patients have on occasion had nose bleeds due to a local effect of paraquat on the nasal mucosa. If paraquat is splashed into the eye, particularly the paraquat concentrate, severe eye injuries have occurred,4 and in
addition, injuries to the fingernails, nail beds and skin have also been reported. All of these injuries are primarily due to the local corrosive effect of this product.

Death following paraquat poisoning is usually the result of progressive fibrosis and epithelial proliferation in the lungs. This effect has been described in human beings, rats, guinea pigs, mice and dogs.

We have studied the effect of paraquat on the lung of the Sherman rat. The early effect of paraquat after a single oral dose of 150 mg/kg by stomach tube, was pulmonary edema within 48 hours.

Examination of the affected lungs with the electron-microscope showed swelling and large vacuoles in the cytoplasm of the membranous pneumocytes. Dark prominent granules were also seen in the cytoplasm and were thought to represent ribosomes. Widening of the basement membrane with separation of the epithelial cells from the basement membrane was noted. Pulmonary edema and red blood cells were observed in the alveoli. A rat which died four days after a single oral dose of 160 mg/kg of paraquat in water showed firm dark lungs on gross inspection, and microscopic exami-

FIGURE 1. Section of lung of rat given 160 mg/kg paraquat by stomach tube. Rat died six days later. Alveoli contain edema fluid, inflammatory cells and macrophages (PAS stain, × 50).

nation revealed edema and hemorrhage.

Rats dying six days after a single dose of paraquat showed an inflammatory reaction in the lungs (Fig 1). Polymorphonuclear leukocytes, macrophages and edema fluid were observed in the alveoli. Evidence of the earlier hemorrhage was still present. A brown pigment which stained positive for iron with Perl’s stain was observed, and probably represented hemosiderin. A highly eosinophilic PAS-positive membrane lining the alveoli was also noted. In rats surviving ten days following a single toxic dose of paraquat the lungs showed beginning proliferation of fibrous and alveolar cells (Fig 2). Rats given a single oral dose of 40 mg/kg body weight, killed eight weeks later showed fairly extensive areas of consolidation. Microscopic examination of these firm grayish areas revealed extensive fibrosis and a brown pigment positive for iron with Perl’s stain (Fig 3), which was consistent with hemosiderin findings. Slight proliferation of epithelial cells was also observed.

When rats were fed 500 ppm paraquat daily in their diet (20-30 mg/kg body weight) an occasional rat developed areas of consolidation. These areas of consolidation contained proliferated epithelial cells which were surrounded by inflammatory cells and fibroblasts. A prominent amorphous material was also observed in the

FIGURE 2. Section of lung of rat given 150 mg/kg of paraquat by stomach tube. Rat died ten days later. Note extensive areas of proliferation of fibrous and epithelial cells. Nuclei of some epithelial cells are enlarged hyperchromatic H&E (× 125).

FIGURE 3. Section of lung from rat receiving 40 mg/kg paraquat, killed eight weeks later. Note pigment staining with Perl stain within macrophages (Perl stain × 500).

FIGURE 4. Electronmicrograph showing electron dense material in alveoli (arrow). Lead citrate and uranyl acetate (× 6,745).

68S 16TH ASPEN LUNG CONFERENCE

CHEST 65: 4, APRIL, 1974 SUPPLEMENT
alveoli. Electronmicroscopic examination (Fig 4,5) showed this material to be striated or to form whorls of electron dense material reminiscent of surfactant and lamellar bodies. Fletcher and Wyatt recently reported a large increase in arachidonic acid and a small increase in the cholesterol esters in the lungs of rats six days after poisoning with paraquat. It is possible that the increase in the fatty acids is due to the material accumulated in the alveoli, which was just illustrated. A hypcholesteremic drug AY9944 apparently shows a similar accumulation of material within the alveoli of the lungs.10

There is a possibility that paraquat is absorbed by the lungs, then may become reactivated in the lung by oxygen. The treatment of patients suffering from paraquat poisoning with oxygen probably aggravates the lung lesion, and oxygen should therefore not be administered. Paraquat is absorbed on bentonite, fuller's earth and related materials, and becomes deactivated. These various absorbents are most effective when given orally within one hour or less after the poisoning by ingestion has occurred. Brown11 has recommended the suspension of 300 gm of finely divided fuller's earth, previously sterilized to kill bacterial spores, in 1 liter of water to be given promptly after the initial lavage. Forced diuresis may also have an effect in treating the poisoning, although this has not been definitely proved. Paraquat is fortunately not well absorbed by the skin or the gastrointestinal tract. Therefore, most patients who have taken the 5 percent solution have survived. If about .05 mg/kg body weight paraquat in water is instilled directly into the trachea in rats, localized fibrosis is produced in the rat lung. This finding illustrates that not very much paraquat needs to reach the lung in order to produce fibrosis.8

In summarizing the sequence of events following a lesion on the lung caused by paraquat, the following seems to take place: the alveolar epithelium becomes vacuolated and degenerated; the alveoli fill with edema fluid; basement membranes are widened; and red blood cells appear in the alveoli. These events are followed by infiltration of the lung with inflammatory cells, fibrosis and epithelial proliferation, and accumulation in the alveoli of electron dense material that resembles lamellar bodies and surfactant.

REFERENCES

8 Kimbrough RD, Linder RE: The ultrastructure of the paraquat lung lesion in the rat. (Environ Res in press)

DISCUSSION

Dr. Corrin: I think it is important to re-emphasize the point that you made, and that is the route by which paraquat reaches the lung. I know of no fatal cases of paraquat poisoning in which the route to the lung has been the respiratory tract. All the fatal cases that have been reported are those in which the paraquat is ingested. In our experimental preparations, the data agree closely with yours in that the epithelium is the major site of damage. There is some endothelial degeneration but the epithelial damage can proceed to complete necrosis with the endothelium being apparently quite normal. This is particularly interesting when one realizes that this toxic substance had reached the alveolar wall via the circulation and not via inhalation. This material is so extremely toxic that one might ask what we can do to reduce the hazard of this material. One might initially suggest that we ban the sale of this material but our farming friends tell us that this is an extraordinarily valuable chemical and a ban would put a great burden on them.

Dr. Kimbrough: I think it would be far better if the law could be changed and the dilute form of paraquat would be the only form available on the market. This would decrease the number of cases of such fatal poisonings.

Dr. K. Fisher: I would like to extend Dr. Kimbrough's remarks on the role of oxygen in paraquat poisoning. We have found that paraquat is far more rapidly and uniformly fatal in animals breathing an atmosphere of oxygen than in those breathing room air. We have also found that concentrations of paraquat which are bacteriostatic to aerobically grown E coli have no effect on anaerobic growth of E coli.

CHEST 65: 4, APRIL, 1974 SUPPLEMENT

16TH ASPEN LUNG CONFERENCE