Control of Australorbis glabratus by Acrolein in Puerto Rico

FREDERICK F. FERGUSON, B.A., M.S., Ph.D., CHARLES S. RICHARDS, B.S., M.S., Ph.D., and JUAN R. PALMER, B.S.

PRELIMINARY field and laboratory studies in Puerto Rico indicate that acrolein (A) is a useful new weapon against aquatic snail intermediate hosts of schistosomiasis, especially those in irrigation systems. Acrolein may be added to the list of satisfactory molluscicides now in use (1-7): pentachlorophenol (PCP), sodium pentachlorophenate (NaPCP), dinitrocyclohexylphenol (DCHP), and copper compounds (CuSO₄, CuCO₄, and others).

The seriousness of schistosomiasis associated with irrigation systems has been cited by the World Health Organization through its Expert Committee on Bilharziasis (8), which stated: "It is a tragic irony that in many parts of the world the vast irrigation schemes constructed with the aim of improving the standard of living have had the effect of undermining the health of the areas they serve. The networks of canals designed to carry water to arid territories have proved ideally suited for carrying bilharziasis." Recent analyses (9) further define the disturbing interaction of irrigation, agriculture, and schistosomiasis.

Dr. Ferguson and Dr. Richards are with the Puerto Rico Field Station, Technical Development Laboratories, Communicable Disease Center, Public Health Service. Mr. Palmer is with the bilharzia control unit, bureau of environmental sanitation, Puerto Rico Department of Health, San Juan.

Technical assistance was provided by Dr. Rene Blondeau, Shell Development Co., Modesto, Calif., and Carlos Costa, Shell Co. (Puerto Rico) Ltd., San Juan. The Puerto Rico Water Resources Authority and Luce and Co., S. en C., Aguirre, cooperated extensively in the study.

Molluscicidal properties of acrolein (CH₂: CH.CHO) were first observed during 1957 field trials in California, coincident to destruction of submersed weeds in the main irrigation canal of the Kern County Land Co. Native species of snails (Lymnaea and Helisoma), as well as the Potamogeton-algae complex, were destroyed for a distance of 20 miles, as a result of a single chemical application at one point in the large canal (with water flow of 300 c.f.s. and over). After 1 week, the carrying capacity nearly doubled, and the canal remained weedfree for 6 weeks. In laboratory trials, the acrolein dosage required to kill Elodea leaf cells was low: 0.5 ppm for 24 hours' exposure (10). Field and laboratory studies were made during 1958-59 to test the effectiveness of acrolein against Australorbis glabratus in Puerto Rico.

Significant Characteristics of Acrolein

Acrolein is a clear liquid with a boiling point of 52.69°C. and a density of 6.99 pounds per gallon at 20°C. The vapor pressure at 20°C. is 213.8 mm. Hg, and the water solubility at this temperature is 22 percent on a weight basis. Acrolein is highly reactive, and in light and air, it slowly forms polymers. However, hydroquinone generally is added to acrolein to inhibit this polymerization (11). In view of its volatility, acrolein is most effective when applied in ponds or channels of considerable depth, since loss by vaporization proceeds rapidly from shallow bodies of water. To hasten solution and thus minimize vapor loss, it is mixed with water during application with a circulating pump or injected into the stream at a point of turbulence.

In an extensive series of aquatic weed control trials in irrigation systems in the western United States, acrolein was applied principally by direct metering from the shipping drum into the discharge side of a circulating pump (personal communication from R. Blondeau, 1959). Advantages of this method are that the acrolein does not enter the body of the pump and there are no positive pressures present. Flow rates can be read directly from several types of in-the-line meters, and uniform mixing with water is accomplished with a minor loss of Because of its high vapor pressure, acrolein cannot be used as a spray or sorbed in the usual dust or granule carriers. A modification of the Klock automatic molluscicide dispenser, developed for NaPCP, also allows satisfactory distribution of the material in running water.

Acrolein is highly irritating to the nose, eyes, and lungs; and since vapor concentrations as low as 0.5 ppm are objectionable, it acts as its own warning agent for men and animals. It is impossible to remain voluntarily in an atmosphere containing dangerous vapor concentrations. Body contact with the compound may produce skin irritations. Despite its chemical and physiological effects, acrolein can be used safely in the field by trained personnel with techniques and equipment designed for the purpose. Because of these properties, the herbicidal-molluscicidal formulation of acrolein is being sold currently for application only by trained and licensed operators.

Acute oral toxicity of aqueous solutions of acrolein for rats at the LD₅₀ level is stated to be 42(37-49) mg. per kilogram of body weight. Rats fed up to 200 ppm of acrolein in their water constantly for 90 days showed no ill effects. Lactating dairy cows given 60 ppm of acrolein in their drinking water for 24 hours showed no change in feed or water intake or milk production. Bioassay and chemical analvses of milk from these cows showed no acrolein present up to the limit of sensitivity of the methods (0.5 to 1 ppm). Individual rabbits immobilized on operating boards and immersed for 1 hour up to their necks in water containing 20 ppm of acrolein showed no ill effects. These data indicate that acrolein applied at herbicidal-molluscicidal dosages can be used without hazard to mammals that drink or contact the treated water. However, acrolein is quite toxic to fish and other aquatic animals such as frogs, shrimp, and crayfish. Harmful effects on fish, shrimp, and crabs were observed in irrigation canals.

Studies on the phytotoxicity of acrolein indicate that no adverse effects occur on most crops with concentrations of less than 25 ppm. In some tests corn, cotton, milo, squash, castor beans, and tomatoes were not harmed when furrow-irrigated with water containing up to 80 ppm of acrolein, and no injury occurred when alfalfa and squash were sprinkler-irrigated with water having 80 ppm (12). In tests in Puerto Rico, young sugarcane was not harmed when furrow-irrigated with water having concentrations up to 70 ppm. Routinely, concentrations of acrolein in irrigation water reaching crops are kept below 15 ppm as an added safety factor. Acrolein leaves no residues in either soils or in plant tissues (10). With a dosage range of 1.5-7.5 ppm, depending upon a water temperature of 60° to 80° F., acrolein killed Hydrodictyon, Cladophora, Spirogyra, Potamogeton, Zannichellia, Elodea, Callitriche, and Ceratophyllum (12); and in trials in Puerto Rico, it destroyed Chara, Najas, and Compsopogon as well. At dosages sufficient to destroy submerged plant species, acrolein shows little effect on emergent weeds, such as tules and Some floating plants, Pistia, Eichcattails. hornia, and Jussiaea, may be controlled by dosages at least double that necessary for submerged forms.

Laboratory Tests

The effects of acrolein on Australorbis eggs and adult snails were determined under laboratory conditions. Acrolein in concentrations of 10, 5, 2.5, 1.25, and 0.6 ppm was tested in 2-liter volumes of pond water in gallon jars. In preliminary tests, eggs and snails were allowed to remain in the acrolein solution with periodic observation of results. In subsequent tests, eggs and snails were exposed to acrolein concentrations from 5 minutes to 24 hours and then removed to jars of fresh water for observation. It should be understood that because of marked evaporation, the starting con-

centrations would not be maintained throughout an experiment.

With continuous exposure in the acrolein solution, the snails retracted almost immediately in 10 and 5 ppm concentrations. After 5 minutes' exposure, all the snails were either floating or on the bottom. These snails appeared dead and never recovered. In later trials, in which the snails were removed to fresh water after the exposure periods, they were not killed at 5-minute exposures; in fact, at 1-hour exposure in 10 ppm, 88 percent of the snails survived. The rapid retraction of snails in acrolein (in contrast with the avoidance reaction in NaPCP) was a significant feature of the tests.

Observed effects on A. glabratus snails and eggs after 24 hours, following exposures of 1, 3, and 24 hours to concentrations of 10, 5, 2.5, and 1.25 ppm of acrolein, are shown in table 1. Data for less than 1 hour of exposure and for less than 0.6 ppm were omitted from the table because only 12 percent of the snails were killed in 10 ppm for 1 hour and none were killed in 1.25 ppm for 24 hours. Partial snail kills occurred in 10 ppm for 1 hour, 5 ppm for 3 hours, and 2.5 ppm for 24 hours. Because laboratory conditions of temperature and air currents varied, evaporation may have resulted

in a rapid loss of acrolein in some tests. Ten ppm for 3 hours killed 88 percent of the snails; 5 ppm for 24 hours killed 58 percent, with the surviving snails appearing moribund; and 2.5 ppm in 24 hours killed 35 percent, with the remainder of snails recovering. These results indicate that 10 ppm of acrolein sustained for 3 hours and 2.5 or 5 ppm sustained for 24 hours probably are lethal to A. glabratus, and that lower concentration-exposure time combinations are partially effective.

Australorbis eggs were more susceptible than the snails to the acrolein. Lower concentration-exposure time combinations gave higher partial kills. The partial survival at 24 hours with an initial 5-ppm concentration was limited to 3 of the 10 tests in which the concentration probably had been critically dissipated by evaporation.

Schistosoma mansoni cercariae were added to shell vials containing 10, 5, and 2.5 ppm of acrolein and to plain water controls. After 3 minutes, actively swimming cercariae were observed in all vials. No activity was observed in 10 ppm after 8 minutes, in 5 ppm after 13 minutes, or in 2.5 ppm after 18 minutes; cercariae were still active in the controls. Microscopic examination after 1 hour revealed that

Table 1. Percentage kill of Australorbis glabratus adult snails and eggs 24 hours after various exposure times to differing concentrations of acrolein

Initial con- centration acrolein, (ppm)	Exposure time ¹								
	1 hour			3 hours			24 hours		
	Number tests	Number exposed	Percent killed	Number tests	Number exposed	Percent killed	Number tests	Number exposed	Percent killed
Adult snails									
10 5 2.5 1.25	4 4 6 1	34 34 50 8	12 0 0 0	4 4 6 1	34 34 50 8	88 6 2 0	10 2 10 12 7	114 114 105 64	98 58 35 0
Eggs 10 5 2.5 1.25	2 4 3		40 40 0	4 6 4		100 50 0	9 2 10 8 5		100 70 40 10

¹ Tests were accompanied by controls of the same number of snails, all of which survived 24 hours in fresh water.

² All survivors appeared "moribund."

all the cercariae in the three acrolein concentrations were dead on the bottom, many with their tails detached. It can be concluded that the cercariae are killed at concentrations of acrolein lower than those required to kill snails or eggs. Miracidia exposed in solutions of acrolein were affected adversely in 10, 5, and 2.5 ppm within 5 minutes and were dead within 10 minutes. They survived for 10 minutes in concentrations of 1.25 ppm, but all died within 20 minutes.

Field Trials

Several types of Australorbis habitats were studied in order to get molluscicidal as well as downstream flow characteristics of acrolein in natural water bodies. Channels chosen included manmade concrete drainways and earthen irrigation canals and ponds which had both dense and medium growth of submerged weeds. The results are summarized in table 2.

The Fort Buchanan trials were conducted in the concrete-lined part of Quebrada El Toro. Normal flow is contained within a basal rectangular inset, 2 feet wide by 4 inches deep. It never reaches more than 0.5 c.f.s. In a preliminary test (July 1958), the objective was to introduce into the channel water a total amount of acrolein equal to the amount of NaPCP used in a standard Puerto Rican application (10 ppm for 24 hours). In a 36-minute period, approximately 23.5 pounds of acrolein was injected; at the mixing point, the concentration ranged from 100 to 650 ppm. Loss of vapor was great in the shallow channel, and concentrations probably dropped as low as 35 ppm 1.2 miles downstream. Caged Australorbis and strands of *Elodea* were killed up to 1 mile, the greatest distance at which cages were placed. In January 1959, approximately 45-50 ppm of acrolein was applied, and it passed smoothly down the course with decreasing concentrations to 14 ppm at 1 mile. Because of considerable dilution from a tributary sidestream as well as vapor loss at a 10-foot-wide, 4-foot-high waterfall, the concentration had fallen to 4 ppm at 1.2 miles. Both snails and snail eggs were destroyed at distances up to 1.2 miles while Elodea was killed up to 2 miles (at an estimated 3 ppm).

Table 2. Summary of field data with acrolein in Puerto Rico

Acrolein	concentration 1					
	(ppm)	$\begin{array}{c} \textbf{Location of test and} \\ \textbf{results} \end{array}$				
Origin	Downstream					
45–50	1 mile, 14	Fort Buchanan: Killed snail eggs at 1.2 miles, not beyond; no snails killed at 1.2 miles or beyond. Vapor loss great plus massive dilutions. Elodea killed				
160	0.7 mile, 35	up to 2 miles in a stream. Daguao: Marisa snails killed to 0.75 mile, not beyond. Australorbis partial kills at 0.75 mile. Elodea damaged at 1.2 miles in a creek.				
160		Roosevelt Roads: Few fish, shrimp, and others killed to 1 mile, not beyond. Neritina snails at 1 mile not killed. No stream				
100	0.25 mile, 25; 0.75 mile, 4.	vegetation. Aibonito, test 1: Treatment wave at least 650 yards long, probably 800 yards at 13-18 hours. No snails in stream section and none at 2 miles. No vegetation				
4-5		harm (grasses). Aibonito, test 2: Wave head detected via smell at maximum distance of 485 yards, 1 week. Never reached Australorbis colonies at 2 miles in stream. Killed				
6–7	0.5 mile, traces.	fish and shrimp en route. Puerto Jobos: Killed abundant Australorbis for 0.7 mile. No effect on emergent vegetation (grasses) in creek.				
15-20	Traces in treated sections.	Faria: Killed Australorbis snails up to 300 yards below farthest downstream injection point. Harmed fish. No effect on emer- gent vegetation in creek.				
45–50	2 miles, 25– 30.	Patillas Canal: Killed sparse vegetation in canal. Killed most Australorbis and Helisoma snails in three receiving ponds (Colonia Fortuna area).				
26	6 miles, 17; 19 miles, 6; 20 miles, 3.	Patillas Canal: Killed vege- tation and Australorbis snails up to 20 miles in canal.				
	4-6 at mar- ginal points.	Arenales: Killed medium dense Chara and Australor-				
10	5, 3, 2, and 1 at 4 points in pond at 3 hours.	bis snails in pond. Colonia Fortuna: Australor- bis snails killed and mini- mal harm to very dense vegetation in pond.				

¹ Concentration at origin calculated; at downstream points determined by chemical test.

In a medium-sized creek, Rio Daguao, acrolein was injected with a circulating pump above an impounded section at 160 ppm for 45 minutes. This impoundment, plus several other large quiescent stream sections, prevented regular chemical passage; at a station 0.7 miles away, a drop in concentration to 35 ppm was noted. The wave passage required 5 hours. Fish and crayfish appeared to be destroyed entirely in the first half mile of treated parts. Heavy masses of Elodea were "burned" but not significantly harmed at a distance of 1.2 miles. Kills of Australorbis lodged in shallow grassprotected margins at 0.75 mile were partial. The operculate snail, Marisa, was also killed up to 0.75 mile.

Downstream movement data were sought in the test at the Quebrada Aguas Claras, Roosevelt Roads Base. The compound was mixed within a deep impoundment (6' x 30' x 15') in the creek by means of a high-speed circulating pump. Although flow beyond was intermittent and went through a large marshy section, no other impoundment in the study section was as large as the impoundment at the injection point. There was no "nasal" evidence that the 160-ppm dose ever left the original impoundment, although a few fish and some shrimp were killed up to the 1-mile station, at which point Neritina snails were unharmed.

Information was sought on adverse hydrologic effects of impounded sections of a stream on the movement of acrolein in two tests in Quebrada Pasto, Aibonito. Although this clear mountain stream is narrow and winds through rather deep cuts for a total of 5 miles, there are numerous ponded sections and the overall average velocity is less than 0.05 m.p.h. The study section consisted of that part of the stream in which Australorbis growth began and extended for 2 miles upstream to the application point. All impoundments as well as flowing subsections were measured for the purpose of estimating the total volume of water under treatment. A total of 719 linear yards (20.4 percent) was essentially non-moving stream in the 49 "ponds." Vegetation was lacking within or on margins of 37 ponds, while vegetated pools had a grass-Caladium complex, which generally formed a surface covering. Two treatments were used (100 ppm for 4 hours, test 1; 4-5

ppm for 28 hours, test 2) in attempts to overcome the combination of still-water pools and emergent vegetation that impeded the passage of acrolein. Although some fish and shrimp were killed in upper sections, the acrolein never reached the snail colony in detectable or harmful amounts. It was volatilized or lost by soil absorption or biological activity during the long time interval required for passage down this slow-flowing stream.

In the Puerto Jobos creek, it was shown that where acrolein moves along in the relatively even flow pattern of a small creek lacking impoundments, complete kills of *Australorbis* may be expected. In this test, all snails were killed for a distance of 0.7 mile, when the material was dispensed at a low rate (6–7 ppm) over a period of 13 hours. Grasses along the creek bank were not harmed.

In the Quebrada Faria test, the material was applied simultaneously at three major impoundments. By use of a constant head siphon principle, acrolein was metered into the three pools, which were spaced about 0.75 mile apart, at a dosage range of 15-20 ppm for 12, 7, and 18 hours, respectively. Only traces of the chemical were found in the test section 24 hours later. Partial kills of Australorbis occurred below the two uppermost injection points, and total kills occurred below the lowermost treatment station for a distance of about 300 yards. Beyond this point, at 24 hours, a mixture of dead and living snails was collected. There was no visible effect on stream-lining Caladium and grasses.

Dispersal characteristics of acrolein in the last 2 miles of the 25-mile-long Patillas Canal (Eastern Irrigation District) were first studied during January 1959, at which time the material passed efficiently downstream in the wellgraded, gravity-flow irrigation channel. dosage of 45-50 ppm was applied for 80 minutes with the use of a high-speed, large capacity circulating pump. Water samples taken at a station 2 miles below the injection point showed a concentration of 25-30 ppm when the wave reached there 2 hours later. Sparse filamentous algae were killed, but no harm was done to extensive mats of Najas in three equalizer tanks of about 1 acre. Most of the Australorbis and Helisoma snails were killed. The dilution factor in these receiving tanks was estimated to be at least in the ratio of 10 to 1, because of the amount of water they contained and the amount of water that continued to flow in after the wave passed.

Another test was made in the same channel during July 1959, when a dosage of 26 ppm was applied for 3.25 hours. The circulating pump method was used within 1 mile of the dam at Lago Patillas (Cacao Alto). In the untreated section upstream and at several stations downstream, there was a dense mat of Najas-Compsopogon, primarily on the earthen bottom which protected an abundance of small- to mediumsize Australorbis (more than 20 per sq. vd.). Treatment time was just prior to the routine quarterly manual cleaning of submerged weeds by the operating agency, the Puerto Rico Water Resources Authority. In order to conserve the chemical, the volume of water had been lowered from 50 to 30 c.f.s. The average speed of the water was 1 m.p.h. Seventy-five gallons of acrolein were used (2.5 gal./c.f.s./day). The water was quite muddy from recent rains and was 82° F. Chemical tests showed 17 ppm. 6 ppm, and 3 ppm at stations 6, 19, and 20 miles, respectively. "Nasal" tests were made by observers at each of 11 stations en route, and the treatment wave passage required an estimated 3 to 4 hours. Vegetation and snails were exposed to a dosage range of 3-26 ppm for 3.25 hours, sufficient to clean the channel of vegetation and kill Australorbis up to the 20-mile station. Beyond the 20-mile station, the concentration dropped below 3 ppm. Vegetation destruction required up to 1 week for maximum effects. Marisa snails were unharmed. After 1 month, green filamentous algae were the first plants to return and snail colonies were recovering. Colonies had been washed in from above the injection point and had been rescued from drying on banksides by prompt return of the channel to normal flow (approximately 50 c.f.s.). This study demonstrated clearly that the application of acrolein from a single point easily removed weeds for 20 miles. Such an operation formerly required expensive manual labor. In addition, acrolein also destroyed snails in the channel.

Two farm pond studies completed this series. With the cooperation of the Puerto Rico Agricultural Experiment Station at Arenales, during the refilling from the Guajataca Division Canal, acrolein was metered at 2-3 gal. per acre-foot into an earthen tank of 0.6 acres. Treated water covered a medium dense mat of Chara on the bottom, which harbored a sparse colony of Australorbis. At the completion of treatment, the concentration was 4-6 ppm at several marginal points. A complete kill of algae and snails, plus fish and toads was observed 1 week later. In another similar test. made at Colonia Fortuna (Amadeo Pond), a larger volume of water (2-3 acre-feet) was treated with 10 ppm of acrolein, intended to kill a very dense mat of Najas-Spirogyra mixed with grasses and cattail. Distribution of the chemical within the mat was poor, and only the surface and edges of the mat were "burned" or decolorized. Snails were killed, however. Application was made at refilling and required 200 The exposure time was approximately 24 hours before it was necessary to use the water. Tests showed 5, 3, 2, and 1 ppm at four marginal points of the earthen tank at 3 hours, but the dosage-time factor was not sufficient for adequate penetration of and harm to the algal mat.

Discussion

The trials in Puerto Rico demonstrated that acrolein is outstanding in those habitats where waterflow is fairly rapid and uniform (for example, irrigation canals and selected streams). Where water is slow moving and ponded, acrolein exhibits the same problem of limited downstream travel as other molluscicides. Equipment for canal injections was well proved, but the problem remains of application for streams with interrupted flow.

Acrolein molluscicide has unusual qualities when compared with either PCP, NaPCP, DCHP, or copper compounds. Proper distribution is necessary for all of them. Based on the Patillas Canal trial, application of acrolein at a minimal rate of 3 ppm appears to kill in the field, whereas the other compounds require a higher dosage in the field than in the laboratory. There is no precipitation in the use of acrolein, and it acts as its own best marker since even a few parts per million in the water

provide sufficient vapor for nasal detection. Biologically, it is most active at higher temperatures. Acrolein kills eggs quickly and efficiently in the laboratory, and this characteristic may possibly have significant carryover for field conditions. Raising the NaPCP dosage above 10 ppm under both field and laboratory conditions increases the avoidance or "crawl out and wait" tendency of Australorbis, but with increased acrolein dosages in laboratory tests there is no such tendency. Although "open jar" tests were used for comparing molluscicidal action of vaporous acrolein with nonvolatile NaPCP as cited, "closed jar" conditions for acrolein are significantly more harmful to Australorbis. This factor probably will be of no advantage under existing field conditions.

Probably of more academic than practical interest is the fact that NaPCP kills schistosome eggs, miracidia, and cercariae at dosages of 10 ppm (13), while there is preliminary indication that acrolein accomplishes kills of schistosomes at somewhat lower dosages. In laboratory tests, 2.5 ppm was effective against cercariae and miracidia in 20 minutes for both acrolein and NaPCP. With reference to toxicity, it is possible that above-molluscicidal dosages NaPCP might not be detected by either persons or animals drinking treated water. Even low dosages (3 ppm) of DCHP exhibit a striking yellow warning color, and it is assumed that the above-molluscicidal dosage would be readily visible to water drinkers. It is inconceivable that any normal individual would not be instantly warned of the presence of an excess dosage of acrolein in domestic water supplies.

There are gross indications that presently used molluscicides are harmful to fish. Acrolein appears to be more rapidly destructive to fish than the other compounds.

The data indicate that the use of acrolein for control of schistosomiasis is worthy of extended field trials under a variety of conditions in other endemic countries where it must compete favorably with the present compounds of choice within the halogenated phenol or copper ion groups (7). Acrolein combines for the first time efficient destruction of underwater vegetation, snails, and snail egg masses, a progressive step in field experimentation for abatement of schistosomiasis. Engineering development of

the technique to widen its application against various intermediate snail hosts is a predictable next move.

Summary

During the search for an efficient, submerged aquatic weed killer in the western United States, acrolein (CH2:CH.CHO) not only demonstrated the singular ability to destroy weed mats for many miles downstream in irrigation systems from a single dosage station but it also obliterated aquatic snails. Preliminary field and laboratory studies of the effectiveness of acrolein against Australorbis glabratus in Puerto Rico confirmed these findings for irrigation channels and farm ponds but indicated the need for further study of application methods in those ponds with excessive mats of vegetation and in streams with impounded sections. The material is volatile and requires careful handling in field applications. However, with techniques and equipment designed for handling and application, trained personnel can safely use it.

Acrolein is a valuable adjunct to other molluscicides in use for control of schistosomiasis. It is characterized primarily by its rapid and efficient action against both adult snails and egg masses and by its ability to destroy snail-protecting vegetation. Thus, it may solve the problem of submerged aquatic vegetation which lowers the carrying capacity of irrigation channels, impedes the regulation of flow, favors sedimentation, and increases loss of water by transpiration and evaporation. At the same time, acrolein may remove the threat of schistosomiasis from expensive plant and animal watering systems.

SUPPLY REFERENCE

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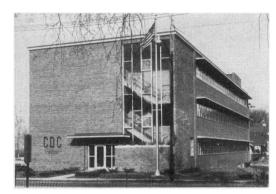
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New Home for Kansas City Field Station



A new laboratory, adjoining the University of Kansas Medical Center at Kansas City, Kans., houses the Kansas City Field Station of the Communicable Disease Center, Public Health Service.

One of 13 in the country, the Kansas City station was established in 1951 under the direction of Dr. M. L. Furcolow to conduct field investigations of fungal infections, especially histoplasmosis, blastomycosis, and cryptococcosis. Intensive studies are also conducted on respiratory and intestinal viruses, with emphasis on poliomyelitis and poliomyelitis-like diseases under the direction of Dr. Tom Y. Chin.

Dr. Furcolow now has a staff of 34 in the new building, including an assistant chief, four epidemic intelligence service officers, a veterinarian, a nurse, two virologists, two mycologists, a biochemist, a statistician, and an administrative officer.