# TECHNICAL PAPERS

## Effect of Urethane on Mouse Myelogenous Leukemia[1]

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It has been reported that the administration of urethane to human leukemic patients produces an effect similar to that following treatment with X-rays (*2*). In preliminary experiments the authors have investigated the effects of urethane on normal and leukemic myeloid tissues of mice.

The leukemia used in this investigation was a transplanted myelogenous chloroleukemia in the fifth transfer generation. The disease developed was a chronic leukemia with the white blood-cell count always rising to more than 100,000 cells/mm.[3] This transfer line was derived from a spontaneous leukemia which had arisen in an $F_1$ hybrid mouse between strains F (high leukemia) and NH (low leukemia), transfer has always been into mice of this hybrid genetic constitution. The disease developed in 76 of 77 mice inoculated, and there were no spontaneous recoveries. Six to eight weeks after inoculation, white blood-cell counts were decidedly elevated, and the spleen was palpable. Mice survived as long as four months following the intraperitoneal inoculation of leukemic cells suspended in isotonic saline. The blood picture was typical of myelogenous leukemia, with stem cells and all intermediate forms down to mature leucocytes. Anemia developed as the disease progressed to fatal termination.

Urethane[3] in aqueous solution was administered intraperitoneally, the daily dose ranging from 0.004 to 1.0 mg./gram of body weight. The dose per gram of mouse was contained in 0.01 cc. of distilled water. After three daily doses of either 0.5 or 1.0 mg./gram of body weight (the latter is an anesthetic dose), white blood-cell counts dropped from over 100,000 cells/mm.[3] to normal levels, the only mononuclear cells of the circulating blood being lymphocytes. Spleen and lymph nodes were definitely reduced in size, although microscopic examination revealed that these organs, the lung, and liver contained leukemic cells. The cellular picture of the bone marrow was altered. In contrast to the decided left shift in white cell development characterizing the marrow of mice with myeloid leukemia, many mature leucocytes were now present. A similar alteration was noted after X-ray therapy (three to five daily doses of 80 r to the whole body), with the counts also dropping to normal levels. Reduction in size of spleen and lymph nodes was greater, however, following urethane treatment. The effect of urethane was similar in all of 13 mice treated with this dose. This transplanted leukemia failed to respond in a similar manner to either Fowler's solution, benzol, or colchicine.

Within three days after daily treatments with urethane in doses of either 0.5 or 1.0 mg./gram of body weight, leukemic mice lost as much as 7 grams (initial weight, 25–28 grams). When the daily dose of urethane was reduced to 0.2 mg./gram of body weight, leucocyte counts still dropped, but weight loss was not appreciable. Counts fell from 150,000–200,000 to 50,000 white blood cells within 7 days, but the lymph nodes and spleen were not decreased in size. This dose of urethane proved ineffective in maintaining the low leucocyte counts previously induced by larger doses. Although the white blood-cell counts rose, and spleen and lymph nodes increased in size, the percentage of mature leucocytes in the blood was greater than that seen in untreated animals. Larger doses of urethane again brought down the white counts and reduced the size of spleen and lymph nodes.

A single dose of 0.1 mg. of urethane/gram of body weight effected a significant dip in the white count in the first 24 hours, followed by a prompt rise to the initial level. There was no further response to the daily administration of this small dose. Doses of less than 0.1 mg. did not alter the white counts.

Experiments are being undertaken to determine whether life can be prolonged when leukemic mice of this transfer line are treated with urethane. In future experiments treatment will also be started earlier in the development of the transplanted disease. Mouse leukemia should provide favorable material with which to determine under controlled conditions the possible effect of urethane on survival time in the human disease.

Two cases of spontaneous mouse leukemia have also been treated with urethane. One, a lymphatic leukemia with a white blood-cell count of 63,000, failed to respond to daily injections of 0.2 mg./gram of body weight. The second, a myelogenous leukemia, responded in the same manner as the transplanted disease, exhibiting a depression in leucocyte count from 82,000 to 20,000 within a week, and decrease in size of the spleen.

It is of interest that in these experiments the response of mouse leukemia to urethane was comparable to that seen in human leukemia (*2*). Urethane has been shown to retard the growth of certain neoplasms of the mouse and human, although the alteration was not permanent (*1*). In the present experiments it was noted that if treatment with urethane was discontinued, the white blood-cell counts became elevated, and spleen and lymph nodes enlarged. In two instances, however, the counts remained depressed for almost two weeks after cessation of treatment. Two weeks is a relatively long time in the course of mouse leukemia.

Urethane induced a depression in the leucocyte count of nonleukemic human subjects (*2*). In normal mice doses of 0.5 or 1.0 mg./gram of body weight produced a similar effect, but neither the drop in leucocyte count nor loss of body weight

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[3] Urethane Merck (ethyl urethane-ethyl carbamate).

appeared as rapidly as in leukemic mice similarly treated. Daily doses of 0.2 mg. or less/gram of body weight given for three weeks proved to be nontoxic for normal mice. Doses of urethane which affected leukemic marrows produced no striking cytologic alterations in the bone marrow of normal mice.

### References


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## The Role of Arsenic in the Production of Alcoholic Polyneuritis


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In view of the experimental work which led to the discovery of BAL (British anti-lewisite) it might be worth while to mention some unpublished results obtained on the urinary excretion of arsenic in patients suffering from alcoholic polyneuritis. The signs and symptoms of arsenic polyneuritis are so similar to those seen in alcoholic polyneuritis that it seemed possible that arsenic as well as alcohol with its concomitant avitaminosis played a role in the latter. Twenty-four patients on the wards and 5 from the Outpatient Department of the Boston City Hospital were studied for urinary arsenic excretion. Fourteen of these suffered from alcoholic polyneuritis, while 15 patients were utilized as a control group.

Six of the 14 patients had paralysis and loss of sensation of all four extremities; 5 had a moderate, and 3 a mild, type of polyneuritis. (The additional diagnosis of Korsakoff's psychosis was made in 5, delirium tremens in 1, and signs of pellagra were present in 2.)

The 15 patients utilized as a control group fell into four groups: (1) those suffering from acute infective polyneuritis; (2) patients suffering from chronic alcoholism without signs of neuritis; (3) a group chosen at random on the ward; and (4) some outpatients who had had alcoholic polyneuritis in the past but who denied the use of alcohol at the time tested.

An attempt was made to collect at least three 24-hour urine specimens from the 24 hospitalized patients. Several had more than 3 specimens tested; the maximum was 11. Only one or more single specimens were collected from the 5 outpatients. The Reinsch test was used on all specimens, and those from 2 patients containing the largest amounts of arsenic were checked by the Gutzeit quantitative test. One patient was excreting as much as .227 mg. of arsenic/1,000 cc. of urine.

The more severe the paralysis, the more positive were these tests for arsenic. Arsenic is not a normal constituent of the body but is present due to ingestion with food. It is possible that alcohol affects the storage of arsenic, which in turn contributes to the interference with enzyme action already present in these malnourished patients.

The 14 patients suffering from alcoholic polyneuritis were consistently excreting significant amounts of arsenic in the urine, while the 15 in the control group showed only an occasional trace of this element.

## Mass Mortality of Marine Animals on the Lower West Coast of Florida, November 1946–January 1947


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Catastrophic mass death involving millions of fish recently occurred on the south Florida Gulf Coast. In the latter part of November 1946, mackerel fishermen noted dead and dying fish and turtles in streaks of discolored water 10–14 miles offshore from Naples. The mortality moved northward and reached Boca Grande by January 10. Fish continued dying in the bays behind Captiva and Sanibel Islands as late as January 29. Investigations carried out since January 15 have resulted in the following observations.

Dead fish, in association with discolored water, were reported from Dry Tortugas to Boca Grande, a distance of 130 miles. At Fort Myers on January 19 the beach was littered with fish in excess of 170/foot of shore line, in addition to those floating on the water in bays and sounds and to a distance of 10 miles offshore. One homeowner on Captiva Island reported burying 60,000 fish from 200 feet of bay beach. The same area had to be cleaned on three other occasions. The total number of dead fish over the whole area was estimated to be over 50,000,000. Oysters, clams, crabs, shrimp, barnacles, and coquinas were also killed. The clam industry at Marco, 50 miles south of Fort Myers, and the sponge industry north of Tampa Bay do not appear to have been involved, nor, in spite of an isolated report of dead fish from a vessel passing Dry Tortugas, does the mortality appear to have reached the Florida Keys. All kinds of fish succumbed. Mackerel seemed to be relatively unaffected, but many mullet were killed. Floating carcasses of black drum, tarpon, groupers, and large jewfish were the most spectacular sights.

Reports of the phenomenon were not received by the writers until the middle of January. From January 18 onward, the water was sampled chemically and with plankton nets near Sanibel Island, where dying fish were encountered; at Fort Myers Beach, where dead fish only were found; and at Naples, where the water was beginning to clear up and the fishermen were making mackerel catches.

Reddish-brown discoloration of the water was observed off Fort Myers Beach. Plankton examination showed this to be unusually rich, consisting predominantly of copepods and invertebrate larvae with little phytoplankton. At Clam Bay dying fish were seen in streaks of greenish-yellow water. These streaks contained large quantities of diatoms with *Coscinodiscus* sp. as the dominant organism, together with considerable detritus and smaller numbers of naked flagellates resembling species of *Gymnodinium*. The water at Naples, which was not discolored, contained copepods and a great abundance of *Rhizosolenia* sp. No one species was overwhelmingly predominant at all stations, but the organisms tentatively identified as *Gymnodinium* sp. were found in varying numbers in samples from the areas where fish were still dying.


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