TECHNICAL PAPERS

Pulmonary Edema in Leucemic Mice Following Treatment With Urethane

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In view of the recent report by Webster (*7*) regarding the toxic potentialities of urethane in the treatment of leucemia, it was felt desirable to present a preliminary report of some of the toxic manifestations noted in a study of urethane and its effect on transplanted leucemia in the mouse.

Until recently, urethane has been described as non-toxic when used in therapeutic doses (*1*) (adult hypnotic dose: 2–4 gm). Because of its feebly hypnotic effect on adult human beings, it was seldom used except as an anesthetic agent for laboratory animals, with the result that few observations on the extended use of urethane in man are available. Certain isolated effects in laboratory animals and in human beings have been reported, however, among them being capillary changes, which are the chief concern of this paper.

Sollmann (*6*) in 1917 reported the formation of wheals when urethane was applied to scarified skin. Krogh and Harrop (*3*) in 1921 described the effects of local applications of 5 and 25% solutions of urethane on capillaries of the frog's tongue. Dilatation and increased permeability were noted, progressing to a stage they called "stasis"—a condition of increased concentration of the blood corpuscles due to rapid loss of plasma. The increased viscosity of such blood caused the flow to be sluggish and finally to cease as the venous capillaries became filled with plugs of tightly packed corpuscles. Krogh and Harrop expressed the belief that the effects were due primarily to capillary dilatation.

Landis (*4*) in 1927, using a micropipette technic, perfused mesenteric capillaries of the frog with dilute solutions of urethane and observed changes in capillary permeability similar to those noted by Krogh and Harrop. He expressed the opinion, however, that this resulted from a direct toxic effect of the urethane on the endothelium and from increased capillary pressure due to blockage of venous capillaries.

Kirschbaum and Bell (*2*) in 1947 reported the induction of glomerular lesions by urethane in mice of the NH strain, a strain in which spontaneous glomerulonephritis develops. In this instance the glomerular capillaries especially seem to be susceptible to the effects of urethane.

We have not encountered in the literature any reference to the state of pulmonary capillaries of experimental animals following urethane anesthesia. However, Moon and Morgan (*5*) in 1936 studied the lungs of dogs in which a state of both continuous and intermittent narcosis by sodium phenobarbital was induced. They likewise found evidence of increased capillary permeability. This progressed to marked pulmonary edema, leading to shock and death in the most severe forms, bronchopneumonia in the less severe, and recovery when edema was very mild.

In our experiments, the effect of urethane on transplanted myelogenous leucemia produced in F–NH hybrid mice was studied. Three groups of mice previously inoculated with suspensions of a leucemic spleen were selected for study when their leucocyte counts had arisen to the neighborhood of 200,000 cells/mm³ of blood. Urethane was administered intraperitoneally to the first group in daily doses of 0.5 mg/gm of body weight. It was administered similarly to the second group at a level of 1.0 mg/gm of body weight. The third group was divided into three subgroups. Subgroup A received 0.75 mg/gm of body weight daily; subgroup B, 1.0 mg/gm every second day; and subgroup C, 1.0 mg/gm every third day.

TABLE 1

DATA ON TREATMENT OF LEUCEMIC MICE WITH URETHANE

Strain of mice	Medication	Dose/gm of body weight*	Percentage incidence		Average weight of lungs (mg)
			Edema	Hydrothorax	
Normal C	Physiologic saline	0.01 cc daily	0.0	Not recorded	140.6
Normal C	Urethane	1.0 mg daily	100.0	Not recorded	384.6
Leucemic F–NH	Physiologic saline	0.01 cc daily	0.0	0.0	237.5
" "	Urethane	0.75 mg daily	100.0	73.7	394.1
" "	"	1.0 mg every second day	81.8	41.6	392.7
" "	"	1.0 mg every third day	83.3	25.0	339.1

* The anesthetizing dose of urethane in the mouse is generally considered to be 1 mg/gm of body weight.

Injections and observations were continued until death of the animal or until the approach of the moribund state, when animals were killed for study.

In addition, urethane was administered daily to normal C strain mice in amounts similar to those given to the second group of leucemic mice.

Among other criteria of the effectiveness of the drug, the life span of each animal was of considerable importance. This measurement was markedly affected in the first series of mice by the development of pneumonia in three-fourths of the treated animals. It was not realized until the pathologic sections were studied that this pneumonia was superimposed on, and probably was secondary to, a relatively mild pulmonary edema, which was present in all the treated animals. In the second series, which received the largest amount of urethane, the life span was also reduced by the development of pulmonary edema of a more severe and more rapidly fatal variety in all of the animals. This occurred not only in the leucemic but also in the normal C strain animals receiving similar doses of urethane. In the third series of animals, graded doses of urethane were used in an effort to attain control of the leucemia without the development of pulmonary edema. Doses sufficiently large to result in a reduction of the total leucocyte count with a shift to the right and a reduction in the sizes of the liver, spleen, and lymph nodes caused pulmonary edema in all of the cases. The smaller doses of urethane were ineffectual in maintaining an initially favorable response, and even these ineffective amounts resulted in the development of pulmonary edema of lesser degree in many of the animals. In this latter group, death resulted, if not immediately from the edema, several days later from pneumonia. Other evidences of capillary damage throughout the body were seen grossly in a large percentage of these cases.

At necropsy, animals with pulmonary edema frequently showed the customary accumulations of pinkish-brown, frothy material about the nares and mouth. On opening the thorax, fluid, usually of a serosanguineous nature, was frequently encountered. The lungs were always tense, completely filling the thorax. Their appearance varied from a pinkish-brown, almost translucent character to a mottled bright and deep red. Petechiae were often seen. On section of the trachea, as well as of the lung parenchyma, large quantities of fluid exuded, after which the lung became quite compressible. Microscopically, the alveoli and the bronchi were distended and filled with a pink-staining homogeneous material. There was marked capillary engorgement with evidence of rupture and release of erythrocytes into the alveoli. Areas of bronchopneumonia were present in animals in which the pulmonary edema was less severe and not immediately fatal.

Although edema in these animals was almost entirely confined to the lungs, other evidences of capillary damage were noted. The axillary and inguinal regions were, in nearly all cases, sites of subcutaneous petechiae and frequently of localized anasarca. The site of intraperitoneal injection (constant because of anatomic reasons) often revealed subcutaneous petechiae progressing at times to ecchymoses. From the low incidence in the untreated controls it may be assumed that these phenomena are not due solely to thrombocytopenia secondary to leucemia. The comparative gross data relative to the foregoing observations are presented in Table 1.

Although we realize that observations on laboratory animals can seldom be applied directly to man, it is the aim of this paper to increase the awareness of the toxic potentialities of urethane, when used over a period of time, particularly with respect to capillary damage.

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Use of Radioactive Diiodofluorescein in the Diagnosis and Localization of Brain Tumors[1]

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The use of fluorescein has recently been suggested as an aid in the diagnosis of malignancy (*1*). In the initial report it was observed that brain tumors appeared to exhibit a consistent special affinity for the absorption of previously injected fluorescein.

In an attempt to extend the clinical usefulness of the fluorescein technique, radioactive derivatives of the dye have been prepared. Since it is considered safe to use, for clinical purposes, only those isotopes with a short half-life, and since the detection of deep-seated intracranial lesions requires the emission of gamma radiation, diiodofluorescein was synthesized to contain I^{131}. The amount of I^{131} added was adjusted to give 1 mc of radioactivity/10 cc of a 2% solution of the final product, sodium diiodofluorescein. An amount of dye calculated to contain 500–600 μc of radioactivity was injected intravenously in each case. In order to give a comparable dose of radioactivity to patients on subsequent days,

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