TECHNICAL PAPERS

Inhibitory Effects of Ethyl Carbamate on Prostatic Cancer[1]

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The antiandrogenic treatment (*7*) of human prostatic cancer is not completely satisfactory, since remission of the disease was less than 5 years in duration in 80 per cent of the cases (*6*) so treated. We have investigated many possible chemotherapeutic agents in patients in whom relapse has occurred after endocrine control, but have encountered only one effective agent in ameliorating the disease when it has become androgen independent and wide spread—ethyl carbamate. Prostatic cancer may be studied simply by enzymatic methods, since the phosphatases of serum constitute sensitive and objective indices of neoplastic activity.

Warburg (*12*) showed that phenyl urethane in small amount caused an arrest of mitosis and cell division in fertilized eggs of the sea urchin with only small change in oxidation in the cells, while a concentration of 0.002N reduced oxygen consumption about one-half. Ethyl carbamate (*4*) nearly abolished mitosis in the cornea of the rat for brief periods. The present interest in urethane in tumors arose through the work of Haddow and his associates; ethyl and isopropyl phenyl carbamates (*5*) were found to cause a temporary but significant retardation of growth of spontaneous mammary cancer in the mouse and in the Walker carcinoma 256 of rats; in the latter tumor, ethyl carbamate also had an inhibitory effect. Paterson and co-workers (*10*) discovered that ethyl carbamate had a very great palliative effect in human leukemia; there was also some regression in 3 of 13 cases of mammary cancer. Ethyl carbamate in doses of 0.1–1 mg./gram caused a decrease in the leucocyte count in myelogenous leukemia (*2*) in the mouse. Goodman and Lewis (*3*) observed that this agent caused regression of cutaneous metastases of a highly anaplastic carcinoma in one patient; the primary site of the tumor was unknown. We wish to present four observations which prove an effect of ethyl carbamate on prostatic cancer and which deal with its mode of action.

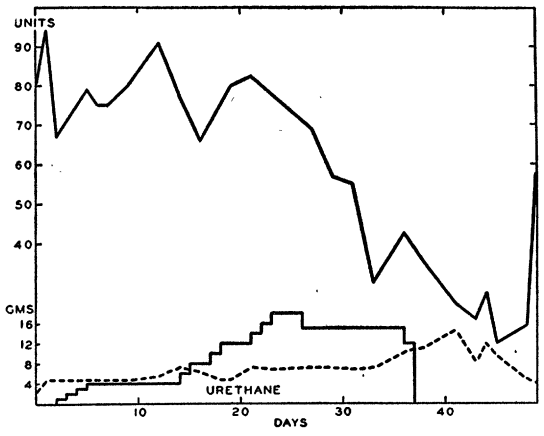

FIG. 1. Changes in acid phosphatase (———) and alkaline phosphatase (----------) of serum induced by toxic amounts of urethane.

(1) *Ethyl carbamate is a toxic drug which causes inhibition of some cases of prostatic cancer.* In one patient an average daily dose of 9 grams of ethyl carbamate was administered for 33 days with fatal effect. At first there was improvement, with a regression in the size of the prostate, return of sensation in a saddle area of anesthesia due to metastatic involvement of the cauda equina, and a decrease of the acid phosphatase level (Fig. 1) of the blood from 90 to 10 King and Armstrong (*9*) units; however, 6 days after discontinuing the medication, icterus and other evidence of liver damage became evident, and the patient died 6 days later from hepatic necrosis, there being a terminal rise of acid phosphatase. In three patients with wide-spread cancer of the prostate there has been a considerable regression of the primary tumor, relief of pain, an improved sense of well-being, and a significant decrease of the acid phosphatase of the serum (Fig. 2).

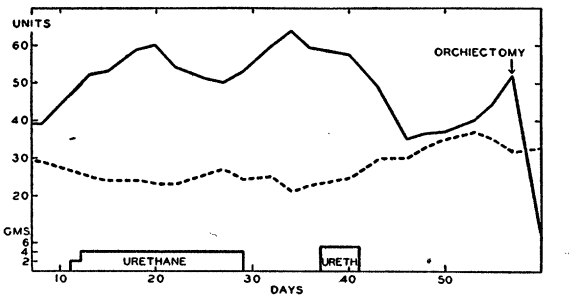

FIG. 2. Decrease in acid phosphatase (———) of serum induced by urethane administration and later by orchiectomy in man. The reciprocal changes in alkaline phosphatase (----------) are indicated.

Effects on the tumor are demonstrable with smaller doses of ethyl carbamate than are required to cause profound changes in the acid phosphatase level where the dosage required approaches the toxic amount. A patient who had an advancing prostatic cancer following a remission induced by orchiectomy, estrogen administration, and roentgenotherapy was found to be bedridden with a large indurated primary tumor and multiple osseous metastasis; 4 grams of ethyl carbamate daily for 5 days, followed by a maintenance dose of 1 gram daily for 6 weeks, eliminated pain and caused great decreases in the size and hardness of the tumor.

Ethyl carbamate must be administered with great caution,

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and the leucocyte count of the blood determined frequently. It is not considered safe to continue ethyl carbamate when the leucocyte content is less than 4,000/mm.^{3} or when nausea and vomiting occur.

(2) *The favorable effects of ethyl carbamate are not due to anti-androgenic action.* The prostatic isolation operation (*8*) was done, and the prostatic secretion collected at 2-day intervals in 3 castrate dogs which received both testosterone propionate (10 mg.) and urethane (4–8 grams) daily for 31 days; the amount of the prostatic secretion increased steadily, proving that the effects of androgen on the prostatic cells were not eliminated.

(3) *Ethyl carbamate does not inhibit glycolysis.* It is well known that tumors are characterized by a high rate of aerobic and anaerobic glycolysis. We have found that urethane in concentrations up to 0.005M had no demonstrable effect on the rate of anaerobic glycolysis in cell-free brain extracts supplemented with glucose and the system described by Utter, Wood, and Reiner (*11*). Lactic acid production was measured manometrically (Table 1). It was concluded that urethane did not inhibit the rate-determining reaction of glycolysis in this particular cell-free system.

TABLE 1

EFFECT OF ETHYL CARBAMATE ON ANAEROBIC GLYCOLYSIS IN RAT BRAIN EXTRACT*

Exp.	Controls	With ethyl carbamate (0.005M)
1	99	107
	106	103
2	100	87
	87	95
3	102	100
	93	95
	104	104

* Results are expressed in microliters of CO_2 liberated in one hour corresponding to lactic acid produced.

Method of Utter, Wood, and Reiner (*11*). The main compartment of the Warburg vessel contained: adenosine triphosphate, 0.0007M; diphosphopyridine nucleotide, 0.0005M; magnesium phosphate, 0.008M; sodium bicarbonate, 0.048M; nicotinamide, 0.04M; phosphate buffer, pH 7.4, 0.01M; 0.05ml. of rat brain extract; and either 0.005M ethyl carbamate or water. The side-arm flask contained glucose and hexose diphosphate to make the final concentration 0.028M and 0.0025M, respectively, in the complete system; final volume, 1 ml.

(4) *Ethyl carbamate has a suppressive effect on the transplantable Sarcoma 39 of rats.* This polymorphous cell sarcoma of albino rats has a 50–90 per cent incidence of takes in our laboratory. The tumor was inoculated subcutaneously in 26 rats, and ethyl carbamate (0.5–1 mg./gm.) was injected daily for 7 days and then at 2-day intervals for 14 days; as a control, 21 rats were inoculated but not injected with urethane. None of the experimental group developed neoplasms, while 12 of the controls grew large tumors. One week after cessation of urethane, 2 of the urethane group developed tumors. Since the dosage of urethane induced anesthesia and weight loss, a further group of controls was established to eliminate the adverse effect of malnutrition on the tumor. Eight rats were inoculated with this tumor and maintained on complete food starvation for 14 days; 4 rats developed tumors on the 9th to the 13th day of starvation.

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Hexokinase Activity and Diabetes Mellitus

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The observations of Price, Cori, and Colowick (*1*) that muscle and liver hexokinase are inhibited, *in vitro* by certain fractions of the anterior pituitary gland and that this inhibition is prevented by the addition of insulin seemed to provide the explanation of the mechanism whereby "diabetogenic" extracts of this gland produce diabetes in susceptible animals. The subsequent observation that a decrease in the hexokinase activity of the muscles and liver occurs in the alloxanized rat (*2*) seemed to imply support to the concept that diabetes is associated with an increase in anterior pituitary activity. During an investigation of the action of various tissue extracts on the activity of muscle and liver hexokinase, we have made observations which question the validity of such a concept.

Hexokinase-containing extracts were prepared according to directions generously furnished us by Colowick (personal communication) and their activity assayed both by the manometric estimation of CO_2 production and by direct analysis of the transfer of easily hydrolyzable phosphate.

The extracts displayed considerable hexokinase activity by either manometric or chemical criteria. In confirmation of the results of Price, Cori, and Colowick, it was possible on occasion to demonstrate the inhibition of such activity after addition of the diabetogenic extracts of pituitary and, further to note the reversal of this inhibition after addition of insulin. Of more than passing interest, however, was the inconstancy of the inhibitory activity of the anterior pituitary extracts. Many apparently identical preparations, known to contain potent diabetogenic factors, failed to produce any inhibition of hexokinase activity (Table 1).

Inasmuch as some anterior pituitary extracts known to possess "diabetogenic" activity may fail to inhibit hexokinase activity (in crude extracts) while other extracts, prepared in an identical manner, may suppress this activity, it is possible that the anterior pituitary gland may contain an hexokinase-inhibiting factor quite distinct from its "diabetogenic" factor