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Phytopharmacological Experiments with Urethane.

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Ethyl carbamate or urethane has been known to pharmacologists for a long time. In fact, its physiological properties were first described by Schmiedeberg,[1] the father of modern pharmacology, who was the first to point out its narcotic properties.

At the beginning of the twentieth century it was quite extensively employed as an anesthetic for laboratory animals and as late as 1929 Macht and Hill[2] called attention to its usefulness for experimenting on small animals such as rats. Clinically, its usefulness was favorably reported towards the end of the nineteenth century as a hypnotic causing very little depression of the circulation and respiration.[3-7] The drug became popular in medical practice until the introduction of the powerful barbiturates which supplanted urethane on account of its rather weak and uncertain hypnotic effects on human beings. Nevertheless, the drug has been occasionally employed in psychiatric practice.

More recently, a new property of this organic chemical compound has been discovered by Haddow and Sexton, who investigated the action of urethane in connection with their studies on the influence of various drugs on the growth of malignant tumors. These studies revealed that while ethyl carbamate produced inconclusive effects on the growth of malignant cells, it did produce a very marked fall in the leucocyte count.

Prompted by this discovery, Paterson, Haddow, Thomas, and Watkinson[8] investigated the effects of urethane on the blood of leukemia and noted in 32 cases of human leukemia a decrease in the total white blood cell count and the return to a normal differential pattern of the white cells. Similar findings were reported by Engstrom, Kirschbaum, and Mixer[9] in their experiments with myelogenous leukemia in mice. More recently, similar studies were reported by Law concerning the effect of urethane on transplantable acute lymphoid leukemia, and further studies were described by Weir and Heinle.

Hirschboeck, Lindert, Chase, and Calvy[10] found little effect produced by urethane on malignant tumors but they did observe marked action of the chemical on leukemic tissues leading to satisfactory remissions in some human patients.

The explanation of this interesting property exhibited by ethyl carbamate is still not clear. Law, studying the effects of urethane on transplantable lymphoid leukemia, concludes that immature lymphoblasts are significantly decreased in leukemia patients and hemoglobin values remain at higher levels in patients treated with the drug. He also found that the growth of the local subcutaneous tumor masses was retarded.[11] Kirschbaum and Lu demonstrated that urethane decreased the number of mitotic figures in the marrow

1 Schmiedeberg, O., *Arch. f. exp. Path. u. Pharmokol.*, 1885, **20**, 203.

2 Macht, D. I., and Hill, P. S., Jr., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 871.

3 Andrews, J. B., *Am. J. Insan.*, 1886, **43**, 256.

4 Huchard, H., *Bull. gen. de therap.*, 1886, **110**, 103.

5 Hübner, C., and Sticker, G., *Deutsche med. Wchnschr.*, 1886, **12**, 236.

6 Sieveking, E. H., and Manifold, C. C., *Brit. M. J.*, 1886, **2**, 108.

7 Binet, P., *Rev. med. de la Suisse Rom.*, 1893, **13**, 540, 628.

8 Paterson, E., Haddow, A., Thomas, I., and Watkinson, J. M., *Lancet*, 1946, **1**, 677.

9 Engstrom, R. M., Kirschbaum, A., and Mixer, H. W., *Science*, 1947, **105**, 255.

10 Hirschboeck, John S., Lindert, M. C. F., Chase, Jules, and Calvy, Thomas L., *J. A. M. A.*, 1948, **136**, 90.

11 Law, L. W., *Proc. Nat. Acad. Science*, 1947, **33**, 204.

myeloid cells and reduced the total number of circulating white cells, especially myeloblasts, in transplanted myeloid leukemia of F strain mice, while Moeschlin[12] claims that in the treatment of leukemia with urethane there is noticed an elective inhibition of the karyokinesis of leukemic neoplastic cells.

The present author for the past 25 years studied the action of various chemicals, drugs, and toxins on plant cells as compared with animal cells. These studies which have been carried on in a quantitative experimental fashion on various plants and especially on the root growth of *Lupinus albus* seedlings have been described in detail.[13,14] It was deemed worthwhile, therefore, to examine the effects of urethane by phytopharmacological methods and the results are reported briefly. This was deemed especially worthwhile because of the recent announcement by Giles that radioactivity produces marked changes in plant cells.[15]

Method. The biological criterion employed was the elongation or growth of the well-defined straight roots of Lupinus seedlings, studied by the author's phytopharmacological methods, details of which have been published. The procedure was briefly as follows:

Seeds of *Lupinus albus,* large variety, were soaked in tap water overnight and planted in finely ground sphagnum moss containing adequate moisture. After germination, the growth of selected seedlings with roots 35 to 50 mm long was studied in a solution consisting of equal parts of distilled water and Shive physiological saline.[16] At least 20 seedlings, placed in hard-glass test tubes, were employed for the control solution and for each drug solution, in the different concentrations used. The sharply defined straight roots were measured at the beginning and end of every experiment, and the ratio of growth of the seedling roots in the various drug solutions is expressed as a percentage of their growth in the control solution according to the formula,

$$\text{Index of growth} = \frac{X}{N} \times 100$$

in which N represents average growth of controls and X the average growth in the drug solution. All of the seedlings were allowed to grow for 24 hours in the dark at 15°C.

Results. In Table I are shown the results obtained with different concentrations of urethane on the growth of *Lupinus albus* seedlings studied in a manner described above. It will be seen that normal seedlings are very little affected by the drug. Even solutions 1:1000 are inhibited only slightly, the index of growth being on the average above 90%.

A very different effect of urethane was obtained by the use of seedlings raised in a different manner. The present writer has been making studies on the comparative reaction of normal seedlings on the one hand and so-called vernalized or "yarovized" seedlings of *Lupinus albus.* Yarovization is a term introduced by Russian plant-physiologists to describe a phenomenon produced by exposing plants to extreme variations of temperature.[17] A simple and efficient method for obtaining yarovized seedlings is to expose them to quite low temperature but not such as would freeze the plant. Such plants obviously are markedly changed in their metabolism and the author has found that such seedlings may respond differently to certain blood sera and drugs from the responses given by normal seedlings, even though both the normal and the previously yarovized plants are allowed to grow at normal temperatures when the phytopharmacological experiment is performed. In the present research, Lupinus seedlings were germinated in sphagnum moss and when they were 2 days old, the jar with the young seedlings was placed in a refrigerator for 24 hours at 42°F (5.5°C). These seedlings were then employed simultaneously with other seedlings reared at a normal temperature of 70° and exposed to the action of various concentrations of urethane. Both sets of plants were placed in a low tempera-

12 Moeschlin, Sven, *Experientia,* 1947, **3**, 195.

13 Macht, D. I., PROC. SOC. EXP. BIOL. AND MED., 1945, **60**, 217.

14 Macht, D. I., and Macht, M. B., *J. Lab. and Clin. Med.,* 1941, **26**, 597.

15 Giles, Norman F., Jr., *Proc. Nat. Acad. Sci.,* 1947, **33**, 283.

16 Shive, J. W., *Physiol. Rev.,* 1915, **1**, 327.

17 Miller, E. C., *Plant Physiology,* 2nd Edition, 1938.

TABLE I.
Influence of Urethane on Growth of Seedlings.

Normal Plants.		Yarovized Plants.		Irradiated Plants, 500 r	
Urethane	Phytotoxic index, %	Urethane	Phytotoxic index, %	Urethane	Phytotoxic index, %
1:10,000	105	1:100,000	86	1:100,000	92
1: 5,000	100	1: 75,000	76	1: 50,000	87
1: 2,000	100	1: 60,000	70	1: 25,000	65
1: 1,000	98	1: 50,000	58	1: 10,000	60
1: 500	90	1: 10,000	49	1: 5,000	50
1: 250	72	1: 5,000	36	1: 1,000	25
1: 100	60	1: 1,000	24		
		1: 500	17		

ture thermostat for 24 hours at 60°F (15°C) and the influence of the drug on root growth was compared in the usual manner.

It was found that vernalized plants were extremely sensitive to the effects of urethane, whereas normal seedlings were very little affected by concentrations of 1:1000. The vernalized seedlings were sensitive to solutions of as little as 1:100,000. This is well illustrated in Table I.

A third method of studying urethane on what may be termed abnormal plant cells was by the employment of seedlings treated with deeply penetrating X-rays. Seeds of *Lupinus albus* were germinated in sphagnum and when 2 days old were radiated with X-rays filtered through a composite filter consisting of 1 mm of aluminum and 2 mm of copper. The apparatus used was a therapy machine operated on 200 Kv on a current of 20 ma, the plants being at a target distance of 50 cm. The dosage employed for the most part in the present experiments was 500 r. The results obtained are shown in the third column of Table I. It will be noted that such irradiated seedlings were also rendered much more sensitive to the effects of ethyl carbamate than normal seedlings.

Comment. There is considerable literature dealing with the inhibitory or suppressive action of urethane, phenyl carbamate, and other carbamates on the growth processes of bacteria, protozoa, and various animal tissues, as well as plant tissues. Templeman and Sexton[18] studied the effects of such compounds upon cereals and other plant species. Lefèvre[19] studied the effects of urethane on wheat seedlings, oats, barley, and rye. Both of the above investigators noted cytological alterations, blocking of mitosis, and other profound changes in the plant soil protoplasm produced by the drugs, not unlike those produced by colchicine. As far as the author is aware, however, no pharmacological studies were reported in which abnormal seedlings were compared with normal ones in respect to their reactions to urethane. The present findings, therefore, are of considerable interest, and are of particular interest in connection with the effects produced by urethane on malignant tumor cells and on leukemia blood cells of animals as compared with normal animal tissue cells. It is not at all uncommon in pharmacology to find a difference in reaction to drugs or poisons between normal animal cells and pathological animal tissues.[20] The same has been shown by the present author to be true of plant cells and is strikingly illustrated in the present investigation which reveals such a marked difference in response to urethane by the normal Lupinus seedlings and seedlings which have been yarovized on the one hand and irradiated on the other. These studies should encourage further investigations on the effects of various chemicals and drugs on abnormal cells both of animals and plants with a view of finding possibly some valuable agents that may inhibit the growth of malignant issues.

Summary. Solutions of ethyl carbamate or urethane were studied in different concentrations as regards their influence on root growth of *Lupinus albus* seedlings. It was found

[18] Templeman, W. G., and Sexton, W. A., *Nature*, 1945, **156**, 630.

[19] Lefèvre, J., *C. R. Acad. Sci. Paris*, 1939, **208**, 301.

[20] Macht, D. I., and Ting, G. C., *J. Pharm. and Exp. Therap.*, 1921, **18**, 111.

that whereas normal seedlings are very little affected by the drug, other seedlings of an abnormal type were very sensitive to urethane. Specifically, it was found that yarovized or vernalized seedlings are extremely sensitive to even dilute solutions of the drug and the same was found to be true of seedlings which were irradiated with hard X-rays in doses of 500 r.*

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An Easily Assembled Pulse-Frequency Recorder.

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Continuous, automatic, kymographic recording of pulse frequency conserves time, recording paper, and workers' patience. The device to be described is easily assembled with ordinary laboratory tools and supplies.

As shown in Fig. 1, a heart-lever fulcrum (F_1) is equipped with a tight-fitting, hollow laboratory straw (carrying lever, L_1) bearing an adjustable counterpoise (WE) on the short arm, and the fulcrum (F_2) of a second straw (contacting lever, L_2) on the long arm. The counterpoise is a weighted heart-lever fulcrum. The fulcrum of the contacting lever is a piece of aluminum (lever) wire, wound about and cemented (Duco) to the carrying lever, and passing as a bearing pin in a horizontal hole through the contacting lever. Into one end of the contacting lever is cemented a soldered-wire slip-yolk (Y) which engages the cross (recording) wire (M) of an ordinary recording mercury manometer. Into the other end is cemented a platinum wire which makes contact (C) with a drop of mercury in a tiny platinum-foil cup soldered onto the fulcrum block of the carrying lever. The long arm of the carrying lever is extended with an aluminum wire shaped (*a*) to serve as an adjustable stop (ST) for the contacting lever, and (*b*) as a lateral friction surface of the carrying lever against a lightly contacting, silk thread (FR) rendered slightly taut by the tension of a rubber band at one end. Contact C closes a circuit between the grounded and insulated (INS) binding posts (BP_1 and BP_2) on the insulation-sheathed supporting rod (SU). The conducting wire (WI) from the platinum wire must be fine and flexible.

The contacting lever is balanced on its fulcrum by a suitable combination of fulcrum position and lengths of wires inserted into its ends. The entire system is then balanced on the carrying fulcrum by adjustment of counterpoise, WE. Engagement with the manometer thus has no significant influence on the latter's mean recording position.

To facilitate adjustments, universally adjustable clamps are used for mounting the device proper and the rods between which the friction thread is strung. The stop (ST) for the contacting lever is adjusted by bending so that the lever's freedom of excursion does not exceed the smallest systolic excursion of the manometer apt to be experienced (short of terminal cardio-vascular failure). The light tension of the friction thread is adjusted to just hold the carrying lever at the position to which it is brought by the excess of the manometer excursion beyond the contacting lever's short range. Automatic compensation thus occurs to changing mean and pulse pressures.

In present use, this contacter activates, through a standard-type electronic relay, a modification of the "counter-recorder" previously described.[1] The modification consists of branching the power end of the recording

[1] Winder, C. V., and Moore, V. A., *J. Lab. and Clin. Med.*, 1945, **30**, 894.