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# IS N-HYDROXYURETHANE AN ACTIVE METABOLITE OF URETHANE IN THE RAT?

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**Abstract**—N-hydroxyurethane, a metabolite of urethane produces increases in plasma corticosterone concentration which are similar, initially, in time course to those induced by urethane itself. N-hydroxyurethane does not produce a depletion of adrenaline and noradrenaline from the adrenal medulla, as does urethane. The metabolite induces an increase in brain 5-HT content which urethane does not, and produces a quicker, greater and more prolonged fall in body temperature than does urethane. It is concluded that urethane does not act through its N-hydroxy metabolite.

URETHANE, in anaesthetic doses, provokes the release of catecholamines from the adrenal medulla and the release of corticotrophin with the subsequent discharge of adrenal corticosterone.[1, 2] After administration of urethane to man, rat or the rabbit, N-hydroxyurethane is found in the urine.[3] It is possible that this metabolite is the active form as it has been shown to possess similar carcinogenic properties to those of urethane.[4] The present experiments were designed to test whether the actions of urethane described by Spriggs and Stockham[1] and Spriggs[2] were mediated by the metabolite, N-hydroxyurethane.

## METHODS

Male Wistar rats weighing 140–200 g were kept in single cages at a constant temperature of 23 ± 2° and fed on a diet of cubes (Diet 41B)[5] with water *ad lib.* Blood samples were collected after quick decapitation and centrifuged at 3000 rev/min for 10 min and the plasma obtained was assayed for corticosterone.[6] The adrenal glands were removed, cleaned and weighed; one was assayed for coricosterone content and the other for adrenaline and noradrenaline by the spectrophotofluorimetric method of Shore and Olin.[7] Brain 5-HT was estimated by the method of Udenfriend, Weissbach and Bogdanski.[8]

Rectal temperatures were recorded with a direct reading thermistor. Urethane or N-hydroxyurethane as a 50% (w/v) solution in water was given intraperitoneally.

## RESULTS

N-hydroxyurethane differed from urethane in its action on the central nervous system. N-hydroxyurethane (1·5 g/kg) did not induce anaesthesia but the rats were sedated. Higher doses (1·75–2·5 g/kg) caused anaesthesia but were toxic. Urethane

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also was toxic at this higher dose range. However, urethane, unlike N-hydroxyurethane in a dose range of 1·0–1·5 g/kg caused central depression ranging from deep sedation to stable surgical anaesthesia. Doses ranging from 0·5–1·0 g/kg of N-hydroxyurethane had no observable effect whereas urethane caused varying degrees of sedation.

*Body temperature*

Within 1 hr of injecting N-hydroxyurethane the rectal temperature fell by 4° whereas only a 1° fall was induced by urethane. After urethane, the rectal temperature fell to 31° after 3 hr before recovering and reaching normal after 8 hr. A further fall in body temperature was observed after 24 hr. The fall in temperature of N-hydroxyurethane-treated rats was greater, reaching 28° after 8 hr and more prolonged than that after urethane (Fig. 1). At 24 hr the body temperature had returned to the pre-injection level.

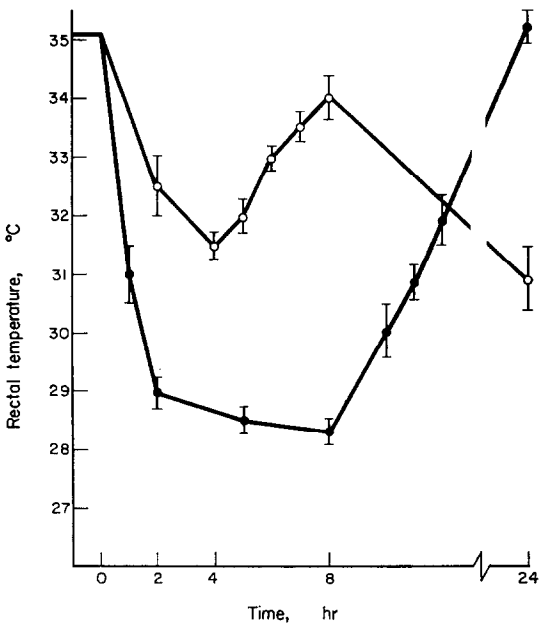


FIG. 1. The hypothermic effects of an intraperitoneal injection of 1·5 g/kg of N-hydroxyurethane (●——●) or urethane (○——○) in rats. Each point represents the mean of at least 6 animals ± the standard error (shown by the vertical lines). Environmental temperature was 23 ± 2°.

*Plasma and adrenal corticosterone*

N-hydroxyurethane induced an increase in both plasma and adrenal corticosterone concentrations. The changes in adrenal corticosterone concentration are qualitatively similar, although quantitatively different from the changes observed after urethane (Fig. 2b). During the first 8 hr after injection of urethane or N-hydroxyurethane the plasma corticosterone responses are both qualitatively similar; after 8 hr, however, the plasma corticosterone level of N-hydroxyurethane-treated animals continued to fall, whereas that of the urethane-treated rats significantly increased above its level after 8 hr (Fig. 2a). The increase in plasma corticosterone at 2 hr—induced by different single doses of urethane—is similar to those caused by the same doses of N-hydroxyurethane (Fig. 3).

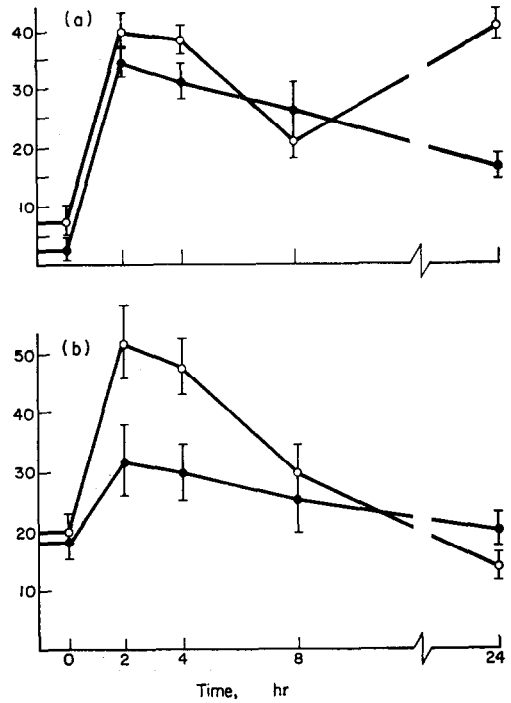


FIG. 2a, plasma corticosterone concentrations, μg/100 ml and b, adrenal corticosterone concentrations μg/g adrenal tissue, after injection intraperitoneally of N-hydroxyurethane (●——●) or urethane (○——○) in a dose of 1·5 g/kg. Each point is the mean ± standard error (vertical line) of at least 4 observations.

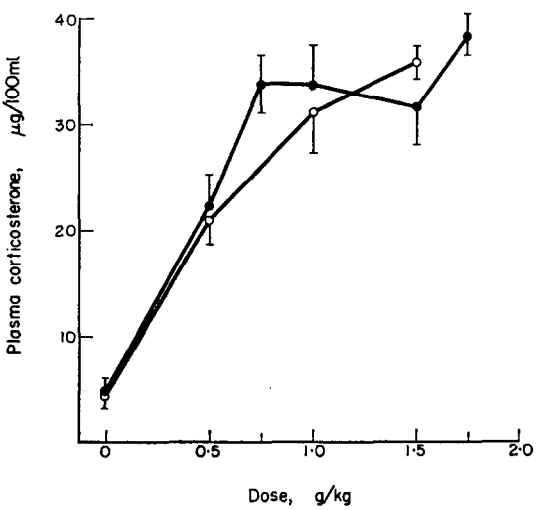


FIG. 3. Plasma corticosterone concentrations 2 hr after the intraperitoneal injection of N-hydroxyurethane (●——●) or urethane (○——○). Each point is the mean ± standard error (shown by vertical lines) of at least 3 observations.

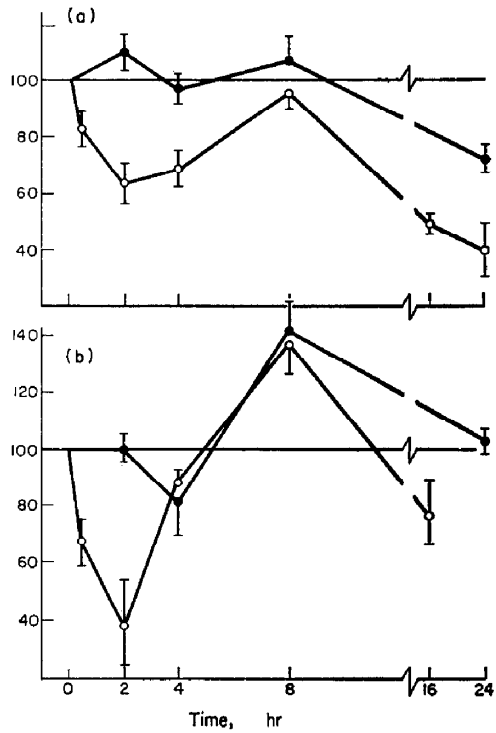


FIG. 4. The effect of intraperitoneal injection of 1·5 g/kg of N-hydroxyurethane (●——●) or urethane (○——○) on the adrenaline content (a, as percentage of control values) and noradrenaline content (b, as percentage of control values) of the adrenal glands. Each point is the mean of at least 4 observations ± standard error (shown by the vertical line).

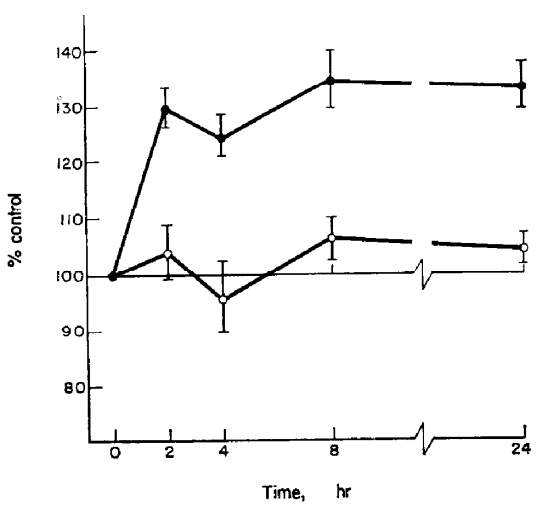


FIG. 5. Brain 5-hydroxytryptamine content (expressed as percentage of control value) after intraperitoneal injection of 1·5 g/kg of N-hydroxyurethane (●——●) or urethane (○——○). Each point is the mean of at least 6 observations ± standard error (as shown by the vertical line).

*Adrenal noradrenaline and adrenaline contents*

There was no change in the adrenaline content of the adrenal gland up to 8 hr after N-hydroxyurethane in contrast to the significant transient depletion occurring after urethane, but a small depletion was present at 24 hr although this was not as great as that found after urethane (Fig. 4a). A small loss of noradrenaline at 4 hr was succeeded by a significant increase at 8 hr, the content returning again to control values by 24 hr (Fig. 4b). An increase in noradrenaline content at 8 hr was also seen after urethane, but it was preceded by a large statistically significant depletion.

*Brain 5-hydroxytryptamine and noradrenaline contents*

After N-hydroxyurethane, a significant increase in 5HT content of the brain occurred at 2 hr and was maintained up to 24 hr. No change in 5HT content was induced by urethane (Fig. 5). Neither drug induced any changes in brain noradrenaline levels up to 8 hr after injection.

## DISCUSSION

The introduction of a hydroxyl group on the nitrogen of urethane is not thought to greatly alter either its lipid or water solubility[9] and thus both urethane and N-hydroxyurethane may be thought to penetrate the blood–brain barrier to a similar extent. Since N-hydroxyurethane does not induce anaesthesia in rats, except in toxic doses, it seems that the anaesthetic effect of urethane does not depend on its metabolism to the N-hydroxy derivative. The possibility may not be excluded that the anaesthesia after high doses of N-hydroxyurethane arose from some reconversion to urethane.

Spriggs and Stockham[1] suggested that urethane exerted a stimulant action on the hypothalamus which had the effect of releasing corticotrophin from the pituitary gland and hence raised plasma and adrenal corticosterone levels, and increased sympathetic outflow to the adrenal medulla so that noradrenaline and adrenaline were depleted. The molecule formed by N-hydroxylation of urethane appears to discriminate between these two actions, retaining the ability to induce a release of corticotrophin but losing the activity reponsible for the increased sympathetic outflow which releases adrenal catecholamines.

The discrepancy between the effects of urethane and of N-hydroxyurethane on the adrenaline and noradrenaline content of the adrenal glands during the first 4 hr is very clear (Fig. 4) but at 8 hr and later, the effects of both drugs appear similar, although urethane retains a stronger depleting activity. This similarity after 4 hr may result from appreciable amounts of N-hydroxyurethane having been converted into urethane.[9]

N-hydroxyurethane induces a quicker, greater and more prolonged fall in body temperature than does urethane. However, urethane also causes a copious release of catecholamines from the adrenal medulla which may antagonise a fall in body temperature by eliciting peripheral vasoconstriction and an increase in basal metabolic rate.[10] It is possible, therefore, that urethane and N-hydroxyurethane operate by the same mechanism to cause body temperature to fall, but that this fall is offset in the urethane-treated rat by the simultaneous release of adrenal catecholamines. 5HT and noradrenaline have been implicated in the hypothalamic regulation of body temperature,[11] but although N-hydroxyurethane increased brain 5HT levels at 2, 4 and 8 hr, when body temperature was low, the 5HT level remained elevated at 24 hr when the temperature had returned to the control value. It is of interest that these elevated

brain 5-hydroxytryptamine levels may be implicated in preventing anaesthesia after N-hydroxyurethane since it has been shown that imipramine[12] and tranylcypromine[13] increase brain 5-hydroxytryptamine levels and reverse the depressant or sedative effects of reserpine treatment. While the change in 5-hydroxytryptamine content over 24 hr does not appear to be related to changes in body temperature, it may alter the degree of sedation, especially as no change in brain 5-hydroxytryptamine level was present after urethane. No change in brain noradrenaline levels were found up to 8 hr after either drug, although Reinhert[14] has reported a loss of hypothalamic noradrenaline 16 hr after urethane.

The changes in plasma corticosterone concentration during the first 8 hr after N-hydroxyurethane or urethane are similar. After 8 hr, urethane provokes a further increase in plasma corticosterone concentration whereas N-hydroxyurethane does not. It is pertinent that in bilateral adrenal demedullated rats no further increase in plasma corticosterone occurs between 8 and 24 hr after urethane.[1] It is apparent from the present experiments that urethane is more active than N-hydroxyurethane in releasing adrenal medullary catecholamines, and the divergence in plasma corticosterone levels after 8 hr may reflect this difference.

The increases in plasma corticosterone induced by N-hydroxyurethane or urethane have similar time courses, and reach similar maxima over the dose range used. It is probable that urethane is not converted to N-hydroxyurethane before activating corticotrophin release but that both molecules are equally able to stimulate the release of corticotrophin and that their effects are independent of changes of brain 5-hydroxytryptamine levels. However, Montanari and Stockham[15] showed that reserpine released corticotrophin only in sedative doses and Brodie and Costa[16] have conclusively shown that brain 5-hydroxytryptamine is implicated in reserpine sedation. The present results with urethane and its N-hydroxy metabolite appear to dissociate brain 5-hydroxytryptamine and sedation from the release of pituitary corticotrophin.

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