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Suitability of urethane anesthesia for physiopharmacological investigations. Part 3: Other systems and conclusions

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Summary. The suitability of urethane anesthesia for physiopharmacological experiments in various systems is briefly reviewed. Urethane anesthesia appears to be suitable for various types of studies on respiratory function and on reflex activation of motility of the urinary bladder and some sections of the intestinal tract. However, urethane produces a variety of potentially disturbing side-effects at endocrine and renal level.
Key words. Urethane; anesthesia; physiology; pharmacology; in vivo experiments; reflexes.

This paper completes a series[48,49] dealing with the suitability of urethane anesthesia for physiopharmacological investigations.

Effect of urethane on respiratory function

Douglas et al.[16] reported a marked decrease (about 40%) of respiration rate and minute volume in guinea pigs receiving i.p. urethane (1.5 g/kg) but Advenier et al.[1], using a lower dose (1.0 g/kg i.v.), found only a small depression of respiratory function.

Sapru and Krieger[67] reported that i.v. urethane (0.75 g/kg) does not affect respiratory rate and tidal volume in decerebrated rats. Urethane (2%) has no effect on ciliary beat frequency while a higher concentration (4%) produced cilioinhibition[39].

We have observed that high concentrations of urethane (70–100 mM) are required to induce a significant depression of field stimulation-induced contractions of the rat isolated diaphragm[66]. Taken together these observations suggest that urethane, at anesthetic doses, has minimal or no depressant effect on resting respiratory function. For this reason urethane-anesthetized animals do not usually require assisted ventilation except during intrathoracic surgery or following administration of substances which depress or impair respiration. Insertion of a tracheal tube is useful to facilitate ventilation, when substances which increase saliva production (eserine, substance P etc.) are administered.

Urethane-anesthetized guinea pigs exhibit an increased pulmonary airway resistance (PAR)[1]. Since urethane has a direct depressant action on the resting tone of the isolated guinea pig trachea[50] it is conceivable that increase in PAR is mediated through a modification of the neural input to the tracheobronchial tree[1,50].

Florez and Borison[25] found that, in decerebrate cats, urethane reduces the slope of the line existing between tidal volume and alveolar pressure of CO_2 with only minimal changes in the apneic point. This was paralleled by a reduced response of the medullary respiratory integrator to electrical stimulation[9]. These findings indicated that urethane depresses the CO_2-tidal volume 'gain' mechanism[9].

Sapru and Krieger[67] reported that in rats receiving i.v. urethane (0.75 g/kg) the respiratory changes produced by injection of NaCN are depressed as compared to decerebrated rats.

Dixon and Brodie[15] reported that, in cats, urethane reduces the muscarine-induced bronchoconstriction. Douglas et al.[16] reported that bronchomotor responses to aerosolized histamine are reduced, as compared to conscious animals, in urethane-anesthetized guinea pigs.

On the other hand Advenier et al.[1] reported that bronchomotor responses to various agonists are potentiated by urethane anesthesia (1 g/kg i.v.). The discrepancy between these two studies could be ascribed to a variety of factors[1]. Urethane has a direct depressant action on contractility of the guinea pig trachea smooth muscle[50]. It appears that the enhanced bronchoconstrictor response to histamine could be due to a depression of tonically active neural bronchodilator influences[1,50]. Such a hypothesis is also supported by the observation that subanesthetic doses of urethane delay the onset of histamine-induced bronchospasm in conscious guinea pigs[50].

Taken together these findings indicate that urethane anesthesia does not interfere with resting respiratory function in various animal species. Hedner[32] concludes that 'urethane appears to affect respiration to a lesser degree (less depressant) than three other anesthetics studied (halothane, enflurane, pentobarbital sodium)' although 'the anesthetic most certainly affects the physiologic and pharmacologic responses in experimental animals'[32]. It appears therefore that urethane anesthesia may interfere with both resting and reflexly activated neural influences at tracheobronchial level in such a way that the physiological relevance of results obtained in these animals should be carefully evaluated.

Effect of urethane on gastrointestinal function

Effect of urethane on gastric and biliary secretion. Urethane is commonly used to study gastric acid secretion in spite of its significant effect on this function[4,12,35]. Lane et al.[35] reported that urethane reduces the basal acid secretion in conscious rats with chronic gastric fistulae. Bralow et al.[12] reported that urethane decreases the secretory response to insulin. The secretory response to histamine and gastrin is lower in urethane-anesthetized as compared to conscious animals[4]. In spite of these adverse effects steady levels of resting gastric acid secretion could be obtained for hours in urethane-anesthetized animals[4]. This makes urethane suitable for testing the effects of substances on gastric acid secretion both in resting and in stimulated conditions[4,36]. Moreover, secretory responses are observed in urethane-anesthetized animals after central administration of stimulating substances[125]. Taken together these findings indicate that urethane may be suitable for pharmacological studies on gastric and secretion although the physiological significance of data obtained in these preparations is uncertain.

Urethane depresses bile flow and increases bile salt concentration and osmolality[65]. In rabbits receiving 1 g/kg of i.v. urethane bile flow was reduced (36%) as compared to pentobarbital-anesthetized animals[23]. Urethane increases (as compared to barbiturates) both bilirubin and sodium excretion[23]. In addition differences between urethane and barbiturate anesthesia were noted in bile salt-activated bromosulphonphtalein excretion[23].

Effect of urethane on spontaneous and stimulated gastrointestinal motility. Dixon and Brodie reported that, in urethane-anesthetized cats the contractile response of the alimentary canal to stimulants was delayed and depressed[15]. Bieger[6] studied the physiology and pharmacology of esophageal motility in urethane-anesthetized rats (1.2 g/kg i.p.) and found this type of anesthesia suitable for investigating the central mechanism(s) regulating primary deglutitive peristalsis in this species. Boullin[10] described that the duodenal peristaltic activity in urethane-anesthetized rats (1.25 g/kg s.c.) occurred with characteristics resembling those observed in the isolated preparation. Perfusion of the stomach produced a coordinated series of movements of the pylorus, the sphincter and the duodenum but sphincteric movements were clearly influenced by depth of anesthesia. Urethane, in anesthetic-like or slightly higher (10–30 mM) concentrations has some depressant effect on spontaneous activity and maximal contractile response of isolated intestinal segments to KCl or carbachol[42a,56]. This emphasizes the need, for in vivo experiments, of using the lowest dose which produces surgical anesthesia[48].

In rats (s.c. urethane, 1.2 g/kg) we obtained reliable contractile responses to topical cholinomimetics or KCl in various parts of the rat gastrointestinal tract, which were suitable for testing the smooth muscle relaxant properties of anticholinergics, spasmolytics and Ca^{++} entry blockers[43,45,47,52].

In urethane-anesthetized guinea pigs (1.5 g/kg s.c.) the distension-induced contractile activity of the hypogastric loop of the distal colon is maintained through a cholinergic neural input[47,51].

Furthermore we observed in the intestine of urethane-anesthetized rats (1.2 g/kg s.c.) or guinea pigs (1.5 g/kg), the occurrence, following saline distension, of motor responses which presumably involve activation of either intrinsic or extrinsic neural structures (see fig. 1). These observations demonstrate that (at least at certain dose levels) some basic functions governing intestinal motility are preserved in urethane-anesthetized animals. As described for gastric acid secretion the potential suitability of urethane anesthesia for physiological studies on gastrointestinal motility remains to be established.

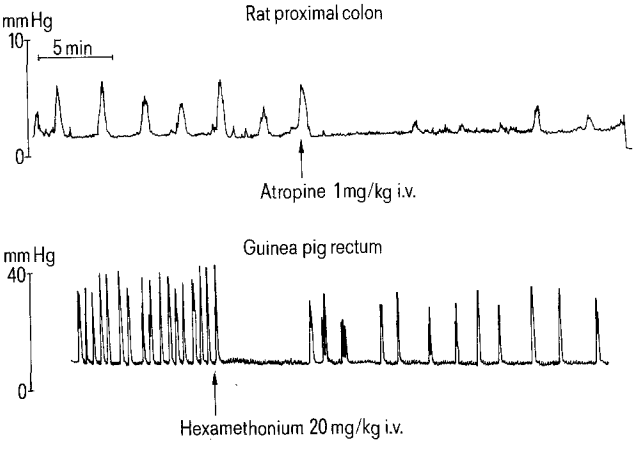

Figure 1. Upper Panel – Typical tracing showing the contractile response following distension in the proximal colon of urethane-anesthetized (1.2 g/kg s.c.) rats and its transient suppression by i.v. atropine (1 mg/kg). The preparation was made as described by Maggi and Meli[45]. Lower Panel – Typical tracing showing the contractile response (recto-rectal excitatory reflex) following distension of the rectum in urethane-anesthetized (1.5 g/kg s.c.) guinea pigs and its transient suppression by hexamethonium (20 mg/kg i.v.).

Effect of urethane on renal function

Only limited information is available on the effects of urethane anesthesia on renal function. However several reports indicate that in urethane-anesthetized animals serum levels of hormones which, like renin and aldosterone, may affect renal function are increased[61,70,73]. Bell et al. reported that i.p. urethane (1.4 g/kg), unlike barbiturates or ketamine, halved renal blood flow in rats[5]. Following i.p. administration urethane induces a marked damage of the mesenteric vasculature[70,78]. No information on a primary nephrotoxicity of i.p. urethane is available; however, Severs et al.[70] found no sign of renal damage (glycosuria, proteinuria) in rats receiving i.p. urethane (1.5 g/kg).

Severs et al.[70] found that the effects of urethane on renal function in rats are markedly influenced by the route of administration. I.p. urethane produces (secondary to the damage of the mesenteric vasculature) a massive leakage of plasma into the peritoneal cavity[78]. The loss of fluid impairs renal function by reducing the glomerular filtration rate. The ability to develop a renal response to a load of NaCl or water was lost[70]. These changes in renal function are independent of factors such as anesthesia per se, angiotensin, aldosterone or vasopressin levels and activity of renal nerves[70].

On the other hand i.v. urethane produces a brisk osmotic diuresis but does not produce fluid leakage in the peritoneum[70]. The level of anesthesia is lower in rats receiving i.v. as compared to i.p. urethane, presumably due to an increased elimination of the anesthetic[70]. Interestingly, i.p. but not i.v. urethane increase plasma osmolality; this effect may complicate the interpretation of experiments on the CNS since hyperosmolality opens the blood-brain barrier[70].

Effect of urethane on micturition reflexes and smooth muscle of the lower urinary tract

Urethane has been used for in vivo studies on the micturition reflex in cats and dogs, frequently in association with chloralose[27,68,69]. Mellanby and Pratt[57,58] described the response of the cat bladder in conditions of constant pressure or volume under urethane anesthesia.

In the past few years we have presented evidence indicating that both the excitatory and inhibitory reflexes concerned with micturition could be elicited in urethane-anesthetized rats (1.2 g/kg s.c.)[41,44,46,52,54] guinea pigs, hamsters and mice[40]. Chemical stimulation of primary afferent fibers by capsaicin[76], activates a supraspinal micturition reflex in urethane-anesthetized rats[52]. Afferent neurotransmission from the periphery to supraspinal sensory centers is unaffected by urethane anesthesia[2]. Therefore we could assume that topical application of capsaicin on the urinary bladder of urethane-anesthetized rats elicited a supraspinal micturition reflex[40,52], involving activation of the same sensory pathways conveying volume information from the detrusor muscle to the CNS[20,34].

Inhibitory neural influences on micturition reflex can also be observed in urethane-anesthetized rats (see also fig. 2). In particular reserpine[46], 6-OH dopamine or bilateral section of the hypogastric nerves[54] produced, in urethane-anesthetized rats, a marked detrusor hyperreflexia[46,54]. These observations support the hypothesis that the sympathetic nervous system contributes to urine storage during the collecting phase of the cystometrogram[7,20,22]. Obviously we cannot exclude that urethane anesthesia might have increased the level of sympathetic tone to the bladder[3,64,74]. Thus the question relative to the physiological relevance of sympathetic inhibition of reflex bladder activation in unanesthetized animals remains open, although such a mechanism(s) have been demonstrated also in barbiturates[22] or chloralose[20] anesthetized animals.

In anesthetic-like concentrations, urethane produces only a moderate (10–15%) depression of detrusor contractility[53]. In urethane-anesthetized rats topical acetylcholine induces an increase of intraluminal pressure (40–50 mmHg) higher than that (20–40 mmHg) of neurogenic contractions elicited by distension[41,52]. A similar ratio between maximal contractile response to acetylcholine and neurogenic response to field stimulation was observed in the isolated rat bladder[40,41]. These observations indicate that the excitatory neurotransmission to the bladder operates, under urethane-anesthesia, within the limits observed in the isolated organ.

In conclusion our findings suggest that urethane anesthesia is suitable for physiological investigations dealing with excitatory and inhibitory micturition reflexes as well as with modification of these responses by drugs.

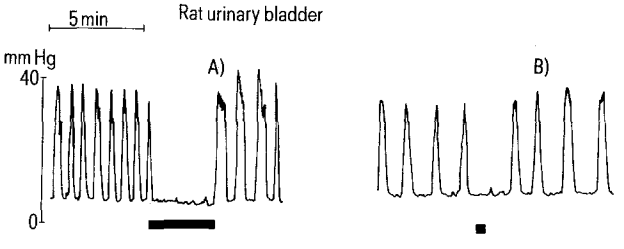

Figure 2. *A* Typical tracing showing suppression of the distension induced rhythmic contractions of the rat bladder[44] by distension of a balloon placed in the distal colon under urethane anesthesia (1.2 g/kg s.c.) (inhibitory colo-vesical reflex). *B* Typical tracing showing the transient suppression of the distension-induced rhythmic contractions of the rat bladder[44] by pinching of the forepaw under urethane anesthesia (1.2 g/kg s.c.) (inhibitory somato-vesical reflex).

Endocrine effects of urethane anesthesia

Urethane anesthesia produces a variety of endocrine effects. The following points should be outlined: a) most available studies on this topic have been performed by using doses larger than the minimal effective anesthetic ones[48]; b) many studies employed the i.p. route of administration whose adverse effects have been outlined previously[48]; c) certain endocrine effects of urethane anesthesia are less pronounced when the i.p. route is avoided[8,70] and d) almost all available information on the endocrine effects of urethane anesthesia has been obtained in rats.

Effect of urethane on prolactin release. After an initial increase[24a] s.c. urethane (2 g/kg) depresses prolactin secretion (about 80% inhibition)[11,24a,62,72]. This effect is paralleled by an increase in levels of dopamine at hypothalamic level, suggesting an increased turnover of this neuro-

transmitter which may be responsible for the genesis of this endocrine effect[24a].

Effect of urethane on release of oxytocin and antidiuretic hormone release. Ginsburg and Brown[26] showed the presence of antidiuretic activity in the blood of urethane-anesthetized rats ascribable to elevated antidiuretic hormone (ADH) levels. Dyball[18] reported that urethane does not alter the resting release of oxytocin from the isolated neural lobes of the rat hypophysis, while it enhanced the stimulatory effect of high K^+. Dyball showed that the potentiating effect of urethane is paralleled by an increased uptake of Ca^{++} from the medium and may be prevented by D-600, a Ca^{++} channel blocker[18]. These observations might help to explain the increased and variable levels of neurohypophyseal hormones found in urethane-anesthetized animals. In fact any type of stimulus may produce the release of larger amounts of these hormones in urethane-anesthetized than in conscious animals[18].

Dyball and McPhail[19] showed that, in urethane-anesthetized rats, the firing rate of hypothalamic neurosecretory neurons was normal in spite of the increased levels of plasma ADH and concluded that the effect of urethane on ADH release is determined through an action at the level of secretory membrane in the neurohypophysis.

Effect of urethane on ovulation and LH release. Urethane has been used extensively in neuroendocrine investigation dealing with control of the pituitary gonadotropic secretion[29,77]. Lincoln and Kelly[38a] reported that urethane administered on the day of proestrus blocked ovulation in approximately 50% of preparations. The dose of luteinizing hormone (LH) required to induce ovulation is lower in urethane- than in barbiturate-anesthetized rats[38a].

On the other hand Blake and Sawyer[8] reported a fivefold increase of the dose of LH required to induce ovulation in urethane (1.3 g/kg i.p.) as compared to barbiturate-anesthetized rats. These authors showed that urethane blocks ovulation by at least two distinct mechanisms, i.e. a central one involving blockade of LH-RH release from the hypothalamus and a 'peripheral' one which was observed after i.p. but not i.v. administration[8].

Dyer et al.[21] showed that the secretory response (increase in FSH and LH plasma levels) following electrical stimulation of the preoptic area of the rat hypothalamus was higher in urethane as compared to barbiturate anesthetized animals.

Carter and Dyer[13] reported that urethane inhibits the release of LH activated by the LH-releasing hormone (LH-RH). Subsequent experiments revealed that the sensitivity of the pituitary to exogenous LH-RH decreases in female (but not male) rats under urethane anesthesia[20a]. Taken together these findings indicate that urethane anesthesia is not suitable for investigating the hypothalamic control of preovulatory gonadotropin secretion since the anesthetic interferes markedly with the brain-pituitary-ovary function.

Effect of urethane on growth hormone release. The study of the control of growth hormone (GH) secretion in the rat was hindered by the large spread of resting plasma levels[14]. Collu et al.[14] reported that under urethane anesthesia (1.5 g/kg i.p.) plasma GH levels are about $\frac{1}{7}$–$\frac{1}{8}$ of those observed in conscious animals. However in urethane-anesthetized rats there was only a minimal spread of individual values around the mean and the preparations were responsive to pharmacological stimulation[14]. It was concluded that urethane-anesthetized rats represent useful preparation for the study of GH secretion, although the mechanism(s) responsible for depression of resting GH levels remain to be elucidated[14].

Effect of urethane on renin release. A variety of anesthetics, among which was urethane, stimulated renin release at doses producing surgical anesthesia[33]. Pettinger et al.[41] reported that in rats anesthetized with i.p. urethane (0.8 g/kg) there is a marked elevation of plasma renin activity (PRA) with mild elevation in blood pressure. The urethane-induced increase in PRA was prevented by blockade of beta adrenoreceptors (propranolol, 1.5 mg/kg s.c.). Leenen and Provost[38] showed that, in rats receiving i.p. urethane (1.25 g/kg) PRA increase was prevented by propranolo, neonatal sympathectomy and/or adrenomedullectomy. It was concluded that, under i.p. urethane anesthesia, PRA was increased through an hyperactivity of the peripheral nervous system.

Effect of urethane on pituitary-adrenal function. It is well known that various anesthetics influence ACTH secretion, sometimes with a biphasic effect during anesthesia[75]. Spriggs and Stockham[75] reported that urethane anesthesia (1.5 g/kg i.p.) increases both plasma and adrenal corticosterone levels while changes in adrenal weight and ascorbic acid content are suggestive of a prolonged hypersecretion of ACTH[75]. This prolonged hyperactivity of the pituitary-adrenal system does not reduce the capacity of the adrenal glands to produce a further increase in plasma corticosterone following administration of exogenous ACTH[75].

Ondo and Kitay[60] and Harmstra et al.[31] showed that this effect of urethane was markedly reduced by previous hypophysectomy. Harmstra et al.[31] showed that i.p. urethane in doses as low as 0.4 g/kg produced a significant increase in plasma corticosterone levels. This effect was observed following hypothalamic isolation, suggesting 'that urethane activates the pituitary-adrenal system by acting on elements within the isolated brain pituitary complex'[31].

Effect of urethane on pharmacological responses in various systems

O'Duffy and Chahl[59] showed that in rats receiving i.p. urethane (1 g/kg) the cutaneous plasma extravasation induced by histamine or 5-HT was similar to that observed in conscious animals. A 20% increase in the dose of urethane produced a significant decrease of response. Griswold et al.[28] reported that urethane, at doses which produced a semiconscious state (0.75 g/kg) or surgical anesthesia (1.25–1.75 g/kg) depressed markedly carrageneen-induced paw oedema development in rats. This effect of urethane anesthesia (which inhibited paw edema by 84%) was partly dependent upon adrenal secretion and anesthesia-induced hypothermia.

Recently we investigated the effects of urethane and sodium thiopenthal on platelet aggregation both in vivo and in vitro[24]. In vitro studies indicate that both anesthetics induce a dose-related decrease of platelet aggregation to collagen, ADP and arachidonate[24]. However this effect was observed only at concentrations higher than those found in plasma during anesthesia[24]. The fall in platelet count produced by i.v. collagen, ADP or arachidonic acid was similar in controls and urethane (1 g/kg i.p.) anesthetized rats[24].

Pharmacokinetic studies in urethane-anesthetized animals

It is apparent that urethane induces (depending upon dose and route of administration, species, sex and age of the animals) variations of unpredictable size in certain body functions (such as renal function and bile excretion)[6,23] which are of paramount relevance in pharmacokinetic studies[48,49]. Changes in resting cardiovascular function and protein serum levels[49,176] make it very difficult to consider pharmacokinetic data obtained in urethane-anesthetized animals as representative of those which may be obtained in conscious ones. Moreover, the massive plasma leakage following i.p. administration of urethane[48,176] in rats is an absolute contraindication to the use of this route of administration for pharmacokinetic studies.

These drawbacks were stressed by Pipkin and Stella[63] who observed that the half life of thiamine was increased, dose dependently, in urethane-anesthetized animals (1.0–2.0 g/kg i.p.).

Concluding remarks: Suitability and contraindications of urethane anesthesia for physiopharmacological studies

In conclusion, urethane produces a plane of surgical anesthesia characterized by: a) a high level of activity of the autonomic nervous system in controlling visceral functions and b) a good preservation of both excitatory and inhibitory reflex responses in a variety of organs and systems[48,49]. When administered by the proper route and avoiding the use of unnecessarily high doses this anesthetic is suitable for a variety of physiopharmacological studies at cardiovascular, respiratory, gastrointestinal and urinary system level.

The major advantages of this anesthetic in animal experimentation could be summarized as follows: a) it produces a long lasting steady level of surgical anesthesia which does not require administration of additional doses; b) it preserves a fairly good level of resting cardiorespiratory function along with an intense skeletal muscle relaxation; this allows experiments which require immobility of the animal to avoid movement artifacts and c), although a certain depression of reflex responses occurs in various systems this drawback can be minimized by avoiding the use of unnecessarily high doses of the anesthetic.

On the other hand, urethane anesthesia produces a variety of hormonal and biochemical changes which alone or in combination may have profound and unpredictable effects on the response under study. Therefore the suitability of urethane anesthesia for any given type of physiopharmacological investigation should be carefully checked. In particular the potential relationship between resting function and degree of response should be investigated, in view of the high autonomic tone regulating visceral function in urethane-anesthetized animals.

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Distribution of metals in annual rings of the beech (*Fagus sylvatica*) as an expression of environmental changes

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Summary. Annual rings of 140–160-year-old beeches (*Fagus sylvatica*) from St. Ingbert, Saarland (FRG) were prepared and analyzed for 14 metals by atomic absorption spectroscopy. According to the chronological variations of their concentrations, the elements could be divided into three groups: 1) Metals without any tendency for chronological changes. This was established for Na, K, Cu, Cr, Co, Ni, Pb, and Cd. 2) Metals with a recent decrease of their concentrations, appropriate for Ca, Mg, Mn, and Zn. 3) Metals with a recent tendency to increase, e.g. Fe and Al. These variations are discussed in connection with the industrial history of the Saarland region and the influence of acid immissions which may alter the soil and thereby the trace element metabolism of the trees with consequences for the vitality of the plants.
Key words. Annual ring; beech; trace metals; environmental pollution.

Introduction

In order to find explanations for the widespread dieback of forest trees in western Europe and the northeastern United States during the last decade, a phenomenon which is now summarized by the German word 'Waldsterben', many hypotheses about the possible causes of these changes have been proposed. Atmospheric pollutants (acid gases, ozone, heavy metal compounds) resulting from the accelerated combustion of fossil fuels are most probably involved in the destruction of our forests[10], but the mechanism of their impact on plants is not clearly understood, and in most cases, a clear relationship between atmospheric pollution and forest decline waits to be scientifically proved. It has been suggested that acid-forming gases (SO_2, NO_x, HCl, HF) may have a direct influence on the parts of the plants above the ground, or that acid rain may penetrate the soil, thereby altering the soil environment; this could lead to an impairment of the roots[15]. Heavy metal compounds may act either directly as dry deposits on the leaf surfaces or, when they reach the soil with the rainwater, they may be toxic for the roots, too[13]. The hypothesis of the effect of photooxidants is based in the effect of UV-radiation on gaseous emissions (NO_x), whereby the phytotoxic ozone is generated[21]. The latter theory is discussed in connection with the dieback of spruces and firs at higher altitudes in the mountains and far from industrial pollution. A detailed summary of the possible causative factors of the Waldsterben and their complex interaction are given by Isermann[5].

During the last years, however, extensive damages are also reported for deciduous trees, like beeches and oaks, which cannot be correlated with special altitudes or industrial locations: In the Saarland region, for instance, where the beech (*Fagus sylvatica*) is widely distributed, damages to this species has increased during the last three years from 7.2 to 13.6 and finally to 41.5% of the total forest stand[16–18]. In this case, an explanation may be given by the general stress hypothesis of Schütt[12]; that is, that the constant presence of relatively small concentrations of atmospheric pollutants impairs the vitality of the forest trees by reducing their photosynthetic capacity.

Whatever the mechanism involved may be observation shows that the affected trees always undergo decisive changes in their metabolism, which finally lead to a total loss of vitality. With respect to conifers, the analysis of needles of several age groups may provide information about short-time alterations in metabolism. Therefore, conifer needles are often used as indicators for immission[11]. Since the leaves of deciduous trees from earlier years are not available, only the annual rings in the wood can reflect to a certain extent metabolic changes in the tree during former periods. This is well documented by a number of morphological studies, and the decline of forests is often accompagnied by a marked reduction of ring-width[8]. Following the trace element patterns of the annual rings should also be useful, especially in connection with the hypothesis of acid rain and its effect on soil pH and an increased mobility of trivalent cations, like Al^{3+} or Fe^{3+}. Provided no secondary movement of the elements across the annual rings takes place, such a 'biochemical dendrochronology' should be suitable for moni-