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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-

toring important environmental changes during the whole lifespan of the tree.
A number of such studies have recently been performed[1,2,5], and it was shown that increases of a variety of elements, including iron, aluminium, copper, cadmium, manganese, could be clearly correlated to an accelerated regional combustion of fossil fuels and associated release of SO_2[2] or other industrial activities like iron- or copper-ore smelters[1]. Evidence was obtained that translocation of metals does not significantly alter historical patterns[2,8]. Therefore, trace element concentrations in tree rings reflect the uptake and availability of the metals in the year when the rings were formed.
In the work presented here, tree ring metal analysis was performed with the beech (*Fagus sylvatica*). The beech is widely distributed in Central European forests, and this species has been especially exposed to danger during the last years, so metal analysis in tree rings and the correlation of the results with environmental pollution could help to provide information about recent alterations in tree metabolism.

Experimental

In March 1984, five 140–160-year-old beeches were felled in the Saarland near the town of St. Ingbert. All the trees were growing at the same site, at a maximal distance apart of 20 m. 1 m above ground level a disk of 15 cm thickness was removed from each trunk. From these disks, segments containing all annual rings were cut by a mechanical saw. The segments were then separated following the annual rings by using a paring-chisel. The tool was only used for tearing off the detached ring zones, whereby care was taken to avoid any contact of the metal with the central parts of the separated chips. The contaminated ends of the chips were eliminated by hand. Only uncontaminated central parts of the chips were used for analysis.

Table 1. Metal contents (mg/kg) in annual growth rings of the beech (*Fagus sylvatica*)

	Metals without trend for temporal change							
	K	Na	Cu	Co	Ni	Cr	Pb	Cr
Lower limit	1130	19	0.4	0.2	0.1	0.3	0.1	0.02
Upper limit	2800	80	2.4	2.5	3.9	3.2	1.6	0.3
	Metals with decreasing trend							
	Ca		Mg		Mn		Zn	
Lower limit	145		74		6		1	
Upper limit	2643		676		405		44	
	Metals with increasing trend							
	Fe			Al				
Lower limit	1			1				
Upper limit	121			109				

The samples were dried at 105 °C (12 h), then about 200–300 mg of each was digested with 2 ml of 65% nitric acid (suprapur, Merck) during 2 h at 170 °C in a teflon autoclave according to Kotz et al.[7]. The clear solutions were diluted with double-distilled water to 10 ml and were then used for metal analysis by atomic absorption spectroscopy in a Perkin-Elmer 420 instrument either by flame or in the graphite furnace HGA-74.

Results and discussion

Fourteen elements were analyzed in tree rings of the beech: Besides sodium, potassium, calcium, and magnesium, the essential trace metals iron, manganese, zinc, copper, cobalt, nickel, and chromium were also determined, and in addition the toxic metals lead and cadmium and aluminium, which is supposed to be important in forest damage[13]. Altogether, tree disks from five 140–160 years old beeches were analyzed, whereby narrow annual rings were sometimes combined into groups of 2–5 years. From the great number of analytical results,

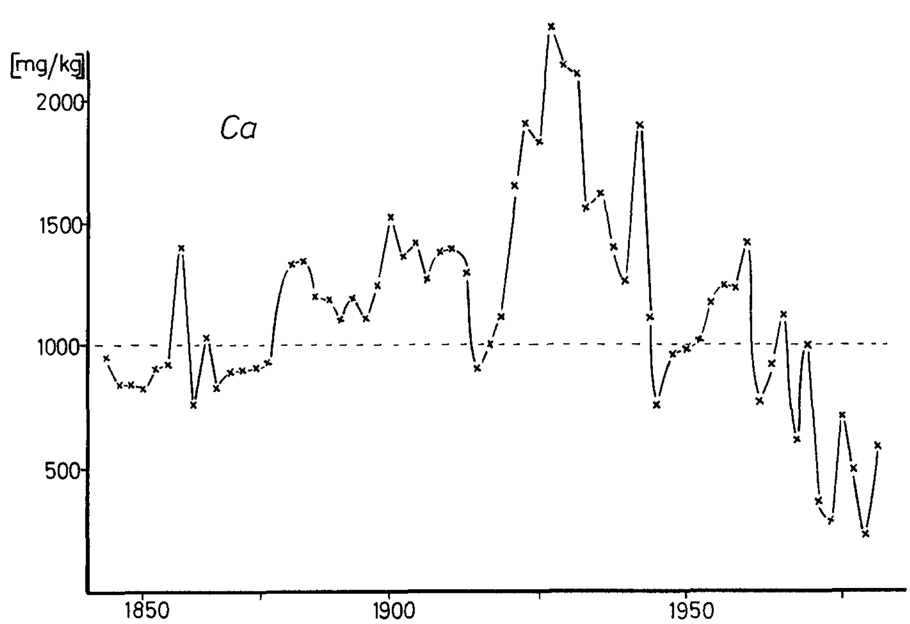

Figure 1. Distribution of calcium in the annual rings of the beech (*Fagus sylvatica*).

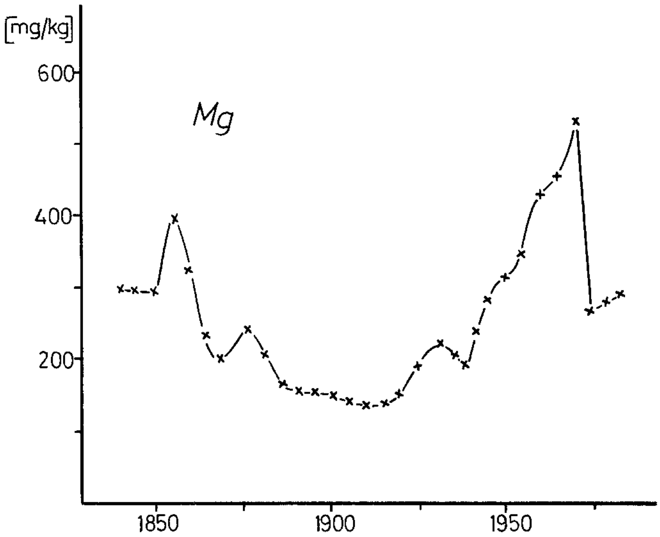

Figure 2. Distribution of magnesium in tree rings of the beech. The five-year means are indicated.

which are presented in full detail elsewhere[6], some general conclusions can first be drawn. Generally, the contents of all metals analyzed show a relatively high variation which is partly of individual origin. However, in view of recent changes, three main groups can be defined: The majority of the elements (K, Na, Cu, Co, Ni, Cr, Pb, Cd) do not show any trend in the course of growth; a second group, formed by Ca, Mg, Mn, and Zn, shows a clear decrease during the last two decades, while the contents of Fe and Al recently seem to have increased. The corresponding analytical data for these three groups, classified according to maximal and minimal contents, are given in table 1. Table 1 shows that the metals of the first group are not suitable for an interpretation of possible metabolic changes as a consequence of emission. The trend observations with the elements of the two other groups, however, seem more promising. With respect to calcium, a tree ring chronology is given in figure 1. Assuming that beech wood contains a mean of 1000 mg Ca/kg dry weight[20], figure 1 shows that between 1880 and 1960, the average value is exceeded for the most part. This can be explained by the industrial phase of minette smelting, combined with a high emission of dust. During this time, maxima or minima of the Ca-contents are correlated with variations in the activity of heavy industry in the Saarland region. Dominating are the 'Saargebiet'-time (1920–1934), the second world war, especially 1940–1944, and the time between 1950 and 1962. During that time, the high rate of production of iron and steel was associated with high levels of dust emission, which are obviously reflected by the calcium content of the beeches. On the other hand, times of reduced industrial production at the beginning of the first world war and after the second world war (1945–1950) can be observed. After 1960, however, a striking change takes place; on the industrial side, extensive dust-removal measures were taken[17], and the beeches start reducing their Ca-uptake, which led to recent concentrations being less than one third of those formed in the young tree (1840–1875). This decrease is clearly expressed in spite of some annual fluctuations which may be of climatic origin.

The chronologies of magnesium, zink and manganese in annual rings of the beech are recorded. For these elements, the calculated five-year means were chosen. This eliminates short-term variations caused by climatic factors, and a sometimes necessary combination of narrow tree rings does not alter the fine-structure of the chronology. Figure 2 shows the chronology of magnesium within the annual rings of the beech. Two distinct peaks can be monitored, the first being between 1850 and 1860, and the second between 1950 and 1965. During these times, the recorded mean value for magnesium in beech wood of 300–400 mg/kg is partially exceeded. In between, there is a larger period of minimal Mg-concentrations which covers several decades at the turn of the century. Striking again is the strong decline of Mg-concentrations after 1965. The interpretation here can only be partly oriented rewards the industrial history of the Saarland. Only the recent period of higher Mg-contents is similar to that for calcium and correlated with the substantial dust emissions of the industry. The dust fallout in the high-load areas of the Saarland continuously increases, as was observed with the Mg-content in the tree rings until 1971; in the city of Saarbrücken up to 0.6 g dust/m^2 and day have been recorded[9].

After introduction of dust-removal measurements, both the dust fallout (1981: 0.1 g/$m^2 \cdot d$) and the Mg-content of the wood decrease, although in the latter case the low values observed at the beginning of the century have not yet been reached.

The distribution of zinc in the annual rings of the beech is given in figure 3. Relatively small Zn-contents of about 15 mg/kg are found during the first decades of the tree's life. Thereafter, an important increase up to Zn-concentrations nearly twice as high are recorded. Between 1860 and 1960, Zn-contents higher than 20 mg/kg are commonly measured. A decrease of Zn-concentrations can be observed between 1925 and 1950 and then again after 1960. During the latter period, the Zn-level in the beech wood drops down to values lower than 10 mg Zn/kg. A correlation with the periods of industrial activities in the region, however, cannot be clearly defined. It is supposed that the generally-increasing industrialization at the end of the last century in connection with the known accumulation of zinc in flue dust might have influenced the Zn-content of the wood, especially between 1875 and 1925. Later on, a relative depletion of the zinc in the soil might

Table 2. Contents of tri- and divalent metals in younger tree rings of *Fagus sylvatica*

Tree No. (age in years)	Tree rings from	Metal contents (mg/kg) Fe	Al	Ca	Mg	Mn
I	1970	7.3	2.0	334	82	82
(173)	1978	23.3	36.1	287	90	51
II	1971/72	5.8	1.6	929	522	30
(143)	1978	6.9	54.0	371	185	24
	1979	21.3	104.2	284	213	12
III	1971/72	7.6	3.5	668	134	147
(41)*	1978/79	9.2	5.9	461	35	99
	1980/82	8.7	42.2	426	51	87
IV	1976	11.9	75	675	208	96
(15)*	1980	37.2	83	603	101	65

*Values taken from Kessler[6].

be superimposed on anthropogenic activities. During the last 15 years, however, very low Zn-concentrations in the beech wood are recorded which stay permanently below 10 mg Zn/kg. This has been observed with a number of other beeches, too[6].

The corresponding chronology of manganese in the tree rings is shown in figure 4. Dominant are concentration maxima around 1880, before and during the second world war (1935–1940) and then again in a less marked form between 1960 and 1970. This has been confirmed also with a number of other beeches from the same region[6]. An explanation may be given by recent changes in iron smelting technologies: In former years, minette was the main ore for the production of pig-iron, while today, the use of high-grade, presintered iron ores, combined with new technologies in steel-refining have contributed to decrease of Mn-containing dust emission. Low Mn-concentrations in the tree rings have been observed at times after the world wars. Noteworthy is, however, as monitored above with Ca, Mg, and Zn, the decline of the Mn-content during the last 15 years; while wood of the young tree contained about 40–70 mg Mn/kg, in recent tree rings only 10–20 mg Mn/kg has been found.

The distribution of iron in the annual rings of the beech shows a completely opposite trend (fig. 5). Until the turn of the century, the Fe-content of the wood is generally not higher than 5 mg/kg. Later on, until 1950, concentrations which sometimes reach 10 mg Fe/kg are observed. But after this time, the Fe-content in the beech wood starts to increase, a remarkable acceleration being recorded during the last decade; the youngest tree rings now contain about 30 mg Fe/kg (see also table 2). This cannot be explained by an increasing efficiency of dust-removal, because an immission-caused iron transfer into the wood should rather lead to decreasing Fe-concentrations.

A similar characteristics is also observed with aluminium (fig. 6). Except for two maxima around 1850 and 1930–1940, the Al-content of the older tree rings is very low (below 10 mg Al/kg). During the last decades, however, an increase can be observed which leads to maximal Al-concentrations of about 50 mg/kg. A similar tendency, sometimes with even higher Al-contents, has been observed with a number of other beeches, too (table 2). Table 2 shows that an increase in iron and aluminium concentrations in the younger tree rings of beeches is very often accompanied by a decrease in the corresponding contents of divalent metals (Ca, Mg, Mn).

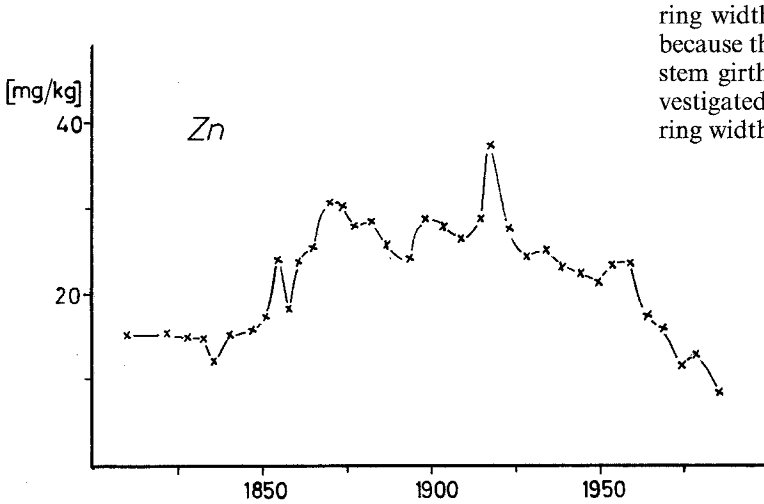

Figure 3. Distribution of zinc in tree rings of the beech. The five-year means are indicated.

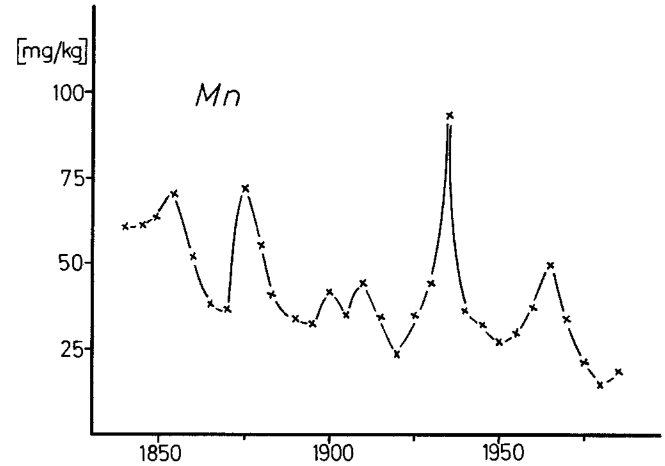

Figure 4. Distribution of manganese in tree rings of the beech. The five-year means are indicated.

On the basis of our metal analyses, we are now trying to add new arguments to the theories of the Waldsterben mentioned above. It seems that the annual rings of the beech are suited to recording temporal changes in the metabolism of several elements. Environmental changes are also reflected in the tree rings, as it is indicated by the connection between times of high industrial activity and the increase of relevant metals in the beech wood. This has been also demonstrated by similar investigations with *Pinus echinata* in the USA, where the industrial history of a whole region could be reconstructed by recording the heavy metal accumulation of the corresponding annual rings[1]. In this case, however, it was found that higher metal concentrations are often correlated with a reduced ring width. This could not be confirmed with *Fagus sylvatica*[6]; the element contents proved to be nearly independent of the annual growth rate. On the other hand, the ring width was estimated to be a less reliable standard, because the former does not run uniformly following the stem girth. The approximately 150-year-old beeches investigated here did not show any reduction of the annual ring width during the last decade.

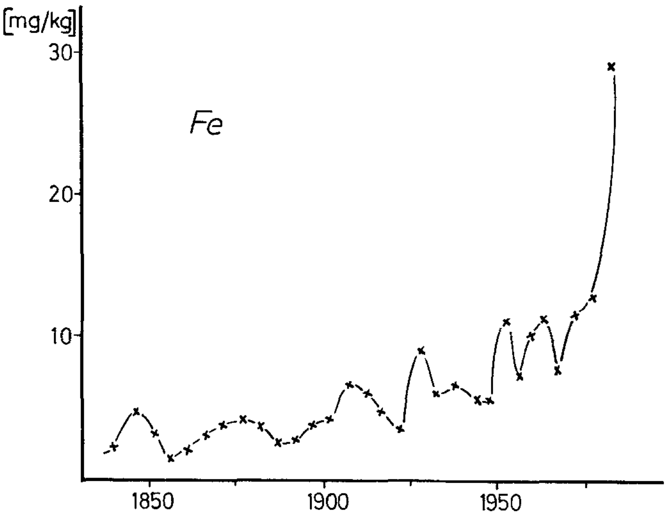

Figure 5. Distribution of iron in tree rings of the beech. The five-year means are indicated.

Except for some spots of bark necrosis, no exterior damage to the beeches could be detected. But according to the analytical data, some important changes in trace element metabolism must have taken place. Especially those essential elements like Ca, Mg, Mn, and Zn, which form divalent cations, show unequivocally decreasing concentrations during the last 15 years. At the same time, the contents of the trivalent metals Fe and Al increase. These criteria satisfy the theory of Ulrich[12], that as a result of acid depositions, the ionic environment of the soil changes in such a way that divalent ions are increasingly washed out, while the usually strongly bound trivalent Fe- and Al-ions are mobilized at a lower pH and are thereby better available for the plants. For the beeches investigated here, this could be of critical importance because they grew on a soil low in calcium (type of soil: brown earth on sand); the exchangeable calcium (extraction with 1 M NH_4Cl) was estimated in the upper 20 cm of the soil to be 100 mg/kg. Similar Ca-contents are also reported from other sandy soils of the Saarland[19], whereby a substantial Ca-loss has been observed during the last 15 years[17,19]. The coincidence of these events is supplemented by the observation that, also in the Saarland, the number of those fungi which are associated with the roots of the forest trees, drastically decreased in the last 15 years[3].

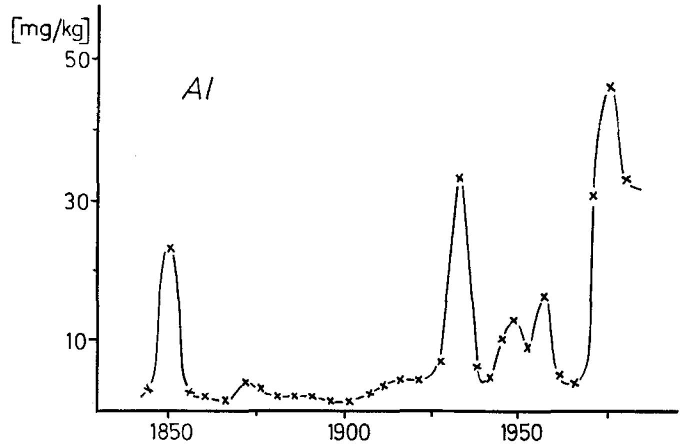

Figure 6. Distribution of aluminium in tree rings of the beech. The five-year means are indicated.

On the other hand, changes in mineral metabolism of the beeches may also be explained in part by the direct action of air pollutants on the leaves of the plant. As a consequence of an impairment of photosynthesis by gaseous emissions, a metabolic change occurs within the plant, whereby the roots are affected, too[12]. This could be lead to a reduced uptake of certain elements, but does not explain the simultaneous increase of iron and aluminium. Independently of what kind of theory may fit in our case, it can be stated that the mineral metabolism of the beeches underwent a remarkable change during the last 15 years which mainly concerns essential trace elements. When we keep in mind that manganese is a constituent of the water-splitting system in photosynthesis and magnesium is the central atom in the chlorophylls, apart from other vital functions of the trace metals during enzymatic conversions, then we should become alarmed about such a decrease. With regard to this, it is not absolutely necessary to implicate in the Waldsterben the toxicity of aluminium[15] which is, moreover, controversial[4]. We discuss the increase of iron and aluminium more as a consequence of a displacement competition which removes other essential elements from the ionic equilibrium, whereby in the plant fatal deficiency symptoms may appear.

Since our investigations, presented here, were performed with old beeches which had reached the end of their lifespan in the sense of forestry, it should be interesting to know whether the same trend will be recorded in younger beeches, too. Preliminary studies showed that young beeches, which have their roots closer to the surface than older ones, are even more affected by environmental changes[6]. This makes us hope that chemical tree ring analysis might be a tool to predict a potential endangering of *Fagus sylvatica* stands independent of the occurrence of exterior damages.

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Short Communications

Distribution of lysozyme in guinea pigs: Implications for the function of gastrointestinal lysozyme in herbivores[1]

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Summary. High levels of gastrointestinal lysozyme were present in the stomach of guinea pigs, but not in other portions of the gastrointestinal tract. Because the cecum is the fermentation organ of guinea pigs, these observations call into question the validity of the current hypothesis that the gastrointestinal lysozyme of herbivores functions in the digestion of bacteria from the anterior fermentation organ.
Key words. Lysozyme; guinea pigs; gastrointestinal tract.

Lysozyme (EC 3.2.1.17) is an enzyme that catalyzes the hydrolysis of the beta (1-4)-glycosidic linkages between N-acetylmuramic acid and N-acetylglucosamine. This substrate is present in the cell walls of bacteria but not in mammalian tissues[3].

The concentration of lysozyme in different tissues varies remarkably among species. Some species have high activity whereas other species have relatively low activity of lysozyme in most tissues. One of the purposes of this study was to determine the lysozyme activity in various tissues of guinea pigs (*Cavia porcellus*).

Recent studies have suggested that the gastrointestinal lysozyme of herbivores functions as a digestive enzyme acting on cellulolytic bacteria originating from the anterior fermentation organ[4,5]. This hypothesized function for gastrointestinal lysozyme in herbivores has been derived indirectly and is based upon observations of the distribution of lysozyme in a limited number of species. It has been stated that '... only mammals with fore-gut fermentation should have high levels of stomach lysozyme ...'[7]. Thus, in cattle, gastrointestinal lysozyme is present in the abomasum, posterior to the fermentation organ, the rumen[4,6]. In rabbits, the gastrointestinal lysozyme is located in the distal colon posterior to the fermentation organ, the cecum[5]. However, rabbit colonic lysozyme is selectively conveyed to the stomach by the unique coprophagic activity of rabbits termed cecotrophy[5]. Thus, rabbit gastrointestinal lysozyme, although synthesized in the distal colon, is transported to the stomach. An alternative hypothesis would be that gastrointestinal lysozyme of herbivores functions in the stomach independently of acting on bacteria from a fermentation organ. The main purpose of this study was to evaluate these hypotheses. For this purpose, guinea pigs, herbivores, in which the cecum is the fermentation organ and in which cecotrophy does not occur, were selected for use.

Materials and methods. Eight healthy young adult male guinea pigs, weighing between 403 and 445 grams, were used. They were anesthetized with ether, blood was obtained from the heart, and they were killed with an i.p. injection of sodium pentobarbital. The tissues listed in the table were collected from each guinea pig immediately after death. The segments of the gastrointestinal tract were separated according to Cooper and Schiller[8] with the cardia and fundus separated from the body and pylorus of the stomach. All tissues were stored individually at $-20\,^{\circ}C$ until thawed for homogenization and assay. Serum was prepared from the heart blood and frozen. Upon thawing, each tissues was weighed, minced, and mixed with 2.33 ml of homogenization buffer per gram of tissue. The buffer consisted of equal parts of 0.067 M sodium phosphate buffer (pH 7.3) and 1% acetic acid in 95% ethanol. The tissues were homogenized in micro plexiglass tissue homogenizers (Bellco Glass Inc., Vineland, NJ, USA) and centrifuged at $3000 \times g$ for 10 min at $5\,^{\circ}C$. The supernatants, less any lipid layer, were collected and recentrifuged. The supernatants were assayed for lysozyme in duplicate by the lysoplate method[9] as previously described[10,11]. The enzymatic activity of the supernatants was determined with chicken egg white lysozyme as the standard and the protein was determined by the method of Lowry et al.[12]. The lysozyme activity in the supernatants was expressed as mean ± SEM chicken egg white lysozyme equivalent µg/mg protein.

Results and discussion. The mean lysozyme activity in each of the tissues of the eight guinea pigs assayed is presented in the table. The nongastrointestinal tissues (bone marrow, lung, spleen and kidney) had lysozyme activities that ranged from 330 to 1008 µg/mg protein. The gastrointestinal lysozyme was localized predominantly in the stomach with approximately equal levels of