Die Schwefeldioxydbegasung während der Anzucht und/oder die Zugabe von Sulfit scheinen die Phosphorylierung etwas herabzusetzen. Die Erniedrigung der K_m-Werte für die beiden Substrate deuten darauf hin, dass die Phosphorylierung durch eine unkompetitive Hemmung der Phosphotransferase leicht verringert wird (Tabelle I). Die Experimente über Aufnahme und Einbau des Thymidins in intakte Kolonien von *Lemna minor* L. unterstützen die vorgängig beschriebenen Befunde (Tabelle II). Die Zugabe von Thymidin zur Nährlösung während der Anzucht erhöht wohl die Aufnahme, nicht aber den Einbau. Die Begasung mit Schwefeldioxyd verringert die Aufnahme auf Kosten des Einbaus. Diese Resultate bestätigen zumindest indirekt die Vermutung, dass den Wasserlinsen eine Thymidinkinase fehlt. Durch die SO_2-Begasung wird der Einbau von Thymidin signifikant gehemmt, was teilweise auf eine Hemmung der Nukleosidphosphotransferase zurückgeführt werden könnte. Höhere Konzentrationen als 0,6–0,8 ppm Schwefeldioxid vermögen die Aufnahme von Thymidin kurzfristig zu steigern, wobei die Werte in Kombination mit einer Thymidinvorbehandlung weit über die 2,45 *pM*/Glied × h (Tabelle II) zu liegen kommen. Es gilt aber zu bedenken, dass die Versuchsorganismen bei maximal 0,6 ppm SO_2 über Monate kultiviert werden können[4]. Schon bei Schwefeldioxydkonzentrationen um 1 ppm sterben sie innert Tagesfrist ab, so dass die Angabe von Messwerten fragwürdig erscheint. Es wird deshalb darauf verzichtet. Immerhin verweisen sie auf die seit langem gehegte Vermutung, dass SO_2 vor allem die cytoplasmatischen Membranen schädigt[2, 13].

Summary. As there appears to be no thymidine kinase in duckweed (*Lemna minor* L.), thymidine seems to be phosphorylated by a nucleoside phosphotransferase. Phosphorylation and incorporation are inhibited by sulfur compounds such as sulfur dioxide and sulfite. The data are discussed in relation to the physiological effect of the air pollutant (SO_2) on plant life.

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Concerning the Extraction of Enzymatically Active Organic Matter from Soil

The most difficult problem in humus studies is to avoid chemical alterations during the extraction of the organic matter from the soil. Though humic substances have been isolated from soils by many procedures[1–3], there are no perfect extractants for such materials. Recently McLaren et al.[4], by analogy with biochemical studies, have postulated that any method for extracting humic material leads to artifacts if enzyme activity is not retained into the extract.

Previous investigations performed in this Laboratory[5] have demonstrated that a 0.1 *M* pyrophosphate solution at pH 7, a typical humic matter extractant, extracts a significative fraction of soil urease without apparently damaging the ureolytic microorganisms.

After incubation in a Dubnoff water bath at 37 °C for 18 h, we have, with a single pyrophosphate treatment, the highest extraction of urease (20% of the total extracellular activity present in the soil) and the highest extraction of humic matter (31.2% of the total).

In order to investigate the differences among the effect of several procedures, in particular on the yield of

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Table I. Organic matter, total amino acid-N and urease extracted

Extracting solution	Total amino acid-N (μg/g soil)[a]	Urease activity (% of the total content in soil)[d]	Organic matter (mg/g soil)[b]
Sol. A	218	N.d.	9.28
Sol. A + USV[c]	202	1.30	8.60
Sol. A (double phase)	231	2.77	10.84
Sol. B	299	N.d.	12.73
Sol. B + USV	311	N.d.	13.23
Sol. B (double phase)	275	N.d.	14.70
Sol. C	351	N.d.	14.94
Sol. C + USV	329	N.d.	14.01
Sol. C (double phase)	331	N.d.	15.09
Sol. D	414	20.00	20.20
Sol. D + USV	403	18.00	17.41
Sol. D (double phase)	350	18.00	16.50

[a] The total amino acid-N content was determined adding up the single values. [b] The organic matter was determined by bichromate oxidation. [c] USV, ultrasonic vibration. [d] N.d., not detectable.

Table 2. Amino acid composition of soil extracts (μmoles/0.5 g soil) [a]

Aminoacids	Sol. A	Sol. A + USV	Sol. A double phase	Sol. B	Sol. B + USV	Sol. B double phase	Sol. C	Sol. C + USV	Sol. C double phase	Sol. D	Sol. D + USV	Sol. D double phase
Lysine	1.02	0.98	1.38	1.74	1.90	1.38	2.06	2.14	2.08	1.84	2.02	1.36
Histidine	0.36	0.34	0.48	0.58	0.70	0.70	0.74	0.74	0.94	1.08	0.98	0.90
Arginine	0.58	0.54	0.56	0.70	0.68	0.74	0.86	0.80	0.84	1.26	1.20	1.00
Aspartic acid	2.40	2.54	2.34	3.38	3.40	3.28	3.66	3.80	3.70	4.58	4.28	3.92
Threonine	0.44	0.60	0.78	1.10	1.20	0.96	1.04	1.00	1.04	1.66	1.54	1.22
Serine	0.80	0.76	0.82	0.98	1.08	0.98	1.34	1.28	1.02	1.78	1.36	1.20
Glutamic acid	1.96	1.16	1.48	2.02	2.16	1.76	2.40	2.14	2.10	2.90	2.68	2.60
Proline	0.38	0.40	0.70	0.62	0.66	0.58	0.64	0.56	0.72	0.98	0.94	0.82
Glycine	1.66	1.58	1.72	2.18	2.24	1.88	2.44	2.12	1.94	2.58	2.32	2.24
Alanine	0.96	0.86	0.88	1.26	1.30	1.02	1.38	1.24	1.24	1.58	1.58	1.46
Valine	0.44	0.36	0.32	0.50	0.32	0.40	0.54	0.50	0.44	0.72	0.66	0.60
Methionine	0.12	0.12	0.12	0.12	0.10	0.12	0.18	0.16	0.18	0.22	0.20	0.20
Isoleucine	0.26	0.24	0.24	0.32	0.32	0.26	0.34	0.30	0.26	0.42	0.38	0.34
Leucine	0.34	0.34	0.34	0.48	0.46	0.34	0.46	0.38	0.36	0.66	0.58	0.52
Tyrosine	0.06	0.06	0.08	0.06	0.04	0.08	0.04	0.04	0.06	0.10	0.10	0.12
Phenylalanine	0.30	0.30	0.26	0.34	0.34	0.28	0.24	0.28	0.30	0.34	0.36	0.32
Glucosamine	0.30	0.34	0.64	0.56	0.62	0.52	0.60	0.50	0.88	0.70	0.68	0.76
Galactosamine	0.22	0.26	0.36	0.48	0.42	0.34	0.48	0.38	0.46	0.54	0.50	0.52

[a] The aminoacids were released from the extracts by boiling with 6 *N* HCl at 110 °C for 24 h. The solutions were analyzed in an automatic amino acid analyzer (Beckman Mod. 121).

enzyme activity and amino acids in the extracts, we planned a set of experiments with a combination of physical and chemical steps.

Material and methods. More detailed information about the selected soil, the extraction procedure and determinations of extracted and total extracellular urease activity have already been reported[5]. The solutions used, besides 0.1 *M* pyrophosphate at pH 7.1 (sol. D), contained the following compounds: 0.1 *M* phosphate buffer pH 7.1, 2 *M* guanidine hydrochloride, 2 *M* NaCl, 0.05 *M* Na_2EDTA, 0.05 *M* Na_2 oxalate and 0.4% polyvinylpyrrolidone. Since the solution containing all the above-mentioned substances has a high inhibiting effect on commercial urease (from Merck), we eliminated a component for every solution: sol. A (-Na_2 EDTA), sol. B (-NaCl) and sol. C (-guanidine hydrochloride).

The percent of inhibition, calculated from determination of the activity of commercial urease dissolved either in phosphate buffer or in the different solutions, was 20, 15 and 14% for the A, B and C solutions respectively; no inhibition occurred in pyrophosphate. We must underline that the effect on the extracted soil enzyme may be lower than on the commercial urease because the soil enzymes are considerably more resistant to the physical agents[6,7].

Three different extraction procedures were performed with the solutions A, B, C and D. In the first one, the above-mentioned solutions were added to the soil without any treatment. The second procedure included a 20-min treatment by ultrasonic vibrations at the beginning of the extraction, in order to investigate if the highly disrupting effect of ultrasonic waves on soil microstructure may substantially improve the yield of enzyme activity. The third procedure was devised to ascertain if a continuous removal of iron and aluminium from the solution during extraction may affect the release process of urease activity from soil. Such a procedure was carried out 'in double phase', by placing an identical volume of acetylacetone in benzene (5:95 v/v) upon the aqueous layer. Iron and aluminium are easily chelated by acetylacetone and soluble and extractable as complexes in the non-polar phase, and so are removed from the solution[8].

Results and discussion. The data reported in Table I show that pyrophosphate extracts the greatest quantity of urease, organic matter and total amino acid-N from soil. Also the values reported in Table II for the inidividual extracted amino acids are higher than for other solutions, with few exceptions. Neither the use of ultrasonic vibration, nor of the double phase, increases the yield extraction by pyrophosphate.

By comparing the values reported for the 4 extracting solutions in Table I, it is possible to see that there is no relation between extracted urease and total-amino acid-N or organic matter contents. In fact, while the values of the release of total amino acid-N and organic matter are always comparable for all the solutions tested, the urease is extracted in valuable quantities only by pyrophosphate. Two distinct inferences arise from these results: there is no definite relation between extraction of enzymatically-active complex and the quantity of extracted total amino acid-N and also organic matter; in order to remove with a single extraction a significant amount of urease activity from the soil, only pyrophosphate solution at pH 7.0, a typical extractant of the organic matter, appears to be effective.

In a previous paper[5], we showed that the extraction of non-active organic matter by pyrophosphate precedes the extraction of the urease-organo-complex. The difference between extraction effectuality of the different solutions A, B, C and D may depend on the necessity of a complete extraction of the bulk of non-active organic matter before the release of enzymatically active complex can start. The extraction of urease activity from the soil has been reported to occur in different steps[9–11].

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Riassunto. Una soluzione di pirofosfato sodico 0,1 *M* a pH 7 estrae da un podzol, rispetto ad altre soluzioni impiegate la maggiore quantità di ureasi, sostanza organica ed azoto aminoacidico. Né l'uso degli ultrasuoni né la tecnica «in doppia fase», in presenza di una soluzione di acetilacetone al 5% in benzene, migliorano la resa di estrazione. I risultati sembrano confermare l'ipotesi che occorra una completa rimozione della sostanza organica non attiva, prima che possa iniziare l'estrazione dei complessi enzimaticamente attivi.

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Über die Hemmung bakterieller Neuraminidasen durch verschiedene Anionen

Inhibition of Bacterial Neuraminidases by Different Anions

Neuraminidasen (EC 3.2.1.18) vieler pathogener Mikroorganismen spielen im Pathomechanismus von Infektionskrankheiten eine wichtige Rolle[1]. Ihre Aktivität lässt sich in vitro durch verschiedene Faktoren beeinflussen. So aktivieren etwa Ca-Ionen manche Neuraminidasen[2,3] und Ca-Komplexbildner wie EDTA inaktivieren sie[4]. Die Enzymhemmung durch strukturanaloge, niedermolekulare N-Acylneuraminsäure-Verbindungen, wie sie besonders von Tuppy et al.[5–8] studiert wurden, erfolgt teils als kompetitive, teils aber auch als nicht-kompetitive Hemmung. Eigene Untersuchungen an *Clostridium perfringens-*, *Pasteurella multocida-* und *Vibrio comma*-Neuraminidase zeigten, dass H_2O_2 in sehr niederen Konzentrationen zu einer Erhöhung der Enzymaktivität führt[9] und auch mit Thioglykol liess sich *Pasteurella multocida*-Neuraminidase aktivieren[10]. Schliesslich beschrieb auch Drzeniek[11] eine Hemmung einiger Neuraminidasen durch Polyanionen.

Die vorliegende Untersuchung beschäftigt sich mit der Beeinflussung der Neuraminidase-Aktivität durch einige Anionen, die z.T. physiologische Bedeutung besitzen. In Figur 1 ist die Enzymaktivität der schon etwas intensiver untersuchten Cholera-Neuraminidase verglichen mit den erst kürzlich neu beschriebenen Neuraminidasen von *Streptococcus viridans*, *Erysipelothrix insidiosa* und *Pasteurella multocida*[3] in Abhängigkeit der Konzentrations-

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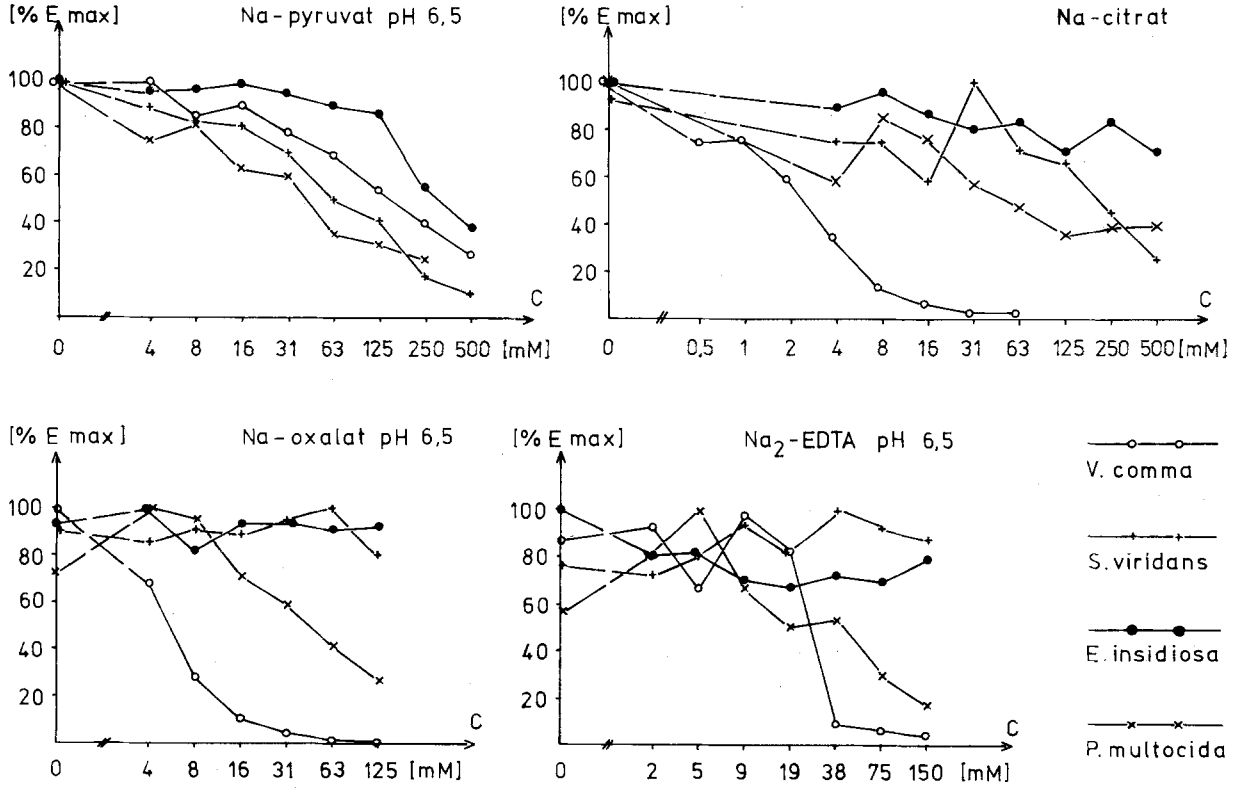

Fig. 1. Der Einfluss von Natrium-Pyruvat, -Citrat, -Oxalat und -EDTA in verschiedenen Konzentrationen auf die Neuraminidase-Aktivität von *Vibrio comma*, *Streptococcus viridans*, *Erysipelothrix insidiosa* und *Pasteurella multocida*.