outlined by membrane-like structures (Figure 2d). At other times only irregularly distributed membrane-like structures were found between the socket and the tubular body (Figure 2e).

The type T1/4 trichobothria respond maximally to deflections in caudal direction[5]. They display a distinct directional sensitivity which here, as well as in some other tactile sensory hairs of insects[7], seems to be influenced among other things by the polar construction of the socket septum. Only deflections of the hair shaft, which press the tubular body against the socket septum and cuticular stick, can result in a compression of the tubular body (= adequate stimulus[8]). This hypothetical idea of stimulus transmission postulates that the socket septa belong to the stimulus transmitting apparatus which influences the transformation of the input stimulus into the effective stimulus[9]. The process of transformation depends on the physical properties of the stimulus transmitting apparatus[2-4,9]. Although it is plainly impossible to see special physical properties of the socket septa in pictures made by the electron microscope, it is possible to say, based on the observed differences in construction, that the socket septum in type T3/4 has different properties (probably less stability) than the socket septum in type T1/4. Therefore in T3/4 trichobothria a different resistance (probably a lesser one or a more rapidly declining one) will be offered to the lateral displacements (caused by the lever effect of the hair shaft) of the tubular body. Although the input stimulus (deflection of the hair shaft through a definite angle) is the same in both cases, different effective stimuli would act upon the tubular bodies. The different patterns of response (T1/4 phasic-tonic; T3/4 phasic)[5] support this assumption. Out of at least 5 fundamentally different processes which possibly cause the decay of excitation in sensory cells[9], in the T3/4 trichobothria in comparison with T1/4 trichobothria, an increased 'dynamical decay of stimulus'[9-11] should be taken into consideration.

[6] K. P. GAFFAL and K. HANSEN, Z. Zellforsch. *132*, 79 (1972).
[7] K. P. GAFFAL, H. TICHY, J. THEISS and G. SEELINGER, Zoomorphologica *82*, 79 (1975).
[8] U. THURM, Science *145*, 1036 (1964).
[9] D. BURKHARDT, Erg. Biol. *22*, 226 (1960). - Fortschr. Zool. *13*, 146 (1961).
[10] D. BURKHARDT, *Wörterbuch der Neurophysiologie* (Gustav-Fischer-Verlag, Jena 1969).
[11] Definition of 'dynamical decay of stimulus' = dynamische Reizminderung[10]: Eine durch die dynamischen Eigenschaften des reizleitenden Apparates bedingte zeitliche Abnahme des vom reizleitenden Apparat zu den sensiblen Endstrukturen geleiteten Reizes.

Prevention of the Formation of Mycotoxins in Whole Wheat Bread by Citric Acid and Lactic Acid (Mycotoxins in Foodstuffs. IX)

J. REISS[1]

Mikrobiologisches Laboratorium, Grahamhaus Studt K.G., D-655 Bad Kreuznach (German Federal Republic, BRD), 22 August 1975.

Summary. Additions of citric acid and lactic acid to whole wheat bread suppress the formation of aflatoxins by *Aspergillus parasiticus* (0.5% citric acid, 0.75% lactic acid) and that of sterigmatocystin by *A. versicolor* (0.25 and 0.5% respectively).

The biosynthesis of aflatoxins is known to be inhibited by several organic acids as carbon sources, e.g. by shikimic, pyruvic, α-ketoglutaric, succinic and citric acids[2-4]. Nothing is known whether or not these acids are also inhibitors of the formation of other mycotoxins, such as sterigmatocystin and patulin. Aflatoxins[5,6] sterigmatocystin[7] and patulin[5,8] can be formed by moulds in bread. Previous studies on the influence of preservatives in bread on the formation of mycotoxins[9] revealed that an acidifying substance for dough, containing citric and lactic acids, suppresses the formation of aflatoxins and sterigmatocystin. This study was continued with pure substances and the results are described here.

Methods. Whole wheat bread (Grahambrot) was prepared with additions of citric acid (DAB 7) and lactic acid (DAB 7) in levels of 0.25, 0.5 and 0.75% (relative to whole wheat). Packages of 3 slices were packed into a cellulose foil (GEB 300; Kalle, Wiesbaden, BRD) and sterilized in hot steam. The total acid content ('Säuregrad') was determined according to the method of Schulerud[5]. The packages were inoculated with spores of one of the following moulds: *Aspergillus parasiticus* (formerly *A. flavus*, strain 89717; Commonwealth Mycological Institute, Kew, Surrey, England), *Aspergillus versicolor* (strain 519; Dr. R. Orth, Karlsruhe, BRD) and *Penicillium expansum* (strain D 19; Institut für Spezielle Botanik, Universität Mainz, BRD). The incubation temperature was 22 °C. The semiquantitative determinations of the toxins were performed as described earlier (aflatoxins[5], sterigmatocystin[7,10], patulin[8,11]). The fungal growth was determined by measuring the radius of the colonies every 24 h.

Results and discussion. a) Aflatoxins. Additions of citric and lactic acids of up to 0.5% do not markedly influence the growth of *A. parasiticus*, whereas the highest concentrations of both acids (0.75%) have a slight growth-reducing effect. The production of the aflatoxins B_1 and G_1, however, is greatly inhibited by low levels of citric acid and by higher concentrations of lactic acid (Table). A comparison of these results with the data of

[1] Acknowledgment. I thank Dr. R. ORTH (Bundesforschungsanstalt für Ernährung, Karlsruhe, BRD) for the strain of *Aspergillus versicolor*.
[2] N. D. DAVIS and U. L. DIENER, Appl. Microbiol. *16*, 158 (1968).
[3] J. D. WILDMAN, L. STOLOFF and R. JACOBS, Biotech. Bioengin. *9*, 429 (1967).
[4] R. I. MATELES and J. C. ADYE, Appl. Microbiol. *13*, 208 (1965).
[5] J. REISS, Eur. J. appl. Microbiol. *1*, 183 (1975).
[6] J. REISS, Food Cosmet. Toxic. *13*, 325 (1975).
[7] J. REISS, Z. Lebensmittelunters. u. -Forsch., in press (1976).
[8] J. REISS, Chem. Mikrobiol. Technol. Lebensmittel *2*, 171 (1973).
[9] J. REISS, Dt. Lebensmittel Rdsch., in press (1976).
[10] J. REISS, Z. analyt. Chem. *275*, 30 (1975).
[11] J. REISS, Mikrochim. Acta *1975*, 473.

Formation of mycotoxins in whole wheat bread with additions of citric acid and lactic acid (incubation time: 10 days)

	Total acid content ('Säuregrad')	Mycotoxins (μg/g)		
		Aflatoxin B_1	Aflatoxin G_1	Sterigmatocystin
No additions	3.4	0.006–0.008	0.01–0.02	0.06–0.08
Citric acid				
0.25%	5.4	< 0.001	< 0.001	— [a]
0.5%	6.6	—	—	no growth
0.75%	7.6	—	—	no growth
Lactic acid				
0.25%	4.7	< 0.001	< 0.002	0.06–0.08
0.5%	6.0	< 0.001	< 0.002	—
0.75%	6.7	—	—	no growth

[a] No toxin detected (detection limits for aflatoxin B_1: 0.001 μg/g; aflatoxin G_1: 0.001 μg/g; sterigmatocystin: 0.02 μg/g).

the optimal environmental conditions for the aflatoxin formation in whole wheat bread[5], show that the influence of citric and lactic acids is not the result of merely raising the acidity of the substrate.

b) Sterigmatocystin. The development of *A. versicolor* is more strongly influenced than that of *A. parasiticus*. No growth occurs under the influence of 0.75 and 0.5% citric acid and of 0.75% lactic acid. The toxin formation is prevented even by 0.25% citric acid and is reduced by 0.5% lactic acid (Table). The total acid content in a wide range does not affect the growth of *A. versicolor* and the formation of sterigmatocystin[7] so that the inhibitory effects of citric and lactic acids are specific.

c) Patulin. Neither the development of colonies of *P. expansum* nor the formation of patulin is influenced by even the highest levels of citric and lactic acids.

The observation that citric acid and lactic acid inhibit the production of aflatoxins and of sterigmatocystin but not the formation of patulin may be interpreted as evidence for the close relation of both toxins in a single pathway of biosynthesis[12].

[12] M. T. Lin, D. P. H. Hsieh, R. C. Yao and J. A. Donkerslot, Biochemistry *12*, 5167 (1973).

Effects of Alanine, Glycine and Glutamic Acid on Nitrogenous Excretion by *Amphiuma means* Liver in Organ Culture

N. Fleming[1] and M. Balls[2,3]

School of Biological Sciences, University of East Anglia, Norwich NR4 7TJ (England), 4 September 1975.

Summary. High concentrations (10 m*M*) of alanine, glycine, and glutamic acid in the culture medium had no effect on urea production in *Amphiuma means* liver in organ culture. Ammonia production was increased in media containing added alanine and glycine, but reduced in medium with added glutamic acid.

In earlier work, nitrogenous excretion in fragments of liver from the urodele amphibian, *Amphiuma means*, was investigated in long-term organ culture in two culture media[4]. Activities of the urea cycle enzymes, arginase and ornithine transcarbamylase (OTC), and of the associated transaminases, glutamic oxalacetic transaminase (GOT) and glutamic pyruvate transaminase (GPT) were higher in Eagle's Minimum Essential Medium (MEM) than in Leibovitz L15 medium (L15), but total nitrogen excretion was twice as high in L15 as in MEM. The two media differ in various respects. The carbohydrate component of L15 is galactose, whereas that of MEM is glucose, and there are minor differences in vitamin and inorganic salt content. L15 contains much greater amounts of free-base amino acids, which are far in excess of the levels required for cell growth and which maintain the medium at the required pH[5]. In addition, L15 includes several amino acids which are absent from MEM, two of which, alanine and glycine, have been investigated with respect to their deamination and the subsequent effects on nitrogenous excretion in the anuran *Xenopus laevis*[6–9]. Balinsky[9] suggested that deamination of the two amino acids may take place at different sites in the cell, and that the fate of their nitrogen may be connected with this separation. He proposed that alanine nitrogen is channeled into urea production in the liver, whereas nitrogen resulting from the deamination of glycine is excreted from the liver in ammonia.

Transamination reactions in the liver lead to the production of large amounts of glutamic acid, which then passes amino groups into the urea cycle. It might, then, be

[1] Present address: Anatomisches Institut der Universität Zürich, Gloriastrasse 19, CH-8006 Zürich, Switzerland.

[2] Present address: Department of Human Morphology, The Medical School, University of Nottingham, Nottingham NG7 2UH, England.

[3] We gratefully acknowledge the support of The Wellcome Trust.

[4] N. Fleming, D. Brown and M. Balls, J. Cell Sci., *18*, 533 (1975).

[5] A. Leibovitz, Am. J. Hyg. *78*, 173 (1963).