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].

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

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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

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expected that high concentrations of glutamic acid in the culture medium would also influence nitrogen excretion. Thus, in the experiments described here, the effects of high concentrations of alanine, glycine and glutamic acid on urea and ammonia excretion from *A. means* liver fragments were investigated.

Materials and methods. Fragments of liver, 1.5–2.0 mm³, from adult *Amphiuma means* were cultured at 25 °C in 50% MEM + 10% foetal calf serum, in 25 ml Erlenmeyer flasks according to the methods described previously[10, 11]. After 20 days, the cultures were divided into 4 groups of 5 flasks and fresh culture medium was added to each group as follows: normal culture medium (controls); MEM + 10 m*M* L-alanine; MEM + 10 m*M* L-glycine; MEM + 10 m*M* glutamic acid. 0.1 ml samples of medium were assayed for urea and ammonia at time 0, then after 3, 6 and 9 days, by the method of FAWCETT and SCOTT[12]. Results are given as the arithmetic mean ± standard error of the mean. Mean values in different groups were compared by the Student *t*-test, with a modification for small samples[13].

Total nitrogenous excretion (urea + ammonia) by *A. means* liver fragments cultured in 4 different media for periods of 3, 6 and 9 days

Medium	mg Nitrogen per g wet weight		
	Day 3	Day 6	Day 9
MEM	3.28	4.14	4.47
MEM + 10 m*M* alanine	3.71	4.79	5.71
MEM + 10 m*M* glycine	3.71	4.45	4.83
MEM + 10 m*M* glutamic acid	1.85	2.55	3.25

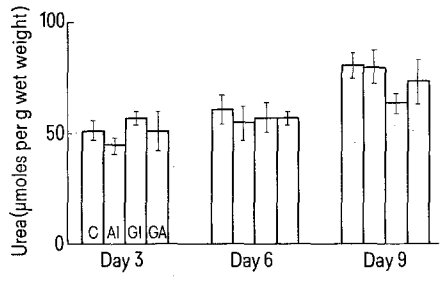

Fig. 1. Urea released by *A. means* liver cultures in 4 different culture media over a 9 day period. C, control (50% MEM + 10% foetal calf serum + 40% double distilled water); Al, control medium *M* + 10 m*M* L-alanine; Gl, control medium + 10 m*M* L-glycine; GA, control medium + 10 m*M* glutamic acid. Values are the mean of 5 samples ± SE.

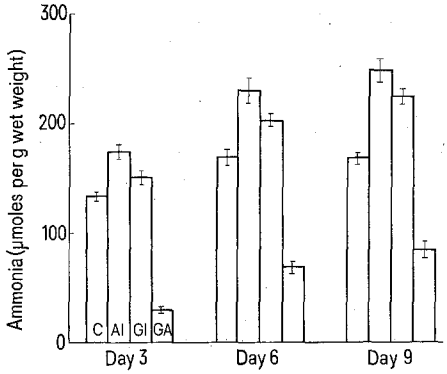

Fig. 2. Ammonia released by *A. means* liver cultures over a 9-day period. Media and symbols as Figure 1.

Results. The amounts of urea and ammonia (μmoles per g wet weight of tissue) produced by cultured *A. means* liver over periods of 3, 6 and 9 days in the 4 different culture media are recorded in Figures 1 and 2. The Table shows the total nitrogen excreted during the same culture period. The greatest release of urea and ammonia occured during the first 3 days. This is probably because fresh culture medium provided a high concentration of substrates, which enabled the reactions involved in amino acid catabolism to proceed at a high rate. The lower rates of urea and ammonia production over the subsequent 3-day periods could be due to the depletion of medium substrates and the build-up of reaction products. There were no significant differences in the amounts of urea released in any of the 4 media over the total experimental period, but the level of ammonia production increased ($p < 0.05$) in media containing alanine and glycine, and substantially decreased ($p < 0.002$) in medium with a high concentration of glutamic acid.

Discussion. High concentrations of alanine and glycine in the culture medium led to an increase in ammonia excretion but not urea excretion by *A. means* liver organ cultures. This suggests that amino acid uptake by the liver cells, and hence the subsequent deamination and transamination reactions are controlled, at least to some extent, by the extracellular amino acid concentration. Glycine is deaminated by a specific flavoprotein, glycine oxidase, to produce ammonia and glyoxylic acid, and alanine may be either deaminated by L-amino oxidases to ammonia and pyruvate or transaminated by GPT to glutamate, which is a central source of excreted nitrogen. The net result of these reactions would be an accumulation of amino groups, which could be fed into the urea cycle or excreted from the cell as ammonia. Cultures in media containing alanine or glycine showed an increase in ammonia production only. It is therefore postulated that control medium (50% MEM) contained a high enough concentration of amino acids to permit the series of reactions comprising the urea cycle to proceed at a maximum rate, under organ culture conditions. Thus, additional amino groups resulting from the deamination of higher concentrations of amino acids could not enter an already 'saturated' cycle and would be excreted directly as ammonia. This explains the observation[4] that *A. means* liver cultured in L15 excreted more ammonia than that cultured in MEM, which has a much lower amino acid content. MONNICKENDAM and BALLS[14] also related the greater release of ammonia by *A. means* kidney fragments in L15 than in MEM to the higher concentration of amino acids in L15.

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Although this hypothesis could account for the increased ammonia production in L15, it does not explain why the levels of activity of the urea cycle enzymes were lower in *A. means* liver cultured in L15 than in MEM (assuming that the level of urea cycle enzyme activity is related to the amount of urea excreted)[4]. The high concentrations of amino acids may themselves produce inhibitory effects. For example, MAAS and CLARK[15] showed that all the enzymes (including OTC) involved in arginine synthesis in *E. coli* were repressed in the presence of arginine by a repressor protein synthesized by a regulator gene. It is possible that a similar type of control mechanism could operate in *A. means* hepatocytes.

On the basis of the hypothesis outlined above, it could be predicted that a high concentration of glutamic acid in the culture medium would result in increased ammonia release from cultured *A. means* liver fragments. However, during the 9-day experimental period total ammonia production was much lower in MEM + 10 m*M* glutamic acid (86 ± 6 µmoles/g wet weight). This finding may also be related to transaminase activity in the cultured tissue. Glutamic acid may be deaminated by glutamate dehydrogenase and the amino groups fed into excretory pathways, or it may be transaminated to other amino acids by transaminase enzymes such as GOT and GPT. The equilibrium constants for transamination reactions are low, so that the reactions are freely reversible, depending on relative substrate concentrations. It is therefore possible that high concentrations of glutamic acid in culture medium stimulate transaminase reactions in the direction: glutamic acid → other amino acids. This implies that, in cultured *A. means* liver, large quantities of glutamic acid are preferentially converted by transaminase enzymes, but that glutamate dehydrogenase activity is sufficient to maintain a maximum level of urea excretion without the production of excess free amino groups which would be released as ammonia[16].

Thus, it is proposed that in *A. means* liver cultures, urea excretion proceeds at the maximum rate under culture conditions; in high concentrations, alanine and glycine are deaminated, either directly or via glutamic acid, resulting in an increase in ammonia production; a high concentration of glutamic acid stimulates its transamination to other amino acids with a corresponding reduction in ammonia production.

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Mast Cells in the Skin of Normal, Hairless and Athymic Mice[1]

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Summary. The skin of congenitally athymic *nu/nu* mice is rich in mast cells which stain metachromatically, contain histamine and 5-hydroxytryptamine, and participate in the PCA reaction. Mast cells of athymic mice have thus the attributes of normal mast cells.

BURNET[3,4] has recently reiterated his view that the tissue mast cell represents an end cell of the T lymphocyte. Congenitally thymus-deprived (*nu/nu*) mice thus provide a convenient test system for analyzing this hypothesis by comparing the mast cell content in the skin of *nu/nu* and normal mice. Since chemical carcinogenesis in normal mouse skin is accompanied by a local accumulation of mast cells[5], skin reactions and mast cell responses following topical application of a chemical carcinogen were also examined in normal Balb/c, in athymic *nu/nu* Balb/c, and in 'hairless' (*hr/hr*) mice.

Material and methods. Normal pathogen-free Balb/c mice, hairless (*hr/hr*) mice (Institut für Biologisch-Medizinische Forschung AG, Füllinsdorf, Switzerland) and congenitally athymic nude mice (*nu/nu*, third backcross generation with Balb/c; Bomholdgård, Ry, Denmark) were painted twice, one week apart, along the centre of the back with 0.2 ml of an 0.25% solution of 7,12,dimethylbenz(a)anthracene (DMBA) in acetone containing no promoting agent; untreated or acetone-treated animals served as controls. Mice had neither been shaved nor epilated before topical application of DMBA. In a few athymic mice, Balb/c thymus was implanted subcutaneously 3 weeks before first DMBA painting.

For histology, samples of skin taken from controls or 3 months after DMBA painting were fixed in Baker's calciumformol, cleared in methylbenzoate, embedded in paraffin, sectioned at 3 to 4 µm, and stained with toluidine blue (pH 3.0) for mast cells. Mast cell counts are expressed per linear cm of sectioned skin. Fixed, unstained sections were examined for 5-hydroxytryptamine (5-HT) fluorescence (excitation 380–415 nm; emission 520 to 530 nm).

For the estimation of histamine, portions of freshly excised dorsal skin were rapidly weighed and extracted with 10% trichloroacetic acid for subsequent assay on the standard guinea-pig ileum preparation.

The capacity to bind reaginic antibody was tested by passive cutaneous anaphylaxis (PCA). Normal Balb/c mice were immunized by an i.p. injection of 100 µg bovine serum albumin (BSA) and *Bordetella pertussis* vaccine[6]. Dilutions of sera with a PCA titer of 1:80 to 1:320 were injected into the skin of normal and *nu/nu* Balb/c mice. 48 h later, the binding of reaginic antibody was assessed by i.v. injection of 1 mg BSA in 0.5 ml 0.25% Evans blue in saline.

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