A given organism, although showing certain growth characteristics under experimental conditions may, under natural conditions, be limited to the exploitation of one definite source of a metabolite (the identity of which may be difficult to establish) and its distribution thus may be affected by the temperature characteristics of an enzyme or enzymes involved in that particular reaction.

Acknowledgments. The elementary analysis of the glucosazone was done through the courtesy of Prof. K. A. JENSEN by Mr. W. EGGER at the Copenhagen University Chemical Laboratory. Thanks are due also to Dr. EVELYN SMITH MILLS for her help with the paper chromatography, to Miss HELEN HOLLESEN for technical assistance, and to Mr. PETER JACOBSEN for taking the photograph.

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Zusammenfassung

Bei *Neurospora crassa* W 2/49 A wird eine extrazelluläre, temperaturabhängige, adaptive Zellulase beschrieben, welche die Anhäufung bemerkenswerter Mengen von Glukose verursacht. Es wird ein möglicher Zusammenhang zwischen der Ökologie des Organismus und dessen Fähigkeit, bedeutende Mengen des Enzyms bei höherer Temperatur zu bilden, kurz diskutiert.

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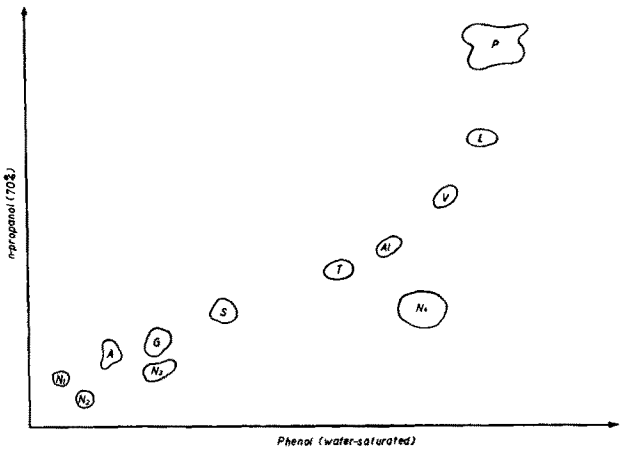
Concerning the Free Amino Acids in Amphibian Development

The paper-chromatographic method has been used by several authors to investigate the changes of free amino acids in amphibian development. LI and ROBERTS[1] were unable to detect any free amino acids in their alcoholic extracts of *Rana pipiens* eggs and concluded that such acids exist in detectable quantity only at later stages. On the other hand, HOLTFRETER, KOSZALKA, and MILLER[2], who also worked on *Rana pipiens*, reported the occurence of a number of free amino acids and other ninhydrin-positive substances in the alcoholic extract of the ovarian eggs. Since the same compounds were found in larvae extracts, the latter authors stated: "As far as the amphibian egg is concerned, the chromatograms give no indication that new amino acids appear and others disappear in the course of embryonic and larval development." Recently, in connection with experiments devoted to studies of protein metabolism in lethal hybrids and in sex hormone treated embryos, we have performed a series of paper chromatograms on the free amino acids at various developmental stages of *Triton palmatus* and *Triton alpestris*. Our preliminary results have led us to conclusions which differ in many respects from those reached by the previous authors.

We used only 5 eggs for each chromatographic separation. The eggs, after being stripped of their jelly, were mashed in about 0·2 cm³ of 80% methyl alcohol in a small test tube and kept in a refrigerator from overnight up to several days. The alcoholic extract together with the protein precipitates was then applied with a fine pipette to a filter paper sheet (WHATMAN No. 1) 28 × 46 cm in size. The starting spot usually had a diameter of about 1 cm. For separating different free amino acids, the two-dimensional chromatography was employed, first ascending in 70% n-propanol and then descending in water-saturated phenol. The sheet was finally dried and sprayed with 0·1% alcoholic solution of ninhydrin.

In the unfertilized eggs, two free amino acids have been identified, namely aspartic acid and glutamic acid. At blastula and gastrula stages, in addition to these two amino acids, four more ninhydrin-positive substances were found. In the young larvae (HARRISON's stage 31–32), two new spots have been recorded, one of which has been identified as serine. During later development (HARRISON's stage 40–44), the chromatogram showed four additional amino acids: threonine, alanine, valine and leucine.



A aspartic acid, *G* glutamic acid, *S* serine, *T* threonine, *Al* alanine, *V* valine, *L* leucine, *P* polypeptide complex, N_{1-4} not yet identified ninhydrin-positive substances.

One fact which deserves special mention is that in *Triton alpestris* a polypeptide complex of high *Rf* values in both directions was found at all stages observed. The hydrolysis of this polypeptide complex with 6 N HCl at 105°C revealed that it consists of aspartic acid, glutamic acid, serine, glycine, alanine, tyrosine and another acid which has not yet been identified.

Furthermore, we have sufficient evidence to believe that there is a change of free amino acid concentration in the course of embryonic and larval development. During neurulation, it seems that there is a reduction of the number and the quantity of amino acids. On the other hand, there is an increase of the acid content during segmentation and during larval development. For example, at least as far as aspartic acid and glutamic acid are concerned, the spots appear to be larger in size and much more intensive in colour in the larvae than those observed at earlier stages.

Considering that during all these investigated stages there was no intake of food and the embryos were still a closed system, this fact is of special importance. It gives us an insight into the chemical changes of the cellular components parallel to the morphogenetic development.

In summary, the results of the present study, show that the free amino acids do exist in "detectable quantity" even in unfertilized eggs and can be chromatographically separated by using only 5 eggs. In the

[1] C. T. LI and E. ROBERTS, Science *110*, 425 (1949).

[2] J. HOLTFRETER, T. R. KOSZALKA, and L. L. MILLER, Exp. cell Res. *1*, 453 (1950).

course of embryonic and larval development, there is a change of the acid concentration and appearance of new amino acids. A more detailed analysis of all ninhydrin-positive materials is still in progress[1].

We are indebted to Dr. ELISABETH STUMM-ZOLLINGER for her valuable help and suggestions in the course of these experiments.

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Zusammenfassung

Wir untersuchten Keime verschiedener Altersstadien von *Triton palmatus* und *Triton alpestris* mit Hilfe der Papierchromatographie auf das Vorkommen von freien Aminosäuren und Polypeptiden. Im Gegensatz zu andern Autoren fanden wir solche in allen Stadien in nachweisbaren Konzentrationen. Ihre Zahl und Menge ändern sich im Lauf der Entwicklung.

Asparaginsäure und Glutaminsäure kommen in jeder Altersstufe vor. Bei jungen Larven (HARRISON 31–32) konnten wir neben andern Ninhydrin-positiven Substanzen auch Serin nachweisen. Bei ältern Larven (HARRISON 40–44) treten Threonin, Alanin, Valin und Leuzin neu in Erscheinung. Bei *Triton alpestris* fanden wir in allen untersuchten Stadien einen Polypeptidkomplex, welcher in beiden verwendeten Lösungsmitteln eine hohe Wanderungsgeschwindigkeit aufwies. Er besteht aus: Asparaginsäure, Glutaminsäure, Serin, Glyzin, Thyrosin, Alanin und einer noch nicht identifizierten Aminosäure.

Mit der Neurulation scheint eine Abnahme von Zahl und Menge der freien Aminosäuren und der Polypeptide verbunden zu sein. Furchung und Larvenentwicklung sind jedoch durch eine Zunahme gekennzeichnet.

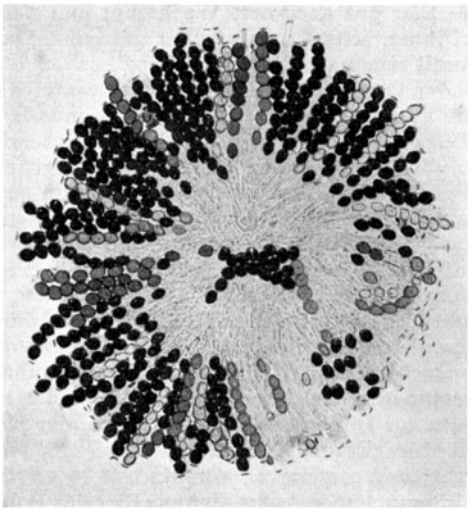
[1] After we sent our manuscript for publication the work of KUTZKY, EAKIN, BERG, and KAVANAU (J. exp. Zool. *124*, 263 [1953]) was made known to us. They worked on *Rana pipiens* and their results concerning the free amino acids are essentially similar to those mentioned in this paper. Since our experiment is still in progress, we refrain from giving a detailed discussion of the minor differences which might be inherent in the materials used in both experiments.

L'acide borique, inhibiteur de la différenciation des ascospores chez *Sordaria*

Les conditions physiologiques présidant à la différenciation des ascospores des champignons Ascomycètes sont encore mal connues[1].

BARNETT et LILLY[2] ont démontré le rôle déterminant joué par la biotine dans la différenciation des ascospores de *Sordaria fimicola*. En présence de doses minima de biotine (0,8 γ par litre), compatibles avec la seule croissance végétative du champignon, ces auteurs ont observé un avortement presque complet des asques et des ascospores.

En cultivant *Sordaria* sp. (espèce homothallique isolé d'un sol de vignoble vaudois) sur milieu optimal[3] additionné de 1/20000 d'acide borique, nous avons eu la surprise d'obtenir des figures d'avortement des asques et des ascospores rappelant de manière frappante celles publiées par BARNETT et LILLY: même absence d'ascospores différenciées dans des asques dégénérés dont le cytoplasme fragmenté est encombré de gouttelettes lipidiques aisément colorables par le bleu de Nil (voir figures ci-dessous).



Rosettes d'asques de *Sordaria* sp. photographiées 20 jours après l'inoculation. Fig. 1. Culture témoin sur milieu optimal (170 ×). Photos J. GAILLARD

Aux doses de 1/5000 et 1/10000, l'action de l'acide borique est plus sévère et, à l'instar d'une forte carence en biotine, se traduit par une stérilité presque totale des cultures dont la croissance végétative n'est par ailleurs même pas ralentie.

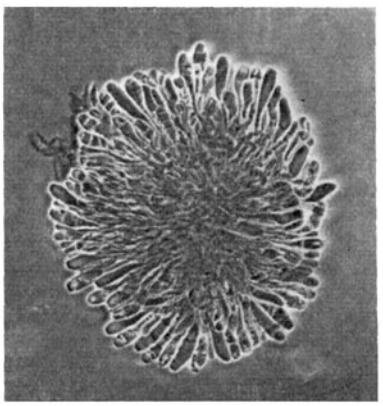


Fig. 2. Culture sur le même milieu additionné de 1/20000 d'acide borique (contraste de phase, 170 ×).

On peut se demander si ce curieux phénomène de convergence morpho-pathologique ne trahit pas une interférence de l'acide borique avec l'utilisation de la biotine[1] au cours des processus sexuels de *Sordaria*.

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Summary

Sordaria sp., when grown on a glucose-starch-yeast extract medium with 1/20000 boric acid, produced only abortive or abnormal asci without differentiated ascospores.

[1] *Sordaria* sp. est hétérotrophe pour la biotine.

[1] V. G. LILLY et H. L. BARNETT, *Physiology of the Fungi* (McGraw Hill, 1951).

[2] H. L. BARNETT et V. G. LILLY, Amer. J. Botany *34*, 196 (1947).

[3] $MgSO_4$ 7 H_2O, 0,5 g; KH_2PO_4, 1,0 g; extrait de levure Difco, 4,0 g; glucose 20,0 g; amidon soluble 20,0 g; agar 15,0 g; eau distillée, 1 litre.