Table II.—Turnover of DNA and PNA phosphorus in growing young rat

Age day	mg Phosphorus per liver		Specific activity count/min/mg P			Total activity count/min/liver		Corrected total activity count/min/liver	
	DNA	PNA	DNA	PNA	inorg. P.	DNA	PNA	DNA	PNA
20	0·291	0·908	6390	5630	3040	1860	5180	1860	5180
30	0·517	2·52	3410	1820	423	1760	4580	1370	1790

Having administered total of 1 mc P^{32}, suckling babies were separated from the mother and delivered to another lactating female rat which had not received P^{32}, in order that the compounds with high specific activity in sucklings should be turned over. The growth of the babies was normal. After ten days of lactation by the foster-rat, 6 radioactive weanlings were obtained. They were then divided into 4 and 2 rats, the ratio of the total body weights of the two groups being just 2:1. The former group was sacrificed directly, the latter group after being allowed to grow for another ten day period. DNA and PNA were purified from pooled livers in each case. The total activity of two nucleic acids per liver did not decrease significantly during the 10 day period of rapid growth of the liver, which corresponded to body weights from 28 to 53 g[1] (Table II). But, considering the pretty high specific activity of inorganic phosphate in the liver of younger rats, correction for the secondary incorporation was made by assuming daily linear increase of PNA and DNA per liver and daily exponential decrease in specific activity of inorganic phosphate as the precursor of nucleic acid-P. The corrected total activity[2] now reveals that there was very probably a breakdown of PNA, and to a lesser extent, of DNA, too, during the growth of the liver, but the extent of this breakdown cannot be correlated in any simple way with the actual increment of PNA and DNA per liver.

These results would, then, suggest that the mechanism of nucleic acid biosynthesis does not necessarily involve a breakdown of the pre-existent molecules of the same compound.

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Microbial Diseases Research Institute, University of Osaka, Doozima-Nisimati 7, Kitaku, Osaka, Japan, August 22, 1953.

Zusammenfassung

Es wurde untersucht, ob die Biosynthese der Nukleinsäuren in wachsenden Systemen mit dem Abbau von ursprünglich vorhandenen Nukleinsäuren derselben Art gekoppelt ist. Die Versuche wurden mit Bakterien (*E. coli*) und mit Rattenleber gemacht. Die in der Zelle vorhandenen Nukleinsäuren waren mit P^{32} markiert, und es wurde der Verlust an Radioaktivität in jeder der beiden Nukleinsäuretypen im Laufe des Wachstums gemessen. Die Resultate wiesen darauf hin, dass das Auftreten von neuen Nukleinsäuremolekeln in der lebenden Zelle ohne den begleitenden Abbau der alten Molekeln stattfinden kann.

[1] M. FUKUDA and A. SIBATANI, J. Biochem. (Japan) *40*, 95 (1953).

[2] The corrected values may be a little too low, because the estimated activity increment due to the secondary incorporation of P^{32} into nucleic acids was subtracted from the observed total activity of the respective nucleic acids obtained from the 30 day old rats, neglecting the loss of activity due to the possible breakdown of nucleic acid molecules containing secondarily incorporated P^{32}.

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Temperature-Dependent Cellulase Production by *Neurospora crassa* and Its Ecological Implications

The ascomycete *Neurospora crassa* SHEAR and DODGE has in recent years become an important tool in biochemical genetics. The mold is usually grown on liquid or agar-solidified synthetic media containing inorganic salts, biotin, and a suitable carbon source. In the course of some recent experiments it became necessary to modify this procedure slightly by growing the mold in a glycerol-containing liquid minimal medium on filterpaper. Under these conditions the mold produces a considerable amount of glucose at 35° C but only a very small amount at 25° C. The sugar is produced only in the presence of filterpaper and its formation is due to the production of an extracellular cellulase.

Neurospora crassa strain W 2/49 A is used; for one of the experiments, strains Abbott a and Chilton a are also used. The medium employed is a WESTERGAARD and MITCHELL[1] medium containing as the carbon source 20 ml of glycerol per litre; 15 ml are used per PETRI dish. To each dish are added 5 sheets of sterile WHATMAN No. 4 filterpaper, 8 cm in diameter. The uninoculated, autoclaved medium gives negative BENEDICT and MOLISCH tests. The filterpaper also is free of reducing substances as well as soluble starch. Inoculation is made by adding to each dish 0·5 ml of a suspension of conidia in sterile distilled water. The temperature of incubation is 35° C or 25° C.

When the organism is incubated at 35°C, a considerable amount of a reducing sugar accumulates in the medium. This sugar gives positive BENEDICT and MOLISCH reactions, and negative BARFOED, SELIVANOFF, mucic acid, phloroglucinol-HCl, and benzidine tests. Microscopic examination of the osazone shows a typical glucosazone. An elementary analysis of this osazone gives the following results:

glucosazone	calculated	C 60·32 H 6·19 N 15·63,
$C_{18}H_{22}O_4N_4$	found	C 60·18 H 6·34 N 15·68.

Final identification of the sugar is made by paper chromatography, using WHATMAN No. 1 filterpaper, butanol-acetic acid-water (4:1:5, v/v) as the solvent, and aniline hydrogen phthalate as the developing agent. The unknown sugar moves at the same speed as glucose, and gives only one spot when mixed with a sample of authentic glucose. The amount of glucose found varies due to its continual utilization by the organism, but it was calculated that in some of the experiments roughly

[1] M. WESTERGAARD and H. K. MITCHELL, Amer. J. Botany *34*, 573 (1947).

Determination of glucose formed from cellulose by filtrates from *Neurospora crassa* W 2/49 A. Organisms grown for 7 days (Nos. 1–3) or 6 days (No. 4) at 35°C. Duration of enzymatic tests, ca. 48 h, temperature of incubation 35°C. In column 4 are given the mean values for the amounts of filtrate needed for the titration, followed by the standard error of the mean; the number of individual determinations are in parenthesis.

No.	Presence or absence of filterpaper during growth	Test conditions	ml of filtrate used for titration	Equivalent to mg of glucose per 10 ml sample	Net production of glucose in mg
1	present	Filtrate + filterpaper	1·33 ± 0·043 (7)	8·4	1·9
2	present	Boiled filtrate + filterpaper	1·71 ± 0·033 (4)	6·5	0
3	present	Filtrate alone	1·72 ± 0·10 (2)	6·5	0
4	absent	Filtrate + filterpaper		0	0

0·5 g of glucose can be isolated from one litre of medium following filtration of the mycelium.

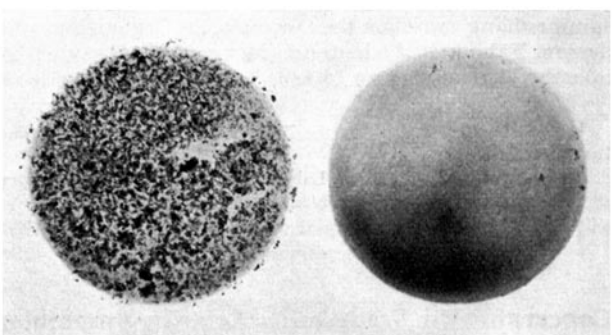
The glucose could originate from either the glycerol or from cellulose present in the form of filterpaper. To test this, the mold is grown at 35°C in shallow layers of liquid medium alone, on perforated rubber pads, and on agar instead of on filterpaper. Under such conditions only traces of a reducing sugar accumulate in the medium. This sugar moves on a paper chromatogram with the same speed as galactose, but the quantities present are too small to permit definitive identification.

The presence of an extracellular cellulase is demonstrated thus: The supernatant from organisms grown on liquid minimal medium on filterpaper at 35°C for 7 days is sterilized by filtration through a bacterial filter. Similar filtrates are obtained from organisms grown on filterpaper in liquid media at 25°C and from organisms grown at 35° C in the absence of filterpaper. 10 ml portions of the filtrate are added to tightly cotton-stoppered 100 ml ERLENMEYER flasks containing pieces of sterile filterpaper and incubated for ca. 48 h at 35° C. Reducing sugar is determined using a slight modification of BENEDICT's method[1]. Results of an experiment are given in the Table. Cellulase is formed by the mold only in the presence of cellulose during growth. It appears, therefore, that this extracellular enzyme is produced adaptively. No tests were done to determine whether other substrates can induce the formation of the enzyme, nor has any attempt been made to see whether mutation and selection or an adaptive enzymatic response is involved; the latter is, however, considered more likely.

Formation of the enzyme is markedly temperature-dependent. When similar filtrates from organisms grown at 25° C are used there is only a much smaller increase in reducing sugar, roughly 20% of that found with the 35° C filtrates; the method becomes inaccurate at such low levels. It might be added that when the mold is grown at 25°C on filterpaper in the presence of amino nitrogen (3,000 mg per litre) not even a trace of reducing sugar is found to accumulate and cellulase is completely absent.

This can be even more convincingly demonstrated by growing the organism in a minimal medium which contains cellulose as the only source of carbon (see also WENT[2] and RYAN *et al.*[3]). On such a medium the mold grows very well at 35°C while only a much slower and poorer growth obtains at 25°C (see Figure). On the other hand, when glycerol is present, the final yield of cell material is approximately 4–6 times greater at 25°C than at 35°C.

With Chilton a, essentially similar results are obtained as with W 2/49 A. With Abbott a, however, the situation is reversed; a small but definite amount of growth is obtained at 25°C while almost no growth occurs at 35°C. (On minimal medium this strain grows well at 35°C.)



Comparison of *N. crassa* W 2/49 A grown for 4 days on minimal medium containing only cellulose as the carbon source. Temperature of incubation: left, 35°C; right, 25°C.

Discussion. *N. crassa* strains have always been collected from tropical or subtropical areas, frequently from burned trees (SHEAR and DODGE[1]; BEADLE and TATUM[2]). Dr. B. O. DODGE (private communication) also has expressed the opinion that *N. crassa* may be considered a tropical or subtropical species. It seems possible that the ability of certain strains to elaborate cellulase at higher temperature ranges is a determining factor in their ability to occur in the wild state in the warmer climates but not in the temperate or cold ones (apart from bakeries, etc.) and thus a direct enzymological explanation can, perhaps, be given for the occurrence of the organism in its own particular ecological niche, be this on a strain or species level.

Another factor which may affect the organism's distribution is that both W 2/49 A and Chilton a, when grown on cellulose media, form aerial hyphae and large numbers of conidia at 35°C, while at 25°C almost no aerial mycelium and few conidia are produced. The Abbott, a strain, however, produces aerial mycelium and conidia at 25°C.

A more detailed enzymological approach to some problems of ecology may prove to be of use elsewhere.

[1] C. BENEDICT, J. Amer. Med. Assoc. *57*, 1193 (1911).

[2] F. A. F. C. WENT, Jb. Wissensch. Bot. *36*, 611 (1901).

[3] F. J. RYAN, G. W. BEADLE, and E. L. TATUM, Amer. J. Botany *30*, 784 (1943).

[1] C. L. SHEAR and B. O. DODGE, J. Agric. Res. *34*, 1019 (1927).

[2] G. W. BEADLE and E. L. TATUM, Amer. J. Botany *32*, 678 (1945).

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.

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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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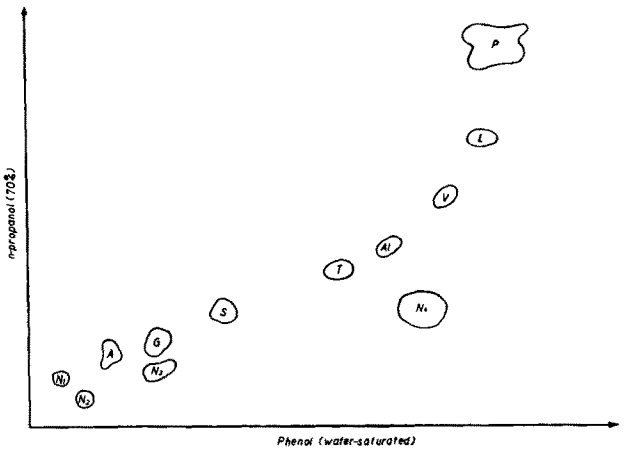
Concerning the Free Amino Acids in Amphibian Development

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).