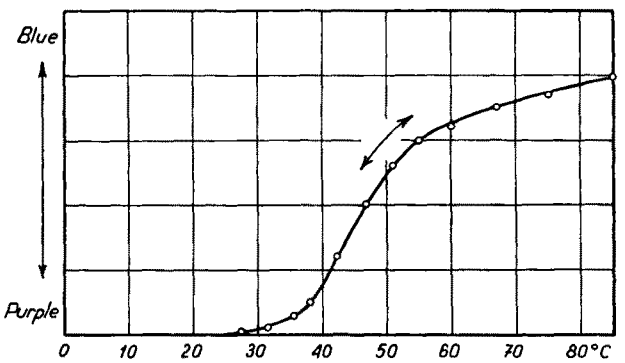
the leukomethylene blue takes place in spite of the presence of ascorbic acid. This fact is proved as follows:

(1) If we make the above-mentioned system containing heparin airtight by a layer of paraffin oil, the effect of heparin on the leukomethylene blue is inhibited completely.

(2) The quantity of leukomethylene blue oxidized by the same amount of heparin depends on the size of surfaces touching the air and the thickness of fluid layers. Mathematically the ratio of the sizes of surfaces referred to the unit of thickness of the layers is equivalent to the ratio of the quantities of oxidized leukomethylene blue. For example: 200 γ heparin oxidized 44 γ leukomethylene blue when the size of surface was 5·5 cm^2 and the thickness of layer 1·5 cm. The same amount of heparin oxidized 7·45 γ leukomethylene blue when the surface was 1·6 cm^2 and the thickness of layer 3·2 cm. The ratio of the quantities of oxidized leukomethylene blue is 5·91. The value calculated theoretically is:

$$\frac{5 \cdot 5/1 \cdot 5}{1 \cdot 6/3 \cdot 2} = 7 \cdot 3.$$

In our experiments the theoretically calculated and experimentally found values agreed as far as 80% (for example: 5·91 and 7·3).



(3) If we added to our system Fe^{+++} ions or H_2O_2 the oxidation of leukomethylene blue was much quicker in the tubes containing γ quantities of heparin than in the control tubes containing no heparin.

The effect of heparin in our system could be inhibited by adding to it small quantities of serum (0·1 ml/5 ml). It has not yet been decided whether this phenomenon is to be explained by the modifying effect of the plasma factor of heparin or by the serum protein causing inactivation of heparin.

We could show the same effect when using toluidine blue instead of methylene blue. This system however, does not yield a pure colour, bluish purple, and was not suitable for colorometric estimation.

The above-mentioned effect of heparin depends much on the p_H. We made our experiments at p_H's between 5–6. The spontaneous oxidation of the leukomethylene blue increases at p_H 7 in control tubes considerably and that of the heparin falls below half. At p_H 7·5, practically no heparin effect can be demonstrated. At higher p_H's there is again a heparin effect, but it could not be measured by colorometric methods, as the colour became greenish blue. In strong basic solutions no spontaneous oxidation could be observed.

As to the mechanism of the heparin effect, it seems probable that heparin with a high molecular weight and high negative charge adsorbes on its surface the basic molecule of leukomethylene blue so that the latter becomes very sensitive for oxidative influences. This supposition is supported by further experiments.

The colour-reaction of heparin-toluidine blue that LISON considered as a specific reaction of sulphuric esters of high molecular weight[1] disappears at higher temperatures and reappears at lower temperatures. As the figure shows, the decomposition begins already at 30° C, and the original purple colour takes a bluish shade. The change of colour between 38–40° C becomes very definite, but complete only at 85° C when the solution is entirely blue. While cooling, the blue turns through transitory colours purple again. Different alcohols (methyl-, æthyl-, propyl-, and butylalcohol, glycerol) and the watery solution of sodium choleinicum also brake the toluidine blue-heparin attachment. If we add propyl alcohol to the system the decomposition begins already at 5% concentration and becomes complete in the presence of 30% of propyl alcohol.

As adsorptive forces decrease and even cease on higher temperatures, and as the desorptive property of alcohols and sodium choleinicum solutions are generally known, our supposition that the heparin toluidine blue attachment is an adsorption seems to be proved.

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IInd Medical Clinic of the Péter Pázmány University of Budapest, Hungary, May 25, 1950.

Zusammenfassung

Wir untersuchten die Heparinwirkung auf Redoxsysteme und stellten dabei fest, daß selbst sehr kleine Mengen Heparin die Oxydation des Leukomethylenblaues fördern. Das Heparin als ein großes, negativ geladenes Molekül gibt diese Wirkung durch die Adsorption und die dadurch hervorgerufene Aktivierung des Leukomethylenblau-Moleküls. Für den Ablauf der Oxydation ist eine Sauerstoffquelle notwendig. Außerdem wurde bewiesen, daß die Heparin-Toluidinblau-Bindung eine adsorptive Bindung ist.

[1] L. LISON, C. r. Soc. Biol. *118*, 821 (1935); Arch. Biol. *46*, 599 (1935); Bull. Soc. Chem. Biol. *18*, 225 (1936).

Lyophilization and Mutation in Yeast

Four years back, chromosomal and genic changes were observed in yeast cultured in a cold room[1]. This was to be expected since temperature shocks have been known to have different effects on animals and plants[2]. Gene mutations in animals are accelerated by temperature shocks while the same agency induces a doubling of the chromosome complement in plants[3]. In yeasts, gene mutations[4], chromosal translocations[5], and tetraploidy[6] have been observed as a result of thermal shocks. The question arose whether lyophilization may not produce similar changes[7]. The most important step during the process of lyophilization is to cool the culture suddenly[8]

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[2] H. J. MULLER, Gen. *13*, 279 (1928). – H. H. PLOUGH, Biol. Symp. *6*, 9 (1942). – TH. DOBZHANSKY, *Genetics and the Origin of Species* (Columbia Univ. Press, 1941).

[3] H. DERMEN, Bot. Rev. *6*, 599 (1940).

[4] M. K. SUBRAMANIAM, B. RANGANATHAN, and S. N. KRISHNA MURTHY, Cellule *52*, 39 (1948).

[5] M. PREMA BAI, Curr. Sci. *16*, 316 (1947).

[6] M. K. SUBRAMANIAM and B. RANGANATHAN, Proc. Nat. Inst. Sci. India *14*, 279 (1948).

[7] B. RANGANATHAN, J. Ind. Inst. Sci. (in the press).

[8] L. J. WICKERHAM and A. A. ANDREASEN, Wallerstein Lab. Commun. *5*, 165 (1942).

to a very low temperature. The dehydration after this step may not inhibit the mutational step in those cells which are viable[1].

To elucidate this possibility a few cultures of our two chromosome brewery yeast[2] at different physiological states were lyophilized at the Haffkine Institute, Bombay, in the second week of January, 1950. The giant colonies of the control grown at Bangalore at the time these experiments were carried out are illustrated in figures 1 and 2. The colonies are mixtures of mutants[3].

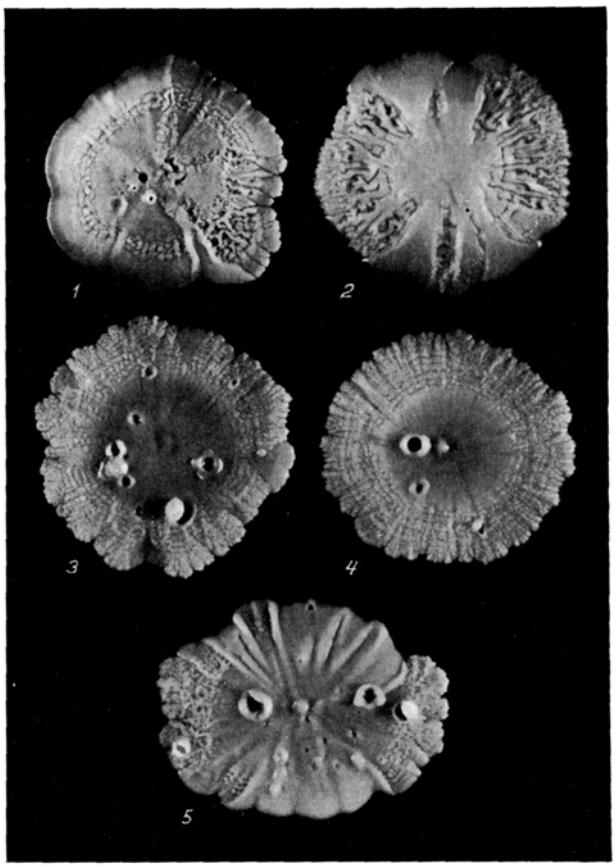

Fig. 1. – By 1 control (12. 10. 45) grown on barley malt agar. 2·9 cm*, 23-day growth, 30. 1. 50.
Fig. 2. – By 1 control (12. 10. 45). 2·1 cm, 16-day growth, 30. 1. 50.
Fig. 3. – By 1 96-hour old culture. Lyophilized on 13. 1. 50. Rejuvenated on 8. 3. 50. 2·8 cm, 11-day growth, 22. 3. 50.
Fig. 4. – By 1 96-hour old culture. Lyophilized on 13. 1. 50. Duplicate ampoule rejuvenated on 8. 3. 50. 2·7 cm, 9-day growth, 22. 3. 50.
Fig. 5. – By 1 control (12. 10. 45). 3·2 cm, 9-day growth, 22. 3. 50.
* Longest Diameter.

A 96-hour old fermenting culture lyophilized and stored in two ampoules was rejuvenated in the first week of March, 1950. The colonies grown from the above are presented as figures 3 and 4. According to the classification given elsewhere[4] the colony in figure 3 belongs to the *Smooth III* while that in fig. 4 is of the *Rough I* category. The resemblance of figures 3 and 4 to figures 14 and 3 respectively presented by SUBRAMANIAM *et al.*[1] is rather striking. These types have already been shown to occur spontaneously as a result of gene mutations[1]. The control from a stock agar slant culture run simultaneously with the colonies of the rejuvenated cultures is illustrated in fig. 5. It is a mixture of *Smooth I*, *Smooth II*, *Smooth III*, and *Rough I* types. *The lyophilized cultures show therefore no similarity to the control at the time of lyophilization or rejuvenation.*

If preservation by freeze-drying produces no genetic changes[2], the rejuvenated cultures should be indistinguishable in their giant colony characteristics from those of the control strain at the commencement of the investigation. Because it is not so, we have to surmise that this method of preservation does produce genetic changes. This is nothing surprising, since it is well known that thermal shocks like an increase in temperature do accelerate the frequency of gene mutations.

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Zusammenfassung

Eine aus einer Mischung von Mutanten bestehende Hefekultur wurde lyophilisiert. Nach Verjüngung hatte diese Kultur keine Ähnlichkeit mehr mit der Kontrollkultur im Zeitpunkt der Lyophilisierung bzw. der Verjüngung. Die genetischen Veränderungen nach Lyophilisierung können auf Genmutationen zurückgeführt werden.

[1] See Note 4, left column.
[2] L. J. WICKERHAM and A. A. ANDREASEN, Wallerstein Lab. Commun. *5*, 165 (1942).

[1] L. ATKINS, W. MOSES, and P. P. GRAY, Wallerstein Lab. Commun. *12*, 365 (1949).
[2] M. K. SUBRAMANIAM, Proc. Nat. Inst. Sci. India *12*, 143 (1946).
[3] M. K. SUBRAMANIAM, B. RANGANATHAN, and S. N. KRISHNA MURTHY, Cellule *52*, 39 (1948). – S. DURAISWAMI and M. K. SUBRAMANIAM, Cellule *53*, 215 (1950).
[4] M. K. SUBRAMANIAM, B. RANGANATHAN, and S. N. KRISHNA MURTHY, Cellule *52*, 39 (1948).

Über den Einfluß antibiotischer Stoffe auf das Wachstum von Grünalgen

Im Rahmen einer größeren Arbeit wurden Versuche über den Einfluß zweier Antibiotika und eines Antiseptikums auf drei Algenklone gemacht. Solche Untersuchungen wurden anscheinend bisher nicht publiziert. Unsere Ergebnisse seien daher hier mitgeteilt.

Wir stellten uns folgende Versuchsfrage: Wie verhalten sich Grünalgen in ihrer Entwicklung auf Agar-Agar-Nährböden gegenüber Streptomycin, Penicillin und Trypaflavin?

Als Versuchsmaterial dienten drei Grünalgenklone: 1. *Cystococcus Placodii saxicolae Nr. 1400* (vorläufige Bezeichnung[1]); 2. *Coccomyxa ellipsoidea* (*Solorinae saccatae*) Zehnder; 3. *Trentepohlia spec.*

Die Klone 1 und 2 sind Gonidien, die aus den Flechten *Placodium saxicola* bzw. *Solorina saccata* isoliert wurden[1]. Der *Trentepohlia*-Klon wurde von Herrn Prof. Dr. OTTO JAAG während seines Aufenthaltes in Buitenzorg in Reinkultur genommen und für meine Untersuchungen freundlicherweise zur Verfügung gestellt.

Die Algen wurden in Erlenmeyer-Kolben auf Malzagarböden (2% Malzextrakt, 1,7% Agar-Agar) geimpft, die nach der Autoklavierung kurz vor dem Erstarren folgende Zusätze erhalten hatten:

a) Kontrollkolben: Kein Zusatz.
b) Streptomycin (Dihydro-Streptomycin Merck, als Sulfat): Je 1 γ, 10 γ, 25 γ pro Kubikzentimeter Substrat.
c) Penicillin (kristallisiertes Kaliumsalz Penicillin G der Firma «Bristol», Syracus, N. Y.): Je 0,2 γ, 1 γ, 5 γ pro Kubikzentimeter Substrat.

[1] A. ZEHNDER, Ber. Schweiz. Bot. Ges. *59*, 202 (1949).