testet werden. Die Versuche wurden bei 84 Kaninchennetzhäuten durchgeführt.

Da der Gedanke naheliegt, dass es sich bei den Plasmafaktoren um Proteine handelt, wurden verschiedene Plasmaeiweissfraktionen geprüft (Alkoholfraktionierung nach KISTLER-NITSCHMANN[4]: Präcipitate IV, D und B, Albumin- und γ-Globulinfraktion). Mit keiner Fraktion gelang es, eine deutliche *b*-Welle zu erhalten. Hingegen blieb die Wirkung des AHP nach Ultrafiltration erhalten (LKB 6300 A, mittlerer Porenradius 24 Å). Damit konnte das Plasma-Eiweiss als wirksamer Faktor ausgeschlossen werden. Durch 10 min Erhitzen des Ultrafiltrats auf 100 °C wurde die Amplitude der *b*-Welle um ein Drittel verkleinert.

Unter den üblichen Bedingungen gewonnenes Plasma und Serum verschiedener Grosstiere (Rind, Pferd, Schwein, Schaf) erwies sich zunächst als wirkungslos. Kontrollversuche ergaben, dass auch Kaninchen- und menschliches Plasma nur dann voll wirksam sind, wenn das Blut unmittelbar nach der Abnahme gekühlt und in der Kühlzentrifuge verarbeitet wird, wie dies beim AHP der Fall war. Von dem unter analogen Bedingungen gewonnenen Plasma anderer Tierarten war das Pferdeplasma in seiner Wirkung auf die isolierte Kaninchennetzhaut dem Kaninchen- bzw. dem menschlichen Plasma gleichwertig, während das ERG mit Schweine- oder Rinderplasma nicht oder nur über kurze Zeit erhalten wurde; der wirksame Faktor des Pferdeplasmas fand sich auch im Ultrafiltrat und erwies sich bei 30 min Erwärmung auf 60 °C bzw. 5 min Erwärmung auf 90 °C als thermostabil (Figur).

Um Störungen durch Fibrinausfällung zu vermeiden, kann das Plasma durch Thrombinzusatz (Topostasin Roche) nachträglich defibriniert werden. Versuche mit ungekühltem Plasma sowie mit Serum, das durch Spontangerinnung bei 4 °C gewonnen wurde, ergaben Anhaltspunkte für das Auftreten toxischer Substanzen, die ebenfalls ultrafiltrierbar sind und in kurzer Zeit das ERG (einschliesslich der negativen Komponente P III) irreversibel auslöschen können.

Die vorliegenden Ergebnisse sind ein Beitrag zur Methodik der isolierten umströmten Warmblüternetzhaut, die gegenüber dem Ganztierversuch eine Reihe von Vorteilen bietet[3]. Weitere Untersuchungen haben eine Isolierung und Identifizierung der niedermolekularen und hitzebeständigen Faktoren zum Ziel, die eine unbedingte Voraussetzung für das Überleben der *b*-Welle in der isolierten umströmten Kaninchennetzhaut bilden[5].

Summary. The *b*-wave in the electroretinogram of the isolated retina of the rabbit can be completely preserved by addition of plasma to the perfusing fluid. The plasma factor is a non-protein (heat-stable, molecular size < 24 Å), and was found in the plasma of rabbit, man, and horse.

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[4] P. KISTLER und Hs. NITSCHMANN, Vox Sang. *7*, 414 (1962).

[5] Das verwendete AHP sowie die Plasmafraktionen wurden freundlicherweise vom Österreichischen Institut für Hämoderivate sowie von Herrn Dr. W. DOLESCHEL zur Verfügung gestellt, wofür an dieser Stelle gedankt sei.

Osmotic Remedial and Osmotic Sensitive Mutants of *Schizosaccharomyces pombe*

In a previous publication I described three mutants of the fission yeast *Schizosaccharomyces pombe* which require high concentrations of potassium for growth[1]. Further investigations of these strains showed that their mutational defect could also be corrected by growing them on media containing high concentrations of glycerol or glucose. In order to show that this effect is not restricted to a specific gene, an attempt was made to isolate additional mutants exhibiting the same property. HAWTHORNE and FRIIS[2] recently published a report in which they described analogous mutants in *Saccharomyces* which they called osmotic remedial. In the present communication the growth behaviour of osmotic remedial mutants of *S. pombe* will be reported. In addition, evidence for the existence of a new class of mutants, called osmotic sensitive, in *S. pombe* will be presented.

Material and methods. The strains of *S. pombe* used in this study were the wild type 975, the mutant 972-G-4[1], an arginine requiring mutant 975-NG-24 and an adenine requiring mutant 975-NG-111 both of which were induced by 1-methyl-3-nitro-1-nitrosoguanidine (NG) and isolated by the 2-deoxyglucose method[3]. The NG-induced, osmotic-sensitive mutant 975-OS-5 was isolated from a mutagenized suspension of wild type cells which were first plated on YEG medium[3] and replica plated on synthetic minimal[4] plates supplemented with 20% (v/v) glycerol on which osmotic-sensitive mutants are strongly inhibited in growth. The growth of the cells was followed by measuring the optical densities of 6 ml suspensions in metal-capped test tubes shaken at 30 °C, with a Lumetron colorimeter equipped with filter 650. Exponential generation times were determined from semilog plots of the time-course of the optical densities.

Results. The effect of added glycerol and glucose to the synthetic minimal medium on the growth of the osmotic remedial mutant 972-G-4 is shown in Figure 1. The growth of the cells in glycerol or glucose containing media starts after a relatively long lag period and proceeds exponentially at a rate comparable to that of the wild type in synthetic minimal medium. An increase in the osmolarity of the medium resulted in a decrease of the lag period. Tests were also made to determine the growth factor requirement of this mutant. However, none of the standard amino acids, vitamins, purines and pyrimidines used in these tests fulfilled its requirement either singly or in combination.

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[3] R. MEGNET, Mutation Res. *2*, 328 (1965).

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The fact that it was possible to isolate a number of different auxotrophic mutants which are also osmotic remedial, shows that the osmotic remedial property is not restricted to a specific gene. Two of these mutants, the arginine-requiring strain 975-NG-24 and the adenine-requiring strain 975-NG-111 were selected for more extensive growth tests. Figure 2 shows a comparison of the growth behaviour of the wild type strain 975 and the osmotic remedial mutant 975-NG-24 in synthetic minimal medium and in the same medium supplemented with arginine, as well as their growth in media containing high concentrations of potassium chloride, ammonium sulphate or glycerol. In Figure 3 the growth of the mutant 975-NG-111 in synthetic minimal medium and minimal medium supplemented with adenine and minimal medium supplemented with glycerol or glucose is shown. The growth behaviour of these two mutants is quite similar to that of the mutant 975-G-4. Their growth rate is proportional and the duration of the lag period inversely proportional to the osmolarity of the medium. The results of tests for the occurrence of revertants in the growing cultures were in all cases negative.

Figure 4 shows a comparison of the relative exponential generation times of cells of the wild type strain 975 and the osmotic-sensitive mutant 975-OS-5 in synthetic minimal medium supplemented with various concentrations of glycerol. The strong inhibitory effect of high concentrations of glycerol on the growth of this mutant clearly differentiates it from the parent strain. Additional preliminary evidence indicates that the growth of osmotic sensitive mutants in glycerol containing media is stimulated by yeast extract.

Discussion. The results obtained in this study show that the mutation in the strain 972-G-4 probably did not result in a defect in the potassium transport system as was suggested in the previous publication[1]. There is

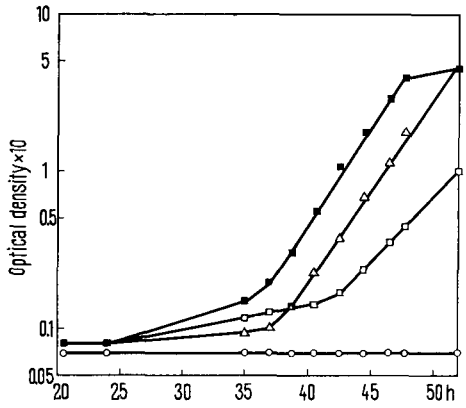

Fig. 1. The effect of high concentrations of glycerol and glucose on the growth of the osmotic remedial mutant 972-G-4 of *S. pombe*. ○, synthetic minimal medium; □, synthetic minimal medium supplemented with 5% (v/v) glycerol; ■, 10% (v/v) glycerol; △, 20% (w/v) glucose.

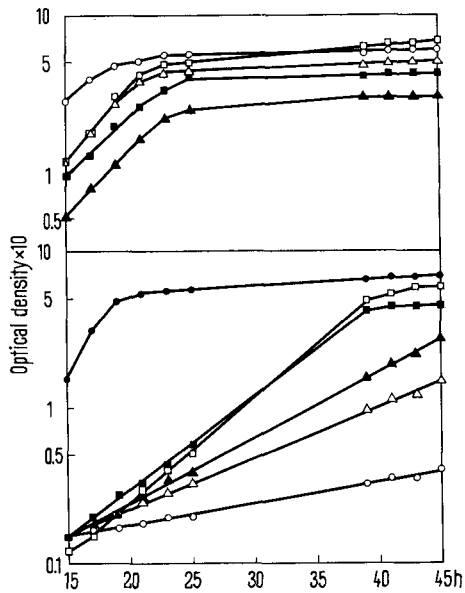

Fig. 2. The effect of high concentrations of potassium chloride, ammonium sulphate and glycerol on the growth of the wild type strain 975 (above) and the arginine requiring, osmotic remedial mutant 975-NG-24 (below) of *S. pombe*. ○, synthetic minimal medium; ●, minimal medium + arginine, 100 µg/ml; △, minimal medium + 0.5 *M* KCl; ▲, minimal medium + 0,75 *M* KCl; □, minimal medium + 10% (v/v) glycerol; ■, minimal medium + 5% (w/v) ammonium sulphate.

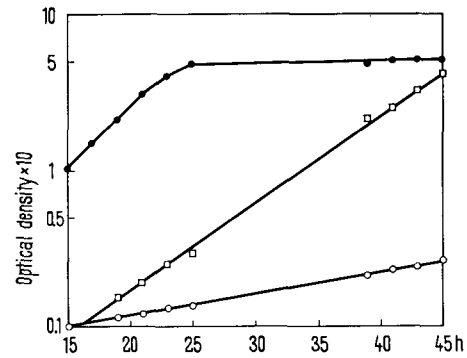

Fig. 3. The effect of high concentration of glycerol on the growth of the adenine requiring, osmotic remedial mutant 975-NNG-111 of *S. pombe*. ○, synthetic minimal medium; ●, minimal medium + adenine, 100 µg/ml; □, minimal medium + 20% (v/v) glycerol.

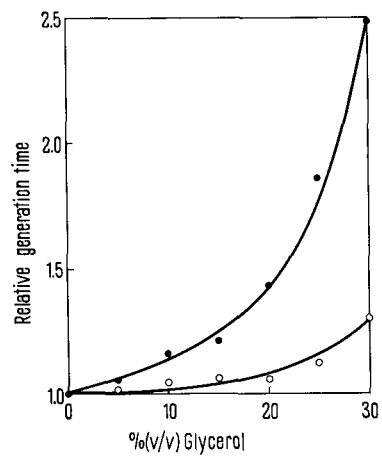

Fig. 4. The effect of glycerol on the growth of the osmotic sensitive mutant 975-OS-5 (●) and the wild type strain 975 (○) of *S. pombe* expressed in relative exponential generation times as a function of the glycerol concentration in synthetic minimal medium.

reason to believe that even though its growth factor requirement could not be determined, this strain belongs to the same physiological class of mutants as do the strains 975-NG-24 and 975-NG-111 which correspond to the definition given for osmotic remedial mutants[2].

It is assumed that the transfer of osmotic remedial, mutant cells into media containing high concentrations of glycerol or glucose causes the activation of a mutationally altered, inactive enzyme. NOSAKI and TANFORD[5] have shown that β-lactoglobulin, in concentrated solutions of ethylene glycol, tends to unfold, thus exposing its hydrophobic but not the peptide groups to the solvent. This type of unfolding, induced by glycerol, could account for the activation of the osmotic remedial enzyme if one assumes that the amino acid replacement in its polypeptide chain resulted in a more compact structure which precludes catalytic activity. The mutant enzyme of osmotic sensitive strains could undergo a similar change in concentrated glycerol solutions. But in this case the unfolding is accompanied by a reduction of activity.

Zusammenfassung. Das Wachstumsverhalten zweier Klassen von Mutanten der Spalthefe *Schizosaccharomyces pombe* in Medien hoher Osmolarität wird beschrieben. Das Wachstum der einen Mutantenklasse wird in solchen Medien gefördert und das der andern gehemmt.

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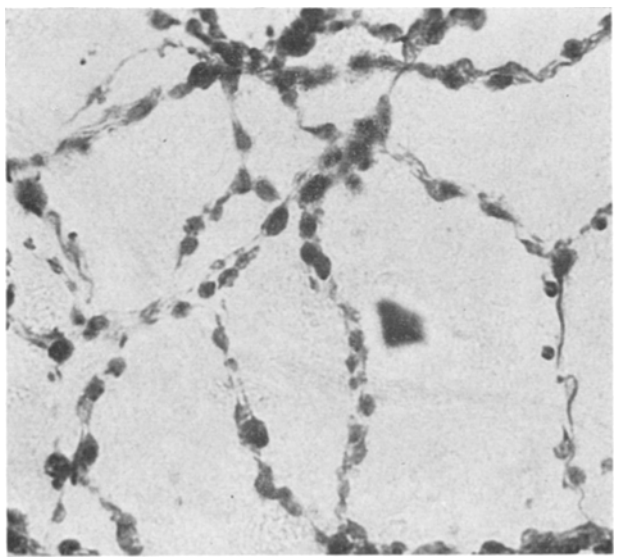
[6] This investigation was supported by Grant No. 3467 from the Swiss National Science Foundation.

Adrenergic and Cholinergic Axons in the Mouse Iris

In the dilator pupillae, which was formerly regarded as purely sympathetically innervated, a dense network of cholinergic fibres has recently been demonstrated[1,2]. Moreover, the adrenergic and cholinergic fibres could be shown to run concomitantly in the Schwann cell strands of the autonomous ground-plexus, demonstrating morphological foundations for a peripheral interaction between the two types of vegetative nerves[1,2]. The observation that there is generally only one adrenergic fibre in each strand of Schwann cells in the mouse iris[3,4] has opened a simple way of demonstrating directly to what extent adrenergic nerve fibres are accompanied by non-adrenergic axons.



Mouse iris, whole mount. Methylene blue stain. Virtually every strand of the ground-plexus contains two or more varicose axons. × 1250.

The adrenergic fibres were demonstrated in whole-mounts of the mouse iris with the fluorescence technique of FALCK and HILLARP[5], cf. also [2]. The total number of axons in the iris was shown with methylene blue staining[2,6].

It was confirmed that, in the mouse, the majority of the Schwann cell strands of the dilator region contained only one adrenergic axon (cf. [3,4]). In the methylene blue stainings, most of these strands contained several beaded axons (Figure) while only a few isolated axons could be seen. Care must be taken to ensure perfect staining, since otherwise the axons will either be obscured by non-specific staining of the Schwann cells, or none at all or only one axon will appear in the Schwann cell strands.

Since there is no reason to believe that sensory fibres run in the vegetative ground-plexus in any significant number, the results excellently corroborate the earlier finding of concomitant adrenergic and cholinergic fibres in the iris[1,2,7].

Résumé. Par la comparaison entre la somme totale des fibrilles nerveuses et celle des fibres adrénergiques des cellules de Schwann de l'iris de la souris, il se confirma que les fibres adrénergiques et cholinergiques terminales sont ici en grande partie concomitantes.

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[7] The research reported in this document has been sponsored by the Air Force Office of Scientific Research under grant AF EOAR 66-14 through the European Office of Aerospace Research (OAR), United States Air Force, by the United States Public Health Service (grant NB 05236-02), by the Statens Medicinska Forskningsråd (grant B 66-320), and by the Faculty of Medicine, University of Lund, Sweden.