

species obtained from collections, the reddish-brown pigment was produced by strains of *Proteus vulgaris*, *P. morgani*, *P. mirabilis*, *P. rettgeri* and *Providencia*, respectively. From the set strains obtained from the clinical material, the pigment was exclusively formed by the *Proteus-Providencia* group under the same conditions. From the total of 367 *Proteus-Providencia* strains, only 3 of them gave a negative or less positive results. The detailed examination showed that these were weakly or later positive strains of *Proteus morgani*. In this species, the pigment production was in general less intensive.

It was found that the pigment was produced also from other media, such as nutrient agar, Endo agar etc., if they contained tryptophane. In nutrient agar the intensity of the pigment was increased in correlation with the contents of *dl*-tryptophane (experiments were made with 0.1 up to 1.0% of the amino acid). The production depended also on the incubation period at 37°C (in nutrient agar with 0.4% of tryptophane the pigment occurred after 6 h) as well as on pH (maximum colour at pH 8). In nutrient broth the pigment was formed more slowly. The same occurred if bacterial cells washed in phosphate buffer (pH 7.2) were incubated for three days at 37°C in the same buffer containing 0.5 or 1.0% of tryptophane.

The isolation of the reddish-brown pigment, its chemical nature, its condition and mechanism of its formations are being studied and the results will be published later.

The authors conclude that this phenomenon might be useful for diagnosis of the *Proteus-Providencia* group. The most convenient procedure is as follows: 0.5% of tryptophane is added to nutrient agar, the medium is poured into small test-tubes (8 × 0.8 cm) to make a very thin slant agar. Inoculated bacteria are incubated one day at 37°C and the results are recorded. A non-inoculated medium is used as control, because some strains of *Proteus morgani* can produce a weaker reaction during this incubation period⁵.

Zusammenfassung. Die Bakterien der Gruppe *Proteus-Providencia* produzieren auf tryptophanreichen Kulturmedien braunrotes Pigment. Die Autoren geben die Bedingungen über diese spezifische Pigmentproduktion und nehmen an, dass diese Pigmentation zur Differentialdiagnostik der Gruppe *Proteus-Providencia* dienen könnte.

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⁵ The authors wish to thank Mr. V. Ostřížek for his technical assistance.

Mutants of the Yeast *Schizosaccharomyces pombe* Requiring a High Concentration of Potassium

In order to test the potentialities of a recently developed method for the selection of auxotrophic mutants of the fission yeast *Schizosaccharomyces pombe*¹, an attempt was made to concentrate mutants of this organism which require a high concentration of potassium ion for growth. Mutants of this type are known in *Escherichia coli*^{2,3}.

The present communication deals with the isolation and characterization, by growth tests, of analogous mutants of *Sch. pombe*.

The potassium-requiring mutant strains, 972-G-1, 972-G-4, and 972-G-5, are of spontaneous origin and are de-

rived from the wild type strain 972. They were concentrated on inositol-free, minimal medium plates which contained potassium ions at a concentration of $7.35 \cdot 10^{-3} M$. These plates were incubated for 7 days at 30°C and later supplemented¹ with inositol and potassium chloride to a final concentration of 0.1 M. After replica plating⁴ and purification by streaking on potassium enriched plates, the mutant strains were transferred and kept on

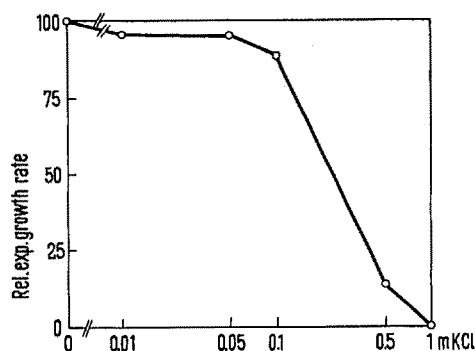


Fig. 1. Relative exponential growth rates of the wild type 972 of *Schizosaccharomyces pombe* on varying supplements of KCl.

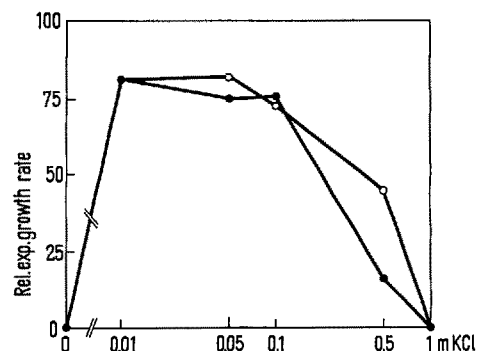


Fig. 2. Relative exponential growth rates of the potassium-requiring mutants 972-G-1 (open circles) and 972-G-5 (full circles).

¹ R. MEGNET, *Experientia* 20, 320 (1964).

² M. LUBIN and D. KESSEL, *Biochem. biophys. Res. Commun.* 2, 249 (1960).

³ S. SCHULTZ and A. K. SALOMON, *Nature* 187, 802 (1960).

⁴ J. LEDERBERG and E. M. LEDERBERG, *J. Bacteriol.* 63, 399 (1952).

yeast extract, KCl slants (Difco yeast extract 5 g/l, glucose 10 g/l, KCl 7.4 g/l, Difco agar 20 g/l). For quantitative growth tests, mutant and wild type cells were grown in 50 ml liquid, synthetic medium⁵ in 200 ml Erlenmeyer flasks. Optical densities were measured with a Lumetron photometer using the filter 650. Flasks supplemented with varying amounts of KCl were all inoculated with the same number of viable cells (10^7) and shaken at 30°C. Exponential growth rates were determined graphically from semilog plots of the optical density values. The relative exponential growth rates were computed, using the value of the growth rate of the wild type in synthetic medium as the standard.

Figures 1-3 summarize the data of the growth experiments. The potassium chloride concentration refers to the initial concentration of the potassium chloride supplement (the basal medium had a potassium ion concentration of

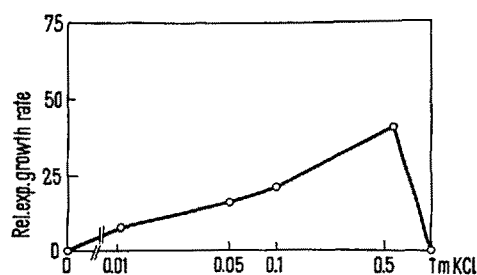


Fig. 3. Relative exponential growth rates of the potassium-requiring mutant 972-G-4.

$7.35 \cdot 10^{-3} M$). The mutants fall into at least two classes. One class, 972-G-1 and 972-G-5, grows optimally at 0.01 M KCl and is inhibited at higher concentrations. The second class with 972-G-4 grows best at 0.5 M KCl, a concentration at which both the wild type and the two other mutants are inhibited in growth.

The mutants 972-G-1 and 972-G-4 can grow at a KCl concentration (0.5 m) at which the wild type is inhibited. This observation suggests a simple method for the isolation of potassium-requiring mutants of *Sch. pombe*, which would involve plating of untreated or mutagenized cells on a medium containing KCl at a concentration of 0.5 m or higher. It is expected that only potassium-requiring mutants would grow on such a medium.

It is planned to determine the nature of the biochemical lesion as well as the genetics of the mutants described. These mutants might also be used as tools to study the mechanisms of potassium ion transport in yeast.

Zusammenfassung. Zwei Klassen von Mutanten der Hefe *Schizosaccharomyces pombe*, welche hohe Konzentrationen von Kaliumionen zum Wachstum benötigen, können auf Grund ihres Wachstumsverhaltens in mit Kaliumchlorid angereichertem Medium unterschieden werden.

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⁵ R. MEGNET, Arch. Jul. Klaus-Stiftung 33, 299 (1959).

The Rate of Biliary Secretion During Flow up Vertical Cannulas of Different Bore

Some time ago it was shown¹ that the perfused rat liver was able to force bile up a vertical tube at a constant rate until a head of about 12 cm was produced, after which the rate fell rapidly to zero. Recently, RICHARDS and THOMSON^{2,3} reported a similar constant rate of bile flow up a wide bore tube attached to a biliary cannula in cholecystectomized dogs, but also showed that the initial rate of flow declined when tubes of narrow bore were used. They suggested that this difference was related to the failure of the hepatic cells to accumulate substrate fast enough to keep pace with the rapidly increasing intrabiliary pressure, and therefore put forward the interesting hypothesis that biliary secretory effort is dependent upon the substrate load until such time as the secretory mechanisms are fully engaged. This hypothesis clearly requires that the rate of secretion of bile is reduced in response to a rapid increase in intrabiliary pressure.

There is, however, another possible explanation of the observations made with tubes of narrow bore, since increased intrabiliary pressure produces distension of the biliary tree^{1,4}. If the distension produced by each unit rise of pressure increases as the intrabiliary pressure rises, and the extra volume contained in the distended biliary tree is significantly large in relation to the volume of bile in the cannula, then the results seen with the narrow bore

tube would be not unexpected. The bile 'lost' from the vertical tube would, in fact, be present in the distended biliary tree and hence the postulated reduction in the rate of secretion of bile need not occur. RICHARDS and THOMSON state that this is not the case with dogs.

Measurement of the true secretory rate during the flow of bile up vertical tubes might be used to confirm or deny one of the above hypotheses. This measurement has been attempted in a series of 15 albino rats, whose common hepatic ducts were cannulated with a cannula having a side arm to which vertical tubes of different bore were attached. Obstruction of the main outflow resulted in bile passing up the vertical side tube. The rate of flow of the bile up tubes of narrow and wide bore (0.5 and 1.0 mm respectively) was measured and similar results obtained to those described by RICHARDS and THOMSON, i.e. the initial rate of flow was constant with the wider bore but declined when the narrow bore tube was used. The volume of the biliary tree was then measured at different pressures by the dye method described elsewhere⁵, and finally

¹ R. W. BRAUER, G. F. LEONG, and R. J. HOLLOWAY, Am. J. Physiol. 177, 103 (1954).

² T. G. RICHARDS and J. Y. THOMSON, J. Physiol. 154, 60 (1960).

³ T. G. RICHARDS and J. Y. THOMSON, Gastroenterology 40, 705 (1961).

⁴ G. BARBER-RILEY, Am. J. Physiol. 205, 1127 (1963).

⁵ G. BARBER-RILEY, Am. J. Physiol. 205, 1122 (1963).