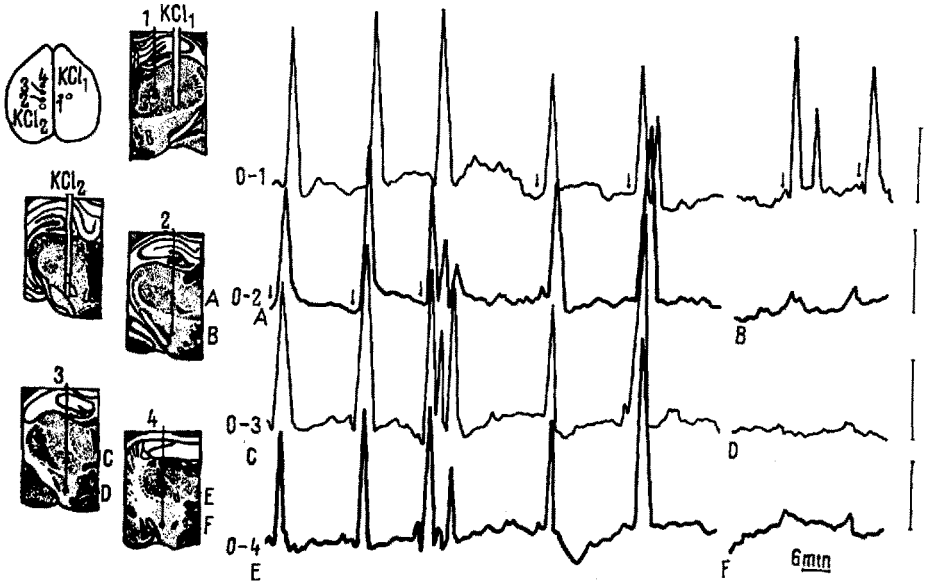

Recording of slow potential changes accompanying SD in the thalamus of rat. 0–1 Nucleus dorsomedialis; 0–2 A nucleus ventromedialis, B area hypothalamica lateralis; 0–3 C nucleus ventralis pars dorsomedialis, D area hypothalamica lateralis; 0–4 E nucleus ventralis, F area hypothalamica lateralis. Arrows indicate KCl application. The reference electrode (O) was placed on neck muscles. Calibration 5 mV.

posterior group. Also the incidence of positive SD reactions in those parts of the thalamus was decreased (Table).

Thus SD can be more readily elicited in the medial part of the thalamus than in the lateral and posterior ones, perhaps because of the fibres penetrating these regions and joining the capsula interna rostrally and lamina medullaris externa occipitally. The impaired transition of SD from the medial to the lateral thalamus may be caused by the lamina medullaris interna enveloping the medial thalamic complex.

Zusammenfassung. Mit KCl-Microinjektion werden im Thalamus langsame Potentialwellen hervorgerufen, deren Amplitudendauer und Ausbreitungsgeschwindigkeit der «spreading depression» von LEÃO (SD) entspricht. Die Bedingungen für SD-Entwicklung sind infolge grösserer Zelldichte in der Gruppe der medialen thalamischen Kerne besser als in den lateralen und posterioren Kernen, was sich in der verminderten Amplitude des langsamen Potentials und in häufigem Wegfall einer positiven Reaktion zeigt.

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Production of Reddish-Brown Pigment from *dl*-Tryptophan by Enterobacteria of the Proteus-Providencia Group

Oxidative deamination of α-amino acids is one of the typical biochemical reactions of the Proteus-Providencia group[1]. The resulting pyruvic acids, especially in the case of cyclic amino acids such as phenylalanine, tryptophane, histidine etc., produce with $FeCl_3$ coloured solutions. This reaction is utilized for the so-called phenylalanine test in routine diagnostic practice[2,3].

Studying the above-mentioned reaction in the basal medium used for phenylalanine test[4], in which this amino acid was replaced by *dl*-tryptophane, the authors observed that in the medium inoculated with bacteria of the Proteus-Providencia group a reddish-brown pigment diffusing in the medium developed after 12 h incubation at 37°C, even though the medium was not treated with $FeCl_3$ solution. As far as the authors know, this phenomenon has not been recorded in the literature. By further incubation (24–48 h) the intensity of the colour increased. Other cyclic amino acids failed to give this production of reddish-brown pigment and it was specific only for the above-mentioned group of bacteria. Therefore a detailed examination of this newly observed phenomenon was carried out.

In order to confirm that the reddish-brown pigment is produced only by bacteria of the Proteus-Providencia group from the *dl*-tryptophane, the authors compared production of the pigment in strains of the Proteus-Providencia group with those from other bacterial genera (all cultures obtained from culture collection), and the characteristic was also examined in 2095 strains of Gram negative bacteria isolated from clinical material; these were mostly enterobacteria, according to their biochemical characteristics, among which the classical phenylalanine test was included. From the total of 41 bacterial

[1] P. K. STUMPF and D. E. GREEN, J. biol. Chem. *153*, 387 (1944).
[2] S. D. HENRIKSEN, J. Bact. *60*, 225 (1950).
[3] Report of the Enterobact. Subcommittee, Int. Bull. of Bact. Nom. and Tax., Vol. 8, No. 1 (1958).
[4] J. SEDLÁK and H. RISCHE, *Enterobacteriaceae-Infektion* (Leipzig 1961), p. 462.

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 period[5].

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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Institute of Microbiology, Faculty of Medicine, University J. E. Purkyně, Brno (Czechoslovakia), June 29, 1964.

[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 derived 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 supplemented[1] with inositol and potassium chloride to a final concentration of $0.1 M$. After replica plating[4] and purification by streaking on potassium enriched plates, the mutant strains were transferred and kept on

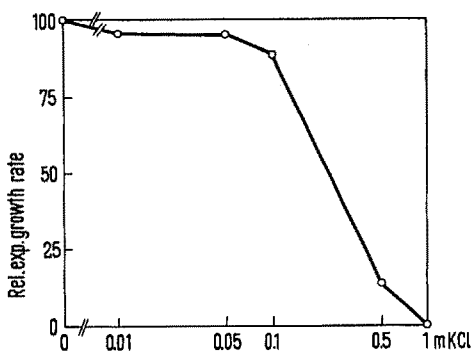

Fig. 1. Relative exponential growth rates of the wild type 972 of *Schizosaccharomyces pombe* on varying supplements of KCl.

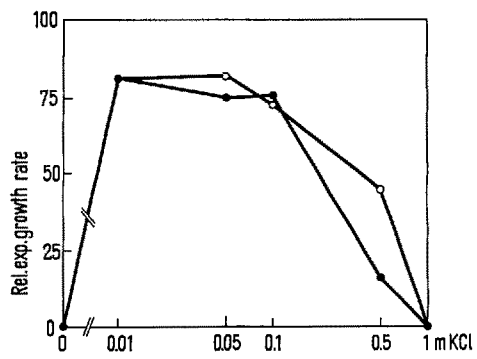

Fig. 2. Relative exponential growth rates of the potassium-requiring mutants 972-G-1 (open circles) and 972-G-5 (full circles).

[1] R. MEGNET, Experientia *20*, 320 (1964).
[2] M. LUBIN and D. KESSEL, Biochem. biophys. Res. Commun. *2*, 249 (1960).
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