Wound forms might be explained by local weakening of the cell wall, the coiled ones by unilateral weakening of cell wall in cells which are growing beyond their normal dimensions. Triangular forms are sometimes found at the beginning of branching, or occur in cases where the branching is later stopped by the bactericidal concentration of the sulfonamides which with time replaces the bacteriostatic concentration, as happens in the sensitivity test.

Ramified forms may be produced by multiple budding of rounded spheroplast-like cells, which were already described in *Bacterium anitratum* under sulfathiazol[1], and by subsequent elongation of these buddings. If the elongations of multiple buddings later fragment at their base but stay in approximately the same position, diphtheroid formations are produced. Perhaps in this way the frequent appearance of diphtheroids (some of which agglutinate in antiserum prepared from the parental strain[6]) from secondary colonies in old bacterial cultures might be explained.

No significant differences were found in the action of different sulfonamides tested.

Zusammenfassung. Neben bereits beschriebenen fusiformen Filamenten und Spheroplasten-ähnlichen Formen entstanden unter dem Einfluss der Sulfonamidpräparate (Sulfathiazol, Sulfadiazine, Sulfamerazine, Sulfamethoxypyridazine, Gantrisin, Elkosin) aus den stäbchenförmigen Zellen des *Bacterium anitratum*, auch andere aberrante Zellformen, wie plektridiumförmige, trianguläre und verzweigte Filamente.

B. BRZIN[7]

Institute of Microbiology, Medical Faculty, Ljubljana (Yugoslavia) Department of Microbiology, and S.U.N.Y., Upstate Medical Center, Syracuse (N.Y., USA), August 23, 1965.

[6] B. R. CHATTERJEE, C. L. GOTT, and R. P. WILLIAMS, Bact. Proc. *3*, 23 (1964).

[7] My thanks are due to Prof. Dr. G. G. HOLZ JR. for helpful discussion and for reading the manuscript.

Succinoxidase Activity of Mitochondria Isolated from the Liver of Rats after Partial Hepatectomy and Hypophysectomy

Whereas a decrease of succinate dehydrogenase activity has been observed in the homogenate of regenerating liver tissue during the first 48 h following partial hepatectomy (PHE)[1], an increase of this activity has been noted in a suspension of mitochondria isolated from regenerating liver[2]. Similar discrepancies, depending on the conditions of assay, have been obtained, for example, in the determination of cytochrome oxidase activity[1–3]. Analogous to the succinate dehydrogenase activity, succinate oxidase activity also falls in the homogenate from regenerating liver[4]. As the respective enzyme system is located exclusively in mitochondria, we have decided to determine succinate oxidase activity in a suspension of isolated mitochondria. Since some processes which take place after partial hepatectomy, such as the incorporation of ^{3}H-thymidine[5], are affected by the removal of the pituitary, succinate oxidase activity was also studied in hypophysectomized rats (HyE).

Fourty-eight albino rats of the Wistar strain, aged 3–4 months, which had had free access to the usual laboratory diet[6], were used. The estimations were performed on unoperated control rats 24 h and 48 h after PHE, i.e. a resection of 65–70% of the liver[7], 24 h after HyE[8], 24 h after HyE and laparotomy (sham operation), and 24 h after HyE and PHE. Mitochondria were isolated from the liver tissue by a modification of the procedure of ALDRIDGE[9,10]. Succinate oxidase activity was determined in the WARBURG apparatus[11–13] from the oxygen consumption by mitochondria suspended in the incubation medium (Krebs-Ringer saline with reduced $NaHCO_3$ content of 0.35 mg/ml)[14] to which sodium succinate had been added (18.3 mg per 3 ml incubation medium). Nitrogen was estimated by the micro-Kjeldahl method[15].

The results are shown in the Table. The left-hand column represents the nitrogen content of mitochondria

Groups	Mitochondrial N mg per 1 g wet tissue	mm^3 O_2 uptake per mg mitochondrial N
Non-operated rats	3.13 ± 0.22	504.70 ± 18.30
24 h after laparotomy	2.76 ± 0.17	404.90 ± 49.00
24 h after partial hepatectomy	2.55 ± 0.14	234.31 ± 52.00
24 h after hypophysectomy	3.22 ± 0.59	520.00 ± 45.07
24 h after hypophysectomy and laparotomy	2.73 ± 0.19	373.00 ± 27.45
24 h after hypophysectomy and partial hepatectomy	3.15 ± 0.65	299.60 ± 60.50
48 h after laparotomy	2.70 ± 0.20	587.50 ± 83.19
48 h after partial hepatectomy	2.84 ± 0.41	190.70 ± 63.70

[1] J. D. PERKINSON and CH. C. IRVING, Cancer Res. *16*, 496 (1956).
[2] A. R. L. GEAR, Biochem. J. *95*, 118 (1965).
[3] J. P. GREENSTEIN, *Biochemistry of Cancer*, 2nd ed. (Academic Press, New York 1954), p. 432.
[4] A. B. NOVIKOFF and V. R. POTTER, J. biol. Chem. *173*, 223 (1948).
[5] H. WRBA, H. RABIS, and H. BRÄNDLE, Naturwissenschaften *61*, 42 (1964).
[6] P. FÁBRY, Čslká Fysiol. *8*, 529 (1959).
[7] G. M. HIGGINS and R. M. ANDERSON, Arch. Path. *12*, 186 (1931).
[8] P. E. SMITH, Am. J. path. Anat. *45*, 205 (1930).
[9] W. N. ALDRIDGE, Biochem. J. *67*, 423 (1957).
[10] I. HRADIL and K. LEJSEK, Biofizika *10*, 171 (1965).
[11] O. WARBURG and E. NEGELEIN, Biochem. Z. *110*, 66 (1920).
[12] A. B. PARDEE, J. biol. Chem. *179*, 1089 (1949).
[13] A. KLEINZELLER (ed.), *Manometrické metody a jejich pouziti v biologii a biochemii* (SZN, Praha 1964).
[14] D. BELLAMY and W. BARTLEY, Biochem. J. *76*, 78 (1960).
[15] A. HILLER, J. PLAZIN, and D. D. VAN SLYKE, J. biol. Chem. *176*, 1401 (1948).

isolated from 1 g wet liver tissue. There was an insignificant decrease of the mitochondrial nitrogen in all experimental groups with the exception of the rats with HyE or HyE + PHE. In the right-hand column, oxygen consumption by mitochondria isolated from the liver tissue is expressed per mg mitochondrial nitrogen. A significant decrease was noted in sham-operated rats (after 24 h, $P < 0.05$), in those with PHE (after 24 h, $P < 0.001$; after 48 h, $P < 0.001$), with HyE plus laparotomy ($P < 0.05$) and HyE plus PHE ($P < 0.05$). HyE alone was without effect; similarly, laparotomy alone showed no influence after 48 h.

The decrease of succinate oxidase activity calculated per unit liver weight in the first 48 h after partial hepatectomy is more pronounced than the respective decrease of mitochondrial nitrogen. This suggests that the drop in activity of the succinate oxidizing system cannot be explained by the decrease of the number of mitochondria. The overall oxygen uptake for the oxidation of hydrogen atoms removed from succinate decreases considerably following PHE, whereas succinate dehydrogenase activity in mitochondria and especially in the fluffy layer[2] has a rising tendency. If one assumes, with GREEN[16], that the relative proportions of the components of the terminal electron transport chain are constant, it would be inevitable to conclude that the concentration of other components does not drop either. The decrease in activity of the overall system might then be explainable by the change of the cofactor concentration or by the presence of inhibitors or, possibly, by the change of spatial orientation of the individual components. Our further analysis of the phenomenon reported here follows these lines. Pituitary function does not seem to be in causal relationship with the decrease of succinoxidase activity. In further study of the metabolism following PHE, it will be necessary to pay attention to possible nervous regulatory links or to the direct influence of the concentration changes of substances in blood or liver. As the results obtained with laparotomized rats show, the drop in succinate oxidase activity after PHE may partly be accounted for by the surgery itself.

Zusammenfassung. Bei den 24 und 48 h nach partieller Hepatektomie (Resektion von 65–70% der Leber) aus der Rattenleber isolierten Mitochondrien wurde eine Abnahme der sukzinoxydaseaktivität beobachtet. Dasselbe wurde auch bei Ratten beobachtet, bei welchen gleichzeitig mit der partiellen Hepatektomie eine Hypophysektomie durchgeführt wurde. Die Veränderung der Sukzinoxydaseaktivität ist nicht auf eine Erniedrigung der Mitochondrienzahl pro Gewichtseinheit Leber zurückzuführen.

J. ŠIMEK, J. SEDLÁČEK,
K. LEJSEK, and I. M. HAIS

Department of Physiology, Department of Pathological Physiology and Department of Medical Chemistry, Faculty of Medicine, Charles University, Hradec Králové (Czechoslovakia), September 17, 1965.

[16] D. E. GREEN, 6th Intern. Congr. Biochem., New York Abstr. VIII-S7 (1964), p. 615.

The Effect of Fluorouracil and Fluorodeoxyuridine on the Genetic Recombination in *Schizosaccharomyces pombe*

Fluorodeoxyuridine, an inhibitor of DNA synthesis[1], is known to have a stimulating effect on the genetic recombination in a number of organisms[2,3]. During a study on the genetics of the Ad_2 locus of the fission yeast *Schizosaccharomyces pombe*, methods were investigated with which the relatively small frequencies of recombination, observed at this locus, could be increased. To this end the effect of fluorouracil (FU)[4] and fluorodeoxyuridine (FdUR)[4] on the frequencies of recombination in an intergenic cross, involving the two closely linked mating type genes[5] and an intragenic cross involving two spontaneous mutants at the Ad_2 locus[6], was investigated.

The strains used for the first set of crosses were the two wild type strains 972 and 975, and for the second set, the mutants ad_2-*R*-67 and ad_2-*R*-113.

The drugs, in sterile filtered solutions, were added to the autoclaved crossing medium containing 3% malt extract and 2% agar. The cells were mixed in equal proportions, spread on the crossing medium slants, and incubated at 25 °C. After 7 days of incubation the crosses were treated with ethanol[7]. The ascospores from the mating type crosses were plated on malt extract plates which were incubated for 6 days at 25 °C. In order to differentiate the recombinant homothallic colonies from the non-recombinant colonies, the plates were treated with iodine vapour[8]. The ascospores from the ad_2 crosses were plated on minimal and on adenine supplemented plates. After 6 days of incubation at 30 °C the colonies were scored and the frequencies of recombinant prototrops calculated.

Figure 1 shows the results of the mating type crosses. The frequency of recombination was increased almost twentyfold at the highest concentration of FU used. The effect of FdUR was less pronounced, only a twofold increase being observed. Figure 2 summarizes the data of the adenine mutant crosses. Here too a stimulation of the genetic recombination by FU and FdUR could be demonstrated.

In control experiments the mutagenicity of FU and FdUR in the strains used in the recombination experiments was tested. The results of these tests were negative. It seems that both drugs are recombinagens and not mutagens for *S. pombe*.

It is assumed that both FU and FdUR, which in vivo probably undergo a transformation to the corresponding

[1] S. S. COHEN, J. G. FLAKS, H. D. BARNER, M. R. LOEB, and J. LICHTENSTEIN, Proc. natn. Acad. Sci. U.S. *44*, 1004 (1958).
[2] N. E. MELCECHEN and P. D. SKAAR, Virology *16*, 21 (1962).
[3] R. E. ESPOSITO and R. HOLLIDAY, Genetics *50*, 1009 (1964).
[4] Supplied by Hoffmann-La Roche & Co. AG, Basle.
[5] U. LEUPOLD, Cold Spring Harb. Symp. quant. Biol. *23*, 161 (1958).
[6] R. MEGNET, in preparation.
[7] U. LEUPOLD, Pathologia Microbiol. *20*, 535 (1957).
[8] U. LEUPOLD, Pathologia Microbiol. *18*, 1141 (1955).