Summary. Reactions of phthalide and 6-chlorophthalide with sodium salts of thiophenols gave *o*-(phenylmercaptomethyl)benzoic acids (III), which were cyclized by the action of polyphosphoric acid to 6,11-dihydrodibenz(b,e)-thiepin-11-ones (I). These ketones were treated with 3-dimethylaminopropylmagnesium chloride and other basic Grignard reagents and the products (IV) dehydrated to a series of 11-(aminoalkylidene)-6,11-dihydrodibenz(b,e)-thiepins (V), the hydrochlorides of which show psychotropic activity.

M. PROTIVA, M. RAJŠNER, V. SEIDLOVÁ, E. ADLEROVÁ und Z. J. VEJDĚLEK

Forschungsinstitut für Pharmazie und Biochemie, Prag (Tschechoslowakei), 26. März 1962.

Cytotoxic Action of Nucleic Acids

In earlier studies, isologous and homologous bone marrow cells were employed to induce survival of mice submitted to a lethal dose of the cytotoxic compound Dimethyl-Myleran (2:5-dimethanesulphonoxyhexane; DMM)[1,2]. In these experiments, practically 100% survival was obtained with isologous cells. The results presented in this note show that the action of nucleic acids on isologous bone marrow cells *in vitro* will diminish their capacity to ensure survival.

Eight week old female mice of the CBA inbred strain were given DMM intraperitoneally in the lethal dose of 17 mg/kg. Isologous donor bone marrow cells were procured from the femora and tibiae of other CBA female mice and incubated with or without addition of nucleic acid preparations. The incubating conditions for these cells varied as shown in the accompanying Table. The nucleic acids were prepared from CBA mouse spleens according to KIRBY[3]. The deoxyribonucleic acid/ribonucleic acid (DNA/RNA) complex obtained after several reprecipitations in 2-ethoxyethanol was finally dissolved in 2–3 ml of 0.15 *M* NaCl and washed 5 times with ether, which then was carefully vacuum-extracted at 35°C. No trace of phenol could be detected in the end product with $FeCl_3$. The protein content of the DNA/RNA preparation extracted with the utilized method is considerably less than 1%. DNA was obtained by exposing the DNA/RNA complex to ribonuclease for 2 h at 22°C. To some of the cell suspensions herring sperm DNA or rat liver RNA was added. After the incubation the cells were washed, resuspended in 0.15 *M* NaCl and injected (0.2 ml) into the tail vein of the recipient mice treated 3–8 h before with DMM. The 15 day survival from the acute bone marrow aplasia was taken to indicate the viability of the incubated cells.

Effect of nucleic acids on the curative action of bone marrow cells against lethal doses of Dimethyl-Myleran

Cells per recipient 10^6	Incubating time	Incubating medium	Nucleic acids	Concentration mg/ml	Survival rate
30	120 min	0.15 *M* NaCl[a]	–		5/5
30	60 min	0.15 *M* NaCl[a]	–		5/5
30	60 min	0.5 *M* NaCl[a]	–		5/5
25	120 min	0.5 *M* NaCl[a]	–		6/6
25	120 min	0.5 *M* NaCl[a]	DNA/RNA	0.35	0/7
5	30 min[b]	0.15 *M* NaCl[a]	–	–	5/6
5	30 min[b]	0.5 *M* NaCl[a]	–	–	2/5
15	30 min[b]	0.5 *M* NaCl[a]	DNA/RNA	0.35	0/4
5	8 h	199[c]	–	–	4/4
15	8 h	199	DNA/RNA	0.35	1/4
15	30 min[b]	0.15 *M* NaCl[a]	–	–	4/4
15	30 min[b]	0.15 *M* NaCl[a]	DNA/RNA	0.35	4/8
3	30 min[b]	0.15 *M* NaCl	DNA	0.7	1/5
0.5	6 h	199	–	–	10/10
0.5	6 h	199	DNA	0.8	5/9
0.5	6 h	199	DNA/RNA	1.2	3/11
0.5	6 h	199	DNA/RNA	0.35	5/7
6	30 min	0.15 *M* NaCl	herring sperm DNA	0.5	1/4
6	30 min	0.15 *M* NaCl	rat liver RNA	0.5	4/4
6	6 h	199	herring sperm DNA	0.5	3/4
6	6 h	199	rat liver RNA	0.5	2/4

The cells were incubated at 37°C, except those annoted below. In the DNA/RNA complex, only the DNA concentration was determined by employing the Diphenylamine method. The herring sperm DNA had been prepared according to E. R. M. KAY, N. S. SIMMONS, and A. L. DOUNCE, J. Amer. chem. Soc. *74*, 1724 (1952).

[a] Plus 0.001 *M* EDTA
[b] Incubated at room temperature.
[c] Tissue culture medium (Glaxo).

The results are given in the Table. Addition of nucleic acids to the incubated cells lowered the survival rate of nearly 100% which occurred in the control animals. From this fact it can be assumed that a cytotoxic effect of the nucleic acids reduced the viable cells to a number which was in many instances insufficient to ensure survival. As it can be seen in the first part of the Table, the effect was accentuated if the cells were incubated in a hypertonic medium. This could be related to studies showing an increased cellular infectivity of viral nucleic acids in media of high osmolarity[4]. As to the effect of hypertonicity *per se* on cells, full survival in control groups was still obtained when cells at a 25×10^6 per animal dose were incubated in 0.5 *M* NaCl for 2 h. With lower cell doses incubated in this medium, the smaller number of cells remaining viable lead to a decreased survival rate. The survival rates of mice receiving different doses of cells incubated with nucleic acids in isotonic saline or under tissue culture conditions were less, but still conspicuously reduced. Dried and stored DNA and RNA from other sources had a weaker cytotoxic effect, demonstrable only after prolonged contact with the cells. The latter finding is not surprising with reference to the fact that dried DNA loses its bacterial transforming activity. It would not be appropriate at this time to attribute the toxic effect to a specific component of the DNA/RNA preparation. Observations supporting the possibility that nucleic acids

[1] G. L. FLOERSHEIM and L. A. ELSON, Acta haemat. *26*, 233 (1961).
[2] G. L. FLOERSHEIM, Med. Exp. *4*, 85 (1961).
[3] K. S. KIRBY, Biochim. biophys. Acta *36*, 117 (1959).
[4] J. S. COLTER and K. A. O. ELLEM, Fed. Proc. *20*, 650 (1961).

might exert a cytotoxic action are recorded. Nucleic acids induced e.g. cytological and cytochemical changes in liver tissue[5] and loss of pathogenicity in ascites tumor cells (after RNA treatment)[6].

In connection with attempts to induce histocompatibility locus transformation in haemopoietic cells by the action of homologous DNA[7], the present experiments indicate the necessity to submit the exposed cells to lower DNA concentrations than those previously used. This condition seems relevant also with regard to the hint of an exposure time–toxicity relation. With the long exposure time necessary for good cellular uptake of DNA[8], the cytotoxicity of the nucleic acid preparation alone at similar concentrations would affect the viability of the cells to be transformed.

Zusammenfassung. Mit einer letalen Dosis von Dimethyl-Myleran vorbehandelte Mäuse überleben nach Transfusion isologer Knochenmarkzellen. Wirken Nukleinsäuren *in vitro* auf die Knochenmarkzellen ein, so ist ihre lebenserhaltende Kapazität vermindert.

G. L. Floersheim[9]

Chester Beatty Research Institute, Institute of Cancer Research: Royal Cancer Hospital, London (England), April 17, 1962.

[5] C. Leuchtenberger, R. Leuchtenberger, and E. Uyeki, Proc. Nat. Acad. Sci. *44*, 700 (1958).
[6] M. C. Nui, Anat. Rec. *136*, 252 (1960).
[7] G. L. Floersheim, Nature *193*, 1266 (1962).
[8] G. L. Floersheim, Nuclear-Med., in press (1962).
[9] Present adress: Pharmakologische Anstalt der Universitat, Basel (Switzerland). This investigation was supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital, London) from the Medical Research Council, the British Empire Cancer Campaign, the Anna Fuller Fund and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

L'activité succino-déhydrogénasique dans le tissu oculaire (étude histo-chimique)

Les méthodes histochimiques modernes, relatives au métabolisme tissulaire, ont permis d'étendre les connaissances que nous possédions grâce aux différentes autres méthodes biochimiques. L'histochimie rend possible, en effet, la localisation, dans la structure, des différentes phases métaboliques. Par conséquent, la compréhension des relations qui existent entre la fonction et la structure de la cellule vivante en est facilitée.

La succino-déhydrogénase (SD), dont la localisation histochimique dans le tissu oculaire fait l'objet de notre travail, est une des déhydrogénases intervenant au cours du cycle de Krebs. Sa présence dans un tissu indique que ce cycle s'y déroule et que ce tissu est, par conséquent, le siège d'un métabolisme aérobique.

Précisons que la SD se distingue des autres déhydrogénases par le fait qu'elle ne transfère pas d'électrons aux coenzymes pyridiniques, mais au contraire directement au coenzyme flavinique.

Méthode. Nos expériences personnelles ont été faites sur le tissu oculaire de l'homme et du lapin. En ce qui concerne la préparation des tissus examinés, nous avons incubé, d'une part, les morceaux de tissu de la cornée, du corps ciliaire et du cristallin et, d'autre part, les coupes en congélation de ces mêmes tissus. La rétine, en revanche, a été incubée en entier, incluse en gélatine et coupée en congélation.

Pour le INT[1] et le NT[2], nous avons employé la méthode de Seligman et Rutenburg[4] modifiée par Rosa et Velardo[5], en milieu aérobie à un pH de 7,6. La méthode de Nachlas et al.[6] a été employée pour le nitro-BT[3].

Pour les contrôles, nous avons incubé les tissus dans le milieu exempt de succinante.

[1] Iodophenyl-nitrophenyl-phenyl tetrazolium (INT).
[2] Neotetrazolium (NT).
[3] Nitro-blue tetrazolium (nitro-BT).
[4] A. M. Seligman et M. Rutenburg, Science *113*, 317 (1951).
[5] C. G. Rosa et J. T. Velardo, J. Histochem. Cytochem. *2*, 110 (1954).
[6] M. M. Nachlas, Tsou Kwan-Chung, E. de Souza, Chao-Shing-Cheng et A. M. Seligman, J. Histochem. Cytochem. *5*, 420 (1957).

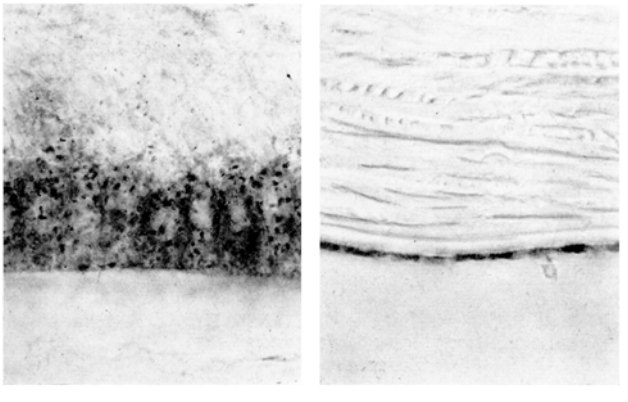

Fig. 1. Cornée humaine. L'activité succino-déhydrogénasique est prononcée dans la couche basale de l'épithelium cornéen (a) et dans les cellules endothéliales (b). (a: Nitro-BT, 500 ×, b: NT, 170 ×).

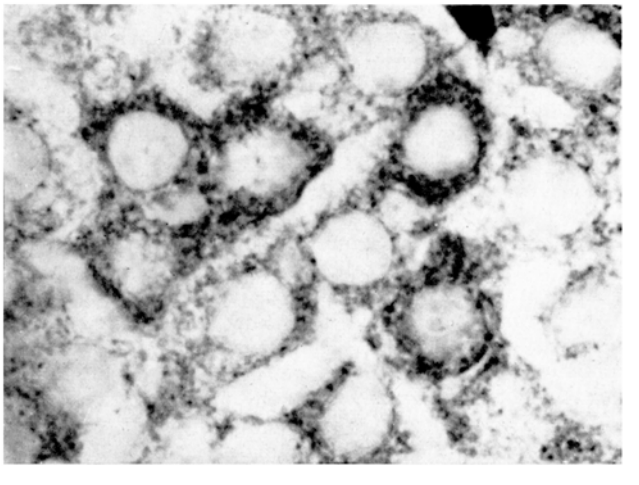

Fig. 2. Epithélium du cristallin humain. L'activité succino-déhydrogénasique est très intense dans le cytoplasme des cellules (Nitro-BT, 1070 ×).