Small intestine of albino male rats (Wistar strain) weighing about 250 g. Length of perfused intestine 30 cm starting from pylorus, fresh weight 1.52±0.08 g. Initial quantity of circulating solution 50 ml. Time of perfusion 1 h. Temperature of perfusing solution 38°C. Oxigenation with a mixture of O_2 95% and CO_2 5%. The number of experiments (n) for each group and the mean values ± S.E. are reported.

Perfusing solution	Transported solution ml/h	Transported Na μE/h	Transported glucose μM/h	Transp.Na(E) Transp.Gluc.(*M*)	Glucose concentration in transp. sol. m*M*/l	Total disappeared glucose[a] μ*M*/h
Krebs + glucose 13.9 m*M*/l n = 8	3.33 ± 0.34	520 ± 50	75.5 ± 12.2	9.5 ± 12.2	27.4 ± 5.1	275 ± 17
Krebs + glucose as above NaCl content reduced to 84 m*M*/l and addition of an isosmolar quantity of urea n = 8	2.08 ± 0.22	290 ± 30	21.1 ± 2.6	14.2 ± 1.2	10.3 ± 0.8	309 ± 6.6
Krebs + glucose as above NaCl content reduced to 54 m*M*/l and addition of an isosmolar quantity of urea n = 7	2.19 ± 0.25	149 ± 19.6	34.8 ± 6.3	6.3 ± 1.5	13.7 ± 3.1	292 ± 33
Krebs + glucose as above NaCl reduced to 54 m*M*/l and addition of an isosmolar quantity of mannitol n = 4	1.56 ± 0.60	108 ± 10	12.2 ± 4.9	13.1 ± 2.5	6.6 ± 1.25	356 ± 45
Krebs + glucose as above Na reduced to 84 mE/l and addition of an isomolar quantity of K n = 8	1.46 ± 0.27	200 ± 28	12.4 ± 2.4	21.1 ± 6.3	8.71 ± 0.4	196 ± 21.5
Krebs + glucose as above Na reduced to 54 mE/l and addition of an isomolar quantity of K n = 8	0.67 ± 0.10	23.5 ± 7.4	3.4 ± 0.4	7.3 ± 0.9	5.3 ± 0.5	133 ± 12.6

[a] Glucose of the initial pool not recovered in perfusing and collected fluids at the end of experiments.

be due to the influence of the increase in potassium concentration rather than to a decrease in sodium.

In conclusion it can be supposed that sodium transport is necessary chiefly for the extrusion of glucose from the cells in the serosal medium. The necessity of sodium transfer for this last step of glucose transport seems not to originate from the restriction of the volume of transported water. In effect water transport does not decrease to the same extent as sodium, especially in the experiments in which urea is employed as substituent, and the glucose concentration in the transported fluids falls below the level present in the controls. There is thus same evidence for admitting that the extrusion of glucose from the cells is determined by a mechanism more complicated than a simple physical diffusion.

Riassunto. Vengono riportati i dati ottenuti sul trasporto di sodio e di glucosio attraverso l'intestino isolato di ratto, perfuso con soluzioni isotoniche a diverso contenuto di sodio. Da questi dati appare che non solo il trasporto di sodio dipende dalla concentrazione di sodio nel liquido mucosale, ma anche il trasporto di glucosio. Si prospetta l'ipotesi, inoltre, che il passaggio di sodio dalla mucosa alla sierosa sia necessario sopratutto per il trasferimento del glucosio dalle cellule al liquido serosale.

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Istituto di Fisiologia generale dell'Università di Milano (Italy), April 16, 1962.

Derivate des 6,11-Dihydrodibenz(b,e)thiepins, eine neue Gruppe von psychotropen Substanzen[1]

Als Fortsetzung unserer vorherigen Arbeiten in den Gruppen der Neuro- und Psychopharmaka, deren Struktur durch das Vorhandensein eines tricyclischen Systems mit einem siebengliedrigen Mittelring charakterisiert ist[2], gingen wir vor fast zwei Jahren zur systematischen Bearbeitung der 6,11-Dihydrodibenz(b,e)thiepinderivate über. Dieses heterocyclische System war bis in neueste Zeit unbekannt. Erst am Anfang dieses Jahres publizierten STACH und SPINGLER[3] eine kurze Mitteilung, in der sie die Darstellung des 6,11-Dihydrodibenz(b,e)thiepin-11-ons (I, $R^1 = R^2 = H$) und seiner 2-Methyl- und 2-Chlorderivate durch Ringschluss der entsprechenden *o*-(Phenylmercaptomethyl)benzoesäuren (III) mit Polyphosphorsäure erwähnen und zugleich die Möglichkeit der weiteren synthetischen Auswertung dieser Ketone andeuten. Diese Arbeit veranlasste uns zur vorläufigen Mitteilung unserer bisherigen Ergebnisse in der vorliegenden Form, obwohl unsere Experimente noch nicht ganz abgeschlossen sind.

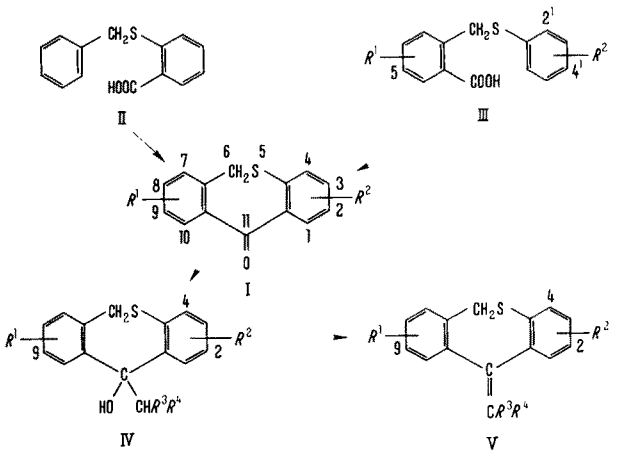
Unsere experimentellen Bemühungen konzentrierten sich in erster Reihe auch auf das Keton I ($R^1 = R^2 = H$), das wir zuerst in niedriger Ausbeute, von der S-Benzylthiosalicylsäure (II)[4] ausgehend, erhielten, und zwar

[1] 6. Mitt. Synthetische Ataractica, 5. Mitt. siehe Československ. farm. *11*, im Druck (1962).

[2] M. PROTIVA et al., Exper. *13*, 291 (1957); Coll. Czechoslov. Chem. Communs. *23*, 1330, 1941 (1958); *24*, 207, 3955 (1959); J. Med. Pharm. Chem. *4*, 411 (1961); Československ. farm. *10*, 459, 506 (1961); *11*, 3 (1962).

[3] K. STACH und H. SPINGLER, Angew. Chem. *74*, 31 (1962).

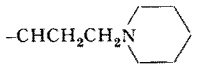
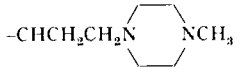
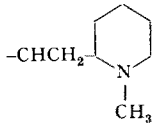
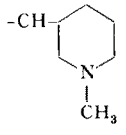
[4] H. APITZSCH, Ber. dtsch. chem. Ges. *46*, 3102 (1913).



Tab. I. Schmelzpunkte der 6,11-Dihydrodibenz(b,e)thiepine I, IV und V ($CR^3R^4 = CHCH_2CH_2N(CH_3)_2$)

Nr.	R^1R^2	I	IV	V-HCl
1	H	86–87°	130–131°	218–221°
2	2-CH_3	119–121°	142–143°	220°
3	4-CH_3	109–111°	164–166°	195–197°
4	2-C_2H_5	52–53°	138–139	200–201°
5	2-*n*-C_3H_7	86–87°		
6	2-iso-C_3H_7	94–95°	169–170	198–200
7	2-*n*-C_4H_9	58–60°	122°	98–101°
8	2-$CH_2C_6H_5$	155–156°	122–123°	
9	2-F	101–105°	155–156°	(A) 229–231° (B) 190–191
10	2-Cl	136°	152–153°	244–247
11	9-Cl	89–90°	144–145°	184–185
12	2,9-Cl_2	135–136°	166–167°	233–236°
13	2-Br	151–156°	164–165°	260–261°

Tab. II. Schmelzpunkte der 6,11-Dihydrodibenz(b,e)thiepine IV und V ($R^1 = R^2 = H$) mit heterocyclischen Substituenten in der Seitenkette

Nr.	$-CR^3R^4$	IV	V-HCl
1		185–186°	260–262°
2		201–206°	256–263° (2 HCl)
3		188–189°	198–201°
4		168–170°	191–194°

durch Cyclisierung ihres Anhydrides (Smp. 105–106°) oder Chlorids (Smp. 117–119°) bei der Einwirkung von Aluminiumchlorid in Nitrobenzol[5]. Neulich haben wir festgestellt, dass weit vorteilhaftere Ausgangsstoffe für Ketone von diesem Typus die Säuren III sind, die wir viel einfacher als STACH und SPINGLER[3] gewannen, nämlich durch Umsetzung von Phtalid und seiner Derivate mit Natriumsalzen der Thiophenole in siedendem Äthanol. Auf diese Weise haben wir bisher die folgenden Säuren III in Ausbeuten von 80–95% hergestellt (R^1R^2 und Smp. angegeben): H 113–116°, 2'-CH_3 109–110°, 4'-CH_3 136–137°, 4'-C_2H_5 89–91°, 4'-n-C_3H_7 96°, 4'-iso-C_3H_7 86–87°, 4'-n-C_4H_9 100–103°, 4'-$CH_2C_6H_5$ 121–122°, 5-Cl 127–129°, 4'-F 131–132°, 4'-Cl 135–137°, 4'-Br 142–145°, 4'-J 149–151°, 5,4'-Cl_2 142–144°, 4'-OCH_3 121–122°, 4'-OC_2H_5 142–143°, 4'-O-n-C_3H_7 117–118°, 4'-O-n-C_4H_9 111–112°, 4'-O-iso-C_4H_9 130–131°, 4'-O-iso-C_5H_{11} 111–112°, 4'-O-n-C_6H_{13} 84–85°, 4'-SCH_3 130–131°.

Die meisten dieser Säuren wurden durch Einwirkung der Polyphosphorsäure cyclisiert (Reaktionstemperatur ungefähr gleich dem Smp. der Säure, Reaktionszeit 1–2 h), wobei in Ausbeuten von 60–95% die Ketone I resultierten. Die Umsetzung dieser Ketone mit 3-Dimethylaminopropylmagnesiumchlorid[6] in einer Äther-Benzolmischung lieferte die kristallinen Carbinole IV, die durch kurzes Aufkochen mit 3 *N* H_2SO_4 dehydratisiert wurden. Die entstandenen Basen V (Gemische von geometrischen Isomeren) führten wir in Hydrochloride über, deren Rekristallisation immer wenigstens zu einem individuellen geometrischen Isomer führte. In der Tabelle I sind die Schmelzpunkte der dargestellten Ketone I, Carbinole IV und Hydrochloride der ungesättigten Basen V angegeben.

Das Grundketon (I, $R^1 = R^2 = H$) wurde daneben noch der Einwirkung von einigen weiteren basischen Grignard-Reagenzien in Tetrahydrofuran unterzogen, die aus 3-Piperidinopropylchlorid, 3-(N'-Methylpiperazino)propylchlorid, 2-(N-Methyl-2-piperidyl)-äthylchlorid und N-Methyl-3-piperidylmethylchlorid dargestellt wurden. Die erhaltenen Carbinole IV wurden ebenfalls dehydratisiert und die Basen V in Hydrochloride übergeführt. Diese Substanzen sind in der Tabelle II zusammengestellt.

Reduktion des Ketons I ($R^1 = R^2 = H$) mit Natriumborhydrid in Methanol liefert das 6,11-Dihydrodibenz-(b,e)thiepin-11-ol (Smp. 107–108°). Die Oxydation des Ketons mit der äquivalenten Menge von Wasserstoffsuperoxid bei Zimmertemperatur ergab das entsprechende Sulfoxid (Smp. 97–100°); das überschüssige Oxydationsmittel in siedender Essigsäure lieferte das Sulfon (Smp. 127–128°).

Die Hydrochloride der ungesättigten Amine V wurden vorläufig von Herrn Prof. Dr. Z. VOTAVA und Frau PhMr. J. METYŠOVÁ im Pharmakologischen Laboratorium unseres Instituts untersucht, besonders vom Gesichtspunkt der Wirksamkeit auf das zentrale Nervensystem aus. Die ausführlichsten Ergebnisse stehen bei der einfachsten Substanz (V-HCl, $R^1 = R^2 = R^3 = H$, $R^4 = CH_2CH_2N(CH_3)_2$)[5] zur Verfügung, die in den grundlegenden Testen (der rotierende Stab, Potenzierung der Thiopental-Narkose) eine milde ataraktische Wirksamkeit aufweist. Diese Substanz wird nun unter der generischen Bezeichnung «Prothiaden» klinisch geprüft, wobei sie bis jetzt einen ähnlichen Wirkungscharakter wie Imipramin zeigt[7].

[5] Tschechoslowak. Patentanmeldung PV 3561-1961.

[6] A. MARXER, Helv. chim. Acta *24*, 215E (1941).

[7] E. VENCOVSKÝ (Psychiatrische Klinik, Pilsen), persönliche Mitteilung.

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.

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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).