Neuartige N,N'-substituierte Bis-(4-hydroximinoformylpyridinium)-dihalogenide als Reaktivatoren für alkylphosphatgehemmte Cholinesterase

Der Einfluss der Stellung der Oximgruppierung bei den N,N'-Polymethylen-bis-(hydroxyliminoformyl-pyridinium)-dihalogeniden auf die Reaktivierung der alkylphosphatvergifteten Cholinesterase ist bereits mehrfach untersucht worden. Verbindungen mit modifizierter Kette, z.B. durch Einbau von Heteroatomen oder funktionellen Gruppen, sind dagegen bisher chemisch oder pharmakologisch wenig studiert worden.

Zur Untersuchung dieser Zusammenhänge sind in der Chemischen Abteilung unseres Instituts folgende Verbindungen synthetisiert worden: S100 Dimethyläther-bis-(4-hydroxyliminoformylpyridinium)-dibromid, Fp. 200–202°C; feine gelbbraune Nadeln (aus A.) – S101 Aceton-bis-(4-hydroxyliminoformylpyridinium)-dibromid, Fp. 217–219°C; gelbbraune feine Nadeln (aus A.) – S102 Propanon-2-oxim-bis-(4-hydroxyliminoformylpyridinium)-dibromid, Fp. 235–237°C; feine gelbbraune Nadeln (aus A.). Weiterhin wurden uns von Herrn Professor PROFFT freundlicherweise folgende Verbindungen zur Untersuchung überlassen: Pro 40 N (β,β'-4-Hydroxyliminoformylpyridinium-diäthylammonium-bromid)-dibromid – Pro 46 Ä Diäthyläther-β,β'-(4-hydroxyliminoformylpyridinium)-dibromid.

ENGELHARD und ERDMANN[1] haben einen dieser Körper (S100) unlängst unter der Bezeichnung Lü H 6 als einen dem TMB-4 überlegenen Reaktivator für alkylphosphatgehemmte Cholinesterasen beschrieben. Wir haben diesen Stoff seit Jahresbeginn ebenfalls geprüft und können uns dieser Feststellung der genannten Autoren anschliessen. Bei der Synthese der Verbindungen entstehen nach unseren Erfahrungen toxische Nebenprodukte, die nur schwer abzutrennen sind. Untersuchungen über dieses Problem sind noch im Gange.

Wird im Pentamethylenderivat dieser Reihe eine Methylengruppe durch ein Sauerstoffatom oder ein Stickstoffatom ersetzt, so bleibt die reaktivierende Wirkung des Moleküls erhalten. Auch beim Diäthylätherderivat wird offenbar durch die Einführung des Sauerstoffatoms die Antidotwirkung günstig beeinflusst.

Die Ergebnisse weiterer tierexperimenteller und chemischer Untersuchungen werden an anderer Stelle ausführlich beschrieben.

Summary. The cholinesterase-reactivating properties of N,N'-substituted bis-(4-hydroximinoformyl-pyridinium)-dibromides are described which bear in the carbonic chain between the pyridine moieties a nitrogen or oxygen atom. The introduction of oxygen into this chain increases the antidotal effects in paraoxon and isodemeton poisoning.

F. HAUSCHILD, M. MASCHHOUR,
R. SCHMIEDEL und W. D. WIEZOREK

Institut für Pharmakologie und Toxikologie der Karl-Marx-Universität, Leipzig (DDR), 11. Juli 1963.

[1] H. ENGELHARD und W. D. ERDMANN, Klin. Wschr. *41*, 525 (1963).
[2] R. AMMON, Pflügers Arch. ges. Physiol. *233*, 486 (1934).
[3] K. D. FRIEDBERG und F. SAKAI, Dtsch. Z. gerichtl. Med. *47*, 580 (1958).
[4] F. HAUSCHILD, M. MASCHHOUR und W. D. WIEZOREK, Med. exp. *4*, 313 (1961).

Reaktivierende Wirkung von TMB-4, S 100, Pro 40 N und Pro 46 A auf die paraoxon- und Tinox-gehemmte Plasmacholinesterase (Pferd). Methodik: Manometrische Bestimmung nach AMMON[2], modifiziert nach FRIEDBERG und SAKAI[3]. Versuchsanordnung und Auswertung nach HAUSCHILD et al.[4]. Als Reaktivierung wird die Differenz zwischen der Aktivität der alkylphosphatgehemmten Cholinesterase und der Aktivität der gehemmten und mit dem jeweiligen Reaktivator behandelten Probe, bezogen auf die Aktivität des ungehemmten Plasmas, bezeichnet. Die angegebenen Werte sind Mittelwerte mit ihren mittleren Abweichungen ($\bar{x} \pm s$). Die Zahlen in Klammern bezeichnen die Anzahl der Versuche. Hemmstoffkonzentrationen: Paraoxon 6×10^{-6} g/ml; Tinox 8×10^{-6} g/ml

Verbindung	LD_{50} mg/kg[a]	Reaktivierung in % (ungehemmtes Serum = 100%)			
		Alkylphosphat	Paraoxon		Tinox
		Konzentration des Reaktivators in g/ml	10^{-3}	10^{-4}	10^{-3}	10^{-4}
TMB-4[4]	131		37 ± 9 (5)	23 ± 3 (5)	46 ± 16 (5)	32 ± 5 (10)
S 100	141		58 ± 16 (5)	28 ± 10 (5)	57 ± 9 (7)	68 ± 8 (7)
Pro 40 N	46		12 ± 5 (6)	-	22 ± 3 (9)	15 ± 3 (7)
Pro 46 Ä	23		47 ± 10 (7)	25 ± 3 (5)	36 ± 4 (6)	25 ± 6 (5)

[a] LD_{50} errechnet nach KÄRBER (männl. Maus, i.p.).

A Study on the Antigenicity of a Human Cell Line Propagated in a Heterologous Medium

Whether living cells, growing *in vitro*, can permanently acquire new antigenic properties is a question that does not appear to be settled. The possibility that this does occur has been suggested by several authors[1–3]. This suggestion has been based upon the repeated observation[1–6] that successful and permanent transplantation of homografts has taken place in those cases where the donor tissue prior to grafting has been cultivated in a media containing serum proteins of the future host. One explanation[1–3] of this observation has postulated that a favourable modification of the cell's antigenic characteristic has occurred during the *in vitro* period. It is further postulated that by assimilating host proteins from the media into its own structural protein the donor cells acquire an antigenic structure similar to the future host. This could favourably diminish the transplantation rejection mechanism. If the foregoing explanation is true then it should

Results of serological tests for horse proteins on HeLa cells propagated in a medium containing horse serum ('HeLa Horse')

Method	Days after removal of 'HeLa Horse' from 'horse medium'							Controls			
	1/2	1	2	4	8	16	32	Rabbit serum	Horse serum	Human serum	HeLa[a]
Ring precipitin test	–	–	–	–	–	–	–	–	+	–	–
Gel diffusion technique	–	–	–	–	–	–	–	–	+	–	–
Capillary method	+	+	+	–	–	–	–	–	+	–	–
Ring precipitin with rabbit anti-human serum	+	+	+	+	+	+	+	–	–	+	+
Gel diffusion with rabbit anti-human serum	+	+	+	+	+	+	+	–	–	+	+

[a] HeLa cells that have never been in contact with horse serum.

be possible to show by immunological methods that *in vitro* cells actually share antigenic characteristics of the proteins of the media. The experiments reported here are directed at this question. A human cell line, which had been cultivated for over 1 1/2 years in a medium containing horse serum, was placed in a similar medium except that the horse serum was replaced by human serum. Following this, extracts of the cells were made at different intervals and tested serologically for horse proteins.

Materials and Methods. HeLa cells, grown in D-7 Carrel flasks were used. The medium was composed of lactalbumin hydrolysate 2.5% – 20 ml; Hanks balanced salt solution – 75 ml; horse serum – 5 ml. To prepare the extracts, cells from 10 culture flasks were washed *in situ* with physiological saline three times, and another three times after mechanical removal from the flasks. Then 2 cm³ of distilled water was added to the packed cells and the suspension was allowed to freeze and thaw three times. After addition of 0.2 ml of an 8.1% salt solution the preparation was agitated for 18 h at 4°C. Hereafter a clear supernatant was obtained by centrifugation. This was tested for horse proteins. The cell extracts were prepared 1/2, 1, 2, 4, 8, 16, and 32 days after replacement of the horse serum in the medium by human serum. Three tests were employed: (1) the ring precipitin method[7], (2) Ouchterlony's gel diffusion technique, and (3) the capillary precipitation method[8]. Only rabbit anti-horse sera with a titre over 1:16000 were used.

Results and Discussion. The results are shown in the Table. It can be seen that only HeLa cells, removed from the horse serum containing medium for two days or less, gave positive results using the capillary precipitation test. All other tests were negative. Tests carried out with rabbit anti-human sera gave positive results with all HeLa cell extracts. LANGMAN[9] has prepared an extract of explants of rabbit gonads, cultivated for three weeks in a medium containing cat serum. On serological testing, this extract appeared to contain cat proteins. LANGMAN[9] concluded that the rabbit cells *assimilated* the foreign proteins, i.e. these proteins became part of the structural proteins of the rabbit cells. MILLER et al.[10] mentioned that HeLa cells, that have been grown in a medium containing horse serum, evoke on injection into rabbits an anti-serum that reacts with horse serum. These data do not disagree with our results, for these tests have been carried out only soon after the removal of the cells from the heterologous medium. The finding of this study that the foreign antigen has been lost rapidly after removal of the cells from the heterologous medium does, however, indicate that cells cultivated in a heterologous medium, may adsorb foreign proteins to their surface and take up 'into' the cell by pinocytosis. It is these proteins that are responsible for the positive serological reaction, which is found with cells that have been removed only for a short time from the heterologous environment. It appears unlikely that foreign proteins of the medium become part of the structural protein of the cell.

Résumé. Des cellules HeLa cultivées dans un milieu qui contient du sérum de cheval, ont été transférées dans un milieu contenant du sérum humain. On a essayé, à différents moments, de détecter la présence de protéines de cheval dans les cellules transférées. Seules les cellules, qui ont été retirées du milieu hétérologue avant 48 h, donnent des réactions sérologiques positives.

G. J. V. SWAEN

Institute of Pathology, Roman Catholic University, Nijmegen (The Netherlands), July 31, 1963.

[1] R. GASSUL, Arch. Entwickl. Mech. Organ. *52–97*, 400 (1923).
[2] R. ERDMANN, Arch. Entwickl. Mech. Organ. *112*, 739 (1927).
[3] J. LANGMAN, *Weelselkweek en Transplantatie*. Proefschr. Amsterdam (1950).
[4] P. J. GAILLARD, Ned. Tijdschr. Geneesk. *91*, 580 (1947).
[5] P. J. GAILLARD, Coll. Internat. Centre Nat. Recherche Scient. *78*, 157 (1957).
[6] G. J. V. SWAEN, Transplantation *1*, 187 (1963).
[7] E. A. KABAT and M. M. MAYER, *Experimental Immunochemistry* (Springfield 1948), p. 59.
[8] G. TEN CATE and W. J. VAN DOORENMALEN, Proc. Kon. Ned. Akad. Wetenschap. *53*, 894 (1950).
[9] J. LANGMAN, Proc. Kon. Ned. Akad. Wetensch. *56*, 219 (1953).
[10] D. G. MILLER and T. C. HSU, Cancer Res. *16*, 306 (1956).