The incidence of recessive lethals in male germ cells of *Drosophila* by oxydemetonmethyl

Experiment	Strain	Treatment	Brood I		II		III		I–III	
		(conc./m*M*)	lethals/chromos. (%)		lethals/chromos. (%)		lethals/chromos. (%)		lethals/chromos. (%)	
1	Hikone-R	0.43 m*M* fed for 2 days	4/998	0.40						
		Control	0/595	—						
2	Hikone-R	3.0 m*M* fed for 26 h	3/819	0.37	1/609	0.16	1/616	0.16	5/2044	0.24
		Control	2/611	0.33	0/614	—	1/620	0.16	3/1845	0.16
3	Hikone-R	1.5 m*M* fed for 3 days	4/607	0.66	2/618	0.32	2/539	0.37	8/1764	0.45
		Control	1/617	0.16	1/616	0.16	1/612	0.16	3/1845	0.16
4	Berlin wild	0.043 m*M* fed for 3 days	1/826	0.12	1/813	0.12	2/820	0.24	4/2459	0.16

brood II, and spermatocytes (and spermatids) in brood III were analyzed.

If the toxic effects of oxydemetonmethyl are analyzed first, both strains exhibit pronounced differences in response to the chemical (Figure). The Berlin wild strain is clearly much more sensitive to the insecticide chemical than the Hikone R strain.

In the sex-linked lethal experiments, a slightly higher incidence of recessive lethal mutations was determined after treatment of Hikone males with concentrations ranging from 0.043 m*M* to 3.0 m*M* (Table). The exposure time had to be shortened at higher doses due to the toxic property of the compound. Most of the lethals induced by oxydemetonmethyl were found in brood I, indicating sensitivity of mature sperm to the chemical.

When the data from all oxydemetonmethyl experiments with HR males are pooled and compared with the pooled data from the control sample, the X^2 test shows that the difference between these 2 samples is just significant ($0.05 > p > 0.01$). However, since a weak rise resulted in all experiments with the insecticide, the test substance may be considered a weak mutagen in Hikone R males. In contrast, no mutagenic effect could be found with Berlin wild males (expt. 4). Failure to recover mutations in Berlin wild males is obviously due to killing effect of the compound in this strain. Only 1/40 of the dose that can be given to Hikone R males for 3 days can be fed to Berlin wild males. Thus, this compound may be classified as non-mutagenic or a weak mutagen in *Drosophila*, depending on the genetic constitution of the test strain.

We plan to continue analyzing variations in response to treatment with chemical mutagens. Such differences promise to provide clues to the mechanism of action and of resistance to mutagens.

Zusammenfassung. Oxydemetonmethyl induziert rezessive Letalmutation im Insektizid-resistenten Stamm Hikone R von *Drosophila melanogaster*. Am Wildstamm Berlin ist, bedingt durch die hohe Toxizität des Insektizids, eine Testung mit höheren Dosen als 0.04 m*M* nicht möglich.

E. Vogel[9]

Zentrallaboratorium für Mutagenitätsprüfung der Deutschen Forschungsgemeinschaft, Breisacherstrasse 33, D–78 Freiburg i. Br. (Germany),
7 September 1973.

[9] The author is grateful to Mrs. M. Klug and Miss G. Kolodziej for excellent technical assistance.

Protein Half-Lives in Neonatal Mice After a Toxic Dose of Cyclophosphamide

Development represents a period of time during which immature cells and tissues of an organism acquire adult structure and function. This orderly transition of form and function ultimately depends on developmentally directed changes in tissue proteins. The protein composition of a tissue differs both quantitatively and qualitatively during development and between various tissue types in the adult. Agents which alter either protein acquisition or composition of a tissue may produce abnormal development.

Cyclophosphamide, a clinically useful alkylating agent, has toxic properties during both embryonic[1,2] and postnatal development[3,4]. One day-old neonatal mice treated with 80 mg/kg cyclophosphamide grew at reduced rate with increased mortality and were morphologically abnormal at maturity[4]. Toxicity, during postnatal development, was correlated with the presence of alkylating cyclophosphamide metabolites[5]. Postnatal toxicity was associated with an inhibition of DNA synthesis in the liver, brain, and carcass and RNA synthesis in the liver and brain during a 5 day observation period after cyclophosphamide[6]. Protein synthesis, however, was not affected in a manner which indicated that drug treatment altered this process of differentiation. Since the protein

[1] J. Wilson, J. Pharmac. exp. Ther. *144*, 429 (1964).
[2] J. Gibson and B. Becker, Cancer Res. *28*, 475 (1968).
[3] H. Nordlinder, Experientia *25*, 1296 (1969).
[4] R. Short and J. Gibson, Experientia *27*, 805 (1971).
[5] J. Bus, R. Short and J. Gibson, J. Pharmac. exp. Ther. *184*, 749 (1973).
[6] R. Short and J. Gibson, Biochem. Pharmac., *22*, 3181 (1973).

Table I. Half-life (days) of proteins from neonatal mice after cyclophosphamide

Tissue fraction		Protein specific activity[a]		Organ specific activity[b]	
		Control	Treated	Control	Treated
Liver					
	Soluble	2.4	1.5[c]	6.0	1.8[c]
	Particulate	2.6	1.6[c]	6.4	2.0[c]
Brain					
	Soluble	3.7	2.3[c]	4.0	4.0
	Particulate	4.1	2.5[c]	2.2	2.2
Carcass					
	Soluble	2.4	1.7[c]	—	—
	Particulate	2.2	1.9	—	—

[a] Half-life values were determined from the least squares regression line of log dpm/mg protein vs. days after treatment for 3 observations made at 1, 3, 5 and 10 days for control liver and brain or 1, 3, and 5 days for carcasses and treated liver and brain. [b] Half-life values were determined from the least squares regression line of log dpm/organ vs. days after treatment for 3 observations made at 1, 3 and 5 days after treatment. [c] 95% confidence limits for the slope of the least squares regression line for control and treated observations do not overlap.

content of a tissue is dependent both on the synthesis and degradation of proteins cyclophosphamide may disrupt normal growth and development by affecting protein degradation. Alkylation of neonatal proteins, for example, may alter the conformation of proteins thus rendering these molecules more susceptable to degradation[7]. These studies were undertaken to determine if the cyclophosphamide induced disruption of postnatal development in mice was associated with a change in the protein content of tissues.

Pregnant Swiss-Webster mice were treated i.p. with 100 μCi/kg of L-arginine (guanido-^{14}C) (59 mCi/mmole, ICN Tracer Lab, Irvine, Calif.) at 22.00 h on day 18 and at 10.00 h and 22.00 h on day 19 of gestation to label fetal proteins before birth. Litters were delivered naturally and 80 mg/kg cyclophosphamide or normal saline was administered s.c. to neonates 24 to 48 h after birth[4,6]. Neonatal mice were sacrificed at 1, 3, 5 and 10 days after cyclophosphamide and the liver, brain, and carcass from 1 or 2 mice were homogenized in 4 volumes of KCl-*tris* (154 m*M* KCl, 10 m*M* *tris*, pH 7.3). The homogenate was separated into a soluble and particulate fraction by centrifugation at 105,000 *g* for 1 h. The supernatant was saved and the precipitate was resuspended in 5ml KCl-*tris* and centrifuged at 105, 000 *g* for 1 h. The supernatants were combined and acidified with concentrated perchloric acid (PCA) to a final concentration of 0.3 *N* PCA. The acid precipitate was washed with successive 5 ml portions of 0.2 *N* PCA, 0.2 *N* PCA, 95% ethanol saturated with sodium acetate, ethanol:ether (3:1), ether and dissolved in 5 ml of 0.3 *N* sodium hydroxide. The pellet and walls of the centrifuge tube were carefully washed with distilled water after the second centrifugation and the pellet was dissolved in 5 ml of 0.3 *N* sodium hydroxide. Radioactivity was measured by liquid scintillation counting after the addition of PCS-Solubilizer (Amersham/Searle, Arlington Heights, Ill.). Protein was measured by the LOWRY method[8] using bovine serum albumin as the standard. Specific activity was expressed as dpm/mg protein or dpm/organ. The least squares regression line of log specific activity vs. time was used to calculate protein half-life which was defined as the time required for the specific activity measured 1 day after treatment to decline by 50%.

Table II. Soluble and particulate protein in the liver of neonatal mice after cyclophosphamide

Tissue fraction	Days after treatment	mg Protein/g tissue	
		Control	Treated
Soluble	1	4.7 ± 0.6[a]	4.7 ± 0.4
	3	7.6 ± 0.4	6.0 ± 0.4
	5	12.2 ± 1.3	6.4 ± 0.5[b]
Particulate	1	8.0 ± 0.8	7.0 ± 0.3
	3	12.6 ± 0.5	10.5 ± 1.0
	5	19.7 ± 1.4	10.2 ± 0.5[b]

[a] Mean ± SE for 3 observations. [b] Significantly different from control by Student's *t*-test ($P < 0.05$).

Protein half-life as calculated by the decline in protein specific activity (dpm/mg protein) was significantly reduced in soluble protein of the liver, brain and carcass and particulate protein from liver and brain of neonatal mice by cyclophosphamide treatment (Table I). The decline in organ specific activity (dpm/organ) indicated that drug treatment significantly reduced protein half-life of the soluble and particulate fraction of the liver. There was a significant reduction in both soluble and particulate total organ protein in the liver 5 days after cyclophosphamide (Table II). There was no significant effect on brain total organ protein or protein/mg tissue in the liver or brain at 1, 3, or 5 days after cyclophosphamide.

Protein half-life values, as measured by the decline in specific activity, were dependent both on the synthesis of new proteins and the degradation of radioactive proteins. The decline in protein specific activity was attributed to the combined effect of synthesis and degradation while the decline in organ specific activity was due primarily to degradation. The validity of the interpretation that the decline in organ specific activity was due to protein degradation depends on the degree to which ^{14}C-guanido-arginine was re-utilized for protein synthesis. Evidence indicates that arginine labelled in the guanido position is

[7] R. SCHIMKE, Mammal. Protein Metab. *3*, 177 (1969).
[8] O. LOWRY, N. ROSEBROUGH, A. FARR and R. RANDALL, J. biol. Chem. *193*, 265 (1951).

not significantly re-utilized for protein synthesis in the livers of adult rats[9].

The drug-induced reduction in half-life of liver proteins was not resolved into separate components of synthesis and degradation since both half-life values were reduced. The larger reduction in organ specific activity half-life values, however, suggested that drug treatment produced a greater effect on protein degradation. As a result of an increased rate of protein degradation relative to protein synthesis the toal amount of both soluble and particulate protein was reduced in the liver.

Brain protein half-life values after cyclophosphamide were reduced as calculated by the decline in protein specific activity and unchanged when measured by the decline in organ specific activity. These observations indicated that cyclophosphamide increased protein synthesis but did not affect degradation. An increased level of synthesis may be indicative of a repair process to drug induced damage. Alkylation of DNA and disruption of the cell cycle[10], alternatively, may have permitted protein synthesis to continue in an uncontrolled fashion. The failure of a 30 min pulse of ^{14}C-leucine to detect a cyclophosphamide effect on brain protein synthesis[6] may be attributed to a drug induced reduction in the specific activity of the precursor pool.

A dose of cyclophosphamide which disrupted normal postnatal development reduced the half-life of proteins from neonatal mice. A regulated balance between protein synthesis and degradation appears to be an important component of normal growth and agents which affect this balance may disrupt subsequent growth and development. The observations with cyclophosphamide, in addition, suggest that alkylating agents may render protein molecules more susceptable to degradation. Since specific proteins were not studied it was not possible to distinguish between selective degradation of alkylated proteins and general protein degradation which resulted from cell death[11].

Zusammenfassung. Untersuchung der Cyclophosphamid-Wirkung bei 1 Tag alten Mäusen. Die Halbwertszeit von Proteinen, die man während der intrauterinen Entwicklung markierte, wurde in verschiedenen Geweben bestimmt. Ermittlung des Gleichgewichts zwischen Proteinsynthese in der Diskussion der Daten über Wachstums- und Entwicklungsbeziehungen.

R. Short[12] and J. Gibson

Michigan State University, Department of Pharmacology, East Lansing (Michigan 48824), USA, 15 October 1973.

[9] I. Arias, D. Doyle and R. Schimke, J. biol. Chem. *244*, 3303 (1969).

[10] J. Roberts, T. Brent and A. Crathorn, in *The Interaction of Drugs and Subcellular Components in Animal Cells* (Ed. P. Campbell; J. & A. Churchill, London 1968), p. 5.

[11] This work was supported by Grant No. 00560 from the National Institute of Environmental Health Sciences and R. Short was supported by NIH Training Grant No. 5T01 GM 01761.

[12] Present address, Faculty of Medicine, Division of Medical Biochemistry, The University of Calgary, Calgary 44, Alberta, Canada.

Tribenoside as an Inhibitor of Chemically Induced Histamine Release

Tribenoside[1], a drug used in the treatment of venous insufficiency of the lower leg, displays a wide spectrum of pharmacological activities, including anti-inflammatory and anti-allergic effects in various test models[2–6].

In addition to suppressing non-immunological and immunological inflammatory reactions, the compound has been shown to exert antinociceptive activity in the mouse[7]. Furthermore, it affords protection against lethal wasp-venom shock in the guinea-pig[8] and against early shock reactions to lethal doses of colchicine and nitrogen mustard in the rat[9], i.e. it possesses 'antitoxic' properties. Of particular interest with regard to its main clinical use is the pronounced venotropic action of tribenoside, which can be demonstrated in various species[10–12] as well as its membrane-stabilizing effect, e.g. protection against silica-induced labilization of lipid bilayer membranes[13] or silica-induced red cell lysis[14]. In order to gain some insight into the cellular or subcellular mechanism of action of tribenoside, we have examined its effect on the histamine release reaction following exposure of rat mast cells to compound 48/80 or C44 680-Ba. The results of this study, in which various known inhibitors of immunological or non-immunological mast cell degranulation were examined comparatively, clearly demonstrate that tribenoside is one of the most potent inhibitors of chemically induced histamine release.

Histamine liberators used: C44680-Ba, a prolyl alkyl derivative of D-Ser[1], Lys[15,17,18] β-corticotrophin, which has previously been shown to be a highly potent histamine liberator[16]. Compound 48/80 (Lot. K4023) was purchased from Wellcome Res. Lab. Beckenham, England.

Methods and materials. Suspensions of unfractionated peritoneal cells were obtained as follows: 10 ml of a modified Tyrode solution (NaCl 110 mM; KCl 2.2 mM; $CaCl_2$ 1.4 mM; Na_2HPO_4 9.4 mM; NaH_2PO_4 0.3 mM; KH_2PO_4 0.3 mM; $NaHCO_3$ 4.8 mM; $MgCl_2$ 0.08 mM; glucose 4.4 mM; pH 7.2), at body temperature was administered i.p. to male rats (Ivanovas, Ra-25) weighing 180 to 200 g. After 10 min the animals were sacrificed, the peritoneal cavity was opened and the fluid containing the cells was transferred into chilled PVC tubes. Cell suspensions from 4 rats were pooled and kept in ice-water until use. Aliquots of 1 ml were mixed with 0.5 ml solution containing the potential inhibitor and pre-incubated in a metabolic shaker at 37 °C. 15 min later, 0.5 ml of a solution with the histamine-releasing agent was added to the mixture, which was further incubated for 15 min. Incubation was stopped by transferring the

[1] Active principle of Glyvenol® (ethyl-3′5′6′-tri-*O*-benzyl-D-glucofuranoside.

[2] M. di Rosa, Archs int. Pharmacodyn. *173*, 162 (1968).

[3] H. Düngemann, Praxis *57*, 643 (1968).

[4] R. Jaques and B. Schär, Schweiz. med. Wschr. *97*, 553 (1967).

[5] L. Riesterer and R. Jaques, Experientia *24*, 581 (1968).

[6] B. Urbaschek, R. Versteyl and D. Götte, Naturwissenschaften *22*, 3187 (1967).

[7] H. Helfer and R. Jaques, Helv. physiol. Acta *26*, 137 (1968).

[8] R. Jaques, Pharmacology *2*, 21 (1969).

[9] G. Egert and L. Lendle, Pharmacology *1*, 154 (1968).

[10] H. Helfer and R. Jaques, Helv. physiol. Acta *25*, 322 (1967).

[11] H. Helfer and R. Jaques, Pharmacology *5*, 23 (1971).

[12] R. Jaques, Pharmacology *4*, 193 (1970).

[13] H. Majer, Agents and Actions *2*, 33 (1971)

[14] H. Majer, personal communication.

[15] L. M. Lichtenstein and S. Margolis, Science *161*, 902 (1968).

[16] R. Jaques and M. Brugger, Pharmacology *2*, 361 (1969).