Table I. Mean values of results obtained in *Macaca mulatta* after i.v. application of endotoxoid

	Before injection		4 weeks after first injection		8 weeks after first injection		12 weeks after first injection	
	Treated	Controls	Treated	Controls	Treated	Controls	Treated	Controls
Body temperature °C	39.8	39.6	39.8	39.5	39.3	39.1	39.6	39.3
Bilirubin mg/100 ml	1.78	1.80	1.58	1.62	1.99	2.05	1.78	2.02
Alk. phosphatase mU/ml	277	272	207	300	269	300	248	289
SGOT mU/ml	31.10	24.93	34.90	31.43	25.17	23.3	31.32	26.91
SGPT mU/ml	21.42	19.28	22.45	23.28	14.45	12.84	23.02	25.29
GLDH mU/ml	3.61	2.71	3.42	3.52	3.24	3.88	3.37	3.33
Urea mg/100 ml	26.6	26.1	37.3	34.6	45.3	36.3	34.3	35.1
Glucose mg/100 ml	85.8	92.5	86.4	91.1	91.9	94.8	91.0	88.9
Protein g/100 ml	9.19	9.91	9.21	9.52	10.15	11.0	10.05	9.87
Prothrombin time (%)	80	89	82	81	81	85	81	89
Hematocrit (%)	49	46	48	48	46	45	47	47
Hemoglobin (%)	55.4	51.5	53.7	49.6	49.5	48.4	49.7	48.8
Erythrocytes mill/mm³	7.21	5.94	6.70	6.0	5.56	5.97	6.16	6.78
Leucocytes mm³	11,858	11,275	10,858	7,438	12,467	11,313	11,675	13,738

Table II. Mean values of blood coagulation studies determined at the end of the experiment

	Macaca mulatta	
	Treated	Untreated
Thrombin time/sec	27.5	22.1
PTT/sec	39.7	43.4
Factor V/sec	36.9	39.5
Prothrombin/sec	37.5	37.1
Thrombocytes/mm³	265,000	296,000
Fibrinogen	249	227

titers slightly increased in monkeys which had received endotoxoid whereas the titers of control animals remained unchanged. With the serological tests employed endotoxoid did not appear to evoke a good antibody response.

Macroscopic observation at necropsy did not reveal any alterations except for pleural adhesions and some little white nodes present in the lungs of one animal of either group. Inflammatory infiltrates in the area of the white nodes especially with eosinophile leucocytes and fibrous tissue obliteration (histiocytes and collagenous fibres) were microscopically observed[21], but obviously had no connection with the endotoxoid treatment[27].

Zusammenfassung. Vor dem Hintergrund der vielfältigen Endotoxoidwirkung wurde diese Substanz in chronischen Toxizitätsstudien bei *Macaca mulatta* geprüft. Endotoxoid wurde *Macaca mulatta* in einer Dosierung von jeweils 100 γ/kg in insgesamt 51 Injektionen innerhalb von 12 Wochen injiziert. Dabei zeigte sich, dass diese Substanz keinen Einfluss auf die geprüften klinisch-chemischen Parameter hatte und bei makroskopischer und histologischer Untersuchung keine Veränderungen auftraten.

B. URBASCHEK[22], R. URBASCHEK[22], G. MAUFF[22], CH. GERLACH[23], K. HUTH[24], F. JUNG[22], R.-H. RINGERT[22], A. NOWOTNY[25] and H. FRITSCH[26]

[21] Kindly supported by a grant from the Deutsche Forschungsgemeinschaft to Dr. FRITSCH.

[22] Institut für Hygiene und Medizinische Mikrobiologie der Universität Heidelberg, Klinikum Mannheim, D-68 Mannheim, D 6, 4–6.

[23] Versuchstierfarm der Universität Heidelberg.

[24] Medizinische Klinik der Universität Giessen, D-63 Giessen, Klinikstrasse 32b.

[25] Temple University, Department of Microbiology and Immunology, Philadelphia (Pa. 19140, USA).

[26] Medizinische Klinik der Universität Heidelberg, D-69 Heidelberg, Bergheimer Strasse 58.

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The Effects of Cyclophosphamide and Nor-Nitrogen Mustard Administration to One-Day-Old Mice

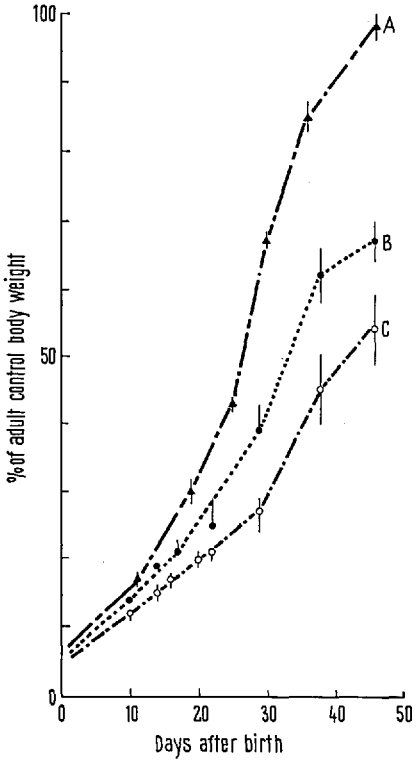
Cyclophosphamide is an antineoplastic agent that can produce abnormal development in both embryonic[1] and neonatal mice[2]. Bioactivation of the parent compound is required for the production of alkylating metabolites[3]. It has been suggested that the teratogenic effects of cyclophosphamide are associated with the parent compound rather than alkylating metabolites[4]. We have shown that the developmental toxicity of this drug, in perinatal mice, occurred at a time when the affected organism had not fully developed an in vitro ability to activate the parent compound[5]. However, since 1-day-old mice had a slight capacity to perform this conversion it became necessary to determine the effects of alkylating activity on neonatal development. If perinatal toxicity arises from an alkylating mechanism then other alkylating agents may produce similar effects. This communication reports the results of experiments in which cyclophosphamide and nor-nitrogen mustard, an agent with in vitro alkylating activity, were administered to 1-day-old mice.

One-day-old Swiss-Webster mice were obtained from timed pregnancies started in this laboratory. 6 litters, born on the same day, were assigned to each drug treatment group. The average number of animals in each litter was 12. Each group was sub-divided into 3 treated and 3 control litters and matched on the basis of litter size and average body weight. The treated mice received 50 µl s.c. of the drug dissolved in normal saline and the controls received only the vehicle. The average doses administered in this study were cyclophosphamide 45 and 80 mg/kg and nor-nitrogen mustard 54 mg/kg. This dose of nor-nitrogen mustard was calculated to be equimolar with the higher dose of cyclophosphamide (MW cyclophosphamide/nor-nitrogen mustard = 0.68).

Cumulative litter mortality produced by cyclophosphamide or nor-nitrogen mustard administration to 1-day-old mice

Treatment	Mortality (%)	
	Control litters	Treated litters
Cyclophosphamide (45 mg/kg)	0[a] (32)	22 ± 15[b] (31)
Cyclophosphamide (80 mg/kg)	15 ± 14 (34)	44 ± 14[b] (37)
Nor-nitrogen mustard (54 mg/kg)	8 ± 4 (36)	11 ± 5 (37)

[a] The values represent the average percent mortality per litter ± S.E. for 3 litters 46 days after birth. Values in parenthesis represent the initial number of animals. [b] Significantly different from control at $p < 0.05$ (Student's t-test).



Growth curves of treated litters. A) The treatments were nor-nitrogen mustard 54 mg/kg; B) cyclophosphamide 45 mg/kg; and C) cyclophosphamide 80 mg/kg. All animals received treatment at one day of age. Points on each curve represent the mean body weight ± S.E. of 3 litters (average number of mice per litter = 12) of neonatal mice expressed as a percent of normal adult body weight. Normal adult body weight was determined from control litters which matured simultaneously with treated litters. The number of animals in each group is indicated in the Table. Values earlier than 10 days are not shown for the sake of clarity. The growth curve for control litters is identical with the growth curve of nor-nitrogen mustard treated litters.

The data in the Table and in the Figure indicate that cyclophosphamide inhibits growth and promotes neonatal mortality. The litters receiving the higher dose exhibited many of the gross morphologic features reported by NORDLINDER[2], e.g., delayed development of hair and short nose, ears, and tail. The litters receiving the lower dose, however, were not affected to the same degree as the above litters. The growth curves for the nor-nitrogen mustard treated litters were identical to control curves and there were no gross morphologic differences between treated and control animals. The treated animals developed a bald spot at the injection site which disappeared with age.

In preliminary experiments nitrobenzyl pyridine (NBP) was used to detect alkylating activity in plasma and urine[4,6]. One-day-old mice were injected with the drug and either isolated (0–4 h) or returned to the mother (8 h) prior to sacrifice. Blood was obtained by exsanguination and the urine was obtained from excized bladders. In cyclophosphamide treated animals the samples were hydrolyzed in 0.5 ml of 1 N HCl for 5 min on a boiling water bath to convert the parent compound to NBP reactive material. As a result of this treatment the NBP reactive material represents total drug. Cyclophosphamide was detected in plasma and urine at the end of 4 h but only in the urine at the end of 8 h. The plasma levels remained relatively constant during this initial 4 h period while the urine levels tended to increase. This is suggestive of an injection depot from which the drug is slowly absorbed. Nor-nitrogen mustard, on the other hand, was detected only in the urine at 1 h.

These observations indicate that the administration of 2 alkylating agents can produce different effects on the growth and development of neonatal mice. The data suggests that these differences may be due to the pharmacokinetic properties of the drugs and/or due to a unique mechanism of cyclophosphamide cytotoxic action. We are not able, however, to exclude the possibility that these agents may affect different biological processes sensitive to alkylating agents. An alternative method to evaluate the role of the parent compound in producing neonatal toxicity involves the techniques of enzyme induction. The practicality of this approach is currently under investigation in our laboratory[7].

Zusammenfassung. Störung der normalen Entwicklung und des Wachstums ein Tag alter Mäuse wurde durch Injektion von Cyclophosphamid verursacht. Verabreichung von Nor-Chlormethin zeigte keine derartigen Effekte und es wird vermutet, dass diese Unterschiede durch die pharmakokinetischen Eigenschaften dieser Stoffe verursacht sein könnten.

R. SHORT and J. GIBSON

Michigan State University, Department of Pharmacology, East Lansing (Michigan 48823, USA), 29 December 1970.

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