Immunodepressive and Leukemogenic Effects of Urethan in C3Hf and SWR Mice* (33665)

GIORGIO PARMIANI, MARIA I. COLNAGHI, AND GIUSEPPE DELLA PORTA
(Introduced by P. Shubik)

Istituto Nazionale per lo Studio e la Cura dei Tumori, 20133 Milano, Italy

Specific antigenicity of neoplastic cells has been demonstrated in several experimental systems, raising the question as to why antigenically changed cells are not destroyed by an immunological reaction before they become established as tumors. In order to explain this phenomenon, the possibility that carcinogenic compounds act both by cell transformation and by interference with the immunological system has been suggested (1, 2). In fact, the carcinogenic chemicals so far examined have caused a persistent decrease of different types of immune response (3–8). In the case of urethan, when doubtful results were found, this appears to be due to different times and doses of drug administration (9–11).

The purpose of the present experiments was to ascertain whether urethan, employed in the same dose which produces malignant lymphomas (12), induced a depression of antibody production against sheep red blood cells, and to investigate the duration of this depression and its relation with the development of lymphoreticular tumors.

Materials and Methods. Two inbred strains of mice, C3Hf/Dp and SWR/Dp, maintained in our laboratory, were used. Urethan (E. Merck A.G., Darmstadt) was dissolved 6% in distilled water; and 1 mg/g of body weight was injected intraperitoneally, starting at 10 days of age, 5 times, at intervals of 2 days. Controls were kept untreated.

Two groups of treated and untreated C3Hf and SWR mice were injected intraperitoneally with 0.25 ml of sheep red blood cells (SRBC) 5% at 30 and 60 days of age, and were bled from the retro-orbital plexus at 35, 50, 70, and 90 days for hemagglutinin titer. Four additional groups of urethan-treated and control C3Hf mice were immunized at 45, 60, 75, and 90 days, respectively, and bled 5 days later.

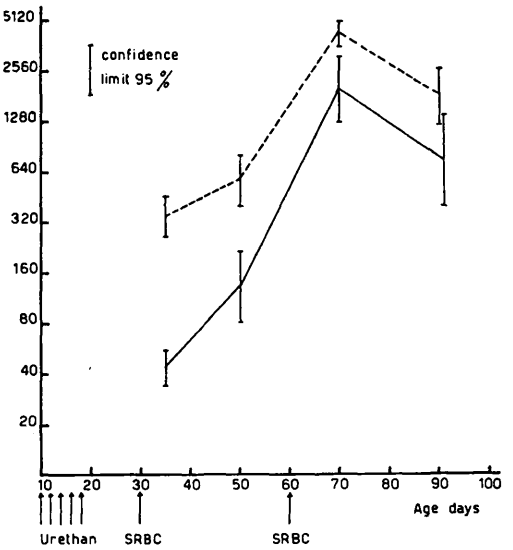

FIG. 1. Effect of urethan on primary and secondary hemagglutinin response in C3Hf mice: (—), urethan-treated mice (9♂ and 14♀); (- - -), untreated mice (7♂ and 7♀).

Inactivated sera were diluted in phosphate buffer, pH 7.2, and serial twofold dilutions were prepared in Takatsy type plates, the first well containing 0.1 ml of 1:10 antiserum. To each well 0.1 ml of three times washed 0.5% SRBC was added; agglutination titers were read after the suspension was settled for 4 hr at room temperature and were controlled after a night.

Results. As shown in Figs. 1 and 2, urethan produced a clear depression of the primary humoral response in both strains, with no sex difference. The secondary response was unaffected by the treatment in SWR mice; in C3Hf the difference between treated and control animals was minimal.

In the four additional groups of urethan-

* Supported by the Consiglio Nazionale delle Ricerche, Rome, Italy.

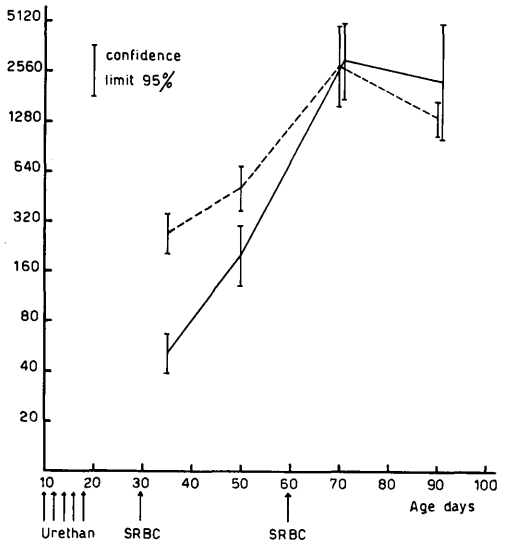

FIG. 2. Effect of urethan on primary and secondary hemagglutinin response in SWR mice. (—) Urethan treated mice (17 ♂ and 19 ♀); (- - -), untreated mice (12 ♂ and 13 ♀).

treated C3Hf mice which were immunized from 45 to 90 days of age, the response remained reduced comparably (or similarly) to the group sensitized at 30 days (Fig. 3).

Forty-three of the 59 urethan-treated C3Hf and SWR mice used for testing the primary and secondary humoral response were kept under observation and 28 died with lymphosarcoma at an average age of 27 weeks. As shown in Table I, the incidence of lymphomas was higher in the groups with the lower hemagglutinin titer. A test for a linear trend between agglutinin titer and lymphomas incidence, calculated from the combined data of the two strains, gave a significant result ($\chi^2_{[1]} = 8.16$; $p<0.005$).

In addition, lung adenomas were observed in all the SWR mice, whereas hepatomas occurred in all the C3Hf mice which survived beyond week 40 of age.

Discussion. The results of the present study show that 5 doses of urethan, administered during infancy to mice, produce a marked reduction of the primary response to erythrocyte antigens injected starting 12 days after the end of treatment. Since urethan is rapidly metabolized within a few hours after injection, the immunodepression should be related to the atrophy of lymphatic tissue, which occurs after injection of urethan to newborn and adult mice (13, 14).

In a dynamic study (13), morphologic recovery of lymphopoietic organs was described to occur 2–3 weeks after a single injection of urethan to adult mice, a treatment which has not been known to be leukemogenic (15). However, we found that the impairment of the immune response still persists 10 weeks after the end of a highly leukemogenic 5-treatment course in infancy. Fur-

TABLE I. Correlation between Depression of Hemagglutinin Titer and Development of Malignant Lymphoma in C3Hf and SWR Urethan-Treated Mice.

Hemagglutinin titer at 35 days	No. of animals[a]		No. of lymphosarcomas			
	C3Hf	SWR	C3Hf	SWR	Total	(%)
≤1:20	8	1	8	1	9	100
1:40	8	12	4	10	14	70
1:80	6	5	3	2	5	45
1:160	—	3	—	1	1	33

[a] Alive at the end of serological tests.

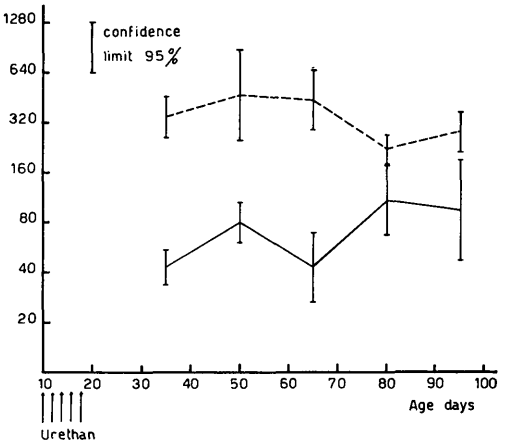

FIG. 3. Time-course study of urethan effect on primary hemagglutinin response, tested in C3Hf mice 5 days after SRBC injection: (—) urethan-treated mice (5 groups immunized, respectively, at 30 days of age (9 ♂ and 14 ♀), 45 days (27 ♂ and 16 ♀), 60 days (4 ♂ and 10 ♀), 75 days (5 ♂ and 8 ♀), and 90 days (4 ♂ and 7 ♀); (- - -), untreated mice (14–19 animals in each of 5 groups).

ther studies are under way to clarify the mechanism of prolongation of immunodepression caused by urethan.

Neonatal thymectomy appears to enhance tumor induction by chemical carcinogens, and this enhancement has been related by some investigators to the immunological impairment caused by the thymectomy (16). The correlation, found in the present study, between the decreased level of hemagglutinin titer caused by the carcinogen itself and the development of malignant lymphomas, further suggests that a persistent immundepression is one of the factors favoring chemical carcinogenesis. It may very well be that the same factor is operating also in chemical carcinogenesis by a single treatment at birth (17), when the immunological system is still in a phase of development.

Summary. A leukemogenic treatment with urethan in infant mice induced a prolonged depression of primary hemagglutinin response against sheep red blood cells. The development of lymphosarcomas was found to be correlated with the degree of immunological impairment.

We wish to thank Dr. Lorenzo Tomatis for stimulation of this research.

1. Prehn, R. T. and Main, J. M., J. Natl. Cancer Inst. **18**, 796 (1957).

2. Berenbaum, M. C., Brit. Med. Bull. **20**, 159 (1964).

3. Malmgren, R. A., Bennison, B. E., and McKinley, T. W., Jr., Proc. Soc. Exptl. Biol. Med. **79**, 484 (1952).

4. Prehn, R. T., J. Natl. Cancer Inst. **31**, 791 (1963).

5. Stjernswärd, J., J. Natl. Cancer Inst. **36**, 1189 (1966).

6. Ball, J. K., Sinclair, N. R., and McCarter, J. A., Science **152**, 650 (1966).

7. Stjernswärd, J., J. Natl. Cancer Inst. **38**, 515 (1967).

8. Doell, R. G., De Vaux St. Cyr, C., and Grabar, P., Intern. J. Cancer **2**, 103 (1967).

9. Berenbaum, M. C., Nature **185**, 167 (1960).

10. Nathan, H. C., Bieber, S., Elion, G. B., and Hitchings, G. H., Proc. Soc. Exptl. Biol. Med. **107**, 796 (1961).

11. Rubin, B. A., Progr. Exptl. Tumor Res. **5**, 217 (1964).

12. Della Porta, G., Capitano, J., Parmi, L., and Colnaghi, M. I., Tumori **53**, 81 (1967).

13. Fiore-Donati, L. and Kaye, A. M., J. Natl. Cancer Inst. **33**, 907 (1964).

14. Haran-Ghera, N. and Kaplan H. S., Cancer Res. **24**, 1926 (1964).

15. Fiore-Donati, L., De Benedictis, G., Chieco-Bianchi, L., and Maiorano, G., Acta, Unio Intern. Contra Cancrum **18**, 134 (1964).

16. Trainin, N., Linker-Israeli, M., Small, M., and Boiato-Chen, L., Intern. J. Cancer **2**, 326 (1967).

17. Pietra, G., Spencer, K., and Shubik, P., Nature **183**, 1689 (1959).

Received Oct. 14, 1968. P.S.E.B.M., 1969, Vol. 130.