Effect of Actinomycin D on White Blood Count and Humoral Antibody Response in Hamsters

Actinomycin D is known to interfere with protein synthesis[1,2] and to display a cytotoxic action on mitotic cells[3,4], qualities which have been used for the suppression of antibody production[5–9]. However, failure to suppress the immune response[10,11] or even stimulation[9,12] have been described. In the course of studies dealing with the alteration of the immune response in hamsters[13,14] it was therefore of interest to investigate the effect of actinomycin D on both white blood count and humoral antibody response.

Materials and methods. Male Syrian hamsters, bred in a closed colony, 10–13 weeks of age, were used; those injected with actinomycin D weighed 101 ± 6 g. They received 30 µg of Lyovac® = Cosmegen® (Merck, Sharp and Dohme) in a single dose, i.p. at the right groin on days 0 or −2. Each animal was injected on day 0 (i.e. 1 h or 2 days after drug administration), together with the controls, i.p. at the left groin with 1 ml of an either 1% or 50% suspension of sheep erythrocytes (corresponding to 2.10^8 and 1.10^{10} cells, respectively). Blood was drawn by retroorbital puncture under ether anesthesia on days 0, +3, +7, +14. Hemagglutinin titrations were performed in glass tubes, using 0.2 ml of a serially 1:2 diluted serum and 0.2 ml of a 1% suspension of sheep erythrocytes. A macroscopic agglutination pattern after 3 h at room temperature was considered as positive. Most of the sera were titrated simultaneously on their resistance against a 0.1 *M* solution of 2-mercaptoethanol. For blood morphology the animals received no antigen. Samples for white blood count were taken on days 0, +3, +7, always in the afternoon. Total and differential counts were made by conventional methods.

Results. 37 animals were treated with actinomycin D; 6 out of them died within the period observed of 14 days, i.e. 16.2%. The surviving ones lost 11.3 g on an average, but regained their original weight (Figure 1). No control animal died during the same period.

The total white blood count, as summarized in Table I and Figure 2, shows a depletion on day 3, followed by a moderate leucocytosis. A similar course is exhibited by the lymphocytes which are decreased on the fourth day (down to 986/mm³) but recover from suppression at the end of the week. The percentage of 40.4 then indicates no more lymphopenia, as absolute count reveals. The number of neutrophils rises without interruption, finally surpassing the original amount about 3 times.

Table II lists the results of hemagglutinin titration. The beginning of the hemagglutinin production takes at least 3 days; peak titers are reached in the second week of the experiment. No differences, neither in the time of actinomycin D administration nor in the dose of sheep erythrocytes, could modify both latent period and peak titer of the hemagglutinins. Sensibility to 2-mercaptoethanol has also been tested for each serum. The results indicate that the initial sensibility of the antibodies turns gradually to resistance, starting from the eighth day.

Discussion. On the one hand the cytotoxic effects of actinomycin D upon bone marrow, spleen, lymph nodes and thymus[15–17] can be made responsible for lymphopenia on the fourth day. At this time surviving lymphocytes may correspond to 'long-lived' types, on the

[1] E. REICH, R. M. FRANKLIN, A. J. SHATKIN and E. L. TATUM, Science *134*, 556 (1961).
[2] I. H. GOLDBERG and M. RABINOWITZ, Science *136*, 315 (1962).
[3] P. SCHOPFER and H. MOHR, Naturwissenschaften *53*, 231 (1966).
[4] P. R. GROSS and G. H. COUSINEAU, Expl Cell Res. *33*, 368 (1964).
[5] H. C. NATHAN, S. BIEBER, G. B. ELION and G. H. HITCHINGS, Proc. Soc. exp. Biol. Med. *107*, 796 (1961).
[6] C. J. WUST and M. G. HANNA, Proc. Soc. exp. Biol. Med. *118*, 1027 (1965).
[7] B. R. JENNINGS, J. inf. Dis. *117*, 116 (1967).
[8] D. M. ĆIRCOVIĆ and M. M. SIMIĆ, J. Immunol. *99*, 327 (1967).
[9] J. DOBBS, I. RIVERO, F. SABB and S. L. LEE, Immunology *14*, 213 (1968).
[10] B. D. GELLER and R. S. SPEIRS, Proc. Soc. exp. Biol. Med. *117*, 782 (1964).
[11] C. J. WUST, C. L. GALL and G. D. NOVELLI, Science *143*, 1041 (1964).
[12] H. N. CLAMAN and E. A. BRONSKY, J. Immunol. *95*, 718 (1965).
[13] S. LAZARY, Pathologia Microbiol. *31*, 65 (1968).
[14] M. GASSER and H. LOEFFLER, to be published.
[15] F. S. PHILIPS, H. S. SCHWARTZ, S. S. STERNBERG and C. T. TAN, Ann. N.Y. Acad. Sci. *89*, 348 (1960).
[16] B. D. GELLER and R. S. SPEIRS, Immunology *15*, 707 (1968).
[17] C. L. MADDOCK, G. J. D'ANGIO, S. FARBER and A. H. HANDLER, Ann. N.Y. Acad. Sci. *89*, 386 (1960).

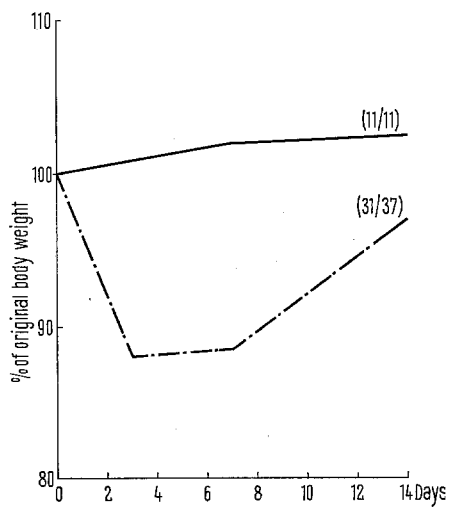

Fig. 1. Body weight of Syrian hamsters. Animals were weighed on days 0, 7, 14 (and 3 with treated animals). In parentheses: number of surviving animals / number of animals at start. –·–·–, with 30 µg actinomycin D treated animals; —, control animals.

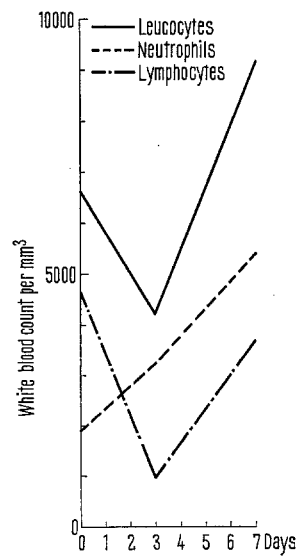

Fig. 2. Influence of Actinomycin D on white blood count. The values from Table I are plotted.

Table I. Influence of actinomycin D on white blood count

White blood count (cells/mm³)	Day of blood taking 0	3	7
Leucocytes	6639 (5550–8050)	4232 (2500–6850)	9136 (3200–16,950)
Neutrophils	1899 (950–3320)	3242 (2480–4060)	5418 (640–14,750)
%	28.6	76.6	59.3
Lymphocytes	4687 (3340–6040)	986 (630–2800)	3691 (792–5650)
%	70.6	23.3	40.4
Other leucocytes	53	4	27
%	0.8	0.1	0.3

Animals were injected on day 0 with 30 µg of actinomycin D. The mean values of 7 animals are plotted, extreme values in parentheses.

Table II. Influence of actinomycin D on hemagglutinin production

Treatment	Immunization with 1 ml of SRBC	Day of blood taking 0	3	7	14	No. of animals
30 µg actinomycin D on day 0	1 pc.	0	0	4.9	4.9	12
	50 pc.	0	0	4.6	5.5	10
30 µg actinomycin D on day −2	1 pc.	0	0	3.8	4.7	6
	50 pc.	0	0	5.6	4.8	5
Control animals	1 pc.	0	0	3.7	3.3	10
	50 pc.	0	0.7	5.0	3.8	6

Animals were immunized on day 0 with sheep red blood cells (SRBC). Hemagglutinin titers were transformed to ^{2}log of $^1/_{10}$ of reciprocal titer + 1. Mean values of these transformed titers are plotted.

number of which actinomycin D exerts no further effect[18]. On the other hand, the cytotoxicity mentioned cannot explain the increasing neutrophilia. PHILIPS et al.[15] have produced in rats, treated with actinomycin D, an enhancement of neutrophils which was highest on day 4; he suggests that enteric toxins would penetrate the damaged intestinal walls and lead to an endotoxin-like shock. The steady rise of the neutrophils in our experiments, however, does not account for this suggestion; nor does it support a reactive regeneration of the depressed bone marrow, as it was concluded from serial investigations of alkylating agents[19]. In mice GELLER and SPEIRS[16] found no bone marrow leucocytosis which normally appeared after antigen injection, under treatment with actinomycin D.

Actinomycin D acts in a more pronounced way upon early immunologic processes, RNA then being unstable and short-lived[20]. Consequently, hemagglutinins[6], hemolysins[8] and antiglobulins[11] appear later than normal. However, though actinomycin D was given at the same time as antigen, our experiments showed no depression of the antibody titers, according to findings of WUST et al.[11], GELLER and SPEIRS[10] and DOBBS et al.[9]. We therefore injected the antigen at the time of lymphatic depletion, but no lowering of antibody titer could be observed either. Since increasing amounts of antigen are known to augment the effect of an immunosuppressive drug[20,21], a further attempt was made by varying the dose of sheep erythrocytes, but results remained unchanged.

Unchanged antibody response in combination with lymphopenia – as it has been observed under the conditions of these experiments – suggests a resemblance with the reaction of adult animals to thymectomy.

Zusammenfassung. Actinomycin D führt bei erwachsenen männlichen Hamstern in einer Dosis, die für 15% der Tiere letal ist, zu einem vorübergehenden Abfall der Lymphozyten und zu einem kontinuierlichen Anstieg der neutrophilen Granulozyten. Die Bildung agglutinierender Antikörper gegen Schaferythrozyten wird dagegen, unabhängig von der Antigendosis und vom Zeitpunkt der Antigenapplikation, durch Actinomycin D nicht beeinflusst.

D. STÜRCHLER

Institut für Mikrobiologie und Hygiene der Universität Basel, 4000 Basel (Switzerland), 3 June 1969.

[18] J. J. MILLER and L. J. COLE, J. exp. Med. *126*, 109 (1967).
[19] L. A. ELSON, Ann. N.Y. Acad. Sci. *68*, 826 (1958).
[20] J. IVÀNYI, M. MALER, L. WUDL and E. SERCARZ, J. exp. Med. *127*, 1149 (1968).
[21] R. S. SCHWARTZ and W. DAMESHEK, J. Immunol. *90*, 703 (1963).

Fluorescent Studies of Antibodies to Rabbit Male Urogenital Tissue

Recent studies initiated in our laboratory employing the methods of tanned cell haemagglutination and gel diffusion precipitation have demonstrated that *multiple* in situ freezing of rabbit male urogenital tissue (coagulating gland and seminal vesicles) by means of a liquid nitrogen-cooled probe at intervals of 30 days elicits a more pronounced and consistent antibody response than that previously reported for a *single* freeze[1,2] analogously to the classical secondary or 'booster response'[3–6].

This report sets forth our initial observations employing the fluorescent antibody (FA) method for the possible histologic localization and identification of the antigen(s) involved in the immunologic response to freezing of rabbit male urogenital tissue.

Surgical methods and in situ freezing of male urogenital tissue of the rabbit by means of a liquid nitrogen-cooled

[1] R. V. JAGODZINSKI, C. YANTORNO and S. SHULMAN, J. Cryobiol. *3*, 456 (1967).
[2] C. YANTORNO, M. J. GONDER, W. A. SOANES and S. SHULMAN, Immunol. *12*, 395 (1967).
[3] W. A. SOANES, M. J. GONDER and R. J. ABLIN, 2nd Ann. Mtg. Soc. Cryosurg. (Miami, Florida 1969).
[4] R. J. ABLIN, 2nd Ann. Mtg. Soc. Cryosurg. (Miami, Florida 1969).
[5] W. A. SOANES, M. J. GONDER, R. J. ABLIN, M. D. MASER and R. V. JAGODZINSKI, J. Cryosurg. *2*, 23 (1969).
[6] R. J. ABLIN, E. WITEBSKY, W. A. SOANES, R. V. JAGODZINSKI and M. J. GONDER, J. Cryobiol., in press (1969).