Synergistic Immunodepressive Effect of 7,12-Dimethylbenz[*a*] Anthracene and Urethan in the Rat[1] (35420)

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The immunodepressive activity of many chemical carcinogens is well known and seems to be related to the oncogenic power, since non-oncogenic analogues are not immunodepressive (1–4).

7,12-Dimethylbenz(*a*)anthracene (DMBA) and urethan (UR) have been demonstrated to act as immunodepressive agents (5–8) even if some doubtful or negative results are also reported (9–11).

As far as we know no data are available on the synergistic effect of concurrently administered chemical carcinogens on the immune response though data exist on the synergism of action of two oncogenic agents (viral and non-viral) concerning oncogenic power (12–13). Therefore the present investigations were designed to examine not only the effect of a double treatment on the humoral antibody response to an antigen (human red blood cells) only indirectly correlated with the immune mechanism involved during tumor induction, but also on homograft reaction against a weak histocompatibility barrier, which is presumably connected with the carcinogenicity of the treatment.

Materials and Methods. Humoral antibody response. Random bred male adults *Wistar* rats, 3 months old at the beginning of the experiment were divided into groups of 10 animals each. Groups I–IV were injected intraperitoneally with DMBA (Sigma) in sterile olive oil at day 1 and intramuscularly with UR (Carlo Erba) in distilled water at days 3, 5, 7, 9, 11, 13. The complete scheme of the treatment is reported in Table I. Another control group (VII) included animals treated with olive oil alone, distilled water alone, or with olive oil and distilled water, since no differences were found within these treatments. At day 8, the rats received iv 1 ml/100 g of body weight of 2.5% suspension of human red blood cells A^+ (HRBC). The rats were weighed, individually bled, and sacrified at day 14 and spleen weight was recorded.

The sera of experimental groups, decomplemented for 30 min at 56°, were immediately tested at the same time as those from corresponding controls. Double dilution of serum (0.5 ml) was made in 0.9% NaCl. An equal volume of a suspension containing 3×10^7 HRBC was added to each tube and the mixture was incubated for 1.5 hr at 37°. The degree of agglutination was observed macroscopically, with gentle agitation, and verified microscopically to establish end points. Titers are expressed as $\log_2$ of the inverse of the maximum dilution showing an appreciable agglutination.

Skin grafting. The technique of skin grafting was essentially that described by Billingham (14). Inbred *F/344* (Ag B-1) and *Lew* (Ag B-1) male rats about 3 months old were divided in three groups of 10 animals each. Group VIII was treated as group II of Table I, but instead of receiving HRBC, the animals were skin grafted (*F/344* as donors and *Lew* strain as recipient) on day 8. Group IX was kept as untreated control and group X received oil and distilled water instead of DMBA and UR. The bandages were removed at day 14, and the grafts were inspected

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TABLE I. The Effect of Combined Doses of DMBA and UR on Hemagglutinin Titers (HT); Final Body Weight minus Initial Body Weight (FBW—IBW) and Final Body Weight/Spleen Weight (FBW/SW).

Group	No. of animals	Treatment[a] DMBA	UR	HT[b]	FBW—IBW (g)	FBW/SW
I	10	50	1	—	—	—
II	10	25	0.5	3.80 ± 0.51[de]	−9.8 ± 5.0[de]	256.37 ± 22.93[de]
III	10	10	0.2	4.70 ± 0.21[e]	+25.4 ± 5.5	165.55 ± 10.06
IV	10	5	0.1	5.70 ± 0.40[e]	+26.0 ± 4.2	179.11 ± 18.44
V	10	25	—	7.55 ± 0.24	+27.1 ± 3.7	168.16 ± 18.03
VI	10	—	0.5	7.40 ± 0.30	+8.3 ± 2.1[e]	227.76 ± 16.22[e]
VII[c]	30	Control		7.70 ± 0.30	+24.0 ± 3.3	178.45 ± 14.40

[a] The doses of DMBA are expressed as μg/g of body wt; UR as mg/g of body wt.

[b] Titers are expressed as $\log_2$ of the inverse of the maximum dilution showing an appreciable hemoagglutination.

[c] Include three control groups injected with (1) olive oil, (2) distilled water, (3) olive oil and distilled water.

[d] ± standard error.

[e] Significantly different from control group VII ($p < .01$).

every day. Mean survival time (MST)[2] is reported taking the endpoint for rejection as the absence of epidermis followed by the formation of an eschar.

Results. The results obtained with the double treatment on antibody formation are reported in Table I. The dose of 25 μg of DMBA/g of body weight, and 0.5 mg/g of body weight of UR, separately injected, did not influence the antibody response. In fact groups V and VI did not significantly differ from the control group VII. On the contrary the same doses concurrently given, depressed the hemoagglutinin titers. The depression was proportional to the dose used and it was also evident with the lower doses administered (groups III and IV). With the higher doses, the mortality rate was 6/10 animals (group I) so that the data obtained with the surviving rats are not reported in Table I.

The weight loss in group II shows that the treatment had some degree of toxicity in this case too. On the contrary there was marked immunodepression in groups III and IV, in which no weight loss was observed. The ratio body weight/spleen weight shows that the decreased spleen weight in group II has to be ascribed to the action of urethan (group VI).

The double treatment did not have any significant effect on MST[2] of *Lew* rats skin grafted with *F/344* (MST = 10.00 ± 0.29

[2] MST = mean survival time (days).

TABLE II. MST[a] of Skin Homografts in Rats Treated with Combined Doses of DMBA and UR.

Group	No. of animals	Treatment[b]	Donor	Host	MST (days)
VIII	10	DMBA + UR	F/344	Lew	10.00 ± 0.29[cd]
IX and X	19	Control	F/344	Lew	10.36 ± 0.48

[a] MST = mean survival time.

[b] Doses injected: DMBA, 25 μg/g of body wt at day 1; UR, 0.5 mg/g of body wt at days 3, 5, 7, 9, 11, 13.

[c] ± standard error.

[d] Non significantly different from controls.

days, group VIII) by comparison with the controls (groups IX and X), which had an MST that was very similar and therefore is shown in Table II as a single group (MST = 10.36 ± 0.48 days).

Discussion. Experimental evidence suggests that both DMBA and UR can act on bone marrow, thymus, and spleen cells (15–21). In view of the cooperative mechanism required for antibody production (22–24), the possibility can be suggested that this synergistic immunodepressive effect of DMBA and UR administered together is correlated with the combined impairment of the activity of bone marrow and thymus. The lack of demonstration of such a synergistic effect on homograft reaction in a strain combination in which it is possible to prolong the MST of skin graft with higher doses of carcinogens separately administered (25) seems to confirm that chemical carcinogens are much more active in depressing the antibody response than cell-mediated immunity. The fact that in our experimental conditions only the humoral antibody response is affected, casts some doubts on the attempts to correlate immunodepression with carcinogenicity.

Summary. Rats concurrently injected with DMBA and UR showed markedly depressed hemagglutinin titers toward HRBC with doses that have no effect when injected alone. The same double treatment with the higher dose failed to prolong the survival time of skin grafts against a weak histocompatibility barrier (*F/344* as donor and *Lew* as recipient).

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