which are more or less identical with those of the controls. As an example VMA (expressed as µg per mg creatinine) goes from 5.41 ± 0.52 in the controls to 12.42 ± 1.10 in the nitrate-treated group and to 14.31 ± 2.58 in the nitrite-treated group. Similarly, the level of HVA expressed in the same way goes from 0.38 ± 0.12 in the controls to 1.15 ± 0.22 and 1.08 ± 0.46 in the nitrate and nitrite-treated groups respectively.

Discussion. Weight variations observed in animals receiving sodium nitrate or nitrite have already been reported[9,24]; the technique used in the present investigation also shows that the presence of these substances in the diet causes a drop in food intake. Pankow et al.[25] and Csallany and Ayaz[26] suggested that the rat modulates its intake of nitrate/nitrite according to its capacity to resist methaemoglobinaemia through adaptation of the corresponding enzyme systems. The results which we obtained with the pair-fed and sodium-equivalent groups indicate that it is the decrease in food intake which is the cause of the reduced growth of the treated animals.

We also observed the well-known diuretic effect of nitrate with concomittant high liquid intake linked to Na^+ ion urinary excretion. Sodium nitrate and chloride are both osmotic diuretics and recent studies have shown that nitrate has no specific effects on filtration in the glomerule or reabsorption in the tubules[27,28]. In our experiment this is confirmed by the fact that a certain number of urinary parameters remain unaffected by nitrate treatment.

The catecholamine study shows that nitrates and nitrites clearly increase the urinary excretion of VMA and HVA. This is probably indicative of a modification of the metabolism of the amines in question. The modification could be related to various vasomotor effects. The variations observed suggest an increase in the metabolism of the amines - this would allow a relationship to be established with known experimental pharmacological effects such as rise in blood pressure in the dog[14] and the rat[15] or increase in the blood supply to various organs in the dog[29] the rabbit[16,17] and the rat[18]. These vasomotor effects already observed under the influence of nitrate or nitrite have sometimes been explained by an action on the muscle fiber[30,31] and also by displacement of the equilibrium of the fluid compartments of the organism. In any case these phenomena can be influenced to a greater or lesser extent by catecholamines. Our results therefore complement these previous observations and it is probable that the action of nitrates/nitrites on catecholamine metabolism (through a mechanism as yet to be elucidated), is one of the key points enabling an explanation to be given of the very numerous effects of these compounds in the various physiological functions mentioned above.

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Host macrophages are involved in systemic adoptive immunity against tumors[1]

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Summary. The positive systemic therapeutic results obtained with adoptive transfer of immune spleen cells could not be reproduced in macrophage depleted mice. Thus, host macrophages are involved in systemic adoptive immunity against tumors.

Immune spleen cells injected into tumor-bearing mice may have a systemic antitumor effect[2]. There are also many data suggesting that host macrophages exert an antitumor effect[3]. These data are consistent with the hypothesis that the systemic antitumor effect of injected immune spleen cells is due to co-operation of the injected lymphocytes with host macrophages. In this study this hypothesis was tested by elimination of macrophages from the tumor-bearing mice before the transfer of immune lymphocytes. The macrophages were eliminated with silica. I.p. silica treatment

results in a wide-spread destruction of macrophages and presumably in a partial destruction of body reserves of these cells[4]. Not only the number of macrophages in silica treated animals is reduced; macrophages that are recovered from animals 2 days after silica treatment also have a decreased ability to survive in vitro, a decreased spontaneous cytotoxicity and a decreased ability to be activated by sensitized lymphocytes[5].

Materials and methods. Animals and tumor cells. DBA/2 mice, 6–10 weeks old, were obtained from the Jackson Laboratory, Bar Harbour, Maine, USA. The SL2 lymphosarcoma was used. This tumor grows as a solid tumor s.c. in syngeneic DBA/2 mice.

Immune spleen cells. Mice were immunized with 2 i.p. injections of 10^7 irradiated (5000 rad) tumor cells with an interval of 10 days. 10 days later the mice received an i.p. injection of 5×10^6 unirradiated tumor cells. Immune spleen cells were collected 6 days after the last immunization. Spleen cell suspensions were prepared by pushing the spleen through a stainless steel sieve.

Depletion of macrophages. Mice were injected i.p. with 1 mg sterile silica (Sigma Chemical Company, USA; particle size 0.012 µm) suspended in 1 ml Fischer's medium over a period of 5 days (total amount of silica 5 mg). About 20 days after the last injection these mice can be regarded as macrophage depleted, as we have described elsewhere[5].

Adoptive transfer. Figure 1 shows the schedule of the experiment. Mice were i.p. injected with 1 mg silica on days 1, 2, 3, 4, and 5. To exclude differences in lymphocyte populations in control and test mice all mice were subjected at day 20 to 850 rad whole body irradiation followed 2 h later by i.v. reconstitution with 5×10^6 nucleated bone marrow cells. On day 22 the mice were inoculated s.c. at the right buttock with 10^5 lymphosarcoma cells. On days 25, 26, 27, 28 and 29 the mice were injected i.p. with 10^8 immune spleen cells.

Results. Figure 2 shows that immune spleen cells injected i.p. into mice with a s.c. growing SL2 lymphosarcoma caused prolongation of the survival time. 30% of the tumor bearing mice treated with immune spleen cells survived tumor free; normal nonimmune spleen cells did not cause any prolongation of the survival ($p < 0.05$). However, in macrophage-depleted mice immune spleen cells could not produce prolongation of the survival time ($p > 0.05$).

Discussion. Elimination of host macrophages by silica treatment abrogated the systemic antitumor effect of adoptively transferred immune spleen cells. So, host macrophages cooperate with the injected immune spleen cells causing a systemic effect. There are various possibilities to explain this co-operation. Macrophages can process and present tumor antigen to the injected immune lymphocytes[6], they can increase the number of Ia molecules on T-lymphocytes[7] or produce lymphocyte activation factors or thymocyte activating factors[8]. The injected lymphocytes may activate host macrophages to cytotoxic effector cells.

This result extends the data of Palladino and Thorbecke[9], who have shown co-operation of host macrophages with adoptively transferred spleen cells in a local system, and also extends our previous data[10] on in vivo co-operation of injected immune spleen cells and injected macrophages in a local and in a systemic system. The latter experiments provide evidence that transferred macrophages and immune spleen cells can interact in normal mice, suggesting that the co-operation of host macrophages with transferred spleen cells in the present experiments is not due to an artefact, resulting from irradiation and bone marrow reconstitution of the host. This is also in line with the fact that normal SL2 bearing mice respond just as well to adoptively transferred immune spleen cells as irradiated, bone marrow reconstituted SL2 bearing mice (data not shown).

We have used this treatment to avoid the side effect of silica on lymphocytes[5,11]. We have irradiated silica treated animals as well as control animals; this was followed by bone marrow reconstitution. This caused a dramatic decrease of the number of lymphoid cells. It is unlikely that some important lymphocyte population was left intact as in a comparable system tumor protective lymphocytes could not be detected after 1000 rad whole body irradiation and tumor protective activity was reduced after 150 or 350 rad whole body irradiation[12].

Residual silica might influence lymphocytes in the irradiated, reconstituted host. However, after i.p. injection of 10^8 spleen cells the number of lymphoid cells in the peritoneal cavity was similar during the following 6 days in silica treated and nontreated mice (data not shown).

A 3 days' growth of SL2 tumor cells may be able to prime the host in a normal situation. In an intact mouse adoptively transferred immune spleen cells may interact with

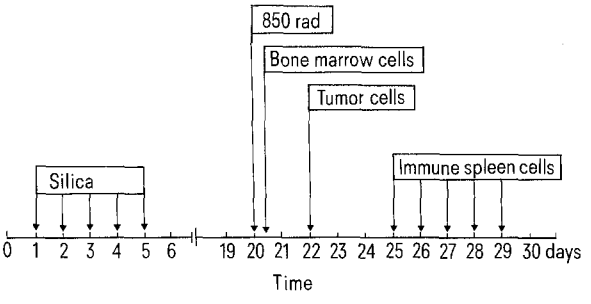

Figure 1. Model to test the involvement of host macrophages in an adoptive immunotherapy.

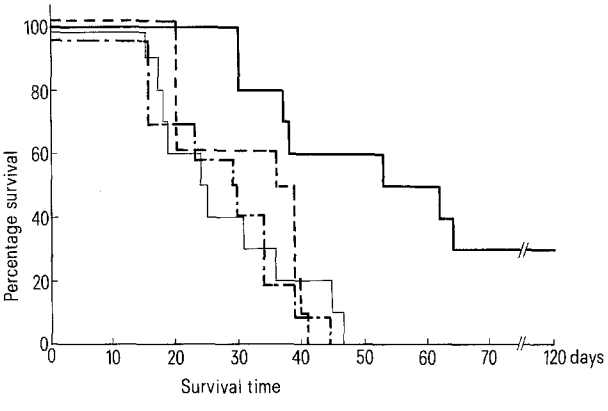

Figure 2. Effect of macrophage depletion in tumor bearing mice on the therapeutic effect of injected immune spleen cells. DBA/2 mice were injected i.p. with 1 mg silica on day 1–5. Both control mice and silica treated mice were irradiated with 850 rad whole body irradiation on day 20, 2 h later they were injected i.v. with 5×10^6 nucleated bone marrow cells. At day 22 10^5 SL2 cells were inoculated s.c. and at day 25–29 10^8 immune or normal DBA/2 spleen cells were injected ip daily. Immunization schedule: 2 i.p. injections of 10^7 irradiated (5000 rad) SL2 cells with an interval of 10 days and 1 i.p. injection with 5×10^6 unirradiated SL2 cells 10 days later. Spleen cells were collected 6 days later after the last injection. 10 animals were used per group. —— Mice were irradiated, injected with bone marrow cells, and treated with immune spleen cells; -.-.- mice were irradiated, injected with bone marrow cells, and treated with normal spleen cells; --- mice were treated with silica, irradiated, injected with bone marrow cells, and treated with immune spleen cells; —— mice were treated with silica, irradiated, injected with bone marrow cells, and treated with normal spleen cells.

primed host cells. However, in the system that is described in this paper, the hosts had a paralyzed immune system, as a result of irradiation. In these animals priming can hardly be expected. Moreover, priming of host cells is not a prerequisite as immune spleen cells, injected simultaneously with tumor cells in normal mice, are able to exert an in vivo antitumor effect[10].

Natural killer cells among the spleen cells may be responsible for the in vivo antitumor effect; these cells can be affected by silica[13]. This option is, however, unlikely, as a substantial antitumor activity of the immune spleen cells remains after in vitro culture during 24 h (to be published), whereas natural killer cells lose their activity within 4 h of culture[14].

Macrophages among the spleen cells do not seem to cause the lengthening of survival time, as elimination of macrophages did not abrogate the effect of the immune spleen cells[10,15]. This shows that the injected syngeneic spleen macrophages cannot replace host macrophages. This could be due to the inability of i.p. injected spleen macrophages to invade the tumor. Another explanation could be that spleen macrophages are not the relevant macrophages. An example of such a difference in relevance is that irritant-induced peritoneal macrophages could give a local antitumor effect in co-operation with sensitized lymphocytes, in contrast to resident macrophages[10].

Macrophages among the injected bone marrow cells can differentiate into mature macrophages, but it is clear that the number of macrophages is smaller in silica treated mice than in control mice. We conclude that host macrophages are involved in the antitumor effect of transferred immune spleen cells.

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Effect of indole-3-acetic acid on the histology of gonads and their development in *Dacus dorsalis* Hendel

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Summary. The paper deals with the effects of indole-3 acetic acid on the histology of the gonads and their development in *Dacus dorsalis* Hendel (Diptera; Tephritidae). Flies were topically treated with solutions of different concentrations. This treatment effectively reduced the size of gonads when applied to newly-emerged flies. Most severely affected parts were the germinal regions of the gonads. The number of sperm bundles was much reduced in the treated testes as compared to controlled ones.

The possibility of using chemicals for sterilizing an insect population has interested every individual learning of the effects of chemosterilants on the reproductive organs. *Dacus dorsalis* Hendel was taken as an experimental insect for the present investigations, since it is a serious pest of most of the fruits and vegetables throughout India. In our present experiment, we tested indole-3-acetic acid as a chemosterilant as an alternative to tepa, metepa, apholate, and tretamine, which have been used to sterilize fruit flies such as the oriental fruit fly, *D. dorsalis,* the melon fruit fly, *Dacus cucurbitae* Cocquillett and the Mediterranean fruit fly, *Ceratitis capitata* Wiedemann[2]. Indole-3-acetic acid has been added to the list of chemosterilants as a compound affecting reproduction in insects[3]. Since then, according to available evidence, no work has been done on the effects of this chemical on the histology of the gonads and their development.

Materials and methods. Laboratory-reared 0-24-h-old adults of either sex of *D. dorsalis* were anesthetized by keeping them in a freezer for 2-5 min and then treated topically with 1 µl of solutions of different concentrations of indole-3-acetic acid in acetone, with the aid of a micrometer controlled calibrated syringe. The chilled flies were easy to handle and mortality was negligible. The concentrations tested were 0.3, 0.5, 2 and 5%. For control purposes, flies were treated with the same amount of acetone alone. 6 replicates of 50 flies each per concentration were studied. Following the treatment on alternative days, 2-5 flies from each replicate were dissected in physiological saline solution. Their reproductive organs were taken out for histopathological study. Testes and ovaries were measured before being permanently mounted, using a monocular microscope equipped with a vernier scale. Measurements included greatest length, width and length of the anterior curved portion, and diameters of ovaries. The average sizes of testes and ovaries was calculated and tabulated (table 1). For histopathological studies, ovaries and testes were fixed in Bouin's fluid (water solution) for 12-24 h. Several sections were taken for histopathological study. Sections were stained in haematoxylin and eosin and examined by a research microscope.

Results. The data obtained showed the effect of indole-3-acetic acid on the size of gonads. Results were statistically analyzed by applying F and Student's t-tests at 5% and 1% levels of significance (tables 1 and 2). It was observed that until 4-6 days after treatment there were no measureable effects on the size of gonads, but thereafter up to maturity a statistically significant reduction in size was observed.