A temporary and mild protective action was only observed after challenging with whole tumor cells carrying polyoma virus. The slight protection decreased progressively as a function of the time, and after 45 days the percentage of tumor takes was essentially the same for both the animal groups.

In another series of investigations it was determined if the tumor was able to produce an appreciable immunological response in isogeneic hosts. 3 different types of experiments were carried out: 1) 4×10^5 trypsinized tumor cells were inoculated into adult C3H/He mice at the dorsal surface of the cartilage plate toward the distal edge of the ear or the extremity of the tail. As soon as the tumor reached an adequate development and before it could cause infiltration into neighbouring tissues, a radical excission was carried out and a challenge was made with isogeneic cells. 2) Adult C3H/He mice were inoculated with homogenized tumor cells and 30 days after a challenge with whole cells was carried out. 3) Adult C3HeB/FeJ mice, born from foster-nursing C3H/He suckled by C57BL/6 and therefore 'theoretically' free from Bittner virus, were incoulated with homogenized cells and 30 days after a challenge with whole tumor cells was carried out.

As shown by data of Table III, positive takes were obtained in all cases, thus indicating that it is impossible to induce any protection in the isogeneic host, at least with the methods employed.

In conclusion, from the data collected until now and briefly described here, 2 points must be emphasized. Firstly, the absence of virus particles and the specific cytopathic effect clearly indicates that mouse salivary gland tumor does not carry polyoma or polyoma-like viruses. In addition, when polyoma virus is artificially introduced into the system a slight decrease of tumor takes was obtained, further confirming that the etiology of this tumor cannot be restricted to polyoma virus. Secondly, it seems apparent that during its development the salivary gland tumor is unable to induce an appreciable immunological response.

Table III. Evaluation of immunological response in relation to the growth of the salivary gland adenocarcinoma in isogeneic hosts

No. of animals	Sites of inoculation	Growth	Days of challenge	Tumor takes 30 days after the challenge
23 C3H/He	Ear cartilage	100%	12[a]	100%
20 C3H/He	Tail	100%	12[a]	100%
30 C3H/He	Neck, s.c.	–	30[b]	100%
23 C3HeB/FeJ	Neck, s.c.	–	30[b]	100%

[a] From the removal of primary tumor. [b] From the inoculation of homogenized cells.

Riassunto. Dai dati riferiti si mettono in evidenza due punti: il primo dimostra nel tumore in esame l'assenza di particelle virali e di effetto citopatico specifico per il virus polioma. Inoltre non si mettono in evidenza anticorpi fissanti il complemento o emoagglutinoinbenti per il virus polioma negli animali portatori di tumori. Il secondo punto dimostra che durante il suo sviluppo il tumore della ghiandola salivare é incapace di indurre una risposta immunologica apprezzabile.

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Histone-Induced Macrophage Disappearance Reaction in Normal Guinea-Pigs

The complex state of delayed type hypersensitivity is represented by different types of specific reactions: The macrophage disappearance reaction (MDR)[1-6,13] and the release from cells of the lymph node permeability factor (LNPF)[7,8] should be cited as examples. A complete functional identity of histones from different sources and LNPF has been demonstrated previously[9,10]. It was of interest to examine whether or not a MDR could also be induced when inoculating normal guinea-pigs with calf thymus histone, instead of treating immunized animals (which exhibit delayed-type hypersensitivity) with the corresponding antigen.

Total histone from calf thymus nuclei was extracted with sulphuric acid (pH 0.7)[11,12]. The preparation was dissolved in saline, shifting the pH value to 7.0–7.2 and was injected s.c. or i.p. into either normal or BCG-sensitized guinea-pigs (Pirbright, 400–600 g). Histone solutions were sterilized by filtration prior to use. Peritoneal exudate was induced by glycogen (Schuchardt). 4 days later the peritoneal cells were harvested and the number of free floating macrophages in the peritoneal exudates was assayed by total and differential cell counts. All these methods, i.e. induction and harvesting of peritoneal exudates as well as differentiating macrophages, were accomplished in exactly the same manner as described by Nelson et al.[2,13].

Pilot experiments indicated that the average value of the total cell count showed much day-to-day variation in normal animals, as previously described by others[2,6]. However, a reliable evaluation of the disappearance of free floating macrophages was achieved when using the relative values of macrophages in differential cell counts only. Guinea-pigs were immunized s.c. by injecting 3 mg of BCG in 0.5 ml saline per animal. 3 or 4 weeks later, the animals were subjected to further experiments. A purified protein derivative of tubercle bacilli (GT, Hoechst) was used as tuberculin.

Intraperitoneal injection of tuberculin (GT) into BCG-sensitized guinea-pigs 2 h before cell harvesting resulted in quick disappearance of most of the free floating macrophages. With non-immunized animals this was not observed. These results are in agreement with the observations of Nelson et al.[1-3,13]. When histone was injected by this route, a marked disappearance of macrophages was observed, even with a dose of only 50 ng (see Table I).

Within 2 h after s.c. injection of a higher dose of 4 mg histone-N per animal, disappearance of macrophages in the peritoneal exudate was not observed, but a marked

decrease was recorded 4 h after histone application. 4 h later, macrophage values were increasing again (Table II).

The induction of MDR by tuberculin in immunized animals can be suppressed by treating the animals with histone on the day of immunization (Table III).

The results of the experiments described above indicate that the free floating macrophages in peritoneal exudates not only disappear after the injection of the corresponding antigen into immunized guinea-pigs with delayed hypersensitivity but also after i.p. or s.c. injection of histone in normal animals. Moreover, histone exhibits the same activities as LNPF with respect to the functional criteria of the latter, and these results lend support to the hypothesis that histone may be a mediator of delayed-type hypersensitivity reactions.

NELSON and NORTH[13] thought that the effect of antigen on peritoneal exudate macrophages in vivo was to render the cells more 'adhesive' so that they stuck to each other and to the lining of the peritoneal cavity. In agreement with these results, we observed in additional experiments in vitro clumping of peritoneal exudate cells following addition of histone.

Total cell content of peritoneal exudate did not decrease significantly after i.p. injection of 1 mg histone-N in 1 ml saline. However, using a dose of 4 mg histone-N in 2 ml saline no cells at all could be observed in the exudate up to 4 h later. This is supposed to be due to toxic effect of histone at this concentration, corresponding to a similar effect of histone on exudate cells in vitro. After s.c. injection of the same dose of 4 mg histone-N in 2 ml saline total cell content of peritoneal exudate remained within normal limits, only the macrophage content decreased.

Histone was well supported by the animals using the doses described. No general signs of toxicity could be observed. Even i.v. injections of 0.4 mg histone-N in 0.4 ml saline (but not 0.5 mg) were tolerated by mice of different ligns weighing 30 ± 2 g. Repeated s.c. injections of histone were equally well supported injecting a total dose of 5 mg histone-N into mice within 10 weeks or up to 15 mg histone-N into guinea-pigs within 20 days, as demonstrated in several other experiments.

The suppression of MDR in BCG-sensitized guinea-pigs by histone treatment at the time of immunization is consistent with the immunosuppressive effect of histone previously described, using transplantation immunity, tuberculin hypersensitivity and humoral antibody formation as experimental models[14–16].

Table I. Changes in the relative macrophage values of glycogen-induced peritoneal exudates by i.p. injected tuberculin (GT) and histone 2 h before cell harvest

Sensitization	Material injected	No. of animals	Relative macrophage values (mean ± standard error)
Normal	Saline	7	43.1 ± 3.2[b]
BCG	Saline	7	38.8 ± 11.2[b]
Normal	GT 50 ng	8	44.4 ± 8.0[b]
BCG	GT 50 ng	6	11.7 ± 9.4[a]
Normal	Histone-N 1000 ng	7	2.9 ± 1.5[a]
Normal	Histone-N 200 ng	8	14.5 ± 8.4[a]
Normal	Histone-N 50 ng	8	3.1 ± 2.8[a]

Statistical significance of differences between macrophage values of 'histone-treated-normal-animals' and 'GT-treated BCG-immunized-animals' respectively[a] and the animals of the other groups[b]: $P < 0.0005$.

Table II. Disappearance of macrophages in glycogen-induced peritoneal exudates of normal guinea-pigs after s.c. injection of 4 mg histone-N

Hours between injection of histone and cell harvesting	No. of animals (duplicate)	Relative macrophage values (mean ± standard error) (duplicate)
2	8	62.2 ± 18.6
4	8	8.5 ± 5.0
5	8	5.8 ± 2.6
	(8)	(5.2 ± 4.7)
8	5	15.6 ± 1.9
	(7)	(22.4 ± 17.1)

Table III. Histone treatment during BCG immunization. Influence on macrophage disappearance reaction by tuberculin (GT)

Immunization	Additional treatment[a]	GT[b]	No. of animals (duplicate)	Relative macrophage values, mean ± standard error (duplicate)
None	Histone-N 4 mg	50 ng	5	41.6 ± 8.6
BCG	Histone-N 4 mg	50 ng	10	45.0 ± 9.9
			(6)	(41.9 ± 4.6)
BCG	None	50 ng	6	11.7 ± 9.4

[a] Beginning on the day of immunization the animals were treated with 8 injections of 1 mg histone-N each at intervals of half a week. 4 weeks after immunization the peritoneal exudate was induced by glycogen. [b] GT was injected i.p. 2 h before cell harvesting.

Zusammenfassung. Histon aus Kalbsthymus bewirkt nach i.p. oder s.c. Injektion bei nichtimmunisierten Meerschweinchen in gleicher Weise eine ausgeprägte Abnahme von Makrophagen in einem mit Glykogen induzierten Peritonealexsudat wie eine Injektion von Antigen bei spätallergischen Tieren. Eine Histonbehandlung während einer Immunisierung mit BCG unterdrückt die sonst mit Tuberkulin auslösbare sogenannte «macrophage disappearance reaction».

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