Influence of Compound 48/80 on Tumors Transplanted to the Hamster Cheek Pouch.* (27694)

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The injection of mouse Ehrlich ascites tumor cells into the cheek pouch of the Syrian hamster usually results in substantial growth of the implant during the first 3 to 6 days following injection, followed by a gradual resorption of the implant. Presumably the growth phase of the implant ceases as the hamsters' natural defenses against the foreign tissue become effective. The present report concerns evidence which indicates that Compound 48/80† increases the rate at which the tumor is rejected by the hamster.

Methods. Adult female hamsters weighing 80-100 g were injected intraperitoneally each day with 60 μg of Compound 48/80 dissolved in 0.1 ml of Hanks' solution(1). Control hamsters received daily, intraperitoneal injections of 0.1 ml of Hanks' solution. On the fourth day these 48/80 treated hamsters and an equal number of controls were anesthetized and 0.1 ml of a suspension of mouse Ehrlich ascites tumor cells was inoculated into the tissue of the cheek pouch. The cell suspension was prepared from the peritoneal fluid of a white Swiss mouse which had been injected 9 days earlier with Ehrlich ascites tumor cells. After removal from the mouse, the cells were washed twice by successive centrifugation and resuspension in 12 volumes of Hanks' solution, and diluted with Hanks' solution so that the final cell concentration was between 4000 and 6000 cells/mm^3. The hamsters in the treated group were inoculated daily with 60 μg of Compound 48/80 throughout the experiment. At 3-day intervals the hamsters were anesthetized and the tumors measured in 3 dimensions with calipers. The approximate volume of the tumor was calculated from the formula

$$\text{Volume} = \frac{\text{length} \times \text{width} \times \text{height}}{2}.$$

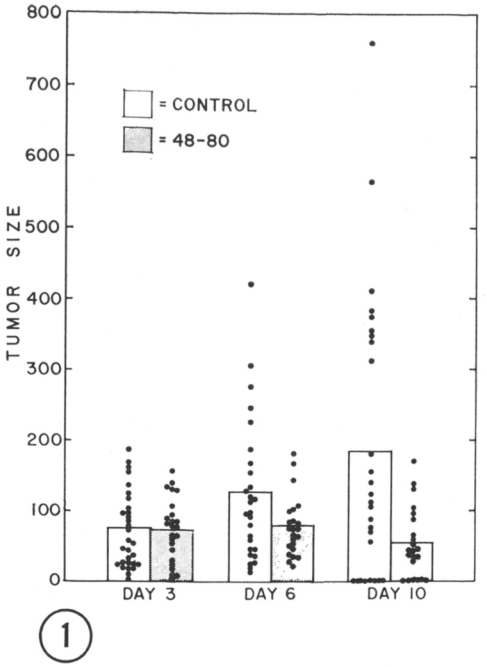
Results. Fig. 1 contains the combined results of 4 experiments, each of which included 6 treated hamsters and 6 controls. Three days after inoculation with the tumor, the control and treated hamsters showed little difference in the average size of their tumors. However, by day 6, enough of the tumor implants had increased in size to raise the average substantially in the controls, while the average tumor size in the 48/80 treated hamsters changed very little. A number of hamsters in both groups had completely resorbed their tumors by day 10, but some hamsters in the untreated group had much larger tumors than at day 6, while no increase in tumor size occurred in the 48/80 treated group. Thus, at each successive measurement the average tumor size was larger in the control group, but was the same or smaller in the 48/80 treated group. The increase in average tumor size in the control group, however, is primarily a result of increases in about ⅓ of the hamsters involved. Thus, the effect of 48/80 seems to be to prevent the tumors in this relatively small portion of hamsters from continuing their growth after day 3.

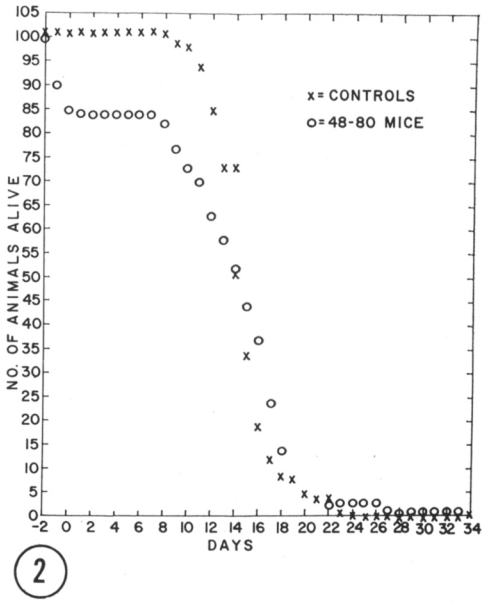
Two statistical procedures were carried out on the data in Fig. 1.‡ A rank correlation test, comparing tumor size in the 48/80 treated hamsters and the controls, revealed no significant difference at day 3, p = less than 0.02 at day 6, and p = less than 0.01 at day 10. The results of an analysis of variance are shown in Table I. When all measurements made, regardless of day, are included, there is a statistically significant difference in tumor size in the 48/80 treated hamsters and

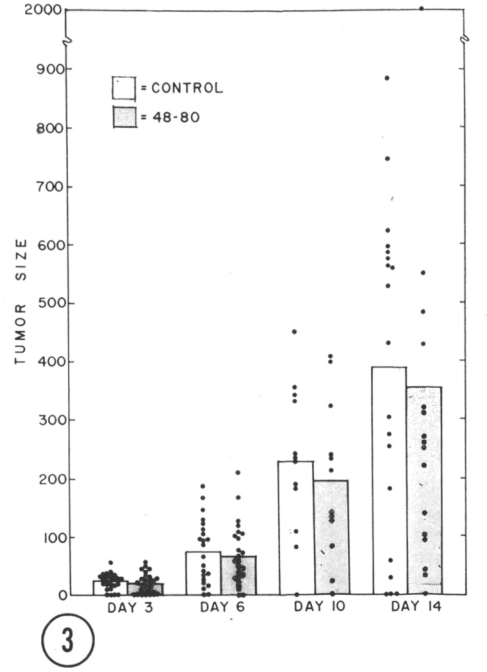
* Work supported in part by grants from New York Foundation and from Nat. Inst. of Allergy and Infect. Dis., U.S.P.H.S.

† Compound 48/80, a histamine release agent, is a condensation product of p-methoxy-phenylethyl-methylamine and formaldehyde. The authors wish to thank Burroughs Wellcome and Co., Tuckahoe, N. Y., for supplying the Compound 48/80.

‡ The authors wish to thank Dr. Albert Pawlowski, Worcester Foundation for Experimental Biology, for advice on statistical procedures.







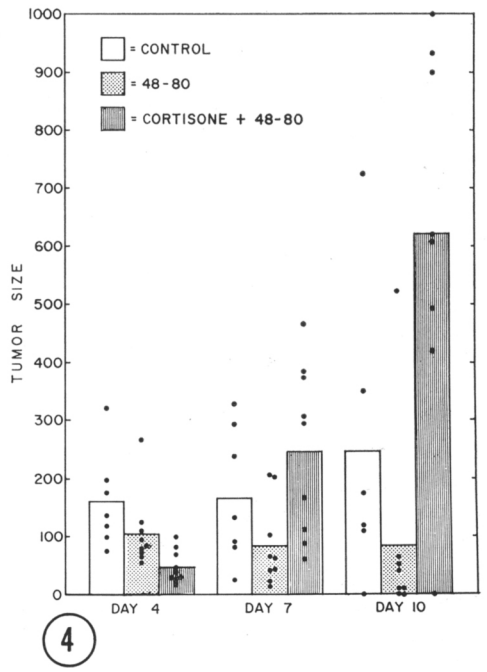


FIG. 1. Effect of Compound 48/80 on growth of mouse ascites tumor cells in hamster cheek pouch. In the control hamsters avg tumor size is larger at each successive measurement, while with 48/80 treatment avg tumor size is the same or smaller at each successive measurement.

FIG. 2. Effect of Compound 48/80 on survival of mice inoculated with mouse ascites tumor cells. Compound 48/80 apparently has no anti-tumor effect.

FIG. 3. Effect of Compound 48/80 on growth of hamster ascites tumor cells in hamster cheek pouch. Compound 48/80 apparently has no effect on these hamster cells.

FIG. 4. Comparison of effects of 48/80 alone and 48/80 with cortisone on growth of mouse ascites tumor cells in hamster cheek pouch. Inhibition of growth caused by 48/80 disappears in presence of cortisone.

TABLE I. Analysis of Variance.

	d.f.	m.s.	F	P
Between groups	1	174	11.89	.01
Error	32	14		
Dates x groups	2	47.42	6.32	.01
Within error	64	7.50		

the controls (between groups). Also, the rate of growth of tumors in the 48/80 treated hamsters was different from that of the controls (Fig. 1). That this difference is statistically significant is indicated in Table I by the value for p of less than 0.01 when tumor size in the 48/80 treated hamsters and in the controls is compared at the 3 different days of measurement (days *vs.* groups).

The above experiments suggested that Compound 48/80 might be directly inhibiting the growth of the mouse ascites tumor cells in the hamster. We therefore tested the effect of Compound 48/80 on survival of mice which were injected with the same strain of Ehrlich ascites tumor cells which had been used in the hamsters. In these experiments a series of female white Swiss mice was injected intraperitoneally with Compound 48/80 according to a schedule of 2 injections per day, totalling 100 μg on the first day, and 200 μg on the second and third days of the experiment. On the fourth day aliquots of mouse Ehrlich ascites tumor cells were injected intraperitoneally into the treated mice and into an equal number of controls. For the duration of the experiment the treated mice were injected with 100 μg of 48/80 once each day. Survival of the 48/80 treated mice was compared with survival of the controls. Five experiments, each including 20 48/80 treated mice and 20 controls, were performed. Several mice receiving Compound 48/80 died before they were inoculated with the tumor (Fig. 2). However, for the remainder of the experiment there appears to be no difference in survival between 48/80 treated mice and control mice.

A further experiment was performed using Wistar strain rats injected with Yoshida ascites tumor cells. Twenty 75 g female rats were injected with Compound 48/80 twice daily, starting at 100 μg per injection, and increasing the dosage by 100 μg each day until 500 μg per injection was reached on the fifth day. After the fifth day each rat received single daily injection of 500 μg with increments of 100 μg every other day to reach 1 mg/day and were continued at this level until the end of experiment. Aliquots of a tumor suspension were injected into the treated rats and into 20 controls on the 4th day after beginning the 48/80 injections. There was no indication of increased survival time among the 48/80 treated rats.

The experiments so far described indicate that mouse Ehrlich ascites tumor cells are sensitive to 48/80 in the hamster, but not in the mouse. Further, rat Yoshida ascites tumor cells were not sensitive to 48/80 in the rat. A further experiment tested hamster ascites tumor cells in the cheek pouch of the hamster. The ascites tumor was derived from a methylcholanthrene induced spindle cell sarcoma. Twelve hamsters were treated with 48/80 according to the schedule described previously, and, along with 12 controls, were inoculated in the cheek pouch with aliquots of a suspension of hamster ascites tumor cells. The results shown in Fig. 3 indicate that Compound 48/80 had little effect on growth of hamster ascites tumor cells in the hamster.

A final experiment was performed to determine the effect of 48/80 on growth of mouse ascites tumor cells in the cheek pouch of hamsters treated with cortisone. One group of hamsters was treated with 48/80 according to the schedule described previously. A second group of hamsters received the same treatment with 48/80, but each hamster also received a subcutaneous injection of 6 mg of cortisone acetate twice each week. A third group of hamsters served as controls. All of the hamsters were inoculated in the cheek pouch with mouse ascites tumor cells. The results shown in Fig. 4 reveal a large difference between the tumors in the hamsters which received 48/80 alone and those which received 48/80 and cortisone. It is clear that 48/80 is ineffective in inhibiting the growth of mouse ascites tumor cells when the hamster is treated with cortisone.

Discussion. When mouse ascites tumor cells are injected into the cheek pouch of the hamster, measurements made at the 10th day

reveal a larger implant than measurements made on the 6th day in about ⅓ of the cases. Such growth does not occur when the hamsters are treated with Compound 48/80. The present evidence indicates that 48/80 has no effect either on mouse ascites tumor cells in the mouse or on hamster ascites tumor cells in the hamster cheek pouch. The evidence therefore does not support the explanation that the growth of tumor cells in the hamster cheek pouch is directly inhibited by 48/80. A more likely explanation appears to be that 48/80 enhances the ability of the hamster to reject a foreign tissue in its cheek pouch.

The increased growth of ascites tumor cells in the cheek pouch of hamsters treated with cortisone presumably results from an inhibition of antibody production by the hamster against the tumor cells(2). The fact that 48/80 does not affect inhibition of antibody production resulting from cortisone treatment suggests that 48/80 has a mode of action not directly associated with antibody production. Billingham *et al.*(3) has associated the persistence of homografts in the hamster cheek pouch with the occurrence in the cheek pouch of certain cells which prevent the arousal of the immunological defenses of the hamster.

The following interpretation, while speculative, seems consistent with the data. In the hamster cheek pouch a heterograft is surrounded by specific cells which render the heterograft less antigenic. These specific cells are sensitive to 48/80, so that treatment of the hamster with 48/80 results in prompt sensitization of the hamster, and rejection of the heterograft. Compound 48/80 is ineffective in cortisone-treated hamsters because, even though 48/80 may allow the heterograft to sensitize the hamster, the hamster's ability to respond to sensitization with antibody production is inhibited by cortisone.

Summary. Implants of mouse ascites tumor cells in cheek pouches of control hamsters showed progressive growth during the first 10 days in about ⅓ of the hamsters. In 48/80 treated hamsters, however, progressive growth of the implant did not occur. Compound 48/80 did not prolong the lives of mice inoculated intraperitoneally with mouse ascites tumor cells, nor of rats inoculated with Yoshida ascites tumor cells. Compound 48/80 did not inhibit the growth of hamster ascites tumor cells in the hamster cheek pouch, nor of mouse ascites tumor cells in the cheek pouches of cortisone treated hamsters. It is suggested that Compound 48/80 alters the cells of the hamster cheek pouch so that the hamster is more rapidly sensitized to foreign cells.

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Received April 18, 1962. P.S.E.B.M., 1962, v111.

Electrophoretic Determination of C-Reactive Protein Which Appears Following Surgery.* (27695)

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The appearance of C-reactive protein (CRP) in serum represents a non-specific response to various types of physiological stress (1). Its absence from normal blood and its peculiar property of precipitating with the C (somatic) polysaccharide of pneumococcus raise the question of its immunological role. In this connection it would be useful to know

* The authors wish to express their appreciation for the cooperation of the surgical staff of Charity Hospital, New Orleans, La. and Dr. Oscar Creech, Dept. of Surgery, Tulane Medical School.