given 1/2 h prior to antigen i.p.) and then primed with a sub-optimal dose of antigen (2×10^6 SRBC i.p.) or primed with a sub-optimal dose of antigen (2×10^6 SRBC i.p.)alone. The mice were killed 21 days later and the spleens of each group pooled separately. Graded numbers of spleen cells, derived either from polyanion-injected and antigen-primed, or from antigen-primed animals, were injected i.v. together with an optimal dose of antigen (4×10^8 SRBC) into irradiated, syngeneic 9-11-week-old recipients. The recipient mice had been irradiated with 600 r (80 r/min) and injected, within less than 4 h after irradiatin, with a mixture of spleen cells and SRBC. Seven days later the number of direct (19 S) and indirect (7 S) plaque-forming cells (PFC) in the spleen, as well as the total and 2-mercapto-ethanol-resistent hemolytic titers in the serum of the recipient mice, were assayed as describetd earlier[2]. For statistical analysis the Wicoxontest was used[5]. The effects were considered significant when $p < 0.05$.

As shown in the Figures 1 and 2, irradiated recipients injected with adequate numbers of spleen cells derived from DS-treated and antigen-primed animals gave a significantly higher ImR to SRBC than recipients injected with the same number of spleen cells derived from animals primed with SRBC alone. Moreover, a typical secondary response (predominantly 7 S antibody formation) could be obtained only in recipients injected with an adequate number of spleen cells derived from DS-treated and antigenprimed mice. Similar results (data not given here) were obtained using spleen cells derived from donors 10 or 42 days after antigen priming or when polyacrylic acid was used instead of DS. It is generally accepted that cooperation of thymus-dependent antigen-carrying lymphocytes (T-cells) and bone marrow-derived lymphocytes (B-cells) is required for primary and for secondary humoral response to SRBC. It has also been shown that memory cells are present in both T-cell and B-cell populations. (for review see[6]). In the present report it has been demonstrated that polyanions enhance the number of memory cells, and it must be assumed that both T- and B-cell memory is enhanced by polyanions, since a profond 'T-cell'-dependent 7 S memory seems to be present only in polyanion-treated mice. If this assumption is correct, polyanions must act on both T- and B-cell populations. Indeed we reported previously that polyanions in general are mitogenic for spleen cells[7]. Moreover it has recently been shown that polyanions activate DNA-Synthesis in B-cells[8] and, to a lesser degree in T-cells[9] in vitro. It seems possible that polyanions act on ImR by increasing the rate of proliferation of immunocompetent B- and T-cells.

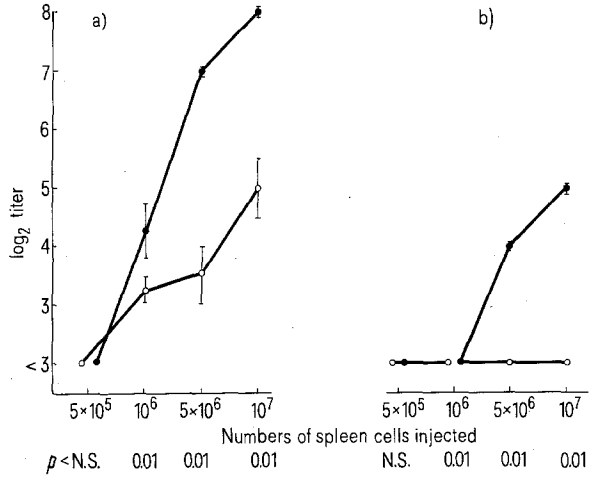

Fig. 2. Reciprocal hemolytic anti-SBRC-titers in irradiated C 57 B1/6J mice injected with graded numbers of spleen cells derived either from dextran sulfate and SRBC primed (●) or form SRBC primed syngeneic mice (○). a) total antibody titers (19S + 7S). b) 2-mercaptoethanol resistent (7S) antibody titers. Antibody titers were assayed 7 days after cell transfer and antigen inoculation. Each point represents average values obtained in 6–8 mice. The standard errors are shown by vertical bars. N.S. = not significant.

Zusammenfassung. Mit 600r bestrahlte Mäuse wurden mit Milzzellen von Spendermäusen, die entweder mit Antigen allein (Kontrollen) oder mit Antigen und einem Polyanion vorbehandelt wurden, injiziert. In den Recipienten, die mit Milzzellen von mit Polyanion vorbehandelten Mäusen injiziert wurden, konnte eine gegenüber Kontrollen signifikant erhöhte (vorwiegend 7 S) Immunantwort gemessen werden. Die Ergebnisse lassen vermuten, dass Polyanionen sowohl auf die B- als auch auf die T-Zellen wirken.

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An Improved Method for the Study of Reagin-Mediated Mast Cell Degranulation in Rats

There are three main methods for demonstrating homocytotropic or reagin-mediated antigen-antibody reactions and their inhibition in rats: Passive cutaneous anaphylaxis (PCA)[1], histamine release[2], and degranulation of mast cells[1] can be studied. The latter method is particularly useful in separating the inhibitory actions on mediator release (antiallergic actions) of drugs from simple antihistaminic ones. Drugs with antiallergic properties are valuable in antigen-induced asthma; they inhibit the PCA reaction as well as the degranulation of mast cells. Antihistaminic drugs inhibit the PCA reaction without

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affecting mast cell degranulation (MCD)[3] and without marked therapeutic effect in asthma.

One drawback to the MCD procedure observed in our laboratory and in others[3–5] is a high rate of unspecific degranulation (by manipulation) in unchallenged control animals. This problem has been overcome by employing our improved method. Furthermore, it has been demonstrated that certain antiasthmatic drugs inhibit the MCD in a dose-dependent fashion.

Methods. Antiserum. Male SIV-50 rats (Ivanovas, Kisslegg) weighing 130–160 g were injected with 5 mg/kg egg albumin (1 × cryst., SERVA) in saline i.m. plus 20×10^9 *B. pertussis* organisms (Behringwerke) i.p. Rats were infected s.c. 10 days later with 2000 larvae of the nematode *Nippostrongylus brasiliensis* to potentiate the reagin response to egg albumin[6]. The antiserum was obtained by bleeding the animals 24 days after sensitisation, passed through millipore filter for sterilisation and stored in 1.5-ml aliquots at −20 °C.

Cell medium. A modified Tyrode's solution was used: NaCl, 123 m*M*; KCl, 2.7 m*M*; hydroxymethyl-aminomethane (*Tris*), 25 m*M*; glucose, 5.6 m*M*; $CaCl_2 \cdot 2\ H_2O$, 1 m*M*; $MgCl_2 \cdot 6H_2O$, 1 m*M*; adding normal HCl to bring pH to 7.6 and glass distilled water up to 1000 ml, sterilized by filtration and kept in the cold.

Improved MCD-method. Male rats (strain: FW 49-Biberach) weighing about 160 g were passively sensitized by i.p. injection of 0.5–1.0 ml antiserum, depending on the activity of the serum. 24 h later the antigen (egg albumin, 50 mg/kg) was given i.v. Control rats were given 0.5 ml/100 g saline i.v. 15 min after antigen challenge the rats were killed with ether and 5 ml absolute alcohol was injected i.p. for fixation. After 5 more min a small piece of mesenterium was dissected out, rinsed in ice-cold cell medium, stained with cold toluidine blue (1 ml toluidine blue 0.1% plus 7.5 ml medium) and rinsed again. The floating mesenterium was then trapped between a slide and a coverslip, the latter dried with a drop of alcohol; magnified 400 times 200 mast cells were counted. Mean values of degranulated mast cells were given in per cent subtracting the value for unspecific degranulation (saline injected animals). Per cent inhibition of degranulation in drug treated animals was calculated using the formula:

$$\% \text{ inhibition} = 100 - \frac{\% \text{ degran. (treated)} - \% \text{ unspec. degran.}}{\% \text{ degran. (control)} - \% \text{ unspec. degran.}} \times 100$$

S.E. of % inhibition was calculated according to the propagation of errors[7].

The other method used included: dissecting out mesenterium with gentle manipulation, challenge with egg albumin (1 mg/ml) at 37 °C in vitro, and fixation in formol-alcohol. Afterwards the preparation was not kept on ice.

Results. The results of either method in terms of specific and unspecific degranulation of mast cells is summarized in the Table. Employing the improved method we obtained a uniformly low background of 3% unspecific degranulation in untreated, antigen-treated and antiserum treated control groups and an antigen induced degranulation of net 37%. On the other hand, the corresponding figures of the method formerly used amounted to 20% unspecific degranulation. In addition, the older method revealed lower figures for specific degranulation (net 24%) than the improved one.

In our further experiments, a dose-dependent inhibition of rat reagin-mediated (specific) mast cell degranulation caused by antiasthmatic drugs could be demonstrated. Comparing quantitatively β-adrenergic drugs such as Fenoterol (Berotec®) and Salbutamol, the former was more active than the latter (Figure). The dose causing a 50% inhibition of the reaction (ED_{50}) was 15 and 82 μg/kg, respectively. Under the same conditions the ED_{50} of disodium cromoglycate, another drug with antiallergic properties[4], was 2 mg/kg i.v.

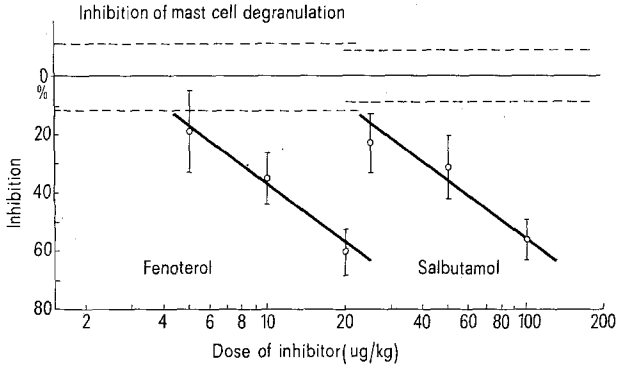
Discussion. Investigating mast cells in rat mesenterium may occasionally be a rather unsatisfying procedure because of the high background of unspecific degranulated cells. According to our observations there are two reasons why older methods may present difficulties: 1. Stretching and folding (although handled gently) of the unfixed mesenterium, and 2. Fixation procedures causing too much shrinking of the collagenous fibres in the mesenterium thus crossing and disrupting mast cells. With these considerations in mind, we used a reaction in vivo, gentle fixation in situ and subsequent cooling of the mesenterium in cell medium in vitro.

Going a step further we inhibited the specific (reagin-mediated) degranulation. The method turned out to be reliable for obtaining a good dose-response relationship using two β-adrenergic stimulants, Fenoterol and Salbutamol.

Reagin-mediated and unspecific (by manipulation) degranulation of mast cells in the mesenterium

Method	Treated with	*n*	Degranulation (%)	S.E.
Improved	—	5	3	0.5
	AG	15	3	0.3
	AB	22	3	0.2
	AG + AB	41	40	1.1
Other	—	6	19	2.0
	AG	6	20	2.0
	AB	6	19	1.4
	AG + AB	6	43	2.8

Mean values of *n* experiments, 200 cells counted, respectively. AG, egg albumin; AB, specific antiserum to egg albumin.



Inhibition of rat reagin-mediated mast cell degranulation by β-adrenergic drugs; dose response relationship; Drugs injected i.v. 1 min before challenge with antigen. Each point is the mean ± S.E. of 5 results.

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As for Fenoterol, introduced as an bronchodilator in asthma, the activity described was observed for the first time. However, the inhibitory action of Fenoterol is not surprising since it is known that various reagin-mediated reactions (see introduction) are inhibited as well by some other β-adrenergic stimulants, i.e. in vitro in human basophiles[8] in human[9–11], and primate[12] lung, and in vivo in man[13] (Prausnitz-Küstner test) and rats[14] (PCA). Thus β-adrenergic stimulants should not only be considered as agents producing symptomatic relief of bronchospasm in asthma[13]; they are potent protective agents against anaphylactic reactions and their action can be demonstrated employing the method described here.

Zusammenfassung. Eine Methode zum Studium der reaginvermittelten Mastzelldegranulation im Mesenterium der Ratte, bei der der Grad der unspezifischen Degranulation sehr niedrig ist, wird beschrieben. Die spezifische (reaginvermittelte) Reaktion ist hemmbar durch die drei Pharmaka Fenoterol, Salbutamol und Natrium-cromoglycat, deren antiasthmatische Wirkung bekannt ist.

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Immunization of Mice Against Transplantable Tumor

In 1965 GOLD and FREEDMAN[1,2] described a carcinoembryonic antigen (CEA) of adenocarcinoma of the human digestive system and of human fetal digestive organs during early periods of gestation. Since then the CEA has been shown to be a glycoprotein intimately associated with the tumor cell membrane[3,4]. The definition of tumor cell antigens as specific products of the tumor cell is, however, still subject to some question since cell surface antigens of certain virus induced tumors are products under virus control[5]. The cell surface antigens which are found are part of the cell membrane and the immune responses which they have been shown to elicit are similar to an allograft reaction. Thus it is recognized that cell mediated immune responses may play a part in tumor rejection. Little is known about how CEA promotes an antibody response in a tumor bearing animal.

It has been recognized for some time that the peculiar properties of tumor cells are dependent to a large extent on the properties of the cell membrane[6–8]. The surface properties are in turn dependent on the membrane composition which includes both proteins and phospholipids. Alteration of the composition of the cell membrane would be expected to alter the metabolite properties of the cell. This has been shown to occur following treatment of cells with Tween 80[9]. Despite the marked alterations in metabolic properties of the cells, and their change in permeability after the use of Tween 80 the cells remain viable and show similar patterns of growth in host mice to that found with untreated cells[10].

The earlier results obtained with the use of Tween 80 prompted an investigation of whether this agent might act as a possible unmasking agent for cell surface antigens in these cells. Such an unmasking of tumor specific antigens, which as indicated are firmly attached to the membrane, should elicit a stronger antibody reaction and allow the host to reject the tumor by a normal antibody antigen reaction.

Cells of the highly malignant Ehrlich-Lettré hyperdiploid strain were used in these experiments and stock tumor was maintained by serial transplant of 0.2 ml of ascites fluid i.p. into host mice. For the immunization experiments ascites fluid was drained from the tumor bearing mice, and the fluid with contained cells was irradiated with a dose of 5000 rad, a dosage required to kill the cells. The fluid was centrifuged at 1000 *g* for 10 min and the packed cells were mixed with 0.25 *M* sucrose containing 1% Tween 80 and allowed to stand for 15 min at room temperature. This procedure has been shown previously to result in marked permeability changes with consequent metabolic alterations in these cells[9]. The suspension was centrifuged at 1000 *g* for 10 min and the packed cells were suspended in 0.9% NaCl, such that 1 ml contained 0.5 ml packed cells. 1 ml of this suspension was injected i.p. into the experimental mice. At the same time, 0.1 ml of complete Freund's adjuvant was injected i.m. Subsequent injections of irradiated and Tween 80 treated cells of the same concentration were made at 1 week and 2 week intervals after the initial insult.

After the third insult the experimental mice developed a marked enlargement and fluid was aspirated under light ether anesthesia 1 week after the third injection of the killed and Tween 80 treated cells. Examination of this fluid showed a marked infiltration of lymphocytes and neutrophils. Many lymphocytes were present attached to

Mortality of mice

	No.	Death (No./day)
Control	10	1/12, 1/13, 2/14, 1/15, 2/16, 3/17
Experimental	10	1/20

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