were fixed with glutaraldehyde and post-fixed with osmium tetroxide. After dehydratation in alcohol the specimens were cleared in propylene oxide and embedded in Durcupan ACM (Fluka). Phagocytic activity was determined in series of thin sections contrasted with uranyl acetate and lead citrate in a Tesla BS 500 electron microscope with the accelerating voltage of 60 kV. Macrophages with 7 or more particles were considered as phagocytic positive cells.

Results and discussion. Macrophages have at least 3 functional types of receptors for attachment and subsequent ingestion of particles: the Fc receptors, the complement receptors and the nonspecific receptors. Phagocytosis via the last type has been studied using 'inert' particles, for example latex or latex-like particles. Under normal conditions, methacrylate copolymer particles adhere neither to the lymphoid cells nor to the peritoneal exudate macrophages obtained from unstimulated donors. After the stimulation with PP or TG-medium the peritoneal macrophages acquire the ability to engulf these particles (table). This result supports the observations[7,8] showing an increased phagocytic activity in stimulated mouse peritoneal macrophages, and is in contradiction with some data on a decreased phagocytic ability[9,10] (especially after the TG-stimulation), although no detailed comparison of the degree of phagocytosis of different types of particles has been done[11]. The addition of sodium azide prevents phagocytosis of these particles. No significant increase of phagocytosis occurs when mouse or calf serum is added to the incubation medium. The experimental data indicate that the stimulation is due either to an increased adhesion and the ingestive power of mouse peritoneal macrophages, or to an increased number of nonspecific surface receptors for this type of particle. The changes in the uptake of particles therefore seem to be accompanying the functional alterations during the stimulation processes and reflecting modifications in the properties of the cell membrane. This suggestion is supported by the numbers of spontaneous rosettes of macrophages with sheep erythrocytes and by changes in intercellular adhesivity[12].

The electron microscopic observations show a smoothened cell surface and a rearrangement of the cortical regions of the cytoplasm of the cells phagocytosing the methacrylate copolymer particles. Pictures show a random distribution of particles over the cell surface and inside the cells (figure 1), often near vacuoles containing stimulating medium (figures 2 and 3). The partial shrinking of the particles during preparation of the specimes for electron microscopy does not seem to be of great importance for our purposes.

Methacrylate copolymer particles could be useful in studies of the phagocytosis of macrophages and, after coating with specific ligands, in specific labelling of cells. In other words, these particles may serve as markers for specific identification of cells by optical as well as by electron microscopy.

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The influence of sodium-8-chlorotheophyllinate (S8CT) on immune processes[1]

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Summary. S8CT injected at the time of immunization significantly enhances specific IgM-production but has no effect on IgG-formation. Mitogen (PHA-P) induced macrophage migration inhibition of cells of S8CT pretreated animals is reduced. The same effect is observed, when normal cells are tested in the presence of S8CT in vitro. Blast transformation of B-lymphocytes but not of thymocytes is significantly stimulated by S8CT. Acid phosphatase activity is also stimulated in B-cells and - to a lesser degree - in cortisone-resistant T-lymphocytes whereas the activity of the total thymocyte population is reduced. No effect was seen on phagocytosis and intracellular bactericidal activity. A stimulatory effect of S8CT for B-cells is postulated.

Cyclic 3′, 5′ adenosine monophosphate (cAMP) and substances enhancing intracellular cAMP-level such as catecholamines or theophylline were described earlier as modulating the immune response[2-5]. Results obtained with theophylline, however, seem not to be homogeneous: no effect on antibody formation or inhibition were found using doses above 10 mg/kg[6-8] when theophylline was applied in vivo. With 1 exception[9], an enhancement of antibody formation[3], depending on the time of contact[10,11], was shown when lymphocytes were antigenically stimulated and treated with this substance in vitro. We report here the results of studies of how different immune processes (antibody formation, mitogen induced macrophage migration inhibition and lymphocyte transformation) are modulated by S8CT. Moreover, the influence of S8CT on the lysosomal acid phosphatase activity of different lymphocyte classes was examined.

Material and methods. S8CT (mol.wt 236.62) was synthesized as described previously[12]. The number of direct (DPFC) and indirect (IPFC) plaque forming (IgM and IgG producing) spleen cells was evaluated on day 3 and 7 after immunization (2×10^8 SRBC in 0.5 ml saline i.p.)[13]. S8CT

in 0.25 ml saline was injected i.v. For all experiments inbred male BALB/c mice (Bomholtgård, Ry, Denmark) of about 20±1 g were used. For macrophage migration inhibition tests[14] peritoneal exudate cells (PEC) were taken 5 days after i.p. injection of 2 ml Bayol (Esso AG).
Thymocytes were taken from BALB/cABom, and B-lymphocytes (spleen cells) from BALB/cBom-nu/nu-mice (Bomholtgård) of about 18–20 g. The cortisone-resistant population of mouse thymocytes (Tcort) described as being suppressor cells[15, 16] were isolated after s.c. injections of 12.5 mg hydrocortisone (Hoechst) on days -2 and -1 respectively. Transformation of lymphocytes was measured by their ^{3}H-thymidine uptake (culture time: 4 days; 0.2×10^6 cells per well; medium: RPMI 1213 (Seromed) with mercaptoethanol and essential amino-acids[17]. For macrophage migration inhibition tests the capillary technique was used[19,20]. Acid phosphatase activity was evaluated as described previously[18] after an incubation time of 1 h using 5×10^6 cells per ml.

Results. When S8CT is injected at the same time as the antigen, the number of IgM-producing cells (DPFC) is enhanced significantly (figure 1). Even later on, i.e. in day 7, DPFC show a tendency towards higher values. S 8CT given on day 4 or on day 0 and day 4 has no influence on the number of late DPFC response on day 7. The number of IgG forming cells does not seem to be influenced. Therefore, the S8CT effect concerns only the primary immune response but not the shift to IgG-formation.
The influence of S8CT on mitogen (purified phytohemagglutinin, PHA-P, Difco)-induced macrophage migration inhibition was examined by using PEC of BALB/c-mice treated on day -3 with 3.95 mg S8CT/0.5 ml saline/animal. Washed PEC were suspended in PHA-P containing medium before packing into the capillary tubes; the PHA-P content of the medium used to fill up the migration chamber was adjusted to the same concentration. As seen in figure 2, PEC of pretreated animals migrate better than those of the controls and the PHA-P induced migration inhibition as per cent of controls without PHA-P is reduced. When PEC of normal animals were assayed in vitro for PHA-P reactivity in the presence of S8CT a comparable result is obtained. In the absence of PHA-P, S8CT resulted in a dose-dependent stimulation of macrophage migration (figure 3, b). The PHA-P-induced migration inhibition in the presence of S8CT is smaller than that in the absence of this substance when the inhibitory effect is evaluated as a per centage of migration values in media with the corresponding S8CT concentration but without PHA-P (figure 3, a).
The direct influence of S8CT on lymphocytes in vitro was examined by measuring their transformation rate (^{3}H-thymidine uptake) and acid phosphatase activity. An enhanced transformation was only seen with B-lymphocytes showing a transformation index of 1.21 at concentrations of 10 and 100 µg S8CT per ml. This stimulation represents a marginal effect. However, the difference between assay and control values ($n = 5$) is statistically significant ($p < 0.01$). In contrast, the influence of S8CT (100 µg/ml) on Tcort cells and total thymocytes is with $n = 5$ and a stimulation rate of 1.18 and 0.87 statistically not significant.
These results are apparently paralleled by the in vitro influence of S8CT on acid phosphatase activity (table 1). B-cells - and to a lesser degree Tcort cells - show a dose-dependent stimulation, whereas thymocytes are inhibited in this respect. In thymocytes and Tcort cells, higher concentrations of S8CT seem to be less effective. Moreover, it could be shown that pulsed thymocytes, i.e. those pretreated with S8CT and washed, give a comparable result.
In other experiments, animals were treated with S8CT on day -1. Lymphocytes taken on day 0 were assayed for acid phosphatase activity and PHA-P reactivity. B-cells were included in these experiments because this lymphocyte class was also shown to react with enhanced phosphatase

Table 1. Influence of S8CT on acid phosphatase activity in vitro

S8CT (µg per ml)	NP (µg)	Difference to control (%)
Thymocytes		
Control	63.8±0.17	-
1	60.0±0*	−5.9
5	58.7±0.12*	−7.9
10^w	50.9±0.12*	−20.3
Control	68.4±0	-
10	44.8±0.36*	−34.3
50	54.9±0.23*	−19.6
100	63.6±0.36*	−7.0
Tcort		
Control	70.2±0.2	-
10	73.4±0.2*	+4.6
50	72.3±0.12*	+3.0
100	70.2±0.2	±0
B-Lymphocytes (spleen)		
Control	65.2±0	-
10	65.2±0	±0
50	70.2±0*	+7.8
100	74.2±0*	+13.8
200	78.2±0*	+19.9

Values: mean of $n = 4$. NP, p-nitrophenol liberated from p-nitrophenyl phosphate. W, cells were preincubated for 30 min with S8CT and assayed after washing. * Significant compared with controls, $p < 0.01$.

Table 2. Influence of sodium-8-chlorotheophyllinate (S8CT) treatment of mice on day −1 on PHA-P reactivity of lymphocytes evaluated by their acid phosphatase activity. Stimulation by 50 µg PHA-P/ml

Treatment S8CT	Treatment S8CT (mg/animal)	Without PHA-P NP (µg)	Difference to control (%)	With 50 µg PHA-P/ml NP (µg)	Difference to control (%)	Stimulation index by PHA-P
Thymocytes	Control	61.5±0.31	-	64.6 ±0.36	-	+5.0*
	3.95	32.0±0*	−48.0	31.2 ±0.13*	−51.7	−2.6
	0.395	29.9±0.38*	−51.4	31.98±0.07*	−50.6	+6.96*
Tcort	Control	36.1±0.14	-	47.3±0.12	-	+31.0*
	3.95	66.2±0*	+83.4	84.0±0*	+77.6	+26.9*
B-Lymphocytes (spleen)	Control	69.5±0.16	-	75.9±0.31	-	+ 9.3*
	3.95	66.7±0.23*	− 4.0	75.8±0.2	− 0.13	+13.6*
	0.395	38.4±0.14*	−44.7	42.4±0.23*	−44.1	+11.2*

NP, p-nitrophenol liberated from p-nitrophenol phosphate. * Significant compared with control, $p < 0.01$.

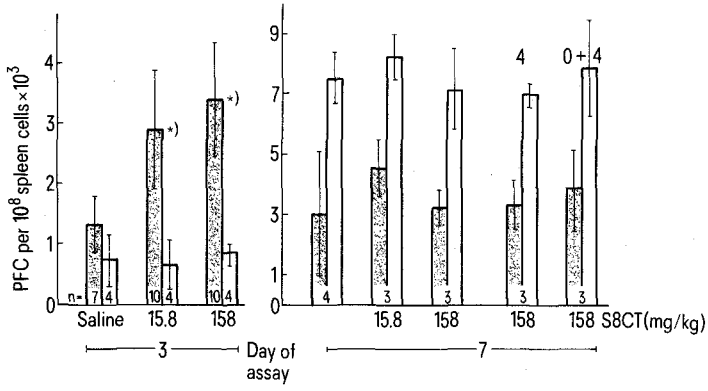


Fig. 1. Anti-SRBC-response of mice treated with sodium-8-chlorotheophyllinate (S8CT). Treatment on day 0, otherwise as indicated on day 4 or on day 0+4.
▨ DPFC; □ IPFC; n = number of animals tested; comparison to control value, $p < 0.01$.

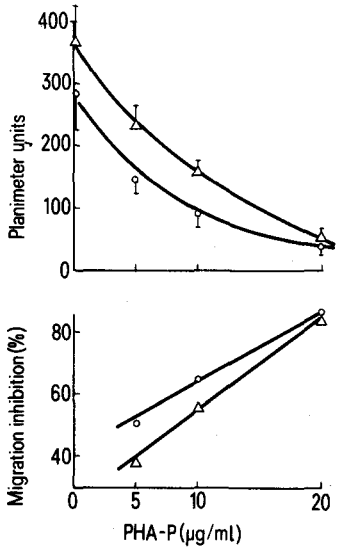
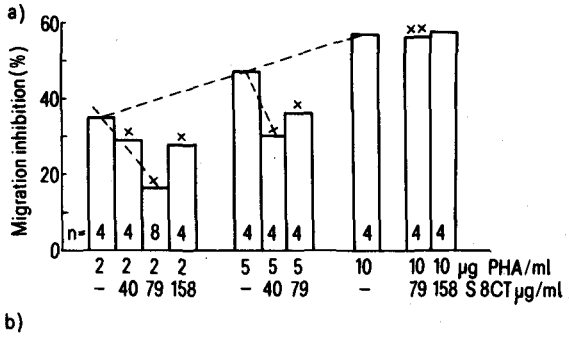


Fig. 2. Influence of S8CT treatment (sodium-8-chlorotheophyllinate) of mice on the PHA-P reactivity of their lymphocytes assayed in the macrophage migration inhibition test. Treatment = 3.95 mg S8CT given i.v. in 0.5 ml saline per animal on day -3. △ = assay; ○ = control.



b)

S8CT (µg/ml)	Migration stimulation (%)
40	8.8
79	33.9
158	42.9

Fig. 3. *a* In vitro effect of S8CT (sodium-8-chlorotheophyllinate) on PHA-P reactivity of lymphocytes from normal mice in macrophage migration inhibition test. *b* Influence of S8CT on macrophage migration in the absence of PHA-P. * $p < 0.01$; ** $p < 0.05$.

values when incubated with PHA-P[21]. As seen in table 2, the basal values of thymocytes and B-cells of pretreated animals are smaller than those of the controls. The same is the case comparing the values after incubation with PHA-P. In contrast, the corresponding values of Tcort cells are always higher. The highest doses of S8CT are less effective than the smaller ones. The PHA-P sensitivity (stimulation index) is as follows Tcort > B-cells > thymocytes.

Phagocytosis and intracellular bactericidal activity of peritoneal macrophages of BALB/c-mice evaluated in vitro with *Staphylococcus epidermidis*[22] are not significantly modulated by pretreatment of animals with 3.95 mg S8CT/0.5 ml saline on day -1.

The experiments show that i.v. application of S8CT at the time of immunization but not thereafter results in enhanced IgM production; however, the shift to IgG production is not altered. This result is comparable with the stimulatory effect of theophylline on antibody production when mouse lymphocytes are immunized in vitro[3,10,11]. This stimulation was supposedly due to an inhibition of suppressor cells[3]. If the drug is present for more than 24 h, the inverse effect, i.e. an inhibition of DPFC response, is observed[9-11]. It is now suggested that S8CT activates B-lymphocytes directly as seen by enhanced blast transformation and acid phosphatase activity. Inhibition may be induced by activating Tcort cells as suppressor cells. It is suggested that S8CT might influence immune regulation in the following way: the substance leads first to an activation of immune reactions and then to an inverse or equilibrating effect by the stimulation of Tcort cells. The latter is thought to be a secondary, i.e. regulatory effect because the relatively small number of Tcort cells, representing only 5% of the normal thymocyte population, may be responsible for a certain delay in the formation of stimulation products. This is compatible with the observation that the phosphatase activity of thymocytes (total population including Tcort cells) and the PHA-P induced macrophage migration inhibition are inhibited. The latter effect was similar to the observation of Pick[23]. Experiments with human lymphocytes are also consistent with this interpretation of a regulation of immune response by S8CT. On the one hand, it was found that Ig production by human peripheral lymphocytes is stimulated by theophylline[24], and on the other that T-suppressor cells are also sensitive to this substance[25]. Theophylline seems also to inhibit the effect of preformed macrophage migration inhibiting factor[26].

In contrast to these observations, it is difficult to explain why theophylline injected i.p., together with erythrocytes as antigen, resulted in an inhibition of antibody response or had no effect[6-8] whereas the SRBC response when poly A:U was given was enhanced. The only difference is the simultaneous application of theophylline and antigen by the i.p. route and the use of a theophylline derivative.

Observations on inhibition of plaque-formation by spleen cells of antigenically stimulated animals on addition of theophylline in vitro[27] are not comparable with the results described above, where the influence of S8CT on sensitization in vivo was tested. The effect of S8CT on immune processes may not only be due to inhibition of cAMP degradation and therefore to a higher intracellular cAMP level because - under the same experimental conditions - antibody formation is described to be inhibited by cAMP[28] and on the other hand to be stimulated by theophylline[3,10,11].

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Viability of rabbit bone marrow after cryopreservation (in vitro and in vivo)[1]

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Summary. A technique for preservation of rabbit bone marrow is described, which preserves viability of stem cells in all 22 animals as tested by autologous bone marrow transplantation and in vitro growth. Erythroid precursors survived better than myeloid precursors as observed by in vitro and in vivo recovery.

Combined antimitotic chemotherapy and high dose irradiation are used for successful treatment of patients with disseminated malignancies[3,4]. These therapies cause a dose-dependent bone marrow aplasia. The period of aplasia can be successfully shortened by an infusion of autologous bone marrow, aspirated before the beginning of therapy and preserved by deep freezing[5,6]. We modified the technique used by Appelbaum and Gorin[6,7] in canine experiments and in human marrow transplantation so that it could be applied in a rabbit model, and we tested the viability of the cryopreserved bone marrow by measuring its ability to repopulate the autologous bone marrow after 1200 rad total body irradiation and by observing in vitro growth of erythroid (BFU_E) and myeloid (CFU_C) precursors.

Materials and methods. In vivo. 1. Animals. Normal outbred New Zealand rabbits weighing 2.5–3.5 kg were used.

2. Bone marrow aspiration. Rabbits were anesthetized with Hypnorm (Philips-Duphar, B.V.). Bone marrow was aspirated from both iliac crests and femurs and put in sterile tubes containing 5 ml TC 199 (tissue culture medium, DIFCO) and 1000 IU heparine (Liquemin®, Roche). After aspiration the bone marrow was filtered and spun at 1000 rpm for 20 min (Hettich Rotanta centrifuge). The fat and plasma were removed and the nucleated cells were counted (Coulter Counter S). No attempt was made to separate the erythrocytes. TC 199 with 20% DMSO (Dimethylsulfoxide, BDH) and 10% autologous plasma was used as cryoprotective agent[8].

3. Freezing. The whole bone marrow volume was devided into 4 portions and transferred into UCAR blood freezing bags. Immediately after adding an equal volume of the cryoprotective agent (resulting in a final concentration of 10% DMSO), the bone marrow was frozen at a rate of 1 °C/min in a biological freezing apparatus (LINDE BF-4, Union Carbide corp.). The frozen material was stored at − 140 °C in the vapour phase of liquid nitrogen for 5–32 days.

4. Total body irradiation. 1 day before transplantation, the rabbits were exposed to a midline tissue dose of 1200 rad total body irradiation (Co^{60} Gammatron) at a rate of 35 rad/min.

5. Bone marrow transplantation. The 4 bone marrow portions of each individual animal were transplanted on the 2 following days. They were thawed quickly in a water bath at 37 °C. The rabbits were anaesthetized with Hypnorm® (Philips-Duphar, B.V.). After an injection of 0.5 ml Sandosten-Ca® (Sandoz) i.v., the thawed bone marrow was slowly