The 7S immunglobulin was separated by chromatography in Sephadex G-200 and DEAE-cellulose column. Peritoneal macrophages were harvested by injecting 3 ml of heparinized Hanks' solution into the peritoneal cavity of exsanguinated mice. $^2/_{10}$ of a suspension containing 1×10^6 cells/ml were added to wells of acrylic rings attached to microscopic slides and incubated at 22–24 °C for 60 min. The adherent macrophages were washed and 0.2 ml of sensitized SRBC with 7S antibody (EA-7S) containing the drugs were then added to each well. After incubation for 30 min at 22–24 °C the slides (test and control) were kept for 45 sec in Hank's solution diluted 1/5 with water to affect lysis of bound but non-ingested SRBC. The macrophages were fixed with glutaraldehyde, treated with benzidine-H202 mixture for erythrocytes staining followed by Wright staining. The number of macrophages with SRBC phagocytized in randomly microscopic fields was estimated by scoring 200 macrophages in duplicated slides. Drug dilution inducing more that 10% of cells taking up Trypan blue were discarded. Solutions of the drugs were prepared each day before use in Hanks' solution, with the final pH adjusted to 7.4 .The following drugs were used: theophylline, 3′,5′-AMP, 5′-AMP, 2-chloradenosine, imidazol and phenylimidothiazol.

Results and discussion. The results are summarized in the Figure. The drugs recognized as capable of raising the levels of intracellular cAMP through a blockade of the phosphodiesterase activity showed a potent inhibition of phagocytosis. In contrast the drugs capable of reducing the levels of intracellular cAMP by activation of phosphodiesterase, such as imidazol and phenylimidothiazol, were found to be potent stimulators of phagocytosis.

The alterations in cellular cAMP levels during phagocytosis, and the influence of adenyl cyclase, specially in PMN leukocytes, have been the subject of recent controversy. Concentrations of cAMP above 10^{-4} *M* induced by theophylline, or prostaglandine and theophylline, have the capacity to retard the phagocytosis of ^{125}I-labelled heataggregated BSA by mouse peritoneal macrophages, whereas very low concentrations of cAMP (10^{-10} *M*) stimulated the taking up of these particles[15]. These data are compatible with the hypothesis that cAMP may exert a byphasic effect on endocytosis. It was also found that increased levels of intracellular cAMP inhibited lysosomal enzyme release of PMN leukocytes exposed either to particles of zymosan or to immune precipitates. This finding raised a possible analogy between the inhibitory effects of cAMP on lysosomal enzyme release and on histamine liberation[15]. It was demonstrated that high intracellular levels of cAMP impair granulocytes's ability to kill *C. albicans* and that this effect could be consistently obtained with theopylline[16]. It has been suggested that at least one consequence of raising the level of cAMP within cells is to impede the traffic of lysosomes to the phagocytic vacuoles, retarding significantly the extrusion of acid hydrolases[15]. It is generally assumed that peritoneal macrophages usually utilize energy from glycolysis for phagocytosis and that oxidative pathways may also be used[17]. Since the only events common to both glycolysis and respiration are synthesis of ATP from ADP, and oxidation and reduction of NAD, it has been widely inferred that membrane invagination and particle interiorization during endocytosis are processed through ATP. Thus, the energy required to induce membrane excitation, for activation of the intracellular actomyosin-like microfilaments[14] and phosphorylation of tubulin[15], are provided by ATP. The results described herein showing that engulfment of antibody-coated erythrocytes proceed optimally in conditions in which the macrophages cAMP content is lower, indicate that the modulation of this process may follow the same mechanism as histamine release from leukocytes[7,8,11,12]. Accordingly, a decrease in the cAMP level of macrophages may follow the interaction of the receptor located on cell surface with the Fc portion of immunoglolulins after antigen-antibody combination. A direct determination of cAMP content in macrophages undergoing immunological phagocytosis has, however, to be made before a final conclusion can be drawn on its role in this process. It has recently been proposed[18] that in a number of cell types, cyclic 3′,5′-guanosine monophosphate (cGMP) promotes cellular events that are antogonistic to those believed to be mediated by cAMP. The influence of cGMP on macrophage phagocytosis is now being studied in our laboratory[19].

Zusammenfassung. Nachweis, dass Drogen, die einen Anstieg des cAMP-Gehalts der Makrophagen durch Verhinderung der Phosphodiesterase hervorrufen, die immunologische Phagozytose verhindern. Andererseits aber wird die Phagozytose durch Drogen gefördert, welche den cAMP-Gehalt der Makrophagen herabsetzen.

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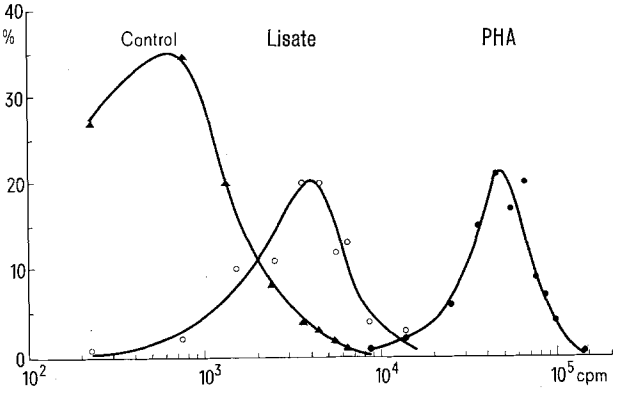
Stimulation of Human Lymphocytes in vitro by Bacterial Hydrolysates[1]

Various antigenic components of microorganisms, such as streptolysins, staphylococcal filtrate and tuberculine amongst others have been reported to induce lymphocyte blastogenesis in vitro[2,3]. The mitogenic activity of non-immunogenic subfractions of such substances, however, has not yet been systematically analyzed. This paper reports the property of a bacterial hydrolysate with a molecular weight below 10,000 to stimulate human lymphocytes in vitro.

Material and methods. Peripheral venous blood lymphocytes were obtained from 16 healthy male volunteers at weekly intervals for 8 weeks. Lymphocyte separation

was done by density gradient sedimentation[4]. Bacterial hydrolysates[5] were prepared by digesting the endo- and exotoxins of a variety of established cultures of pathogenic bacteriae[6] with a protease of *B. subtilis* as described elsewhere[7]. The chemical composition was: oligopeptides 1.6 mg, polysaccharides 0.02 mg and nucleic acids 0.085 mg per 1 ml of hydrolysate. Lymphocytes were cultured in vitro for 110 h, followed by ^{3}H-thymidine labelling for additional 12–16 h, DNA-precipitation and liquid scintillation counting as described previously[8]. Separate lymphocyte cultures were stimulated with either 0.1 ml phytohemagglutinin (Difco) or 0.1 ml hydrolysate which in pilot studies at this concentration had its maximal stimulating effect. No stimulant was added to the control tubes. The cultures were done in triplicate, giving a total of 384 cultures for each mitogen. The results were expressed in cpm radioactivity of DNA precipitates. The data were statistically evaluated by an analysis of variance and a *t*-test adapted to multiple comparisons by DUNNET[9].

Results. The range of cpm was 367 to 2,138 for the controls, 1,583 to 9,357 for the hydrolysates and 20,445 to 120,830 for the PHA-cultures. The distribution of the counts is presented in the Figure. The difference between the background and each of the cultures stimulated by PHA and hydrolysate is significant at the level of $p < 0.01$.



Frequency distribution of counts per minute (cpm). Ordinate indicates % of all experiments done for stimulants or control.

Discussion. In contrast to PHA-P or bacterial endotoxins, the bacterial lysate is only a weak mitogen. Whether the mitogenic activity is due to the whole lysate as such, or to one or another of its components, remains to be shown. Because lymphocyte transformation occurred in almost 100% of the cultures it can be concluded that the substrate responsible for stimulation exerts an immunologically unspecific action. Further preliminary work suggests that this weak mitogen might be suitable for the demonstration of minor impairments of lymphocyte transformation in vitro.

Zusammenfassung. Hydrolysate, hergestellt mit einer *B.-subtilis*-Proteaseaus Endo-und Exotoxinen pathogener Mikroorganismen, stimulieren menschliche Lymphozyten in vitro. Die Stimulation ist schwach, verglichen mit Phytohämagglutinin, und wahrscheinlich immunologisch unspezifisch.

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Increased Immunogenicity of TSTA on Heterokaryocytes of Syngeneic Tumoral and Allogeneic Normal Cells

The immune response to tumour specific transplantation antigens (TSTA) is generally weak, thereby limiting both the efficiency of the spontaneous defence and the attempts of immunotherapy. Many efforts have been made in order to increase the immunogenicity of TSTA by means of aspecific adjuvants[1,2] or by the introduction of new antigenic determinants on tumour cells[3]. In 1967 SCHIERMAN and MCBRIDE[4] showed that a very weak humoral response to erythrocyte isoantigens was increased by the presence of stronger antigens on the same

Table I. Sequence of immunizing treatments and serological tests

Experiment 1						
Day 0 1st immunization 2×10^6 cells	Day 19 CI test	Day 25 Booster 2×10^6 cells	Day 33 Challenge 5×10^5 cells	Day 53 CI test	Day 67 CI test	Day 90 Final assessment of the number and volume of tumors
Experiment 2						
Day 0 Immunization 2×10^6 cells		Day 5 Challenge 5×10^5 cells		Day 20 CI test		Day 90 Final assessment of the number and volume of tumors