Table 3. Effects of intravenously administered NCO-700 (20 mg · kg^{-1}) on FMLP-induced CL in rabbit PMNs.

	Chemiluminescence (mV)			
	Data control	Data drug	Peak control	Peak drug
Means (6)	11.23	6.23*	15.79	7.75*
SD	1.91	3.13	4.99	4.57

* $p < 0.05$ (Mann-Whitney U test).

inhibited this CL production, NCO-700 may act by scavenging superoxide anions. The high concentrations employed in this study did not affect urate formation by XOD (data not shown).

The effect of NCO-700 on FMLP-stimulated CL production was further examined in PMNs in vivo. Table 3 shows that PMNs from NCO-700 pre-treated rabbits exhibited a decreased CL production response to FMLP compared to cells prepared from control rabbits. Although we have demonstrated that NCO-700 can quench luminol-dependent CL generated in both NaOCl-H_2O_2 and hypoxanthine-XOD systems, the scavenging effect of the free NCO-700 does not explain its ability to interfere with drug-treated and washed PMNs or PMNs treated in vivo. Indeed, the ability of NCO-700 to interfere with PMNs under these conditions suggests that its primary effects are independent of scavenging and due to a direct, but undetermined, effect on PMNs.

While the present results indicate that FMLP-triggered active oxygen production is prevented by NCO-700, they do not identify the mechanism through which the inhibition is achieved. Although luminol-amplified CL in phagocytosing PMNs depends largely on the presence of myeloperoxidase (MPO) released from the cells, it has been shown that FMLP-triggered CL production was independent of the presence of MPO [9]. Moreover, NCO-700 did not affect MPO activity or FMLP-stimulated MPO release (unpublished observation) in spite of the significant inhibitory effect of NCO-700 on CL production.

Although the evidence in this study is not wholly conclusive, it suggests that NCO-700 may inhibit the production of reactive oxygen. A plausible explanation is that the thiol proteases, presumably CANP, may play important roles in activating NADPH-oxidase, responsible for superoxide anion production, and/or hydrogen peroxide generation. Other factors which could alter the sensitivity of PMNs to FMLP include changes in FMLP-binding to specific receptors on the cell surface and changes in post-receptor activation.

Weiss and others suggested that the critical PMN oxidants are hypochlorous acid or its amino adducts, and/or the N-chloramines, which is consistent with his and other previous studies [10–13]. The MPO-derived hypochlorous acids are probably the most potent oxidants generated by stimulated PMNs. Since NCO-700 scavenges several reactive oxygen species generated in both NaOCl-H_2O_2 and hypoxanthine-XOD systems and inhibits reactive oxygen production, NCO-700 may have some beneficial effects on oxidant-induced tissue injury triggered by activated PMNs.

Interestingly, NCO-700 has recently been described as a protective agent against cardiac muscle degradation induced by coronary artery occlusion in rabbits [14]. There is experimental evidence that drugs which impair PMNs function may reduce the size of acute myocardial infarction [15]. Since NCO-700 inhibits oxidant formation by PMNs, its cardioprotective effect in animals may partly be due to the decrease in cytotoxic oxygen radicals produced by PMNs which have migrated into the ischemic area.

It is tempting to assume that thiol protease, but not serine protease, has some role in generating active oxygen production in FMLP-triggered rabbit PMNs. Although further studies are required to support this hypothesis, the present data indicate that the direct action of NCO-700 is distinct from its scavenging effects.

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Specific radioimmunoprecipitation of histone H2A antigens by protein A conjugated sepharose

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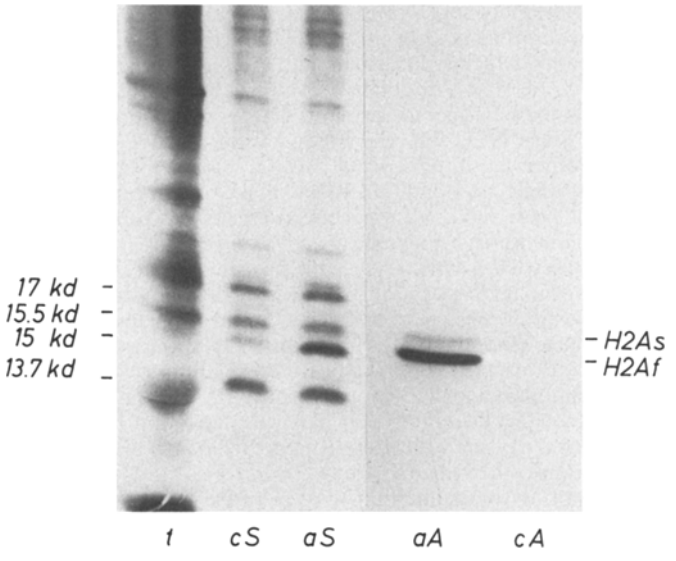
Summary. A modified radioimmunoprecipitation technique is described which allows the specific detection of histone H2A antigens. The technique circumvents unspecific binding of histones to the bacterial adsorbent.
Key words. Protein A; radioimmunoprecipitation; histone H2A.

Protein A, a cell wall constituent of certain *Staphylococcus aureus* strains, binds specifically to the Fc regions of many IgG molecules [1, 2]. Because of its high adsorption capacity, rapid binding and advantageous sedimentation properties, fixed cells of the Cowan strain of the bacterium *Staphylococcus aureus* are widely used as an adsorbent for antigen-antibody-complexes and for rapid isolation and characterization of labeled antigens in radioimmunoassays [3, 4]. The use of this precipitation technique is limited by the fact that fixed *St. aureus* cells bind radiolabeled proteins non-specifically in the



Fluorography of histone H2A antigens after radioimmunoprecipitation. Total RNA (10 µg) of *Sciara coprophila* embryos was translated in vitro and translation products were labeled with ^{35}S methionine [6]. An aliquot was taken for analysis of total translation products (lane t). The remainder was incubated with the monoclonal antibody By 187 directed against *Drosophila melanogaster* histone H2A (lanes a) [7], or a control monoclonal antibody which showed no specific crossreactivity with *Sciara* proteins (lanes c), and immunocomplexes were precipitated with fixed *Staphylococcus aureus* cells (10 mg) (lanes S) or with protein A-conjugated sepharose beads (2 mg, Pharmacia, Uppsala, Sweden) (lanes A) and washed five times in washing solution (1% Nonidet P40, 0.01% deoxycholic acid, 0.01% SDS, 5 mM potassium iodide, 2 mM methionine in PBS). Isolated radiolabeled protein fractions were separated by SDS-polyacrylamide gel electrophoresis (15% acrylamide) and bands were visualized by fluorography as described [6]. Molecular weights and positions of the H2A antigens are indicated.

absence of specific antibodies, causing problems in the detection of specific proteins present in small amounts. Platt et al. [5] tried to overcome these difficulties by a complicated and elaborate modification of the precipitation technique, accepting lower recoveries of the radiolabeled antigens.
In our recent work [6] we analyzed radiolabeled histone H2A in vitro translation products of total RNA of *Sciara coprophila* embryos using a monoclonal antibody directed against *Drosophila melanogaster* histone H2A. Here, in similar radioimmunoprecipitation experiments we compare, after fluorography, results obtained by precipitation of the histones H2A-anti-H2A antibody complexes either with protein A-containing *St. aureus* cells (fig. lane aS) or with protein A-conjugated sepharose (lane aA). As a control we repeated the precipitation experiment using a monoclonal antibody which showed no specific reaction with *Sc. coprophila* proteins (lane cS, precipitated with *St. aureus*, and lane cA, precipitated with protein A-conjugated sepharose). Total radiolabeled translation products are shown in lane t. As can be seen, specific H2A antigens (H2As and H2Af) are selectively precipitated by protein A-conjugated sepharose and in the presence of the H2A specific antibody (compare lane aA with lane cA). In contrast, fixed *St. aureus* cells (lanes aS and cS) precipitate not only these H2A antigens, but also additional proteins non-specifically (compare lane aS with cS). These might, as judged by their electrophoretic mobility [6], correspond to the other core histones. With these latter experiments specific H2A antigens cannot be reliably analyzed. The unspecific binding of core histones could result from the interaction of the histones with the bacterial DNA in fixed staphylococcal cells.
As a consequence, in performing radioimmunoprecipitation experiments with histone antigens, protein A-conjugated sepharose should be used as an immunoadsorbent, since this technique displays high adsorption capacity, rapid binding, and advantageous sedimentation properties, but prevents unspecific binding of other proteins and low recovery of antigens.

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Reversion of *Lactobacillus lactis* protoplasts

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Summary. More than 99% of *L. lactis* cells have been converted to protoplasts upon digestion of cell walls with mutanolysin (N-(acetyl)muramidase). Functional protoplasts were obtained even with the lowest level of the enzyme that was used (0.1 $U \cdot ml^{-1}$ of the cell suspension) and after incubation at 37 °C for 2 min. The regeneration of the polymerized cell wall appears to be induced by a cell homogenate of the same organism.
Key words. L. lactis; protoplasts; protoplast regeneration.

Contrary to other species of lactic acid bacteria [2] *L. lactis*, which plays an important role in food science and technology has attracted little attention in genetic and biosynthetic studies. The degradation of the cell walls and membrane polymers of Gram-positive bacilli is facilitated by the fact that these organisms possess only one membrane system. The supramolecular aggregates of the bacterial surface can be dissociated by muralytic enzymes, e.g. mutanolysin or