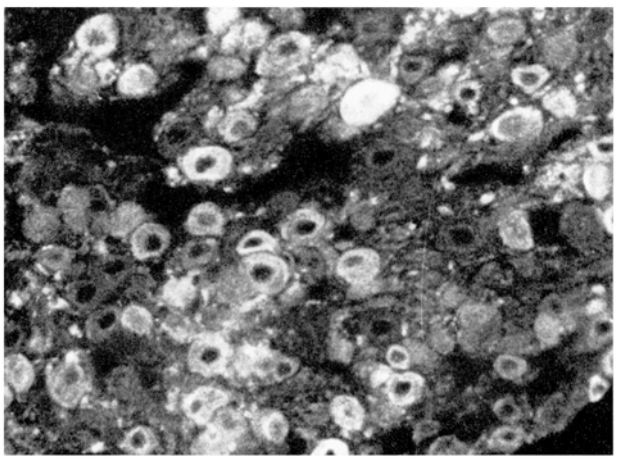
The ganglion cells in animals, treated with the monoamine oxidase inhibitor nialamide[6] (500 mg/kg, i.p., $5\frac{1}{2}$ h), showed a general slight increase of the fluorescence level. Particularly cells with a low or medium degree of fluorescence displayed increased intensity. However, even with this treatment, there was considerable variation in intensity among the cell bodies (see Figure) and some cells showed no fluorescence at all. Specific fluorescence did not occur in animals given reserpine[6] (5 mg/kg, i.p., 2–6 h), and was of very low intensity after α-methyl-*m*-tyrosine (400 mg/kg, i.p., 24 h) and L-aramine[6] (25 mg/kg, i.p., 6 h).

The results obtained in the experiments with the above-mentioned drugs strongly support the view that the fluorescence is due to a monoamine, probably a catecholamine (CARLSSON et al.[2]). Since the histochemical criteria (see FALCK[3]) were also satisfied, there seems to be little doubt that the fluorescent material is derived from a primary catecholamine. As most of the neurons in the sympathetic ganglia are adrenergic, it may be concluded that the amine, demonstrated in the cells, is, in fact, noradrenaline.

One possible explanation of the variation in fluorescence intensity among ganglion cells might be that the catecholamine content of some of the postganglionic neurons is in some way affected by means of preganglionic impulses. This, however, does not appear to be the case, since animals in which preganglionic denervation of the superior cervical ganglion had been performed 14–18 days before dissection, showed about the same degree of variation in fluorescence intensity among the ganglion cells, as did normal animals.



Superior cervical ganglion from a rat given nialamide 500 mg/kg, $5\frac{1}{2}$ h previous to autopsy.

Zusammenfassung. Sympathische Ganglien wurden mit einer spezifischen und besonders empfindlichen histochemischen Methode zum Nachweis gewisser Monoamine studiert. Die meisten Zellkörper der Ganglienzellen enthalten im Cytoplasma mehr oder weniger einer primären Monoamine, wahrscheinlich Noradrenalin. In prävertebralen Ganglien wurden auch adrenerge Nevenendigungen nachgewiesen, die synaptische Verbindungen mit den Ganglienzellen eingehen.

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The Use of Lytic Enzymes of *Micromonospora spp.* to Prepare Protoplasts of Yeasts

In the past few years great progress has been made in the field of bacterial anatomy, due largely to the utilization of the cell wall lytic activity of various enzymes, mainly the egg white lysozyme. More recently, striking advances have been obtained with other groups of organisms due to the introduction in this practice of enzymes from the gut of the snail *Helix pomatia*[1] and from growth media filtrates of *Streptomyces spp.*[2,3].

For the past months, studies have been made in the author's laboratory on cell wall lytic agents active against yeast and moulds. Microorganisms capable of lysing the cell walls of these organisms were isolated from soil samples. Some of the results obtained have already been described in previous papers[4–8]. So far as the authors' knowledge goes, the work reported here is the first specifically designed to obtain protoplasts of yeast using the lytic enzymes produced by species of *Micromonospora*. Recently a group of Japanese workers has reported in a preliminary note the lysis of BCG cell walls by extracts of *Micromonospora sp.*[9].

The method of obtaining a partially purified lytic enzyme preparation has been described elsewhere[10]. Further purifications of the enzyme(s) has been attempted using various absorbants, and although some improvement was obtained, satisfactory results have not yet been found. Further studies on this point are in progress.

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The strain of yeast used in these experiments was *Candida utilis* due to the facility of distinguishing the formation of protoplasts and to the remarkable sensitivity that these cells exhibited to the enzyme preparation. Naked protoplasts were prepared using young cells of the yeast suspended in 0.1 *M* phosphate buffer pH 6.8 containing 0.8 *M* mannitol. For conversion of the yeast cell to protoplasts, 0.2 ml of solution of the enzyme containing between 15–20 mg per ml was added per ml of incubation mixture. The digestion of the cell wall was allowed to take place at 30°C for a few hours with gentle agitation. The cells of this organism can be seen to separate into discrete spheres under the influence of the enzyme preparation which is capable of digesting their cell walls. If the treatment is carried out in media of appropriate solute concentrations, stable forms are produced which will lyse if the suspension medium is diluted. The spherical forms lysed in distilled water leave a delicate membrane or wall.

The *Micromonospora* enzyme system was better for the release of the protoplasts than the commercial preparations obtained from *Helix pomatia* since the snail mixture ultimately attacked also the protoplast membrane. The activity of that system seems to be higher than the 'streptzyme'[2], not only when acting on the intact cells but also on the isolated cell walls.

That true naked protoplasts were formed was suggested by the osmotic fragility of the preparations and by the absence of most of the cell wall components (unpublished observations). Isolated cell walls were not completely dissolved by the enzyme treatment and it may be that some structural component still remained. The reduction in optical density of isolated yeast cell walls or intact cells was actually due to lysis of the cell walls as demonstrated by microscopic observation and by the release of cell wall components which reacted with rabbit anticell-wall serum. Details of these studies will be published later, together with the results of chemical investigations on the lysates and residual materials.

The susceptibility of different strains of yeast to cell wall digestion by the *Micromonospora* enzyme(s) is variable. While some strains will yield protoplasts readily from others under similar conditions, very few or no protoplasts can be obtained even after prolonged digestion. With strains of *Torulopsis aedis*, *C. utilis*, and *Schizosaccharomyces zambesi*, it is found that in general it is much easier to obtain protoplasts from young vigourously growing cultures, which is in good agreement with results found previously using other enzyme preparations. In *C. utilis* the cell wall seems to be attacked very uniformly with practically no release of untacked wall structures in the suspending medium.

Résumé. On met en évidence pour la première fois l'action lytique d'une espèce de *Micromonospora* qui a le pouvoir de digérer la paroi cellulaire des levures, donnant lieu à la formation de structures possédant des propriétés de protoplastes. Cette enzyme que nous avons déjà partiellement purifée, montre plus d'activité que d'autres systèmes semblables quant à l'obtention de protoplastes ainsi que de meilleures propriétés.

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Experimental Observations on the Participation of Macrophages in the Mechanism of Antibody Production[1]

It is a well known fact that the antibody formation persists for a long time after the penetration of the antigens into the organism, even after particulate antigens are no longer recognizable in it. Recently the persistence of fragments of labeled soluble antigen for many months, and perhaps for years, has been affirmed in the cells of an immunized animal[2].

It therefore seems likely that such antigenic derivatives are the carriers of the antigenic information or are the antigenic information itself. Nevertheless, there is no agreement on the possible antigenic activity or antigenic information activity of these derivatives of the partial disintegration of the antigen molecules[2–5].

We have investigated whether it is possible to demonstrate any antigenic information activity in the macrophages which have ingested a bacterial antigen, even after the taken-up bacteria are no longer visible in the cytoplasm of the macrophages themselves. We used the transfer of peritoneal-exudate macrophages from an intraperitoneally immunized animal to a healthy animal. Before the transfer, the macrophages were washed in order to prevent the transfer of any antibodies, and were also injured so as not to allow any antibody-producing action on their part in the host.

HARRIS and HARRIS[6] have, however, emphasized that, both with the transfer of injured cells to a healthy animal, and with the transfer of living cells to an irradiated animal, it is not possible to show in the serum of the host any antibodies against the antigen used to immunize the donor. Our attempts to demonstrate agglutinating antibodies in the serum of the host also gave negative or debatable results.

We therefore used the agglutination test *in vitro*, described by REISS, MERTENS, and EHRICH[7], to investigate the antibody activity of individual lymph node cells of the host.

Here we briefly report some indication about the material and the method used.

The donor rabbits were immunized with intraperitoneal injections of *Salmonellae Ballerup* killed with formol; the suspension of *Salmonellae* was emulsified with sterile vaseline oil. Three injections were made with a time interval of 48 h between each; in the first two there were 2 ml of bacterial suspension (titre 8×10^9) and in the third 6 ml; in each there were 10 ml of vaseline oil.

The peritoneal exudate was taken 5–10–15 days after the last immunizing injections. It contained on the

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