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was suggested by the osmotic fragility of the preparations and by the absence of most of the cell wall components (unpublished observations).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.

A. G. OCHOA, I. GARCIA ACHA,
S. GASON, and J. R. VILLANUEVA

Instituto 'Jaime Ferrán' de Microbiologia, Centro de Investigaciones Biologicas, Madrid (Spain),
June 26, 1963.

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

[1] This work has been supported by a grant from the C.E.C.A. – European Community of Coal and Steel.
[2] J. S. GARVEY and D. H. CAMPBELL, J. exp. Med. *110*, 355 (1959).
[3] F. J. DIXON, J. cell. comp. Physiol. *50*, Suppl. 1, 27 (1957).
[4] D. H. CAMPBELL, Blood *12*, 589 (1957).
[5] P. D. MCMASTER and J. L. EDWARDS, J. exp. Med. *106*, 219 (1957).
[6] T. N. HARRIS and S. HARRIS, in *Ciba Foundation Symposium on Cellular Aspects of Immunity* (Churchill Ltd., London 1960), p. 172.
[7] E. REISS, E. MERTENS, and W. E. EHRICH, Proc. Soc. exp. Biol. Med. *74*, 732 (1950).

average 1 000 000 cells/ml, with about 70% macrophages; no *Salmonellae* were seen in the macrophages. The cells were repeatedly washed in a saline solution, and then frozen. 3 ml of suspension of cellular material were then injected, immediately after it had been heated to 38 C, into the foot-pad of the hosts; two more injections were made, after 5 and 10 days, respectively.

Five days after the last injection into the foot-pad, the popliteal lymph node was taken; this was partly used for histological examination and partly dilacerated into a saline solution to obtain a cellular suspension. We observed histologically a reactive hyperplasia with plenty of pyroninophilic cells. By bringing the suspension of lymph node cells into contact with a *Ballerup Salmonellae* suspension, we observed agglutination of *Salmonellae* against lymphoid or transitional cells, and sometimes even in the medium. The agglutination was quite evident in the fresh state, but it could also be spotted in dried specimens stained by the Giemsa method. The agglutination test was positive after transfer of macrophages taken from the donor 5–10–15 days after the last immunizing injection.

To check the specificity of the test, it was repeated each time, using the suspension of popliteal lymph node cells from the host, and a bacterial suspension different from the one used to immunize the donor; we used in the controls a suspension of *B. subtilis*. No agglutination occurred in these controls, nor in other controls when the suspension of *Salmonellae* was brought into contact with a suspension of lymph node cells from an untreated animal. It must be emphasized that research with the usual methods of antibodies agglutinating the *Salmonellae Ballerup* in the serum of the recipient rabbits always gave negative or debatable results.

From the results of this research, we believe that there is an antibody activity in the popliteal lymph node cells of the host after injection of macrophagic material from the immunized donor into the foot-pad, and that this antibody activity is specific against the bacteria used to immunize the donor. It is therefore probable that the macrophages of the latter, after ingestion of the bacterial antigen, are capable of transferring an antigen action or an antigenic information action; this is possible even after the bacterial antigen is no longer microscopically visible in the macrophages. The antibody response in the host is nevertheless limited, and does not reach an hematic rate detectable by common serological methods. The agglutination test between popliteal lymph node cells of the host after transfer by injections into the foot-pad, and the bacteria used to immunize the donor, is useful in bringing to light such a subliminal antibody response.

Riassunto. Gli autori hanno iniettato nella zampa di conigli riceventi materiale macrofagico di essudato peritoneale di conigli donatori immunizzati con *Salmonellae* per via intraperitoneale; hanno poi osservato agglutinazione *in vitro* fra cellule di linfonodo popliteo degli animali riceventi, e *Salmonallae* del ceppo usato per l'immunizzazione. Gli autori ritengono quindi probabile che i macrofagi abbiano la capacità di trasferire stimoli antigenici.

F. Mollo, M. Governa,
C. Citati, and M. Dellepiane

Istituto di Anatomia e Istologia Patologica dell'Università di Torino (Italy), February 27, 1963.

Partielle Sprengung der Erythrocyten[1]

Tomcsik und Litschel[2] haben unlängst beschrieben, dass Hyaluronsäure in einem pH-Bereich von 2 bis 3,6 anstelle von Hämolyse eine Reihe überraschender Änderungen in der Erythrocytenstruktur hervorruft, von denen die wichtigsten sind: (a) augenblickliche, ruckartige Vergrösserung der Zelle, (b) plötzliche Sprengung des grössten Teiles des in Körnchen fixierten Hämoglobins, (c) Ruhestadium der nach der partiellen Sprengung zurückgebliebenen und in mehreren Schichten gespaltenen Membranen.

Wir konnten in der hämatologischen Literatur der letzten 60 Jahre keine ähnliche Beobachtung finden. Zu unserer Überraschung wurde aber genau vor 100 Jahren ein prinzipiell ähnliches Phänomen mit einer anderen Substanz entdeckt und in den folgenden 40 Jahren von zwei Autoren bestätigt.

Hensen[3] fand, dass nach Zugabe von Harnstoff die Erythrocyten quellen und ihre Membran «weicher» wird. Nach Roberts[4] entstehen «macula» an der Erythrocyten-«Zellwand», wenn gesättigtes Rosanilinnitrat einer Erythrocytensuspension zugegeben wird. Roberts entdeckte vor 100 Jahren in der gleichen Arbeit ein hochinteressantes Phänomen nach Zugabe von Tannin: «pullulation» des Erythrocyteninhaltes; ein Phänomen, welches in gewissen Details wahrscheinlich der Hyaluronsäurewirkung entspricht. Kaliumferrocyanid, Magnesiumsulfat, Jod, Alkohol, Glycerin, Zucker, Gallussäure, Karbol, Atropin, Morphiumderivate, Gummi und Kaffeeinfusion waren unwirksam; Pikrinsäure verursachte weniger deutliche Änderungen als Rosanilinnitrat. Kollmann[5] bestätigte die Befunde von Roberts mit 0.5% Tannin. Teilerscheinungen (wahrscheinlich Membranspalten) erhielt er auch mit 15% Harnstofflösung. Weidenreich[6] beschrieb und illustrierte mit dem Zeißschen Zeichenapparat die Wirkung einer $^3/_4$prozentigen Tanninlösung auf Erythrocyten. Nach Erweiterung der «Erythrocyten-Hülle» erfolgte ihr Platzen und dann erschien ganz plötzlich die zuerst von Roberts beobachtete «pullulation» des Zellinhaltes. Weidenreich beobachtete gewisse Teilerscheinungen der Tanninwirkung auch bei Verwendung von Pikrinsäure und von Jodsäure.

Manche Ausdrücke der hier zitierten älteren Arbeiten über Erythrocyten sind heute unverständlich oder mindestens unklar. Die Existenz von «Stroma» und diejenige einer echten oder künstlichen Membran, die Glockenform und Isotonie der Erythrocyten wurden ausgiebig diskutiert; doch scheint es wahrscheinlicher zu sein, dass 0,5 bis 1% Tanninlösung ein prinzipiell ähnliches Phänomen an Erythrocyten auslöst, wie wir es vor kurzem mit Hyaluronsäure beobachtet haben. Der Zweck der vorliegenden Arbeit ist, die Wirkung der Hyaluronsäure mit derjenigen des Tannins zu vergleichen.

Die Methoden und Materialien wurden bereits in unserem ersten Bericht[2] beschrieben.

[1] Diese Arbeit wurde von der H.-Buss-Stiftung (Basel) unterstützt.

[2] J. Tomcsik und Edith Litschel, Proc. Soc. exp. Biol. Med., im Druck.

[3] Hensen, Z. wiss. Zool. *11*, 253 (1862).

[4] W. Roberts, Quart J. micr. Sci. *3*, 170 (1863).

[5] J. Kollmann, Z. wiss. Zool. *23*, 463 (1874).

[6] F. Weidenreich, Arch. mikr. Anat. *61*, 459 (1902).