found in the sub-fractions eluted by buffers of higher molarity than in fractions eluted by buffer of lower molarity. In the different experiments, the largest difference in this respect between sub-fractions originating from the same γ-globulin preparation varied 2–5 titre steps.

The possibility that this difference could be explained by an enrichment in the more active sub-fractions of aggregated 7*S* γ-globulin, which may have a high agglutination-inhibiting activity in the *Gm* grouping test system[5], was excluded by the following experiment. γ-Globulin sub-fractions obtained by hydroxyl apatite chromatography were submitted to zone ultracentrifugation in a sucrose gradient. The top fractions in this way freed from any fast-sedimenting 7*S* γ-globulin aggregates were recovered and tested in the *Gm* grouping system. The same difference in agglutination inhibition was observed as before ultracentrifugation.

The serological investigation of the γ-globulin sub-fractions of the serum containing incomplete *Rh*-antibodies is shown in Table 1. Besides the differences mentioned above, an enrichment of the *Rh*-antibodies in the sub-fractions eluted by buffer of high molarity was observed.

These observations thus indicate that hydroxyl apatite chromatography of human 7*S* γ-globulin may give sub-fractions differing in serological character.

Ulf Nilsson

Department of Clinical Chemistry,
University of Uppsala.

[1] Grubb, R., *Acta Path. Microbiol. Scand.*, **39**, 195 (1956).
[2] Grubb, R., and Laurell, A.-B., *Acta Path. Microbiol. Scand.*, **39**, 390 (1956).
[3] Hjertén, S., *Biochim. Biophys. Acta*, **31**, 216 (1959).
[4] Heremans, J., *Les globulines sériques du systéme gamma*, 119 (Editions Arscia, S.A., Bruxelles, 1960).
[5] Kunkel, H. G., Müller-Eberhard, H. J., Fudenberg, H. H., and Tomasi, T. B., *J. Clin. Invest.*, **40**, 117 (1961).

PATHOLOGY

Intracellular Response to an Iso-immune Reaction at the Surface of Ascites Tumour Cells

The cytotoxic effect on mouse ascites tumour cells treated *in vitro* with various antibodies has been shown to require the presence of complement[1,2]. In the absence of complement the cells appear to be normal when viewed by phase contrast microscopy[3]; they retain their capacity to exclude trypan blue[2] and to metabolize glucose[4]. The only changes which have been reported are slight alterations in the fine structure of the cell surface, which were thought to favour agglutination by rabbit antibody directed against ascites cells[3].

There is strong evidence, from hæmagglutination work, that some iso-antigens of the *H*-2 system[6] are present on the surface of mouse erythrocytes[5]. The present investigation was undertaken to determine whether such iso-antigens could be demonstrated on the surface of *EL4* ascites cells, and whether histochemical methods could be used to reveal the effect on the cell of an iso-immune reaction at its surface, in the absence of complement.

Iso-antisera were prepared in *BALB/c* strain mice against *EL4* tumour cells resident in *C57BL* strain mice by immunization with tumour cells mixed with Freund's complete adjuvant. The mice and tumour were the generous gift of the late Dr. P. A. Gorer. The cytotoxic titres[2] of these antisera, in the presence of complement, varied from 1/64 to 1/256. In the absence of complement, iso-antibody caused negligible agglutination of cells even when present at as high a concentration as 1 vol. antiserum: 1 vol. cell suspension containing 2×10^7 cells/ml. This was in marked contrast to the strong agglutinating effect of rabbit antibody which was observable even with rabbit antiserum diluted 256 times.

It was first necessary to confirm that the mouse iso-antibody did react with surface antigens. This was effected by incubating suspensions of *EL4* cells with mouse antiserum (preheated for 30 min. at 56° C.) in the absence of added complement. That the antibody was located exclusively on the cell surface was observed by subsequent staining of air-dried smears of washed cells with a rabbit anti-mouse globulin which had been conjugated with fluorescein isothiocyanate.

It was remarkable that in these circumstances, which were expected to produce purely surface effects, marked cytoplasmic changes were observed when histochemical techniques were applied to air-dried smears. Thus, exposure of the cells to iso-antiserum alone, at 37° C. for 15 min., in the absence of complement, resulted in marked activation of lysosomal acid phosphatase[7]; there was also simultaneous unmasking of cytoplasmic phospholipid, particularly in discrete granules, as shown by the acid hæmatin technique[8]. The stimulation of glucose dehydrogenase activity observed under similar conditions could have been a non-specific effect since it was also observed after incubation of these cells with normal serum.

The immunofluorescent results indicated that this iso-antibody acted solely at the surface of the cells under these experimental conditions. If this were true, then the effect of the immune reaction at the cell surface in causing changes in intra-cytoplasmic particles was surprising.

In homograft rejection, circulating antibody may act synergically with immunologically competent host cells, though the extent to which complement is involved is uncertain. It is possible that intra-cytoplasmic effects of iso-antibody acting at the donor cell surface may play some part in the mechanism of rejection.

We are indebted to Prof. E. G. L. Bywaters, Dr. L. E. Glynn and Dr. E. J. Holborow. One of us (L. B.) wishes to thank the Trustees of the Prophit Fund for a research studentship.

D. C. Dumonde
Clarice M. Walter

Medical Research Council Rheumatism Research Unit,
Canadian Red Cross Memorial Hospital,
Taplow, Maidenhead, Berks.

Lucille Bitensky
G. J. Cunningham
J. Chayen

Department of Pathology,
Royal College of Surgeons,
Lincoln's Inn Fields,
London, W.C.2.

[1] Wissler, R. W., and Flax, M. H., *Ann. N.Y. Acad. Sci.*, **69**, 773 (1957).
[2] Gorer, P. A., and O'Gorman, P., *Transplantation Bull.*, **3**, 142 (1956).
[3] Goldberg, B., and Green, H., *J. Exp. Med.*, **109**, 505 (1959).
[4] Bickis, I. J., Quastel, J. H., and Vas, S. I., *Cancer Res.*, **19**, 602 (1959).
[5] Gorer, P. A., *Adv. Cancer Res.*, **4**, 149 (1956).
[6] Snell, G. D., *J. Genet.*, **49**, 87 (1948).
[7] Bitensky, L., *Quart. J. Micro. Sci.* (in the press).
[8] Baker, J. R., *Quart. J. Micro. Sci.*, **87**, 441 (1946).