Table 1. Lactic dehydrogenase activity and isoenzyme distribution in muscle of mice bearing a mammary carcinoma and of controls

Weeks after transplantation	Total LD (IU/g)	Isoenzyme (%) LD 1	LD 2	LD 3	LD 4	LD 5
0	443±47	11±1.2	12±1.6	15±1.8	22±2.1	40±2.1
1	440±30	6±1.6	12±1.6	14±0.7	19±1.9	40±2.8
2	459±28	5±0.75	9±1.04	14±0.5	19±1.1	53±2.4
3	588±48	5±0.55	8±0.85	11±0.9	20±1.2	56±2.7
4	441±42	0	5±0.5	6±0.7	29±0.9	60±1.5
5	402±43	0	6±0.2	8±0.2	26±1.2	60±2.3
6	340±49	0	0	5±0.5	33±0.67	62±3.9
2 weeks after resection of a 4-week tumor	394±44	1±0.6	6±0.7	10±1.5	28±1.9	55±2.5

Table 2. Lactic dehydrogenase activity and isoenzyme distribution in the mammary carcinoma

Weeks of growth of tumor	Total LD (IU/g)	Isoenzyme (%) LD 1	LD 2	LD 3	LD 4	LD 5
3	245±28.6	0	0	5±0.5	36±2.1	59±2.7
4	213±27.1	0	0	9±0.3	25±1.1	66±1.8
5	139±18	0	0	6±0.2	31±0.2	63±1.5
6	180±27.5	0	0	6±0.3	34±1.5	60±1.9

1 This study was supported by a grant from the Minnesota Masonic Memorial Cancer Research Fund and, in part, by Grant No. 16-P-5681015-17 from the Rehabilitation Services Administration, U.S. Dept. of Health, Education and Welfare.
2 G. Pfleiderer and E.D. Wachsmuth, Biochem. Z. *334*, 185 (1961).
3 R. Richterich and A. Burger, Enzymol. Biol. Clin. *3*, 65 (1963).
4 R.D. Goldman, N.O. Kaplan and T.C. Hall, Cancer Res. *24*, 389 (1964).
5 H. Poznanska-Linde, J.H. Wilkinson and W.A. Withycombe, Nature *209*, 727 (1966).
6 R. Hilf, W.D. Rector and R.A. Orlando, Cancer *37*, 1825 (1976).
7 F. Schapira, Adv. Cancer Res. *18*, 77 (1973).
8 N.Q. Hanson and E.F. Freier, Clin. Chem. *20*, 769 (1974).
9 Lactic Dehydrogenase Isoenzyme Assay, Manual No. 55339, Beckman Instruments, Inc. 1973.
10 A.L. Latner, D.M. Turner and S.A. Way, Lancet *2*, 814 (1966).
11 R. Yasin and F. Bergel, Eur. J. Cancer *1*, 203 (1965).
12 E. Langvad, Int. J. Cancer *3*, 17 (1968).
13 R. Woollams, P.J. Barratt, R.L. Orwell and D.W. Piper, Digestion *14*, 20 (1976).
14 R. Tayler, V.H. Cumberland and D.W. Piper, Gut *18*, 45 (1977).
15 P. Carda-Abella, S. Perez-Cuadrado and J. Mate-Jimenez, Cancer *42*, 490 (1978).
16 E.V. Parina, S.A. Mykhailova and V.S. Antonov, Biokhimia *43*, 706 (1978).
17 K. Lundholm, S. Edstrom, L. Ekman, I. Karlberg, A.C. Bylund and T. Schersten, Cancer *42*, 453 (1978).
18 C.P. Holroyde, R.S. Axelrod, C.L. Skutches, A.C. Haff, P. Paul and G.A. Reichard, Cancer Res. *39*, 4900 (1979).

Estimation of nonspecific lectin-mediated staining of glutaraldehyde-fixed cells

N. Gilboa-Garber and L. Mizrahi

Department of Life Sciences, Bar-Ilan University, Ramat-Gan (Israel), 17 March 1980

Summary. Lectin-mediated stainings are widely used for the visualization of carbohydrate-carrying cellular components using the electron microscope. The use of glutarladehyde-fixed cells for these stainings introduces the possibility of low nonspecific lectin-trapping by the glutaraldehyde which coats the cells. This trapping was estimated by means of peroxidase-binding to human leukocytes, *Tetrahymena pyriformis* and *Escherichia coli* cells and was shown to be prevented by rinsing the glutaraldehyde-fixed cells in an amino acid solution before exposure to the lectin.

The use of concanavalin A-mediated peroxidase-binding to cells for ultrastructural cytochemistry was first described by Bernhard and Avrameas[1]. The method is based on the specificity of Con A for α-D-glucopyranosyl, α-D-mannopyranosyl or β-D-fructofuranosyl residues[1,2] and on its ability to react at one of its active sites with a cell-bound sugar and at the other active site with the sugar of horseradish peroxidase. The bound peroxidase is revealed by the diaminobenzidine (DAB) method of Graham and Karnovsky[3]. In the controls, 0.2 M α-methyl mannoside is added to the Con A and peroxidase solutions[1]. The sugar addition inhibits the specific binding of Con A to the cells and the peroxidase-binding to nonspecifically bound Con A. It does not prevent nonspecific Con A-binding to the cells. While nonspecific Con A trapping is negligible when untreated cells are used, glutaraldehydefixed cells[1,4,5] may trap some Con A due to remaining free aldehyde groups[6,7]. This trapped lectin will then be stained similarly to the specifically bound lectin and will not be detected in those controls which contain the free sugar in the peroxidase solution[1].

Con A-mediated peroxidase-binding to human leukocytes, tetrahymenas and *E.coli* cells as revealed by the peroxidase elution from the cells by D-mannose and examination of its activity according to Huet and Bernadac[11]

Treatment			Peroxidase activity*		
Glutaraldehyde fixation	Glycine treatment	D-mannose addition	Leukocytes	Tetrahymenas	*E.coli*
−	−	−	80.0	119.0	2.8
−	−	+	0	1.5	1.7
+	−	−	94.0	136.1	14.0
+	−	+	11.2	14.0	11.2
+	+	−	81.6	118.1	1.9
+	+	+	0	3	1.7

* Peroxidase activity is expressed as nmoles/ml/min dianisidine oxidized by the peroxidase which was eluted to the supernatant by addition of 0.2 M D-mannose to the cells. Values represent means of 3 experimental results.

Similar methods are also used for the ultrastructural visualization of cell-coat components by means of other lectins[8], with peroxidase, ferritin[9,10] or colloidal gold[11,12].

The aim of the present study was to examine the extent of the nonspecific lectin trapping to glutaraldehyde-fixed cells as compared to that of glutaraldehyde-fixed cells which were rinsed in an amino acid solution after the fixation. The lectin-binding was assessed by determination of Con A-mediated peroxidase-binding[13]. The cells used were human peripheral leukocytes and *Tetrahymena pyriformis,* both known to contain Con A-binding receptors[14], as well as bacteria which lack such receptors.

Materials and methods. Leukocytes were isolated from healthy human bloods drawn into heparinized tubes[15] and washed 3 times in phosphate buffered saline (PBS). *Tetrahymena pyriformis* GL cells were cultured aseptically in 2% proteose peptone +0.1% yeast extract at 28–29 °C for 2 days without shaking. After 3 washings, the tetrahymenas were treated with 0.1 M Na fluoride (to arrest their vigorous movement) and again washed 3 times in PBS. *Escherichia coli* cells were grown on nutrient broth (Difco) at 23 °C for 20 h and washed 3 times in PBS.

The washed cell suspensions were divided into 3 equal parts. After sedimentation by centrifugation, the cells in the 1st sample were resuspended in 1 ml of either PBS or Cacodylate-HCl buffered saline and the other 2 samples were fixed in 1 ml of 2% (v/v) glutaraldehyde solution in the same buffered saline. After 1 h the cells were washed in the respective buffered saline and 1 of the 2 glutaraldehyde-treated cell samples was suspended in 1 ml of 0.2 M glycine solution. After 30 min in the glycine solution, these cells were again washed in the buffered saline. The 3 cell samples were then equally divided into 2 tubes (to make 3 pairs of tubes). In each pair 0.3 ml of saline was added to one tube and 0.3 ml of 0.2 M D-mannose solution to the other tube. Then 0.3 ml of Con A solution (1 mg/ml, purchased from Miles-Yeda, Ltd, Rehovot, Israel) was added to all the tubes. After 1 h the cells were washed 3 times in PBS and exposed to 50 µg of horseradish peroxidase (from Sigma, type II) in 1 ml of PBS for 1 h. After 5 washings in PBS, the specifically bound peroxidase was eluted from the cells by 0.8 ml of a 0.2 M D-mannose solution. The supernatant fluid (0.5 ml) obtained after 30 min at room temperature was examined for peroxidase activity[13] by the addition of 2 ml of dianisidine solution (containing 0.1 mg 3,3′-dimethoxybenzidine 2 HCl in 1 ml of 0.12 M phosphate buffer at pH 7) and 0.1 ml of 0.005% H_2O_2. The reaction was terminated after 1 min by 0.1 ml of 6 N HCl and absorbance at 420 nm was recorded.

Results and discussion. The table demonstrates peroxidase elution from Con A-bound to unfixed, as compared to glutaraldehyde-fixed cells. The results presented in this table indicate that glutaraldehyde fixation of leukocytes and tetrahymenas leads to additional Con A-binding which is not prevented by 0.2 M D-mannose in contrast to the specific Con A-binding to unfixed cells. After glycine-treatment of the glutaraldehyde-fixed cells, Con A-binding is similar to that obtained with the unfixed cells and is abolished by D-mannose. Glutaraldehyde-fixed bacteria also trap Con A, in contrast to unfixed bacteria, which do not exhibit any significant Con A-binding. The extent of this nonspecific binding is similar to the additional binding observed with the glutaraldehyde-fixed leukocytes and tetrahymenas and is also resistant to D-mannose addition. Essentially the same results were obtained whether phosphate or cacodylate was used for buffering. The described results indicate that the use of glutaraldehyde-fixed cells without neutralization with an amino acid solution for the electron microscope stainings[1,5] may suffer from some nonspecific staining due to Con A trapping by the glutaraldehyde. Therefore, some of the differences described between the findings obtained when Con A was added to fixed, as compared to unfixed cells[1], may also be due to such nonspecific binding. The controls in which D-mannose or methyl α-D-mannoside are added to the Con A and peroxidase solutions[1] do not reveal this binding, since the bound Con A cannot bind the peroxidase and be stained in the presence of the sugar. If peroxidase without sugar is added, the nonspecific Con A trapping may be revealed. A weak nonspecific staining with peroxidase in such controls, or in controls treated with peroxidase without Con A, was described[4] and could probably be prevented by neutralization of the free aldehyde groups by glycine or NH_4Cl[6,7]. We therefore suggest introducing the herein suggested correction to these very important cytochemical methods for localization of lectin binding sites on glutaraldehyde-fixed cells.

1 W. Bernhard and S. Avrameas, Exp. Cell Res. *64,* 232 (1971).
2 I.J. Goldstein, L.L. So, Y. Young and Q.C.I. Callies, J. Immunol. *103,* 695 (1969).
3 R.C. Graham and M. Karnovsky, J. Histochem. Cytochem. *14,* 291 (1966).
4 E. Wyroba, Ann. med. Sect. Pol. Acad. Sci. *20,* 143 (1975).
5 W. De Souza, M.M. Bunn and J. Angluster, J. Protozool. *23,* 329 (1976).
6 S.M. Brown and J.P. Revel, J. Cell Biol. *68,* 629 (1976).
7 I. Nir and M.O. Hall, Exp. Eye Res. *29,* 181 (1979).
8 C.H. Huet and J. Garrido, Exp. Cell Res. *75,* 523 (1972).
9 I. Virtanen and J. Wartiovaara, J. Cell Sci. *22,* 335 (1976).
10 P.A.M. Eagles, L.N. Johnson and C. Van Horn, J. Cell Sci. *19,* 33 (1975).
11 M. Horisberger and J. Rosset, J. Histochem. Cytochem. *25,* 295 (1977).
12 G. Csaba and B. Madarasz, Experientia *35,* 1181 (1979).
13 C.H. Huet and A. Bernadac, Exp. Cell Res. *89,* 429 (1974).
14 A. Frisch, W. Bessler, H.J. Lipps and D. Ammermann, J. Protozool. *23,* 427 (1976).
15 D. Nelken, N. Gilboa-Garber and J. Gurevitch, J. clin. Path. *13,* 266 (1960).