Characterization of deoxyribonuclease activities derived from control and inflammation-associated mouse peritoneal macrophages

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Native DNAase (deoxyribonuclease) activities derived from mouse peritoneal cavity and peripheral blood components were separated, detected, and characterized by electrophoresis into polyacrylamide gels containing DNA, followed by incubation of the gels, and staining of the substrate to reveal only the DNAase activities. Resident peritoneal macrophages contained 12 DNAase-II-like activities that were characteristic of that cell type, whereas lymphocytes and granulocytes each contained five DNAases. Induction of inflammation by peritoneal injection of thioglycollate resulted in changes in macrophage DNAase expression, including: increased total DNAase activity, a decrease in the number of activities from 12 to 11, increased activity of a specific subset of the enzymes, and a change in the apparent size of a specific subset of the enzymes. Electrophoretic and enzymic properties and sensitivity to endo- β -N-acetylglucosaminidase H indicated that the macrophage activities probably represented charge variants of one or two parent peptide chains.

DNAases have been identified in alveolar, peritoneal (Meyers *et al.*, 1970) and liver (Berg & Boman, 1973) macrophages. However, the enzymes were observed only as gross activities in unfractionated extracts by using convenient colorimetric assays and were characterized simply as having acid pH optima. The number of macrophage DNAases, their biochemical and enzymic properties, their genetic relatedness, and their biological significance were not examined.

A variety of macrophage hydrolytic enzymes are increased by peritoneal injection of inflammation-inducing agents such as thioglycollate (Karnovsky & Lazdins, 1978). This altered enzyme expression may correspond to an enhanced ability of the macrophages to combat infection. In apparent conflict with this hypothesis has been the observation that some enzyme classes exhibit activities that are essentially unchanged or even depressed during inflammation (Karnovsky & Lazdins, 1978). However, it is possible in these cases that inflammation accentuates the expression of individual isoenzymes or that the enzymes are modified in some manner that enhances their biological role.

Characterization of individual enzymes usually

Abbreviations used: Bes, 2-[bis-(2-hydroxyethyl)-amino]ethanesulphonic acid; DNAase, deoxyribonuclease; Mes, 4-morpholine-ethanesulphonic acid; SDS, sodium dodecyl sulphate.

requires laborious purifications and large amounts of starting material, and often results in minor activities and quantitative relationships between enzymes being overlooked. Fortunately, proteins present in minute quantities of material can be resolved easily by polyacrylamide-gel electrophoresis. Inclusion of substrate within the gels permits detection of individual isoenzymes within enzyme classes after incubation of the gels under appropriate conditions and staining either substrate or product. Although most groups use SDS or other protein denaturants to minimize interactions between nucleases and substrate during electrophoresis (Rosenthal & Lacks, 1977), such a procedure is undesirable, owing to the uncertainty of full renaturation and the loss of information resulting from the simplification of electrophoretic properties (Blank & Dekker, 1981). However, by careful attention to electrophoretic conditions, one-dimensional polynucleotide/polyacrylamide-gel electrophoresis has been used in the absence of protein denaturants to characterize the individual native DNAase activities in human leucocytes (Zollner *et al.*, 1975; Brown *et al.*, 1982; Karpetsky *et al.*, 1982). In the present study, we have used this electrophoretic technique in the identification and partial characterization of the native DNAase activities in control and inflammatory peritoneal macrophages.

Experimental

Reagents were purchased from the following sources: Flow Laboratories, Rockville, MD, U.S.A. (minimum essential medium, and foetal-calf serum); Health Research Inc., Albany, NY, U.S.A. (endo- β -*N*-acetylglucosaminidase H). Disease-free mice and sterile thioglycollate broth were obtained through National Institutes of Health, Bethesda, MD, U.S.A. Single-stranded DNA was prepared by heating calf thymus DNA in distilled water to 105°C for 10 min, followed by rapid cooling. Denaturation of DNA was verified by measuring the decrease in hypochromicity (Felsenfeld & Sandeen, 1962). Peritoneal cells were collected from AKR mice before the development of leukaemia. Other materials were obtained from the sources given by Brown *et al.* (1982).

Sample preparation

Mouse peritoneal cells were harvested by peritoneal lavage with minimum essential medium as described by Adams (1979). Cells were processed at 0–4°C. Cells were washed twice with minimum essential medium, counted with an electronic cell counter (model ZBI; Coulter Electronics, Hialeah, FL, U.S.A.), and pelleted (15600g) for 2 min in a bench-top centrifuge (model 5412; Eppendorf, Hamburg, Germany). Cell pellets were stored at –80°C. Inflammation-associated macrophages generally were obtained by giving each mouse a peritoneal injection of 1 ml of thioglycollate (or other agents) and harvesting peritoneal cells 5 days later. Macrophages were not purified routinely, and 'peritoneal macrophages' in this paper refers to unfractionated peritoneal cells.

Peripheral blood was obtained by retro-orbital bleeding into 30 U.S.P. units of heparin per mouse. Total peripheral leucocytes were prepared as described for human cells (Brown *et al.*, 1982). Mononuclear cells (95–100% pure) were prepared by centrifuging whole blood over Ficoll/Hypaque as described by Böyum (1968). The pellets were used for purification of neutrophils (85–95% pure) by the procedure used for total leucocytes. Cell preparations were checked for aggregates at the time of cell counting. Cells were pelleted on slides (Cytospin Centrifuge; Shandon Southern Instruments, Sewickley, PA, U.S.A.), and stained (Harleco Diff-Quik; Dade Diagnostics, Aguada, PR, U.S.A.). Duplicate slides were developed for non-specific esterase with α -naphthyl butyrate (Li *et al.*, 1973).

Cells were assayed for 5'-nucleotidase activity (Edelson & Cohn, 1976) by using 200 μ l reaction mixtures containing supernatant obtained from 10^5 macrophages. Total DNAase activity was measured by preincubating cell-free extracts in 50 mM-citrate/phosphate (50 mM- Na_2HPO_4 , pH

adjusted to 4.0 with citric acid) containing 0.5 mM-phenylmethanesulphonyl fluoride, for 1 h at 37°C to degrade endogenous DNA. ^3H -labelled *Escherichia coli* DNA was added to a concentration of 3 $\mu\text{g}/\text{ml}$ and the reactions were continued. Initial rates of degradation, determined from the decrease in 5% (w/v) trichloroacetic acid-precipitable radioactivity, were used to determine relative enzyme units per cell.

Polynucleotide/polyacrylamide-gel electrophoresis

Gel and sample preparation, electrophoresis and development of enzyme activity were performed as described previously (Brown *et al.*, 1982; Karpetsky *et al.*, 1982). The enzyme extraction procedure, involving sonication of the cells, was efficient and yielded reproducible patterns, since sonication for longer times, freeze-thawing the cells ten times, or adding 1% Nonidet P-40 to the crude sonicated preparations did not increase the numbers or quantities of DNAases observed. Re-extraction of the pelleted debris resulted in recovery of only trace amounts of DNAase activity. The stacking gels (2 mm $\times$ 10 mm) contained 3.1% (w/v) acrylamide, 0.08% (w/v) *NN'*-methylenebisacrylamide, 0.08% (v/v) *NNNN'*-tetramethylethylenediamine, 0.06% (w/v) ammonium persulphate and 0.367 M-Tris/HCl, pH 7.4. The sieving gels (2 mm $\times$ 80 mm) contained 6.8% (w/v) acrylamide, 0.18% (w/v) *NN'*-methylenebisacrylamide, 0.035% (v/v) *NNNN'*-tetramethylethylenediamine, 0.05% (w/v) ammonium persulphate and 0.315 M-Tris/HCl, pH 8.9. Samples were placed on top of the gels and electrophoresed towards the anode. For the development of enzyme activity, electrophoresed gels usually were incubated in 50 mM-citrate/phosphate, pH 4.0, for 18 h at 37°C, stained for 6 h with 0.1% (w/v) pyronin Y in 7% (v/v) acetic acid at 37°C and destained overnight in 7% acetic acid at room temperature.

Results and discussion

Resident macrophage DNAases

Resident peritoneal macrophages from all mouse strains investigated (AKR/J, AKR/N, CDF-1, DBA/2N, C3H/HEN, C3H/HEJ, BALB/c) exhibited similar DNAase patterns. Representative polyacrylamide gels containing activities derived from increasing amounts of resident AKR/J peritoneal macrophages are shown in Fig. 1. The R_m values of the activities ranged from 0.064 to 0.56, with the enzymes having the most activity located near the top of each gel. As sample size increased, a total of 12 activity bands were observed. Although the number and R_m values of activities for macrophages

Fig. 1. DNAase activities derived from resident mouse peritoneal macrophages

The samples of cell-free extract applied to the gels were equivalent to: (a) 5×10^5 cells; (b) 2.5×10^5 cells; (c) 10^5 cells; (d) 5×10^4 cells; (e) 2.5×10^4 cells.

from different strains were identical, reproducible minor differences were found among the relative intensities of corresponding bands. The nutritional status, age and sex of the mice did not appear to affect activity patterns.

The clear to lightly stained discs in the gels were due to enzymic hydrolysis of substrate. First, the major Coomassie-Blue-stained protein bands did not correspond to the DNAase activity bands. Thus high concentrations of proteins were not prohibiting dye uptake. Secondly, the development of the clear bands required conditions associated with enzymic activity. The development of DNAase activity required incubation of gels in buffers of proper ionic strength and pH. Increasing the duration of incubation increased the hydrolysis of DNA, as indicated by progressive loss of colour in narrow regions of the gels. Either boiling the electrophoresed gels before incubation or electro-

Fig. 2. Comparison of DNAase activities derived from resident peritoneal macrophages and peripheral blood leucocytes

The samples of cell-free extract applied to each gel were equivalent to 5×10^5 cells. (a) Peritoneal macrophages; (b) unfractionated leucocytes; (c) lymphocytes; (d) neutrophils.

phoresis of samples of the buffers used in cell purification and enzyme extraction always resulted in uniformly stained gels.

Potential contaminants exhibited different, but characteristic, DNAase patterns. Total peripheral leucocytes, lymphocytes and neutrophils exhibited identical patterns, containing five bands of activity (Fig. 2). The fact that acid DNAase expression in lymphocytes (a non-phagocytic cell) and neutrophils appeared identical indicates that these enzymes are primarily involved not in degrading phagocytosed materials, but in cellular DNA 'housekeeping'.

Serum and plasma yielded two bands (R_m 0.26 and 0.70) that corresponded to regions of high protein concentration (gels not shown). A rinse (1.0ml) of the peritoneal cavity with phosphate-

buffered saline (Dulbecco & Vogt, 1954) yielded one activity band (R_m 0.71), which corresponded to a region of high protein concentration. All of these activity bands were eliminated by boiling the electrophoresed gels before incubation. The slowest-migrating activity in serum and plasma might have been represented by the fastest lymphocyte and neutrophil activity, since the R_m values were similar (0.26). The R_m of the fastest-migrating serum and plasma activity (0.70) did not correspond to any other activity investigated in this study, with the exception of the peritoneal-rinse enzyme (0.71). The DNAase activity present in the peritoneal rinse was not altered after thioglycollate-induced inflammation, which is consistent with the macrophage DNAases not being ectoenzymes (Morahan *et al.*, 1980). The different R_m values of DNAases from different sources suggest that the serum, plasma and peritoneal-fluid DNAases do not originate from the cell types investigated.

We chose to examine unfractionated peritoneal cells for several reasons. First, crude macrophages (70% macrophages, 8% neutrophils, 14% lymphocytes, 8% mast cells) were purified by using a LACS Cell Separator (De Konen BV, Arnhem, Holland) as described by Bont *et al.* (1979) to give a population consisting of 91% macrophages, 1% neutrophils, 4% lymphocytes and 4% mast cells. Enriched macrophage preparations yielded patterns identical (results not shown) with those shown in Fig. 1. Since crude and purified control macrophages yielded identical DNAase patterns, and since these patterns were markedly different from those of potential contaminants, the contribution of DNAase from contaminating cell types apparently was negligible. Secondly, there were only very low amounts of non-macrophage cells in inflammatory macrophage preparations (usually <1%). Thirdly, the method best suited for obtaining purified macrophages from large numbers of samples is adherence of the cells to a substrate during incubation for many hours. Such manipulations complicate data analysis, since they result in large alterations of many of the enzyme activities (Adams, 1979).

The optimal DNAase activity conditions of all of the individual activities within macrophages appeared identical with respect to any of the criteria investigated. The pH and ionic-strength optima were investigated in several buffers in the presence of EDTA or Mg^{2+} plus Ca^{2+} (Table 1). The pH optima in each of four buffers were different, and varied by as much as 1.5 pH units. In addition, the presence of EDTA in three of the buffers resulted in a shift of the pH optima to 0.5 unit higher than that found in the presence of Mg^{2+} and Ca^{2+} . The exception was citrate/phosphate

Table 1. *pH and buffer concentration optima of mouse peritoneal macrophages*

Optima were obtained by overnight incubations of the electrophoresed gels in the indicated buffers containing either 5 mM-EDTA or 10 mM- $MgCl_2$ plus 1 mM- $CaCl_2$. For pH optima, a range of at least 4 pH units in 0.5-unit increments was investigated. Concentration optima were investigated at 0.05, 0.1, 0.15, 0.175, 0.2, 0.3, 0.4, 0.5, 0.6 and 0.7 M. The pH optima were obtained at the buffer concentration optima indicated. Concentration optima were independent of the presence of EDTA or Mg^{2+} plus Ca^{2+} .

Buffer	pH		Concn. (M)
	EDTA	$Mg^{2+} + Ca^{2+}$	
Bes	6.5	6.0	0.175
Citrate/phosphate	5.0	5.0	0.05–0.10
Mes	6.0	5.5	0.5
Sodium acetate	5.5	5.0	0.175

phosphate buffer, which did not exhibit a change in pH optimum, possibly owing to its intrinsic chelation capacity. There was no apparent difference in maximum activity in EDTA as compared with Mg^{2+} plus Ca^{2+} . Specific buffer effects on activity were noted in that the use of citrate/phosphate buffer under optimum conditions reproducibly yielded the greatest maximum activity and Mes gave the least activity of the buffers investigated. No DNAases with alkaline pH optima were detected with 0.1 M-Tris/HCl, pH 7.35–8.5, as the incubation buffer. All of the DNAase activities had broad buffer-concentration requirements. This consistency of enzymic properties suggested that the individual DNAases were closely related and may be derived from a single parental activity.

Relationships among resident DNAases

Even though the DNAase patterns were reproducible under a variety of sample-preparation and electrophoresis conditions, it was possible that the 12 activities represented different oligomeric states of a single protein. This possibility was checked by re-electrophoresis of 2 mm slices of the sieving portion of a gel (Fig. 3). One to three closely migrating DNAase bands per gel were found in each of the second sets of gels. The DNAases had the same R_m values as those in the gel slices. There was no re-equilibration of DNAase activities to other regions of the gels. In addition, band intensities for enzymes electrophoresed once or twice appeared to be similar, indicating minimal loss of activity resulting from the increased sample manipulation. Thus, if oligomers were present, they were very stable.

The relationship between the activities was

Fig. 3. Re-electrophoresis of DNAases contained in 2mm slices of the sieving portion of a gel

Cell-free extract, equivalent to the contents of 3×10^6 resident peritoneal macrophages, was electrophoresed into a polyacrylamide gel. After electrophoresis the resolving gel was cut into 2mm-thick discs. The discs were placed directly on to a second set of gels that then were electrophoresed and processed as usual. The gels contain: (a) cell-free extract from 10^6 cells; (b)–(j) discs (numbered from the top of the gel): (b) 1; (c) 4; (d) 7; (e) 10; (f) 13; (g) 16; (h) 19; (i) 22; (j) 25.

⊖ investigated further by Ferguson analysis, in which identical samples were electrophoresed into gels of different %T [concentration (w/v) of acrylamide plus *NN'*-methylenebisacrylamide] and plotting $\log R_m$ against %T (Ferguson, 1964; Hedrick & Smith, 1968) (Fig. 4a). In this type of analysis, different polymeric states of a single protein yield a series of lines that intersect at a single point. However, charge variants of a single protein yield a series of parallel lines. The results with the macrophage DNAases were a series of parallel lines (slope = -0.03 ; $P < 0.05$) that intercepted the $\log R_m$ axis at equal intervals ($P < 0.05$). Thus, on the basis of identical activity optima, reproducibility of R_m values on re-electrophoresis, and Ferguson analysis, the individual DNAases probably represent charge variants of one or a few closely related parent polypeptides.

⊕ *Changes in macrophage DNAase expression associated with induced inflammation*

The inflammatory response was efficiently induced by intraperitoneal injection of thioglycolate, as indicated by time-dependent changes in the peritoneal cell populations (Beelan *et al.*, 1978). After changes in macrophage numbers and purity from about 2 million cells/mouse and 75% macrophages before injection to about 9 million cells/mouse and 13% macrophages at 5h, there was a rapid change to about 12 million cells/mouse and 99% macrophages by 3 days after injection. The composition remained stable until about day 6 or 7,

Fig. 4. Ferguson plot of peritoneal-macrophage DNAase activities

Supernatants from sonicated macrophages were electrophoresed into gels. The percentage of *NN'*-methylenebisacrylamide was maintained at 2.6% of the total concentration of monomer, but the %T was varied from gel to gel (4.1, 5.13, 6.16, 7.18, 8.21, 9.23%). DNAase activity positions were computed relative to that of the Bromophenol Blue marker. (a) Resident peritoneal macrophages; (b) inflammation-associated peritoneal macrophages.

Fig. 5. DNAase activities derived from inflammation-associated peritoneal macrophages

The samples of cell-free extract applied to each gel were equivalent to: (a) 10^6 cells; (b) 5×10^5 cells; (c) 2.5×10^5 cells; (d) 10^5 cells; (e) 5×10^4 cells; (f) 2.5×10^4 cells; (g) 10^4 cells.

when the numbers of macrophages decreased and the distribution of cell types began returning to normal. Further evidence for the induction of an inflammatory response was the greatly decreased content of 5'-nucleotidase in macrophages after administration of thioglycollate (Morahan *et al.*, 1980). As expected, the content of 5'-nucleotidase rapidly decreased from 6.3 units (nm-AMP/min per 10^6 macrophages) to 0.30 unit at 24h to undetectable values by 2 days.

The inflammatory response was associated with several changes in peritoneal-macrophage DNAase expression (Fig. 5). Reaction mixtures containing extract derived from control cells degraded $0.27 \mu\text{g}$ of DNA/min per 10^6 cells under our standard assay conditions. At 5 days there was a 70% increase in total DNAase activity on a per-cell basis (average of two experiments: 60%, 81% increases). This increase in activity was manifested in the slower-migrating activities. The changes appeared to be general phenomena in that similar changes in DNAase expression were obtained by using intraperitoneal injection of foetal-calf serum,

Fig. 6. DNAase activities derived from peritoneal macrophages at different times after intraperitoneal injection of thioglycollate

Cell-free extract equivalent to 3×10^4 cells was applied to each gel. (a) Zero time; (b) 5h; (c) 1 day; (d) 2 days; (e) 3 days; (f) 4 days; (g) 5 days; (h) 6 days.

casein, or highly purified L subunits of phytohaemagglutinin (generously given by R. Felsted, NIH) to induce inflammation of all six mouse strains investigated (AKR/J, AKR/N, CDF-1, DBA/2N, C3H/HEN, C3H/HEJ). The intraperitoneal injection agents had no effect on DNAase expression when they were added to control macrophages at the sonication step. In addition, the altered DNAase expression was specific for the inflammatory response, since it was induced by known inflammation-inducing agents, but not by agents known not to induce an inflammatory response (iso-osmotic NaCl, mineral oil).

Changes in macrophage DNAase expression became evident as early as 2 days after thioglycollate injection (Fig. 6). The apparent decrease in activity at 5h presumably was due to the transient decrease in macrophages and increase in neutrophils at that time. At later times, the enzyme changes included increased activity in the slower-migrating bands and decreased activity in the faster-migrating bands. Maximum changes in DNAase activity were observed by 3 days and appeared to remain stable for at least 7 days after injection, even though cell numbers had returned

to pre-treatment values and appearance by this time. Thus the time course of the development of altered DNAase expression links the changes in DNAase expression to the inflammatory response.

The changes in DNAase expression after thioglycollate injection probably were due to the macrophages, not to contaminants, since similar patterns of 11–12 bands were never found in any other cell type investigated, including peripheral leucocytes from control and thioglycollate-injected mice. Furthermore, samples from 5 h after injection, which, in agreement with the observation by Beelan *et al.* (1978), contained increased numbers of neutrophils (65–75% of total cells), did not show stronger DNAase-activity bands. The DNAase patterns 5 h after injection resembled patterns obtained by mixing peripheral neutrophils with control peritoneal macrophages. The stronger DNAase-activity bands were only found at 2 days or more after injection, at which time there was natural enrichment for macrophages, resulting in over 95% purity. It was quite unlikely that a minor cell contaminant was responsible for the increase in activity, since the source would have to have been incredibly rich in DNAase.

Characteristics of DNAases derived from thioglycollate-associated macrophages

The possibility that the inflammation-associated DNAases had properties different from those derived from resident macrophages was examined first by investigating buffer requirements for enzyme activity. Activities from both types of macrophages had the same pH and concentration optima in Bes, Mes and sodium acetate buffers. Furthermore, each DNAase had identical activity on double-stranded DNA and single-stranded DNA in the gels.

A subtle difference between thioglycollate-associated and resident macrophage DNAase activities was detected by Ferguson analysis of the enzymes. The plots for activities derived from control (Fig. 4a) and from thioglycollate-associated (Fig. 4b) macrophages were superficially similar. However, the lines representing all the DNAases were parallel to each other (slope = -0.03 ; $P < 0.05$), except for activities 1–6 (broken lines) of the inflammation associated cells (slope = -0.028 ; $P < 0.05$). These latter activities formed a second parallel set of lines. Thus, as a consequence of the inflammatory response, a subset of the DNAases appeared to be modified in such a way as to cause the same small change in the apparent molecular weight of each of the component enzymes.

DNAases are glycoproteins

Since the control and thioglycollate-associated

Fig. 7. Effect of incubation with or without endoglycosidase H on the electrophoretic behaviour of DNAases derived from resident peritoneal macrophages

For treatment with endo- β -N-acetylglucosaminidase H, cells were sonicated in half the usual volume of buffer. To $10\mu\text{l}$ of cell-free extract, equivalent to the contents of 3×10^6 cells, were added $15\mu\text{l}$ of enzyme (equivalent to 0.005 unit of enzyme/ 10^6 cells), $5\mu\text{l}$ of 1 M-sodium acetate, pH 5.8, and $15\mu\text{l}$ of water. Incubation was at 37°C for 2 h. (a) Cell-free extract incubated with enzyme; (b) cell-free extract incubated without enzyme.

macrophage DNAases appeared to contain one or two molecular-weight families, as indicated by Ferguson analysis, it was possible that all the DNAases were derived from multiple post-translational modifications of one or two polypeptide species, such as branched glycosylation at one or a few amino acid residues. This possibility was investigated by preincubating crude cell-free extracts derived from macrophages with endo- β -N-acetylglucosaminidase H

that was proteinase- and neuraminidase-free. This enzyme requires a tetrasaccharide structure $\text{Man}\alpha(1\rightarrow3)\text{Man}\alpha(1\rightarrow6)\text{Man}\beta(1\rightarrow4)\text{GlcNAc}$ as its specific glycone and will hydrolyse sugar chains with $\text{GlcNAc}\rightarrow\text{Asn}$ as the aglycone moiety (Tai *et al.*, 1977; Tarentino & Maley, 1974; Tarentino *et al.*, 1972), leaving only *N*-acetylglucosamine attached to the asparagine. The preincubation resulted in the disappearance of the more negatively charged DNAase activities derived from resident and inflammation-associated macrophages. Data for the resident macrophages are shown in Fig. 7.

The charge series could have resulted from moieties analogous to the hybrid high-mannose branched structures bearing *N*-acetylneuraminic acid detected by Hunt & Wright (1981) on a Rous-sarcoma-virus protein, to the high-mannose structure containing phosphate described by von Figura & Klein (1979), or to other charged groups such as sulphate, which has been detected on *N*-glycosidic moieties (Heifetz & Prager, 1981). Alternatively, some of the charged modifications could have been directly linked to the polypeptide. Since many of these modifications have been implicated in sub-cellular localization and control of enzyme activity, changes in them may be associated with the enhanced metabolic state of inflammatory macrophages.

References

- Adams, D. O. (1979) *Methods Enzymol.* **58**, 484–506
- Beelan, R. H. J., Broekhuis-Fluitsma, D. M., Korn, C. & Hoefamit, E. C. M. (1978) *J. Reticuloendothel. Soc.* **23**, 103–110
- Berg, T. & Boman, D. (1973) *Biochim. Biophys. Acta* **321**, 585–596
- Blank, A. & Dekker, C. A. (1981) *Biochemistry* **20**, 2261–2267
- Bont, W. S., de Vries, J. E., Geel, M., van Dongen, A. & Loos, M. A. (1979) *J. Immunol. Methods* **29**, 1–16
- Böyum, A. (1968) *Scand. J. Clin. Lab. Invest.* **21**, Suppl. **97**, 77–89
- Brown, G. E., Karpetsky, T. K., Rahman, A., McFarland, E. & Haroth, M. B. (1982) *Electrophoresis* **3**, 151–157
- Dulbecco, R. & Vogt, M. (1954) *J. Exp. Med.* **99**, 167–182
- Edelson, P. J. & Cohn, Z. A. (1976) *J. Exp. Med.* **144**, 1581–1595
- Felsenfeld, G. & Sandeen, G. (1962) *J. Mol. Biol.* **5**, 587–610
- Ferguson, K. A. (1964) *Metab. Clin. Exp.* **13**, 985–1002
- Hedrick, J. L. & Smith, A. I. (1968) *Arch. Biochem. Biophys.* **126**, 155–164
- Heifetz, A. & Prager, M. D. (1981) *J. Biol. Chem.* **256**, 6529–6532
- Hunt, L. A. & Wright, S. E. (1981) *J. Virol.* **39**, 646–650
- Karnovsky, M. L. & Lazdins, J. K. (1978) *J. Immunol.* **121**, 809–813
- Karpetsky, T., Brown, G. E., McFarland, E., Rahman, A., Rictor, K., Roth, W., Haroth, M. B., Ansher, A., Duffey, P. & Levy, C. (1982) in *Electrophoresis '81* (Allen, R. C. & Arnaud, P., eds.) pp. 647–654, W. de Gruyter, New York
- Li, C. Y., Lam, K. W. & Yam, L. J. (1973) *J. Histochem. Cytochem.* **21**, 1–12
- Meyers, O. T., Dannenberg, A. M., Mizunoe, K. (1970) *J. Reticuloendothel. Soc.* **7**, 15–31
- Morahan, P. S., Edelson, P. J. & Gass, K. (1980) *J. Immunol.* **125**, 1312–1317
- Rosenthal, A. L. & Lacks, S. A. (1977) *Anal. Biochem.* **80**, 76–90
- Tai, T., Yamashita, K. & Kobata, A. (1977) *Biochem. Biophys. Res. Commun.* **78**, 434–441
- Tarentino, A. L. & Maley, F. (1974) *J. Biol. Chem.* **249**, 811–817
- Tarentino, A. L., Plummer, T. H. Jr. & Maley, F. (1972) *J. Biol. Chem.* **247**, 2629–2631
- von Figura, K. & Klein, U. (1979) *Eur. J. Biochem.* **94**, 347–354
- Zollner, E. J., Klepsch, D. M., Zahn, R. K. & Knepper, R. (1975) *Enzyme* **19**, 60–64