ADP-ribosyltransferase in *Plasmodium* (malaria parasites)

Eugene Ezenna OKOLIE and Nkechi I. ONYEZILI
National Institute for Medical Research, P.M.B. 2013, Yaba, Lagos, Nigeria

(Received 10 August 1982/Accepted 24 November 1982)

The nuclei of *Plasmodium yoelii nigeriensis* contain an enzyme, ADP-ribosyltransferase, that will incorporate the ADP-ribose moiety of NAD⁺ into acid-insoluble product. The time, pH and temperature optima of this incorporation are 30 min, 8.5 and 25°C respectively. Maximum stimulation of the enzyme activity is obtained with 1.0 mM-dithiothreitol or 2.0 mM-2-mercaptoethanol. Ca²⁺ and Mg²⁺ ions at optimum concentrations of 5 mM and 10 mM respectively stimulated the activity of the enzyme by 21% and 91%. The enzyme activity is, however, inhibited by 24% in the presence of 10 mM-MnSO₄. The substrate, NAD⁺, exhibits an apparent K_m of 500 μ M, and the activity of the enzyme is inhibited by four chemical classes of inhibitors: nicotinamides, methylxanthines, thymidine and aromatic amides. The inhibitors are effective in the following increasing order: nicotinamide < 3-aminobenzamide < thymidine < 5-methyl-nicotinamide < theophylline < *m*-methoxybenzamide < theobromine. The enzyme activity is also inhibited by some DNA-binding anti-malarial drugs.

Poly(ADP-ribosyl)ation is one of the post-synthetic covalent modifications of nuclear proteins (Shall *et al.*, 1977). The exact function of this modification is not clear, but available evidence suggests that it may play an important role in the regulation of gene expression (Burzio & Koide, 1970; Coyer *et al.*, 1973; Shall *et al.*, 1977; Caplan *et al.*, 1979a), DNA repair (Benjamin & Gill, 1980; Durkacz *et al.*, 1980; Nduka *et al.*, 1980) and in cell differentiation and development (Caplan *et al.*, 1979b; Berger *et al.*, 1979; Farzaneh *et al.*, 1980).

The enzyme responsible for this modification, ADP-ribosyltransferase, is tightly bound to chromatin. It catalyses the transfer and elongation of the ADP-ribose moiety of NAD⁺ on to acceptor proteins to form a polymer ranging in size up to 65-ADP-ribose units (Tanaka *et al.*, 1978). The transferase has been detected in the nuclei of various mammalian tissues and in a variety of tissue-culture cells. It has also been detected in the nuclei of higher plants as well as in the primitive nucleated organism *Physarum polycephalum*, but not in bacteria (for review see Hilz & Stone, 1976).

The present paper reports the detection of ADP-ribosyltransferase activity in partially purified nuclei of the rodent malaria parasite *Plasmodium yoelii nigeriensis*. It describes also some of the properties of the protozoan enzyme, which are compared with those of the mammalian and plant enzymes. Preliminary reports of parts of this work have already been published (Okolie, 1980, 1981).

Materials and methods

Chemicals

Adenosine, AMP, NAD⁺ and snake-venom phosphodiesterase (type 1; no. P-6877) were products of Sigma Chemical Co. (St. Louis, MO, U.S.A.). GF/C glass-fibre filters were purchased from Whatman (Maidstone, Kent, U.K.). [adenosine-³H]NAD⁺ was from the Radiochemical Centre (Amersham, Bucks., U.K.). Dithiothreitol, nicotinamide, 5-methyl-nicotinamide, 3-aminobenzamide, *m*-methoxybenzamide, thymidine, theophylline and theobromine were gifts from Professor Sydney Shall (University of Sussex, Brighton, U.K.). The PEI (polyethyleneimine)-cellulose t.l.c. plates were obtained from Dr. Gwyn Williams (St. George's Hospital Medical School, London, U.K.). Quinacrine hydrochloride, primaquine phosphate and quinine sulphate were supplied by Dr. Nsirim Nduka (Liverpool School of Tropical Medicine and Hygiene, Liverpool, U.K.). Proguanil hydrochloride (paludrine), chloroquine sulphate and amodiaquine hydrochloride (camoquine) were obtained from the Staff Clinic, National Institute for Medical Research, Yaba, Nigeria. Unlabelled poly(ADP-ribose) of average chain length 20 ADP-ribose units was prepared as previously described (Okolie & Shall, 1979).

Organisms

A laboratory strain of *Plasmodium yoelii nigeriensis* was maintained by syringe passage in

Swiss albino mice. At about 9–12 days after the infection, the mice were anaesthetized with ether and blood was collected by cardiac puncture into cold phosphate/saline/glucose (2 g of glucose per litre of 0.15 M-NaCl/10 mM-sodium phosphate buffer, pH 7.2). Sufficient parasite material was usually obtained from blood collected from 10–20 infected adult mice.

Isolation of free malaria parasites

The infected blood was passed through four layers of absorbent gauze to remove any tissue debris or fibrin clots. The blood cells were then washed three times with cold phosphate/saline/glucose by centrifuging at 1800 *g* for 5 min, and the white-cell buffy-coat was removed with a pipette during each washing. Parasites were released from infected erythrocytes by incubation of the blood cells with 40 times their volume of saponin solution (0.1 g of saponin per litre of phosphate/saline/glucose). The suspension was kept at room temperature (26°C) for 20 min, with occasional mixing. The released parasites were collected by centrifuging at 1800 *g* for 10 min and then washed two times with cold phosphate/saline/glucose. A film of the pellet stained with Wright's Giemsa stain showed intact parasites, leucocytes and erythrocyte stroma. The parasite pellet was then diluted 1:5 with phosphate/saline/glucose and layered over a discontinuous gradient made up of cold 10%, 23% and 37% (w/v) sucrose solutions (Siddiqui *et al.*, 1978). The sample volume was one-third of the volume of the gradient. The loaded gradients were centrifuged at 250 *g* for 7 min at 4°C in a Beckman TJ 6R refrigerated centrifuge. Two distinct bands were obtained, and these were removed with a pipette and pelleted at 1800 *g* for 10 min. Stained films made of these bands showed that the upper brownish band (in the 10%-sucrose layer) contained free parasites, whereas the lower band contained some parasites still attached to the erythrocyte membranes, leucocytes and erythrocyte stroma.

Isolation of partially purified parasite nuclei

The free parasite pellet was washed twice with phosphate/saline/glucose and was then taken up in a cold solution of 250 mM-sucrose containing 10 mM-MgCl₂. The parasites were homogenized with 20 strokes of a Dounce glass homogenizer, and samples of the homogenate were removed for enzyme and protein assays. The remainder of the homogenate was centrifuged at 1000 *g* for 20 min at 4°C, and the crude nuclear pellet was washed twice with the sucrose solution. The protein contents of the parasite materials were measured by the method of Lowry *et al.* (1951), with bovine serum albumin as a standard.

Marker enzyme

The enzyme used as a marker for contamination of the nuclear preparation with cytoplasmic contents was malate dehydrogenase. It is a cytoplasmic enzyme and has been reported to be present in *Plasmodium yoelii nigeriensis* (Momen, 1979). The enzyme was assayed by the method of Falk *et al.* (1980).

Assay of ADP-ribosyltransferase activity and identification of the reaction product

The basal assay mixture contained 10 mM-MgCl₂, 1 mM-dithiothreitol, 50 mM-KCl, 4 mM-NaF, 100 mM-Tris/HCl buffer, pH 8.5, and 0.26–0.5 μ Ci of [³H]NAD⁺. The parasite nuclear suspension was used as the enzyme source, and incubation was for 30 min at 25°C except where indicated otherwise. The legends to the individual Tables and Figures indicate variations in the other conditions of the assay. The reaction was usually terminated by addition of an equal volume of cold 20% (w/v) trichloroacetic acid, and the precipitates were allowed to form in ice for 1 h. The precipitates were then collected on Whatman GF/C glass-fibre filters, washed with cold 10% trichloroacetic acid and dried. The dried filters were placed into counting vials, and 6 ml of the Beckman Ready-Solv scintillation fluid was added to each vial. The radioactivity in the acid-insoluble product was measured in a Beckman LS-3150T liquid-scintillation counter. Poly(ADP-ribose) was identified as the reaction product by the isolation of phosphoribosyl-AMP, a unique product liberated by the action of snake-venom phosphodiesterase, by using PEI-cellulose t.l.c. (Stone *et al.*, 1973). Unlabelled poly(ADP-ribose) standard (see above) was digested with the phosphodiesterase and spotted on the t.l.c. plates along with adenosine and AMP standards in order to identify the transferase reaction product.

Results and discussion

Isolation of malaria parasite nuclei

The sucrose-density-gradient separation of the saponin-lysed infected erythrocytes resulted in two bands: an upper brownish band (in the 10%-sucrose layer) containing free parasites, and a lower band containing some parasites, leucocytes and erythrocyte stroma. Malaria parasites prepared by this technique have been successfully used in vaccination studies in *Aotus* monkeys (Siddiqui *et al.*, 1978). Although the nuclear fraction was only partially purified, it was almost free of cytoplasmic contamination, as judged by the low malate dehydrogenase activity (Table 1). The maximum contamination was less than 4% of the total activity in

Table 1. *Malate dehydrogenase and ADP-ribosyltransferase activities in parasite fractions*

For full experimental details see the text. The unit of malate dehydrogenase activity is defined as the amount of enzyme required to oxidize 1 μ mol of NAD⁺/min. The unit of ADP-ribosyltransferase activity is defined as pmol of NAD⁺ incorporated into acid-insoluble product/min.

Fraction	Protein content (mg)	Malate dehydrogenase			ADP-ribosyltransferase activity			
		Total activity (units)	Specific activity (units/mg of protein)	Yield (%)	Radioactivity incorporated (c.p.m.)	Total activity (units)	Specific activity (units/mg of protein)	Yield (%)
Parasite homogenate	2.10	5.00	2.38	100	32 625	14.55	6.93	100
Nuclear preparation	0.81	0.15	0.19	3	28 141	12.55	15.49	86
Supernatant	1.05	4.80	4.57	96	2185	0.97	0.93	7

Fig. 1. Time course of poly(ADP-ribose) formation when parasite homogenate (a) or nuclear suspension (b) is used as enzyme source

The reaction mixture contained, in 1.5 ml: 100 mM-Tris/HCl buffer, pH 8.5, 10 mM-MgCl₂, 4 mM-NaF, 50 mM-KCl, 1 mM-dithiothreitol and 0.26 μ Ci of [³H]NAD⁺. At specified time intervals, two 100 μ l portions were removed, precipitated with 20% trichloroacetic acid and assayed for the acid-insoluble product.

the parasite homogenate. The nuclear preparation was not examined for contamination with host-cell or parasite components by electron microscopy owing to lack of facility.

However, since control uninfected mouse blood subjected to the same purification procedures did not show ADP-ribosyltransferase activity (results not shown), it is reasonable to conclude that the detected enzyme activity was of parasite origin. The activity of this enzyme has been shown to be localized exclusively in the nucleus (Nishizuka *et al.*, 1969), and the higher activity detected in the partially purified nuclei (Table 1) confirms this

observation: 86% of the ADP-ribosyltransferase activity was recovered in the nuclear preparation.

Effect of time on the activity

The time course of the activity of the protozoan enzyme showed that when the nuclear suspension was used the incorporation of NAD⁺ attained a plateau after about 30 min at 25°C that lasted for over 60 min. However, when the free-parasite homogenate was used, there was a peak of incorporation at about 20 min, followed by a decrease in incorporation (Fig. 1). The difference in the kinetics of NAD⁺ incorporation observed with the homo-

Fig. 2. Effect of temperature on ADP-ribosyltransferase activity

The reaction mixture was as described in the Materials and methods section, except that $0.45 \mu\text{Ci}$ of $[^3\text{H}]\text{NAD}^+$ was used and the incubation was at the temperatures indicated.

genate may be due to the presence of cytoplasmic enzymes capable of degrading the polymer or the substrate in this fraction. The pattern of incorporation obtained with the nuclei is similar to that of mouse fibroblast nuclei (Stone & Shall, 1973), but differs from that obtained with rat liver nuclei, where there is a peak of incorporation at about 10 min followed by a decline in incorporation (Burzio & Koide, 1970).

Effect of temperature on the activity

Plasmodium yoelii ADP-ribosyltransferase exhibited optimum activity at 25°C (Fig. 2). Similar temperature optima have been obtained with mouse fibroblast and rat liver enzymes (Stone & Shall, 1973; Burzio & Koide, 1970), whereas the *Physarum* enzyme shows a temperature optimum below 10°C (Brightwell *et al.*, 1975).

Effect of pH on the activity

The pH optimum of NAD^+ incorporation is 8.5 (Fig. 3). This is similar to that obtained with the mouse fibroblast enzyme (Stone & Shall, 1973). Enzymes from rat liver and *Physarum polycephalum* have pH optima of 8.0 and 8.2 re-

Fig. 3. Effect of pH on ADP-ribosyltransferase activity

The reaction mixture was as described in the Materials and methods section, except that $0.26 \mu\text{Ci}$ of $[^3\text{H}]\text{NAD}^+$ was used and the pH of the 100 mM-Tris/HCl buffer was varied.

spectively (Burzio & Koide, 1970; Brightwell *et al.*, 1975).

Effect of substrate concentration on the activity

The substrate NAD^+ exhibited an apparent K_m of 0.50 mM (Fig. 4). This is somewhat higher than the reported values of 0.27 mM for Ehrlich-ascites-cell nuclei (Romer *et al.*, 1968) and 0.28 mM for *Physarum polycephalum* (Brightwell *et al.*, 1975), but is lower than the reported values of 0.85 mM for rat liver nuclei (Clark *et al.*, 1971) and 1.47 mM for LS-cell nuclei (Stone & Shall, 1973). These K_m values are, however, much higher than the $23 \mu\text{M}$ and $100 \mu\text{M}$ values obtained for purified enzymes (Tsopanakis *et al.*, 1978; Mandel & Hilz, 1976).

Requirement of the enzyme for bivalent ions

The protozoan ADP-ribosyltransferase required the presence of bivalent ions for activity, a feature common to the enzyme from other sources. Mg^{2+} ions at an optimum concentration of 10 mM stimulated the activity of the enzyme by 91%, and Ca^{2+}

Fig. 4. Double-reciprocal plot of the variation of ADP-ribosyltransferase activity with substrate concentration. The reaction mixture was as described in the Materials and methods section. [³H]NAD⁺ (0.5 μ Ci) was present at various concentrations up to 1 mM.

ions stimulated the enzyme by 21% at an optimum concentration of 5 mM (Fig. 5). Mn²⁺ ions, however, had an opposite effect. The enzyme activity was inhibited by 24% in the presence of 10 mM-MnSO₄. Mg²⁺ and Ca²⁺ ions are known to stimulate the enzymes from various sources (Nishizuka *et al.*, 1967; Stone & Shall, 1973; Brightwell *et al.*, 1975; Mandel & Hilz, 1976), and Mn²⁺ ions have been found to stimulate the purified enzymes from calf thymus (Mandel & Hilz, 1976).

Requirement of the enzyme for reduced thiol reagents

Maximum stimulation of the enzyme activity was obtained with 1 mM-dithiothreitol or with 2 mM-2-mercaptoethanol (Fig. 6). This requirement for reduced thiol reagents has been reported with partially purified rat liver enzyme (Yamada *et al.*, 1971), mouse fibroblast nuclei (Stone & Shall, 1973), purified calf thymus enzyme (Mandel & Hilz, 1976) and *Physarum polycephalum* nuclei (Brightwell *et al.*, 1975).

Effect of inhibitors on the activity

The enzyme was inhibited by four chemical classes of inhibitors: the nicotinamides, the methyl-xanthines, thymidine and aromatic amides (Table 2). They were effective in the following increasing order: nicotinamide < 3-aminobenzamide < thymidine < 5-methylnicotinamide < theophylline < *m*-methoxy-

Fig. 5. Effect of bivalent ions on ADP-ribosyltransferase activity.

The reaction mixture was as described in the Materials and methods section, except that various concentrations of MgCl₂ (O), CaCl₂ (Δ) and MnSO₄ ($\bullet$) were used; 0.5 μ Ci of [³H]NAD⁺ was also used.

benzamide < theobromine. Although these compounds are known inhibitors of ADP-ribosyltransferases, their effectiveness as enzyme inhibitors appears to be greater in mammalian systems (Hilz & Stone, 1976; Durkacz *et al.*, 1980; Purnell & Whish, 1980; Petzold *et al.*, 1981).

Effect of some anti-malarial drugs on the activity

Table 3 shows the effect of 1 mM concentrations of known anti-malarial drugs on the enzyme activity. These drugs belong to five chemical classes, namely the cinchona alkaloids (quinine), the 4-aminoquinolines (chloroquine, amodiaquine), the 8-aminoquinolines (primaquine), the 9-aminoacridines

Fig. 6. *Effect of thiol compounds on ADP-ribosyltransferase activity*

The reaction mixture was as described in the Materials and methods section, with $0.5 \mu\text{Ci}$ of $[^3\text{H}]\text{NAD}^+$ present. Dithiothreitol (●) or 2-mercaptoethanol (○) was added as indicated.

Table 2. *Effect of inhibitors on ADP-ribosyltransferase activity*

The assay mixture contained, in a total volume of $100 \mu\text{l}$: $126 \mu\text{g}$ of partially purified nuclei, 10 mM-MgCl_2 , $1 \text{ mM-dithiothreitol}$, 50 mM-KCl , 4 mM-NaF , 100 mM-Tris/HCl buffer, $\text{pH } 8.5$, $0.52 \mu\text{Ci}$ of $[^3\text{H}]\text{-NAD}^+$ and 1 mM of each inhibitor. The control tube contained the assay buffer and no inhibitor. The transferase assay was conducted as outlined in the Materials and methods section. The unit of ADP-ribosyltransferase activity is defined as pmol of NAD^+ incorporated into acid-insoluble product/min.

Inhibitor (1 mM)	Specific activity (units/mg of protein)	Inhibition (%)
Control (buffer)	7.60	0
Nicotinamide	5.71	25
3-Aminobenzamide	5.30	30
Thymidine	4.42	42
5-Methylnicotinamide	4.20	45
Theophylline	4.00	47
<i>m</i> -Methoxybenzamide	3.50	54
Theobromine	3.10	59

Table 3. *Effect of some anti-malarial drugs on ADP-ribosyltransferase activity*

The reaction conditions are similar to those given in Table 2 legend, except that the assay mixture contained $268 \mu\text{g}$ of nuclei and 1 mM concentrations of anti-malarial drugs instead of inhibitors. The unit of ADP-ribosyltransferase activity is defined as pmol of NAD^+ incorporated into acid-insoluble product/min.

Drug (1 mM)	Specific activity (units/mg of protein)	Inhibition (%)
Control (buffer)	15.05	0
Proguanil hydrochloride	14.94	0.7
Chloroquine sulphate	12.69	16
Primaquine phosphate	10.86	28
Quinine sulphate	10.70	29
Amodiaquine	10.10	33
Quinacrine hydrochloride	9.98	34

Table 4. *Effect of incubation of some anti-malarial drugs with infected blood cells on ADP-ribosyltransferase activity*

Infected blood was collected into tubes containing equal volumes of phosphate/saline/glucose and then filtered, and each drug was added to give a final concentration of 1 mM . The control infected blood sample contained phosphate/saline/glucose but no drug. The tubes were incubated for 1 h at 37°C with occasional mixing. The blood cells were then washed three times with phosphate/saline/glucose, and the isolation of parasite nuclei and assay of ADP-ribosyltransferase activity were performed as indicated in the Materials and methods section.

Drug (1 mM)	Inhibition (%)
Control (phosphate/saline/glucose)	0
Chloroquine sulphate	77
Primaquine phosphate	59
Quinacrine hydrochloride	37
Proguanil hydrochloride	0.2

(quinacrine) and the biguanides (proguanil). They were effective in the following increasing order: proguanil hydrochloride < chloroquine sulphate < primaquine phosphate < quinine sulphate < amodiaquine hydrochloride < quinacrine hydrochloride. Table 4 shows the effect on the enzyme of incubating 1 mM concentrations of chloroquine, primaquine, quinacrine and proguanil with parasitized mouse erythrocytes for 1 h at 37°C before the isolation of parasite nuclei and enzyme assay. The enzyme activity of the drug-treated infected cells was compared with that of control infected cells incubated with phosphate/saline/glucose only. Chloroquine, primaquine and quinacrine were found to inhibit the enzyme activity by 77, 59 and

37% respectively, whereas proguanil hydrochloride had little or no effect. All the anti-malarial drugs used in these experiments, except proguanil, are believed to bind to DNA by intercalation, presumably diminishing the effectiveness of the parasite DNA to act as a template (Gutteridge & Coombs, 1977; McVie, 1977; Richards, 1978). However, since ADP-ribosyltransferase is known to have an absolute requirement for DNA, it is possible that the inhibitory effects of these anti-malarial drugs on the enzyme activity may be due to their ability to intercalate DNA. Perhaps the difference in the effect on the activity of the enzyme between proguanil hydrochloride on one hand, and the DNA-binding drugs on the other, may be explained, in part, by differences in their modes of action. Proguanil hydrochloride is inactive in its original form, but is metabolized in the host to the active dihydrotriazine form, which competitively antagonizes the utilization of folic acid by the parasite (Franklin & Snow, 1975).

This investigation received support from the Federal Ministry of Science and Technology, Lagos, Nigeria, and the U.N.D.P./World Bank/W.H.O. Special Program for Research and Training in Tropical Diseases. The technical assistance of Mr. Emmanuel Osho and Mr. Samuel Umobong is gratefully acknowledged.

References

- Benjamin, R. C. & Gill, M. D. (1980) *J. Biol. Chem.* **255**, 10493–10501
- Berger, N. A., Petzold, S. J. & Berger, S. J. (1979) *Biochim. Biophys. Acta* **564**, 90–104
- Brightwell, M. D., Leech, C. E., O'Farrell, M. K., Whish, W. J. D. & Shall, S. (1975) *Biochem. J.* **147**, 119–129
- Burzio, L. & Koide, S. S. (1970) *Biochem. Biophys. Res. Commun.* **40**, 1013–1020
- Caplan, A. I., Niedergang, C., Okazaki, H. & Mandel, P. (1979a) *Arch. Biochem. Biophys.* **198**, 60–69
- Caplan, A. I., Niedergang, C., Okazaki, H. & Mandel, P. (1979b) *Dev. Biol.* **72**, 102–109
- Clark, J. B., Ferris, G. M. & Pinder, S. (1971) *Biochim. Biophys. Acta* **238**, 82–85
- Coyler, R. A., Burdette, K. E. & Kidwell, W. R. (1973) *Biochem. Biophys. Res. Commun.* **53**, 960–966
- Durkacz, B. W., Omidiji, O., Gray, D. A. & Shall, S. (1980) *Nature (London)* **283**, 593–596
- Falk, E., Akinrimisi, E. O. & Onoagbe, I. (1980) *Int. J. Biochem.* **12**, 647–650
- Farzaneh, F., Shall, S. & Zalin, R. (1980) in *Novel ADP-ribosylations of Regulatory Enzymes and Proteins* (Smulson, M. E. & Sugimura, T., eds.), pp. 217–225, Elsevier/North-Holland, New York
- Franklin, T. J. & Snow, G. A. (1975) *Biochemistry of Anti-microbial Action*, pp. 139–159, Chapman and Hall, London
- Gutteridge, W. E. & Coombs, G. H. (1977) *Biochemistry of Parasitic Protozoa*, pp. 121–145, Macmillan, London
- Hilz, H. & Stone, P. (1976) *Rev. Physiol. Biochem. Pharmacol.* **76**, 1–58
- Lowry, O. H., Rosebrough, N. J., Farr, A. L. & Randall, R. J. (1951) *J. Biol. Chem.* **193**, 265–275
- Mandel, P. & Hilz, H. (1976) *Proc. Int. Symp. Poly(ADP-ribose) and ADP-ribosylation of Proteins 4th*, p. 8
- McVie, J. G. (1977) in *Clinical Pharmacology* (Girwood, G. H., ed.), pp. 56–141, Baillière Tindall, London
- Momen, H. (1979) *Ann. Trop. Med. Parasitol.* **73**, 109–115
- Nduka, N., Skidmore, C. J. & Shall, S. (1980) *Eur. J. Biochem.* **105**, 525–530
- Nishizuka, Y., Ueda, K., Nakazawa, K. & Hayaishi, O. (1967) *J. Biol. Chem.* **242**, 3164–3171
- Nishizuka, Y., Ueda, K., Yoshihara, K., Yamamura, H., Takeda, M. & Hayaishi, O. (1969) *Cold Spring Harbor Symp. Quant. Biol.* **34**, 781–785
- Okolie, E. E. (1980) *Proc. Eur. Multicolloq. Parasitol. 3rd* p. 19
- Okolie, E. E. (1981) *Proc. Int. Congr. Protozool. 6th*, p. 277
- Okolie, E. E. & Shall, S. (1979) *Clin. Exp. Immunol.* **36**, 151–164
- Petzold, S. J., Booth, B. A., Leimbach, G. A. & Berger, N. A. (1981) *Biochemistry* **20**, 7075–7081
- Purnell, M. R. & Whish, W. J. D. (1980) *Biochem. J.* **185**, 775–777
- Richards, W. G. H. (1978) in *Malaria Chemotherapy* (*Proc. Sci. Semin. W. Afr. Pharmaceut. Fed. 1st*) (Adenika, F. B., ed.), pp. 52–82, Literamed Press, Ikeja, Lagos
- Romer, V., Lamprecht, J., Kittler, M. & Hilz, H. (1968) *Hoppe-Seyler's Z. Physiol. Chem.* **349**, 109–115
- Shall, S., Goodwin, P., Halldorsson, H., Khan, G., Skidmore, C. & Tsopanakis, C. (1977) *Biochem. Soc. Symp.* **42**, 103–116
- Siddiqui, W. A., Kramer, K. & Richard-Crum, S. M. (1978) *J. Parasitol.* **64**, 168–169
- Stone, P. R. & Shall, S. (1973) *Eur. J. Biochem.* **38**, 146–152
- Stone, P. R., Whish, W. J. D. & Shall, S. (1973) *FEBS Lett.* **36**, 334–337
- Tanaka, M., Hayaishi, K., Sakura, H., Miwa, M., Matsushima, T. & Sugimura, T. (1978) *Nucleic Acids Res.* **5**, 3183–3194
- Tsopanakis, C., Leeson, E., Tsopanakis, A. & Shall, S. (1978) *Eur. J. Biochem.* **90**, 337–345
- Yamada, M., Miwa, M. & Sugimura, T. (1971) *Arch. Biochem. Biophys.* **146**, 579–586