recovery of tritium as this metabolite (Table 3) and multiplying by 100 and by 0·99. The latter figure is a correction factor equal to the ratio of the molecular weights of progesterone and 3α-hydroxy-5β-pregnan-20-one (314 and 318 respectively).

In the equation employed by Pearlman (1957) for calculating P on the basis of the specific activity of urinary 3α:20α-diacetoxy-5β-pregnane, the correction factor was incorrectly given as 0·98, although the values for P were correctly stated. The correction factor in that particular form of the equation should be 1·28, which is the ratio of the molecular weights of 3α:20α-diacetoxy-5β-pregnane and progesterone (404 and 314 respectively).

SUMMARY

1. [16-³H]Progesterone was injected intramuscularly into two women in the last trimester of pregnancy and into two oophorectomized–hysterectomized patients who served as a control group. The urinary recovery of tritium in the various components of the neutral ketonic fraction is reported.

2. The major ketonic radiometabolite in both experimental groups is 3α-hydroxy-5β-pregnan-20-one; a minor constituent is 5β-pregnane-3:20-dione.

3. A significant portion, 10–14%, of the radioactivity in the ketonic fraction in pregnancy urine is in an unidentified highly polar material. This radioactive material was not detected in the urine of oophorectomized–hysterectomized subjects.

4. The daily endogenous production of progesterone in advanced pregnancy was estimated from measurements of the specific activity of urinary 3α-hydroxy-5β-pregnan-20-one; these estimates were in fair agreement with those previously obtained on the basis of the specific activity of urinary 5β-pregnane-3α:20α-diol.

5. Procedures for the separation of certain urinary progesterone metabolites by reverse-phase partition column chromatography are described.

REFERENCES

Arnold, J. R. (1957). *Symposium on Tritium in Tracer Applications*, p. 12. Boston: New England Nuclear Corp.

Avivi, P., Simpson, S. A., Tait, J. F. & Whitehead, J. K. (1954). *2nd Radioisotope Conference*, vol. 1, p. 313. Ed. by Johnston, J. E. London: Butterworths Scientific Publications.

Ayres, P. J., Pearlman, W. H., Tait, J. F. & Tait, S. A. S. (1958). *Biochem. J.* **70**, 230.

Bradlow, H. L. & Gallagher, T. F. (1955). *Fed. Proc.* **14**, 185.

Carpenter, F. H. & Hess, G. P. (1956). *J. Amer. chem. Soc.* **78**, 3351.

Davis, M. E. & Plotz, E. J. (1958). *Amer. J. Obstet. Gynec.* **76**, 939.

Davis, M. E., Plotz, E. J., LeRoy, G. V., Gould, R. G. & Werbin, H. (1956). *Amer. J. Obstet. Gynec.* **72**, 740.

Howard, G. A. & Martin, A. J. P. (1950). *Biochem. J.* **46**, 532.

Pearlman, W. H. (1957). *Biochem. J.* **67**, 1.

Sandberg, A. A. & Slaunwhite, W. R., jun. (1958). *J. clin. Endocrin. Metab.* **18**, 253.

Wiest, W. G., Fujimoto, G. I. & Sandberg, A. A. (1958). *J. clin. Endocrin. Metab.* **18**, 972.

The Effect of *Plasmodium berghei* Malaria on Mouse-Liver Mitochondria

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Recent investigations into the pharmacological or toxic actions of various drugs, hormones and steroids have been concerned with a possible correlation of such actions with impairment of the normal functions of the mitochondria of tissue cells. To this end, the properties of isolated liver mitochondria, after treatment *in vivo* or *in vitro* with the substance under investigation, have been employed as test systems by comparison with the same properties of untreated mitochondria. By means of this approach, for example, Christie & Judah (1954) have shown that fatty degeneration and necrosis caused by oral administration of carbon tetrachloride are accompanied by

disorganization of the mitochondria with consequent loss of integrated enzymic activity. Aldridge & Cremer (1955), studying the toxic trialkyltins, demonstrated that the site of action of the drug was upon the oxidative-phosphorylation processes of the mitochondria, and Zetterström & Ernster (1956) found a similar effect in incubation of bilirubin with liver mitochondria *in vitro*, in connexion with a possible explanation of the causes of kernicterus (bilirubin encephalopathy) in children. Disintegration of mitochondrial activity by progesterone was also shown in studies *in vitro* by Wade & Jones (1956) and similar findings were reported by Salmony (1956) and by Dickens & Salmony (1956) for the action of stilboestrol. The well-established uncoupling action of thyroxine with respect to oxidative phosphorylation in liver mitochondria (Lardy & Feldott, 1951; Martius & Hess, 1951; Lardy, 1955; Tapley, Cooper & Lehninger, 1955; Tapley & Cooper, 1956) is another example of the same type of phenomenon.

It was considered that similar processes might be contributing towards the liver damage occurring in malarial infections. That the centrilobular damage which occurs (Maegraith, 1948) is not due to a severe decrease in the oxygen-carrying capacity of the blood, even when the blood is heavily parasitized, has been shown by Maegraith, Jones & Andrews (1951). In the present work, the experiments were undertaken to determine the type and extent of the biochemical changes, if any, detectable in liver mitochondria isolated from mice during the progress of infection with *Plasmodium berghei* malaria.

P. berghei infection in rats and mice induces an erythropoietic response (Deegan & Maegraith, 1958) with appearance in the circulation of large numbers of reticulocytes, which serve as host cells for about 95 % of the parasites (Thurston, 1953). The metabolic character of these reticulocytes, with their associated respiratory activity (Jones, Maegraith & Gibson, 1953), necessitated the removal of as much blood as possible from the livers before treatment for the isolation of mitochondria. Accordingly, the viscera of all the infected animals were viviperfused with ice-cold 0·9 % sodium chloride solution, and a similar procedure was applied to the normal animals killed as controls for each group of infected mice.

The erythropoietic response accompanying *P. berghei* infection does not prevent an increasing anaemia of infection. In order to determine the contribution of a highly anaemic condition towards the effects observed in malarial infection, normal mice were made anaemic by daily bleedings over a period approximating to the duration of the malarial infection. The characteristics of the mitochondria of the livers of these animals, again isolated

after viviperfusion of the viscera, were also determined.

Some aspects of these investigations have been reported briefly by Maegraith, Deegan & Riley (1959).

METHODS

Malarial infection. Mice (Glaxo GFF strain; 20–25 g.) were injected intravenously with blood from strain mice infected with *P. berghei* (strain originally obtained from the London School of Hygiene and Tropical Medicine). Infected animals and control animals, of the same weight, were maintained at 70–75° F on a cube diet (M.R.C. Diet no. 41) and water without restriction.

The course of the infection was followed by daily examination, under the $\frac{1}{12}$ in. oil-immersion objective, of Leishman-stained thin blood smears from the tails of the mice. The parasitaemia was expressed as the percentage of infected cells in the circulation; 1000 cells were counted in each examination. The extent of the associated anaemia of infection was determined by counting the red-cell content of blood samples from the tail; standard procedures were adopted.

Production of anaemia in normal mice. Groups of normal mice, of the same strain and weight as those infected with malaria and housed under identical conditions, were bled from the tail daily over a prolonged period (15–18 days). About 0·2–0·3 ml. of blood was removed at each bleeding. The fall in red-cell count was followed in the same manner as in the malarial infection.

Preparation of mitochondria. Before the removal of the livers from the infected, control and anaemic mice each animal was anaesthetized with ether and its viscera were perfused with ice-cold 0·9 % sodium chloride solution. For this purpose, an extensive mid-line incision was made, a cannula was inserted into the thoracic aorta and the vena cava cut above the liver. With practice the perfusion was complete in 5 min. After perfusion the livers were removed, blotted lightly and immersed in ice-cold sucrose solution. With all types of animal studied the livers from two to four animals were combined to provide sufficient weight of tissue.

Mitochondria were isolated by one of two techniques. In the work on oxidative phosphorylation, the particles were obtained from homogenates in 0·25M-sucrose by the method of Schneider & Hogeboom (1950); when adenosine triphosphatase (ATP-ase) activity was estimated, mitochondria were isolated by the procedure of Aldridge (1957), with 0·3M-sucrose as the homogenizing medium.

Manometric measurements. Oxygen uptake together with associated phosphorylation was measured with the three substrates, succinate, glutamate and β-hydroxybutyrate. With air as the gas phase, at 28°, the basic flask constituents were orthophosphate buffer (AnalaR; 32 mM; pH 7·2), hexokinase (Sigma Chemical Co.; 200 units), glucose (AnalaR; 24 mM), sodium adenosine triphosphate (ATP) (Sigma Chemical Co.; 2 mM), MgSO₄ (AnalaR; 6 mM) and MnCl₂ (AnalaR; 0·5 mM). Succinate was employed in a concentration of 60 mM and glutamate and β-hydroxybutyrate in a concentration of 16 mM. Dependence of respiration upon added diphosphopyridine nucleotide (DPN) (C. F. Boehringer und Söhne; 0·9 mM) was tested with the two last-named substrates. Mitochondria (0·4 ml., equivalent to 200 mg. wet wt. of the livers) were added last

to the flasks, which were pre-cooled in ice. The total volume in each flask was 2·5 ml.; 0·2 ml. of 2 N-KOH was added to the centre wells.

The flasks were equilibrated for 10 min. and oxygen uptake was measured for a further 20–40 min. Reactions were stopped by addition of 2 ml. of 10% (w/v) trichloroacetic acid at 0° and orthophosphate was measured by the method of Martin & Doty (1949). Oxygen uptakes were expressed as μl. of oxygen/mg. of N/hr. (q_{O_2}); the nitrogen content of the mitochondria was estimated by the micro-Kjeldahl technique.

Measurement of adenosine-triphosphatase activity. For this series of measurements mitochondria (0·2 ml., equivalent to 60 mg. wet wt. of liver) were suspended at 28° in a medium consisting of ATP (Sigma Chemical Co.; 6 mM) and sucrose (0·2 M), to which were added MgCl$_2$ (AnalaR; 8 mM) or 2:4-dinitrophenol (0·25 mM) in a final volume of 2 ml. The particles were incubated for 15 min. and the reaction was then stopped by addition of 2 ml. of 10% (w/v) trichloroacetic acid at 0°.

Comparison was made of the ATP-ase activity of freshly prepared mitochondria and of mitochondria aged in 0·3 M-sucrose at 28° for 20 min. Orthophosphate was determined by the method of Martin & Doty (1949). The results were expressed in terms of μg.atoms of phosphorus released/mg. of N/15 min.

In addition, the orthophosphate contents of the freshly prepared suspensions of mitochondria in 0·3 M-sucrose were determined, and also after aging at 28° for 20 min. in the absence of any supplements. The same method of determination was employed.

RESULTS

Course of the malarial infection

Infected blood cells were detectable in thin blood films on the third day after inoculation and the parasitaemia rose with appearance of increasing numbers of parasitized reticulocytes until 8–10 days after inoculation (Fig. 1). After this period of time the infection either maintained itself at a maximum parasitaemia equivalent to 30–40% of the circulating blood cells, as in Fig. 1, or increased to the region of 50% parasitaemia.
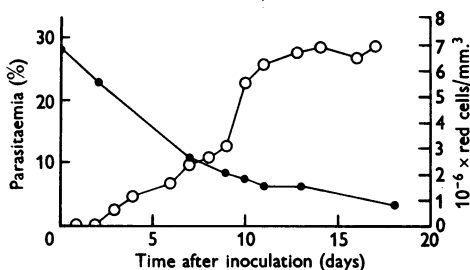

Fig. 1. Relationship between parasitaemia (○), expressed as the percentage of circulating red cells infected, and the associated anaemia of infection (●), evaluated in terms of the red-cell count (millions/mm.³ of blood), in a typical case of *P. berghei* infection in mice.

In the former case, which was typical of the infections when the oxidative phosphorylation of isolated liver mitochondria was studied (Table 1), the static parasitaemia was accompanied by a progressively increasing anaemia of infection (Fig. 1). The animals became anorexic from about 8–10 days after inoculation, moribund and cold to the touch from the twelfth day and death usually occurred between 14 and 18 days.

When parasitaemia increased to 50% the general symptoms were similar but the increasing parasitaemia throughout the entire course of the infection resulted in an earlier death, in 12–14 days after inoculation. These conditions were typical of the infections when ATP-ase activity of the isolated liver particles was measured.

In groups of animals bled to induce anaemia, the amounts removed, equivalent to 10–20% of the blood volume of the mice at each daily operation, produced in the final stages a fall in the circulating red cells similar to that shown in the course of the longer-term malarial infection. The physical condition of the bled animals also closely resembled that of the infected animals which followed this course.

Oxidative phosphorylation of liver mitochondria

The results of the manometric experiments, in terms of rates of oxidation and associated P/O ratios for each enzyme system studied, are expressed in relation to the duration of the malarial infection in Table 1. A marked difference in behaviour was apparent between normal liver mitochondria and infected liver mitochondria after the infection had progressed for 14 days after inoculation, but no appreciable change in either oxidation rates or associated P/O ratios was detected in the mitochondria of livers from infected mice killed before this period had elapsed. The affected mitochondria showed a lowered oxidative capacity together with a lowered P/O ratio. Addition of DPN to the glutamate and β-hydroxybutyrate systems produced a slight increase in the rate of oxidation but had no effect upon the P/O ratio. No similar effects were observed with the mitochondria from the control animals or the animals killed in the earlier period of infection. The results of a typical experiment illustrating the effects of DPN supplementation upon glutamate oxidation by normal and long-term infected-liver mitochondria are shown in Fig. 2.

The larger standard deviations associated with the results from the groups of animals killed after 14 days deny any absolute significance to the findings. However, it must be remembered that the variation in the physical condition of each individual animal was greater after infection had lasted for 14 days and thus the degree of mitochondrial disturbance would be expected to vary

more within this group than within the other groups studied.

The liver mitochondria from the animals which had been suffering prolonged severe anaemia, and whose physical condition resembled that of the malarial mice, showed an interesting property (Table 1). Although the rates of oxidation with respect to glutamate and succinate were lower than the corresponding results for normal mitochondria, glutamate oxidation was insensitive to added DPN and the associated P/O ratios for both substrates were unchanged. This was in contrast with the sensitivity of glutamate oxidation to added DPN and the lowering of the P/O ratios experienced in the same oxidations by mitochondria from mice which had been infected for a similar period (15–18 days). Mitochondria from one group of mice which were bled for 20 days did exhibit the effect of a lowered P/O ratio in addition to a lowered rate of oxidation. Data from this source are not included in Table 1 as malarial infections lasting 20 days were not experienced, and hence a direct comparison with data from liver mitochondria of comparably infected mice could not be made.

The insensitivity of glutamate respiration to added coenzyme together with the normal P/O values for both substrates indicate that the mito-
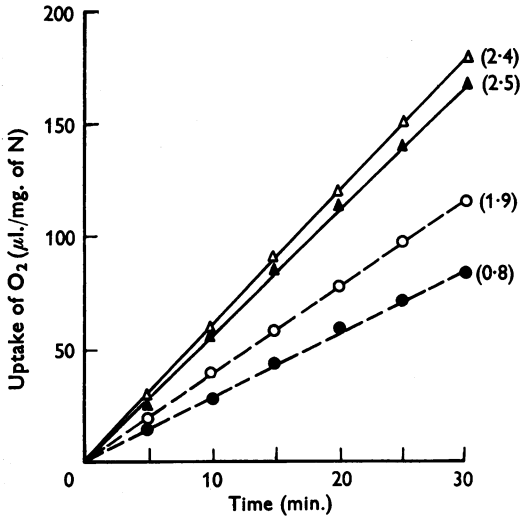

Fig. 2. Oxygen consumption and associated phosphorylation during glutamate oxidation by liver mitochondria from normal mice and mice infected with *P. berghei* for more than 14 days. ▲, △, Respective characteristics of unsupplemented and DPN-supplemented liver mitochondria from normal mice; ●, ○, respective characteristics of unsupplemented and DPN-supplemented liver mitochondria from infected mice. Conditions employed are described in the text. Figures in parentheses represent P/O values.

Table 1. *Effect of duration of malarial infection and of anaemia upon oxidative phosphorylation of liver mitochondria*

Figures in parentheses refer to the number of groups of mice killed for each value presented and results are expressed as means ±S.E. Conditions were as described in the Experimental section.

Source of mitochondria	Succinate q_{O_2}	Succinate P/O	Glutamate Without added DPN q_{O_2}	Glutamate Without added DPN P/O	Glutamate With added DPN q_{O_2}	Glutamate With added DPN P/O	β-Hydroxybutyrate Without added DPN q_{O_2}	β-Hydroxybutyrate Without added DPN P/O	β-Hydroxybutyrate With added DPN q_{O_2}	β-Hydroxybutyrate With added DPN P/O
Normal mice	590 (16) ±45	1·5 ±0·1	360 (11) ±40	2·5 ±0·3	370 (10) ±38	2·4 ±0·2	150 (9) ±24	2·2 ±0·3	160 (8) ±22	2·1 ±0·3
Infected mice Less than 14 days	585 (9) ±68	1·4 ±0·1	330 (4) ±46	2·3 ±0·3	340 (3) ±15	2·3 ±0·3	145 (6) ±21	1·9 ±0·6	155 (6) ±19	2·0 ±0·5
More than 14 days	435 (7) ±117	0·9 ±0·5	215 (7) ±53	1·5 ±0·9	260 (7) ±47	1·6 ±0·7	115 (3) ±14	1·4 ±0·6	135 (3) ±12	1·2 ±0·4
Anaemic mice	475 (3) ±65	1·4 ±0·1	300 (3) ±75	2·5 ±0·1	310 (3) ±80	2·3 ±0·2				

chondria from the anaemic animals possessed integrated enzymic activity. The lower rates of oxidation, however, suggest that the mitochondria had a lowered metabolic capacity, which might possibly have been due to a lowered working enzyme concentration.

Adenosine triphosphatase of liver mitochondria

The results of the experiments upon ATP-ase activity (Table 2) indicate, in confirmation with other workers (Kielley & Kielley, 1951; Lardy & Wellman, 1953; Potter, Siekevitz & Simonson, 1953), a low rate of ATP breakdown in freshly prepared normal mitochondria, except in the presence of added dinitrophenol. On aging, the latent and Mg^{2+} ion-stimulated ATP-ase effects were apparent whereas the dinitrophenol-stimulated breakdown decreased.

The mitochondria from the livers of the infected animals, which, it will be recalled, were from the group showing a continuously increasing infection, showed characteristics, when freshly prepared, which were similar to those exhibited by the aged particles from the control livers. Higher basic and Mg^{2+} ion-stimulated activities were coupled with a lower dinitrophenol-stimulated ATP breakdown. The changes on aging were less marked than with the mitochondria from the control mice.

The orthophosphate content of the two preparations of mitochondria also differed. Initially the orthophosphate content of the control particles was low but increased upon aging; that of the particles from the infected livers gave an initially higher value which also increased on aging, the final figure being in the same range as that for the aged normal mitochondria. Such increases in the orthophosphate content of the mitochondria may reflect the breakdown of high-energy-phosphate compounds or structural-phosphate compounds in the aged and infected mitochondria.

DISCUSSION

The biochemical changes which are apparent in the properties of the liver mitochondria from mice which had been infected for more than 14 days are similar in many respects to those described in the investigations on liver mitochondria cited at the beginning of this paper. A lowered oxidative capacity, associated with a lowered P/O ratio; an inability to carry out DPN-linked oxidations, partially reversed by addition of DPN; increased ATP-ase and Mg^{2+} ion-induced ATP-ase activities–all these symptoms correspond, as indicated by Ernster & Lindberg (1958), to the 'aging' symptoms of normal mitochondria, and, as such, cannot be considered as specific to the condition investigated. These factors may be described as a biochemical syndrome accompanying and contributing towards liver damage, but, with the limited data available, cannot be designated the initially active factors promoting the appearance of the damage. It is conceivable that, especially in malaria, other processes such as localized circulatory disturbance (Maegraith, 1948) may possibly provide the initial stimulus to liver degeneration, the final manifestations of which, in the biochemical sense, are changes of the type described.

Although no changes in mitochondrial function were detectable in livers from animals killed earlier than 14 days after inoculation, it cannot be assumed that a measure of disturbance had not occurred by this time. It must be remembered that the technique used sampled the entire liver, whereas the liver changes occurring in malaria are localized in the centrilobular regions. Hence it is probable that signs of early damage were masked by the preponderance of normal tissue over damaged tissue in the mitochondrial preparations, and differences in activity became apparent only

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Table 2. *Effect of malarial infection upon the adenosine-triphosphatase activity and orthophosphate content of liver mitochondria*

Conditions were as described in the Experimental section. Infected mice taken in the terminal stages of infection were from the group dying in 12–14 days (see text). Figures in parentheses refer to the number of groups of mice killed for each value presented and results are expressed as means ± S.E.

	Mitochondria from normal mice		Mitochondria from infected mice	
	Freshly prepared	Aged 20 min. at 28°	Freshly prepared	Aged 20 min. at 28°
ATP-ase activity (μg.atoms of P liberated/mg. of N/15 min. at 28°)				
Basic incubation medium (6)	1·6 ± 0·8	14·8 ± 5·7	4·2 ± 1·6	8·2 ± 3·0
Basic + Mg^{2+} ions (3)	0·6 ± 0·5	16·0 ± 6·0	9·0 ± 4·3	13·2 ± 5·5
Basic + dinitrophenol (6)	46·0 ± 6·2	21·8 ± 5·2	19·8 ± 8·5	13·6 ± 4·9
Orthophosphate content of mitochondria (μg.atoms of P/mg. of N) (3)	0·14 ± 0·02	0·40 ± 0·02	0·27 ± 0·05	0·48 ± 0·06

when the damaged area constituted a marked proportion of the whole.

At this stage in the infection the animals had been grossly anaemic for some time and the results obtained from normal mice which had been bled to simulate this condition indicate that anaemia of this magnitude acting over this duration can itself produce some effect upon liver mitochondria. However, the changes appeared less advanced in the liver mitochondria from the anaemic animals, since the P/O ratios were normal and glutamate oxidation was insensitive to added coenzyme. It would appear certain that malaria produces effects of a more severe nature when acting over the same period, but the contributory effects of the prolonged anaemia of infection towards the changes described in the behaviour of the liver particles from the infected mice cannot be ignored. The extent of this contribution may be more clearly defined in similar experiments with a more rapidly acting strain of malaria and preliminary results suggest that some changes in liver-mitochondrial metabolism are produced within a period when anaemia is neither as severe nor as long-acting as in the reported work.

Although the strain of mouse, method of transmission, housing and diet were identical in all respects, the course of the malarial infection in the groups for the study of oxidative phosphorylation and of ATP-ase activity was different. Such changes in the degree of response to malarial infection by the same type of animal to the same species of *Plasmodium* are by no means uncommon. In relation to the results obtained, however, it is not considered that the change in the course of the infection would influence the nature of the terminal biochemical changes reported, and, as such, both the results of the experiments upon oxidative phosphorylation and those upon ATP-ase activity may be presented in a complementary fashion as descriptions of the overall effects of malaria upon the metabolism of the liver particles.

SUMMARY

1. Infection of mice with *Plasmodium berghei* malaria produces demonstrable disturbance in the behaviour of liver mitochondria.

2. Oxidation rates of succinate, glutamate and β-hydroxybutyrate are depressed and addition of coenzyme to the diphosphopyridine nucleotide-linked oxidations effects only partial reactivation; P/O ratios are lowered with each substrate oxidized.

3. Both latent and Mg^{2+} ion-stimulated adenosine-triphosphatase activities are increased, whereas dinitrophenol-stimulated activity is decreased.

4. The relation of these changes to the duration of the infection and to the degree and duration of the accompanying anaemia is described, and the similarity of the findings to mitochondrial disturbance in toxic liver damage is discussed.

REFERENCES

Aldridge, W. N. (1957). *Biochem. J.* **67**, 423.

Aldridge, W. N. & Cremer, J. E. (1955). *Biochem. J.* **61**, 406.

Christie, G. S. & Judah, J. D. (1954). *Proc. Roy. Soc.* B, **142**, 241.

Deegan, T. & Maegraith, B. G. (1958). *Ann. trop. Med. Parasit.* **52**, 232.

Dickens, F. & Salmony, D. (1956). *Biochem. J.* **64**, 645.

Ernster, L. & Lindberg, O. (1958). *Annu. Rev. Physiol.* **20**, 13.

Jones, E. S., Maegraith, B. G. & Gibson, Q. H. (1953). *Ann. trop. Med. Parasit.* **47**, 431.

Kielley, W. W. & Kielley, R. K. (1951). *J. biol. Chem.* **191**, 485.

Lardy, H. A. (1955). *Proc. 3rd. int. Congr. Biochem., Brussels*, p. 287.

Lardy, H. A. & Feldott, G. (1951). *Ann. N.Y. Acad. Sci.* **54**, 636.

Lardy, H. A. & Wellman, H. (1953). *J. biol. Chem.* **201**, 357.

Maegraith, B. G. (1948). *Pathological Processes in Malaria and Blackwater Fever.* Oxford: Blackwell Scientific Publications Ltd.

Maegraith, B. G., Deegan, T. & Riley, M. V. (1959). *Trans. R. Soc. trop. Med. Hyg.* **53**, 3.

Maegraith, B. G., Jones, E. S. & Andrews, W. H. H. (1951). *Trans. R. Soc. trop. Med. Hyg.* **45**, 15.

Martin, J. B. & Doty, D. M. (1949). *Analyt. Chem.* **21**, 965.

Martius, C. & Hess, B. (1951). *Arch. Biochem. Biophys.* **33**, 486.

Potter, V. R., Siekevitz, P. & Simonson, H. C. (1953). *J. biol. Chem.* **205**, 893.

Salmony, D. (1956). *Biochem. J.* **62**, 411.

Schneider, W. C. & Hogeboom, G. H. (1950). *J. biol. Chem.* **183**, 123.

Tapley, D. F. & Cooper, C. (1956). *J. biol. Chem.* **222**, 341.

Tapley, D. F., Cooper, C. & Lehninger, A. L. (1955). *Biochim. biophys. Acta*, **18**, 597.

Thurston, J. P. (1953). *Exp. Parasit.* **2**, 311.

Wade, R. & Jones, H. W. (1956). *J. biol. Chem.* **220**, 547, 553.

Zetterström, R. & Ernster, L. (1956). *Nature, Lond.*, **178**, 1335.