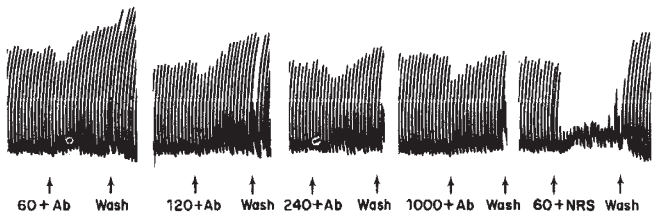

Fig. 2 Effect on the electrically-induced contractions of isolated guinea pig ileum of adding 0.4 ml. anti-M-6-HS-BSA globulin (Ab) preincubated with either 60 nM, 120 nM, or 1,000 nM morphine; and the effect of adding 0.4 ml. normal rabbit globulin (NRS) preincubated with 60 nM morphine.

action, antibody was added to the muscle bath 1 min after addition of morphine when the induced inhibition of muscle contraction was maximal. Typical results are illustrated in Fig. 3*a*. Anti-M-6-HS-BSA added for 1 min after morphine completely reversed the inhibition of muscle twitch caused by 120 nM morphine. The recovery of the twitch response was complete within 2 min and this effect appeared to be identical to that observed after addition of 10 nM naloxone. (Fig. 3*b*). Neither NRS globulin or anti-BSA globulin (Fig. 3*c*) had any effect on the morphine-induced inhibition of the muscle twitch. Reversal of the morphine effect was about 80% complete when a stoichiometrically equivalent amount of antibody was added to the muscle bath.

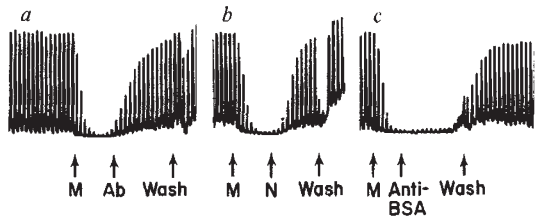

Fig. 3 Effect on the electrically-induced contractions of isolated guinea pig ileum of 120 nM morphine (M) present in the muscle bath for 1 min followed by the addition of either: *a*, 0.5 ml. of anti-M-6-HS-BSA (Ab); *b*, 10 nM naloxone (N); or *c*, 0.5 ml. of anti-BSA.

Similar experiments have been carried out with globulin obtained from several rabbits immunized with morphine-6-succinyl-BSA, with identical results. We suggest that because anti-BSA globulin has no effect on the morphine activity the inhibition of biological action of morphine is the specific effect of antibodies reactive with morphine.

Results of our experiments indicate that sepecific antibodies are capable of antagonizing the inhibitory effect of morphine on the electrically stimulated contractions of guinea pig ileum. The antibody molecules apparently bind morphine more strongly than the tissue receptor site as antibodies were able to prevent and reverse the inhibitory effect of morphine on smooth muscle contraction. The use of this sensitive *in vitro* assay together with immunochemical measures of antibody avidity should allow determination of the molar concentrations of antibody required for morphine antagonism and the relative affinities of morphine receptors in tissues for their drug substrates.

This work was supported by US Public Health Service training grant and research grant.

B. H. WAINER,
F. W. FITCH

Department of Pathology,

R. M. ROTHBERG

Department of Pediatrics,

C. R. SCHUSTER

Department of Psychiatry and Pharmacology,
University of Chicago,
Chicago, Illinois 60637

Received September 20; revised November 6, 1972.

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Glucose Catabolism by the Simian Malaria Parasite *Plasmodium knowlesi*

ALTHOUGH the parasitology of malaria has been extensively studied[1], biochemical aspects of the relationships between the intra-erythrocytic parasite and its host erythrocyte are poorly understood. The main source of metabolic energy for the erythrocytic stages of the parasite is the breakdown of glucose with formation of lactic acid[2–6], which it may carry out independently of the red cell[2]. Catabolism of glucose by metabolic pathways other than glycolysis may occur, although the overall pattern of glucose breakdown appears to vary with the species of malaria parasite[7]. The ability of the parasite, as distinct from the erythrocyte, to carry out the reactions of the pentose phosphate pathway is unresolved[8–11,19].

Here we have attempted to estimate the flux of glucose through the glycolytic pathway, in normal rhesus monkey erythrocytes, in erythrocytes infected with *P. knowlesi* and in parasites "freed" from their host erythrocytes. *P. knowlesi* shows synchronous growth with a 24 h asexual life cycle and parasites may therefore be conveniently prepared from the monkey host in relatively large quantities at well defined life

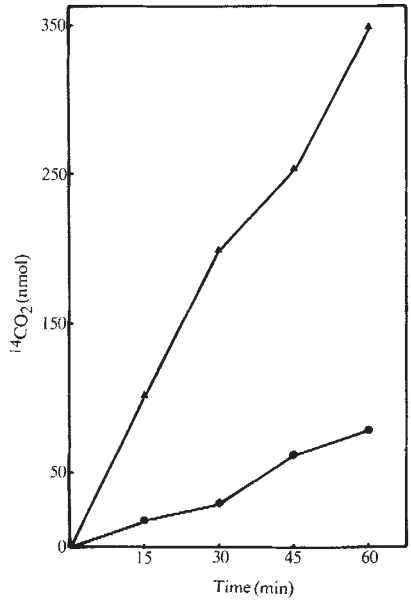

Fig. 1 Production of $^{14}CO_2$ from 1-^{14}C- and 6-^{14}C-D-glucose in erythrocytes of the rhesus monkey infected with *Plasmodium knowlesi*. ▲, 1-^{14}C-D-glucose; ●, 6-^{14}C-D-glucose.

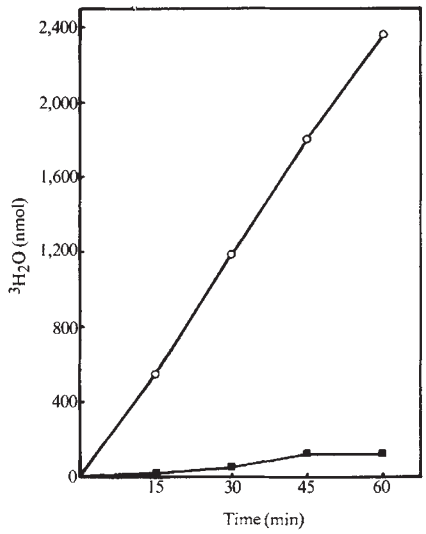

Fig. 2 Release of ^3H_2O from 2-^{3}H-D-glucose by erythrocytes from the rhesus monkey. ■, Normal; ○, infected with *Plasmodium knowlesi*.

cycle stages. Parasites were prepared when they had grown to the late trophozoite and schizont stages.

The flux of glucose through the glycolytic pathway and the primary dehydrogenases of the pentose phosphate pathway was measured radiochemically by the method of Hutton[12] which estimates glycolytic flux as the release of ^3H_2O from 2-^{3}H-D-glucose in the hexosephosphate isomerase (EC 5.3.1.9) reaction. Flux through the primary dehydrogenases of the pentose phosphate pathway was estimated from the release of $^{14}CO_2$ from 1-^{14}C-D-glucose and 6-^{14}C-D-glucose.

The results (Figs. 1 and 2) show that the release of ^3H_2O from 2-^{3}-H-D-glucose and of $^{14}CO_2$ from 1-^{14}C- and 6-^{14}C-D-glucose were linear over the experimental time. The flux of the glucose through the glycolytic pathway obtained by radiochemical investigation agreed with the values obtained by measuring production of lactic acid, and was greatly increased in the parasitized erythrocytes as compared with normal erythrocytes (Table 1). A high value for glycolytic flux was observed in the free parasites.

The results presented in Table 1 show also that the flux of glucose through the primary dehydrogenases of the pentose phosphate pathway was very much greater in parasitized erythrocytes than in normal erythrocytes. This large increase in metabolic activity was retained with the parasite when "freed" from its host erythrocyte.

The high $^{14}CO_2$ production from 6-^{14}C-D-glucose indicated the presence of an oxidative metabolic process in the parasitized erythrocyte and in the "free" parasite preparation. This value is higher than can be accounted for by recycling through the hexose phosphate pool[16,17]. Randomization of the C_6 position into the C_1 position of the hexose phosphate pool indicates that the specific activity of the C_1 position may be up to 20% of the initial specific activity of the C_6 position[18]. A reasonable estimate of the maximal rate of $^{14}CO_2$ production from 6-^{14}C-D-glucose recycling through the pentose phosphate pathway would be about 20% of the rate of $^{14}CO_2$ production from 1-^{14}C-D-glucose assuming 100% recycling. Observed values would probably be lower than 20%. Although extensive recycling of hexose phosphate in the erythrocyte has been reported[20] it is most unlikely that 100% of the hexose phosphate produced in the pentose phosphate pathway would be recycled by glucose-6-phosphate. Our results show that the rate of production of $^{14}CO_2$ in the parasitized erythrocyte was between 19–28% of the rate of production of $^{14}CO_2$ from 1-^{14}C-D-glucose. In the "free" parasite preparation the corresponding figure lay between 22% and 50%. This suggested that $^{14}CO_2$ was produced from 6-^{14}C-D-glucose by processes other than recycling through the pentose phosphate pathway.

We believe that our results represent a valid assessment of the flux through the glycolytic pathway and through the primary dehydrogenases of the pentose phosphate pathway, though the method yields no information of the fate of end products and intermediates of these pathways. Glycolytic flux is measured as the flux through the first enzyme peculiar to the glycolytic pathway. It is an accurate measurement of the flux of glucose through to pyruvate only if the pentose phosphate pathway is of small activity compared to the glycolytic activity. In our system, 20% or less, of the glucose appears to be metabolized by the initial enzymes of the pentose phosphate pathway. We consider that this, together with the agreement between radiochemical measurements of the glycolytic flux and measurement of lactate production provides good evidence that the radiochemical measurement is giving an acceptable assessment of glycolysis and that the method is a valuable tool for the investigation of metabolic characteristics in malaria infection.

Our results show therefore that the breakdown of glucose in the parasitized erythrocyte, by both glycolysis and the pentose

Table 1 Glycolytic Flux and Lactate Production

	^3H_2O release nmol/10^9 cells h^{-1}	Lactate production nmol/10^9 cells h^{-1}	$^{14}CO_2$ production from: 1-^{14}C-D-glucose nmol/10^9 cells h^{-1}	$^{14}CO_2$ production from: 6-^{14}C-D-glucose nmol/10^9 cell h^{-1}
Normal erythrocytes	75– 155 (4)	180– 224 (4)	9– 16 (3)	Not detected
Parasitized erythrocytes	800–2,360 (3)	1,988–4,912 (5)	348–410 (3)	67–116 (3)
"Free" parasites	1,080–3,600 (3)	None available	368–478 (3)	78–196 (3)

Values given are upper and lower values in the observed range. Number of experiments is shown in parentheses. Both parasitized erythrocytes and free parasites for the radiochemical experiment were prepared from blood from the same infected rhesus monkey. Erythrocytes were prepared from freshly drawn heparinized blood by centrifugation at 1500*g* for 10 min, washed twice under the same conditions in Krebs glucose saline, and separated from white cells and platelets using a sucrose gradient[13]. Parasites were freed from host erythrocytes by immune lysis[14]. Erythrocytes and parasites were washed three times by centrifugation at 1500*g* for 10 min with the medium used in the glycolytic incubation[15]. Erythrocytes and parasites were finally suspended in the glycolytic medium to 50% haematocrit. The cells were incubated in closed Warburg flasks containing: 2.0 ml. glycolytic medium, 0.5 ml. erythrocyte or parasite suspension with 0.5 μCi 1-^{14}C or 6-^{14}C-D-glucose and 1.5 μCi 2-^{3}H-D-glucose. The reaction was terminated by tipping 0.5 ml. of 20% TCA from the side arm and carbon dioxide evolved was trapped in 0.3 ml. hyamine (Koch–Light Labs, Bucks) in the centre well. The flasks were pre-incubated 15 min at 37° C before addition of the radioactive substrate in 10 μmol of carrier glucose. At the end of the experiment the flasks were further shaken in the 'New Brunswick Controlled Environment Incubator' for 1 h at 50 to 60 cycles per min to remove final traces of $^{14}CO_2$. Recovery of $^{14}CO_2$ was calculated by trapping $^{14}CO_2$ evolved from a known addition of $Na_2{}^{14}CO_3$ after the addition of TCA. This was estimated as 70±2%. ^3H_2O was sublimed from the incubation mixture under vacuum in Thunberg tubes, trapping the sublimed ^3H_2O by immersing one end of the tube in a dry-ice-methanol bath. Counting of the $^{14}CO_2$ was performed in toluene based scintillator (6 g PPO, 0.12 g POPOP $l.^{-1}$) and the ^3H_2O was counted in a Dioxan based scintillator (180 g $l.^{-1}$ naphthalene 4 g PPO, 0.1 g POPOP $l.^{-1}$). All counting was performed on a 'Packard liquid scintillator spectrometer'. Erythrocyte and parasites were counted using a 'Thoma' haemocytometer with Neubauer ruling after dispersing parasites by drawing through a syringe needle several times.

phosphate pathway, was very much increased compared with the normal erythrocyte. These activities were recovered, at least in part, with the parasite when it was "freed" from its host erythrocyte. We have not made a quantitative assessment of this recovery, nor does this observation indicate that the activity observed is of parasite origin. The immune lysis method used to "free" the parasites may not remove the erythrocyte membrane from the parasite-erythrocyte complex although the host cell haemoglobin was removed, and it is possible that host erythrocyte glycolytic enzymes may be retained in the "free" parasite preparation associated with this[21–23]. It is essential to discover from the point of view of the host-parasite relationship, if the increase in metabolic activity is due to the elaboration of an independent metabolic capability by the parasite, or if the parasite can enhance and utilize the metabolic processes of its host erythrocyte.

P. I. T. received financial assistance from the World Health Organization. We thank our colleagues for critically reading the manuscript and Mrs S. J. Burt and Mr P. Schofield for technical assistance.

P. G. SHAKESPEARE
P. I. TRIGG

Division of Parasitology,
National Institute for Medical Research,
Mill Hill, London NW7 1AA

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A Preliminary Investigation of the Swine Vesicular Disease Epidemic in Britain

ON December 11, 1972, an outbreak of vesicular disease in pigs was reported from Staffordshire. It was diagnosed as foot-and-mouth disease (FMD) on clinical grounds but this was not confirmed on initial serological tests. Investigations were carried out to determine the nature of the aetiological agent.

Outbreaks of disease have occurred in pigs on two previous occasions which have been clinically indistinguishable from FMD, but from which different causal agents have been isolated. Nardelli *et al.*[1] desbcribed such a syndrome in Italy in 1966, and suggested that the agent responsible was a porcine enterovirus, and this has been confirmed by Newman *et al.*[2]. Another outbreak in pigs in Hong Kong in 1971 was reported by Mowat *et al.*[3] and the causative agent was again described as a porcine enterovirus (Hong Kong 1971), serologically similar to the Italian enterovirus.

The disease as observed in Britain closely resembles that described in these two reports (A. J. G. Stubbins, R. S. Hedger and G. N. Mowat, personal communication). It occurred only in pigs although on several premises cattle or sheep were in close contact with large numbers of the infected animals. Fifteen outbreaks were confirmed in four weeks, eight being in the first week. Several outbreaks occurred in pigs which were fed on swill collected from the Birmingham area, and in a number of instances spread from the first reported outbreak could be attributed to the movement of pigs or of people. The pattern of disease spread suggested an incubation period of four to six days. Morbidity rates of up to 60% were recorded and the lesions could not be differentiated from those of FMD.

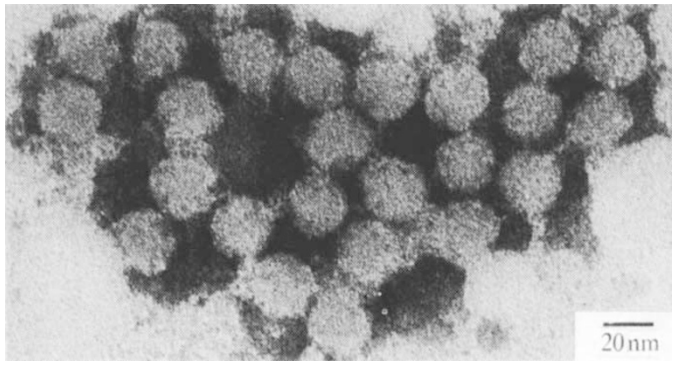

Fig. 1 Electron micrograph of complexes of the swine vesicular disease virus (England 1972) with antiserum to the Hong Kong 1971 enterovirus.

Vesicular fluid, extracts of vesicular epithelium and fluid from infected tissue culture monolayers were tested by complement fixation for the presence of FMD virus or enterovirus antigens. Hyperimmune guinea-pig sera were used which had been prepared against all seven types of FMD virus and against the Italy 1966 enterovirus. The tests were performed in microplates using a fixed complement dose of five 50% haemolytic units and a constant amount of antigen. The antisera were diluted in 1.5-fold series from starting dilutions of 1/16; plates were incubated for 18 h at 4° C. In all of the samples tested there was no reaction with any of the FMD virus antisera. Positive results, however, were obtained with the Italy 1966 enterovirus antiserum, in many instances using material from infected pigs and in all tests in which tissue culture fluid was used from monolayers showing extensive cytopathogenic effects.

To further verify the identity of the virus, virus from infected tissue culture fluid was purified, mixed with serum from a pig convalescent from Hong Kong 1971 enterovirus and examined in the electron microscope ('Siemens Elmiskop I'). Complexes of virus and antibody were seen (Fig. 1), which were also present in mixtures of virus and the Italy 1966 enterovirus antiserum, but were not observed when the virus was mixed with FMD antiserum.

FMD virus differs from enteroviruses in being acid-labile, exposure to *p*H 5 causing a reduction in infectivity by degradation of the virion to its RNA and protein subunits[4]. Swine vesicular disease virus (England 1972) when so treated showed