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The Metabolism of *Plasmodium berghei*, the Malaria Parasite of Rodents

2. AN EFFECT OF MEPACRINE ON THE METABOLISM OF GLUCOSE BY THE PARASITE SEPARATED FROM ITS HOST CELL*

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(*Received* 25 *August* 1960)

Glucose is essential for the growth of the erythrocytic forms of several malaria parasites *in vitro*, and certain antimalarial drugs such as mepacrine inhibit development and multiplication and cause a rapid decrease in the utilization of added glucose and lactate (McKee, 1951). Although the relation of these latter effects to the primary action of the drug is obscure, mepacrine is known to have an inhibitory effect on several enzymes of the Embden–Meyerhof glycolytic pathway present in the parasites of avian malaria (Speck & Evans, 1945*a*; Marshall, 1948) and on the oxidation by the same parasite of intermediates of the tricarboxylic acid cycle (Speck, Moulder & Evans, 1946; Moulder, 1948, 1949; Bovarnick, Lindsay & Hellerman, 1946; Marshall, 1948). It is not, however, known whether glucose is metabolized exclusively by these pathways in the intact parasite.

In the present work, *Plasmodium berghei* has been used as a readily available mammalian parasite. The metabolism of specifically and uniformly labelled [^{14}C]glucose by the parasite separated from its host cell, the reticulocyte, has been studied and it has been concluded that an enzyme concerned with the further catabolism of hexose 6-phosphate is the most sensitive to approximately therapeutic concentrations of mepacrine. This appears to be a selective inhibitory effect since an appreciable increase in drug concentration was without effect on the uptake of glucose by the reticulocyte.

* Part 1: Bowman, Grant & Kermack (1960).

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Part of this work has already been briefly reported (Bowman, Grant, Kermack & Ogston, 1959).

EXPERIMENTAL

Suspension of infected cells. The strain of *P. berghei* used was obtained from Dr J. D. Fulton (see Fulton & Spooner, 1956). Infected blood was obtained by cardiac puncture from white mice (about 20–25 g.) which had been inoculated intraperitoneally 5–7 days previously with 0·2 ml. of blood containing about 10^7 infected cells.

White cells were removed by percolation of freshly shed blood through small columns of filter-paper powder (1 g./10 ml. of blood; Fulton & Grant, 1956). The blood was then centrifuged at 1000 *g* for 10 min., the supernatant was discarded and the cells were resuspended in an equal volume of a phosphate-buffered iso-osmotic salt solution, pH 7·2 (Krebs & Eggleston, 1940). This washing procedure was repeated twice. The suspension was then centrifuged at 1000 *g* for exactly 10 min. in 10 cm. × 1 cm. graduated centrifuge tubes and the sedimented cells were resuspended in 19 times their volume of phosphate-buffered salt solution which contained 2 μmoles of $NaHCO_3$/ml. Suspensions prepared in this way contained about 10^6 cells/ml., of which not less than 70% were infected.

Suspensions of free parasites. Free parasites were obtained by haemolysis of infected red cells with the serum of rabbits which had been immunized against mouse red cells. Fresh guinea-pig serum was used as a source of complement. The washed parasites were centrifuged at 1000 *g* for exactly 10 min. and resuspended in 19 times their volume of a buffered iso-osmotic salt solution, pH 7·2, which contained K^+ and Na^+ ions in the ratio 7:1. Details of these procedures have been given previously (Bowman, Grant & Kermack, 1960). The final suspension contained in 1 ml. the free parasites originally present in about 10^9 infected red cells, and such preparations will subsequently be referred to as 'standard suspensions' of free parasites.

Suspensions of reticulocytes. Mice were treated with acetylphenylhydrazine as described by Jones, Maegraith & Gibson (1953). When 80–90% of the red cells in the blood were reticulocytes, the animal was killed by cardiac puncture and suspensions of reticulocytes were obtained as described for infected red cells.

Cell-counting methods. Total red-cell counts were made in an 'improved' Neubauer haemocytometer chamber. The percentage of infected cells was determined by counting 500 red cells in blood films treated with Giemsa stain. The number of reticulocytes was determined by supra-vital staining with brilliant cresyl blue followed by counter-staining the blood film with Giemsa stain for 2 min.

Labelled compounds. Uniformly labelled glucose ([U-^{14}C]-glucose) and [1-^{14}C]- and [6-^{14}C]-glucose were obtained from The Radiochemical Centre, Amersham, Bucks.

Incubation procedures and isolation of the products of metabolism of [^{14}C]glucose

Carbon dioxide. Suspensions of cells (3 ml.) containing 6 mg. of [^{14}C]glucose were incubated with gentle shaking for 1 hr. at 37° in stoppered Warburg flasks (volume 15 ml.), fitted with a centre well containing 0·3 ml. of 20% (w/v) KOH and a side arm containing 0·5 ml. of 40% (w/v) trichloroacetic acid. At the end of the incubation period, the side-arm contents were added to the suspension and, after 30 min., the alkali in the centre well was quantitatively transferred to a centrifuge tube, 0·1 m-mole of $NaHCO_3$ was added and the carbonate was precipitated as barium carbonate. This precipitate was washed three times with carbonate-free distilled water, twice with ethanol and twice with diethyl ether.

Other compounds. Suspensions of free parasites (10 ml.) containing 20 mg. of [^{14}C]glucose were incubated with gentle shaking for 1 hr. at 37° in stoppered conical flasks (volume 50 ml.). When organic acids were to be isolated the reaction was stopped by the addition of 2 ml. of 10% (w/v) metaphosphoric acid, otherwise 5 ml. of 10% (w/v) trichloroacetic acid was used. To facilitate identification and estimation of any product of catabolism of [U-^{14}C]-glucose a known amount of the non-radioactive compound was added, dissolved in the acid used to stop the reaction.

(*a*) Organic acids. The protein-free medium was continuously extracted with diethyl ether for 36 hr. and, after removal of the solvent, the acids in the extract were separated by partition chromatography on columns (20 cm. × 2 cm.) of Hyflo Supercel (Johns-Manville and Co. Ltd., London) with 0·5N-H_2SO_4 as the stationary phase. A preliminary separation was obtained by using mixtures of butan-1-ol in chloroform as the mobile phase (Phares, Mosbach, Denison & Carson, 1952) and each acid fraction was then further chromatographed on a similar column with diethyl ether as the mobile phase. Acid in the eluate was detected by titration of each fraction (5 ml.) with 0·01N-NaOH and phenol red as an internal indicator. The appropriate fractions were combined and the separated aqueous phase was treated with charcoal (Norit Ultra, United Norit Sales Corp. Ltd., Amsterdam) to remove the indicator. After acidification the organic acid was isolated by continuous extraction with diethyl ether and its identity was confirmed by paper chromatography (Jones, Dowling & Skraba, 1953) with the spray reagent containing glucose and aniline that was described by Carles, Schneider & Lacoste (1958).

(*b*) Phosphate esters. The protein-free medium was diluted with a solution of sodium borate so that the final concentrations of chloride and borate were 0·025M and 0·01M respectively. This solution was then allowed to percolate slowly (0·5 ml./min.) through a 12 cm. × 1 cm. column of Dowex-1 (200–400 mesh, X 8) in the chloride form. The column was then washed with 10 ml. of aq. 1 mM-NH_3 soln. containing 20 mg. of unlabelled glucose, then with 50 ml. of aq. 1 mM-NH_3 soln. and subsequently the hexose phosphates were eluted with the solvent systems described by Khym & Cohn (1953). The identity of these esters in bulked fractions was confirmed by paper chromatography (Bandurski & Axelrod, 1951).

Preparation of compounds for radioassay

Lactic acid and other organic acids. These compounds were oxidized in the combustion apparatus described by Claycomb, Hutchens & van Bruggen (1950) with the combustion fluid of van Slyke & Folch (1940). The evolved CO_2 was absorbed in 2N-NaOH, which was subsequently quantitatively transferred to a small centrifuge tube, and the carbonate was precipitated as $BaCO_3$. The latter was successively washed with carbonate-free water, ethanol and diethyl ether.

The distribution of ^{14}C in lactic acid samples formed from [1-^{14}C]- or [6-^{14}C]-glucose was determined by the procedures detailed by Sakami (1955). This involved the oxidation of lactic acid to acetaldehyde and CO_2 (containing C-1 of lactate). A portion of the acetaldehyde was counted directly as the 2:4-dinitrophenylhydrazone derivative and the remainder was oxidized to iodoform (C-3), and formic acid (C-2). The iodoform was oxidized to CO_2 by the procedure given by Shreeve, Leaver & Siegel (1952), and the formic acid was oxidized with Hg^{2+} ions to CO_2 (Pirie, 1946).

Hexose 6-phosphates. Bulked fractions of these phosphate esters from Dowex-1 columns contained appreciable amounts of Cl^- and borate ions and were oxidized with the van Slyke–Folch reagent, the evolved gases being first passed through a condenser, an ice-cold trap and a bead tower containing 25 ml. of 0·1M-$AgNO_3$ to remove chromyl chloride and boric acid.

The CO_2 was then trapped in saturated $Ba(OH)_2$ and the precipitate of $BaCO_3$ was washed and dried as previously described. These $BaCO_3$ samples were treated with cold perchloric acid and the CO_2 evolved was reprecipitated as $BaCO_3$. The specific radioactivity was unchanged by this last-named treatment, showing that these samples were free of barium chromate or borate.

Measurement of radioactivity

All samples were plated at infinite thickness on 1 cm^2. polythene disks (Popják, 1950) and counted with a halogen-filled end-window Geiger–Müller counter with a type N530D Autoscaler (Ekco Electronics Ltd., Essex). At least 1000 counts were recorded in each assay and the combined standard error of sample preparation and statistical variation of radioassay was not greater than 6%.

Glucose utilization by cell suspensions

At the beginning and end of the experimental period portions (0·4 ml.) of the cell suspensions were removed and mixed with 0·4 ml. of 0·3N-$Ba(OH)_2$. Water (4·8 ml.) was

then added, followed by 0·4 ml. of 0·32N-$ZnSO_4$. The suspension was centrifuged and 3 ml. of the supernatant was percolated through columns (3 cm. × 1 cm.) of Zeo-Karb 215 (The Permutit Co. Ltd., London) in the H^+ form. The column was washed with 3 ml., then with 2 ml., of water and the combined eluate was made up to 10 ml. Glucose was determined in samples (1 ml.) by the method of Nelson (1944), with the modified colorimetric reagents of Somogyi (1952). Mepacrine, which interferes with the development of colour in the glucose estimation, is completely retained by the ion-exchanger.

RESULTS

Pathways of glucose catabolism by Plasmodium berghei *separated from the host cell.* Lactate is the major product of glucose catabolism by the free parasite (Bowman *et al.* 1960), and the distribution of ^{14}C in lactate formed during incubation with [1-^{14}C]- or [6-^{14}C]-glucose is consistent with that predicted by the Embden–Meyerhof glycolytic scheme (Table 1). By this pathway, one molecule of glucose is cleaved into two molecules of triose phosphate, which are equivalent to one another as far as lactate formation is concerned. In this way, both C-1 and C-6 of glucose become the methyl carbon atom C-3 of lactate. The traces of ^{14}C in the other carbon atoms of lactate (Table 1) suggest either contamination during the chemical-degradation procedure or the existence of alternative pathways of glucose catabolism in the parasite. The latter explanation is supported by the fact that significantly more $^{14}CO_2$ was evolved from [1-^{14}C]- than from [6-^{14}C]-glucose (Table 4), which would indicate the oxidative pathway. In this pathway one of the first reactions is the oxidative decarboxylation of 6-phosphogluconic acid to yield CO_2 from C-1 of glucose and a pentose phosphate, so that C-1 and C-6 of glucose are not metabolized in an identical manner. The yield of $^{14}CO_2$, however, only accounted for 2% of the [^{14}C]glucose utilized (Table 4) so that the contribution of the oxidative pathway was not appreciable under the experimental conditions.

Table 1. *Distribution of radioactivity in lactate formed during the incubation of free parasites with* [1-^{14}C]- *or* [6-^{14}C]-*glucose*

'Standard suspensions' of free parasite (10 ml.) were incubated with either (Expt. 1) [1-^{14}C]glucose (20 mg., 47·55 μc/g.) or (Expt. 2) [6-^{14}C]glucose (20 mg., 31·02 μc/g.) for 1 hr. at 37°. Metaphosphoric acid containing unlabelled lactic acid (91 and 123 mg. respectively) was added and the lactate was isolated and degraded as described in the Experimental section.

Compound	Carbon atom of lactate	Radioactivity (μc/mole) Expt. 1	Expt. 2
$BaCO_3$	1+2+3	93·00	27·20
Acetaldehyde 2:4-dinitrophenylhydrazone	2+3	—	27·02
$BaCO_3$	1	0·05	0·00
$BaCO_3$	2	0·09	0·21
$BaCO_3$	3	94·00	28·10

Table 2. *Effect of mepacrine on the utilization of glucose by cell suspensions*

Suspensions of cells (3 ml.) containing glucose (6 mg.) were incubated at 37° for 1 hr. At the beginning and end of the experimental period, samples (0·4 ml.) were removed for the determination of glucose. Suspensions of normal and parasitized reticulocytes contained respectively 10^9 cells, of which 87% were reticulocytes, and $1·6 \times 10^9$ cells, of which 72% were infected. The free parasite suspension contained the parasites originally present in 2×10^9 infected reticulocytes. —, Value not determined.

Concn. of mepacrine	Glucose utilized (μg./ml. of suspension) Free parasites	Parasitized reticulocytes	Reticulocytes
0	711	630	156
2·5 μM	696	640	150
12·5 μM	466	606	—
35·0 μM	44	340	—
0·2 mM	6	146	152
0·5 mM	0	90	—
1·0 mM	—	38	157

Effect of mepacrine on the utilization of glucose and the conversion of [^{14}C]*glucose into* $^{14}CO_2$ *by cell suspensions.* The rate of utilization of glucose by all cell suspensions was reasonably constant for 60 min., and this incubation time was accordingly used in all subsequent experiments. Mepacrine (2-chloro-5-[1-methyl-4-diethylaminobutyl]-7-methoxyamino-acridine dihydrochloride), even at low concentrations, had a marked effect on the utilization of glucose by free parasites (Table 2). It may be noted that 14 times the concentration which just failed to produce a significant effect resulted in almost complete inhibition, and the drug at a concentration of 12·5 μM in this and subsequent experiments (Tables 4–6) consistently decreased glucose utilization by 30–40%. This inhibitory effect would appear to be selective for the parasite since an equivalent number of reticulocytes was unaffected by the drug even at a concentration of 1 mM.

The ratio of $^{14}CO_2$ obtained from [1-^{14}C]- or [6-^{14}C]-glucose was not significantly altered by the presence of mepacrine (Tables 3 and 4), and this showed that the contribution of the alternative pathway involving pentose phosphate to the amount of glucose metabolized was unaffected by the drug. At the same time, mepacrine did not significantly alter the percentage of [U-^{14}C]glucose which was converted into $^{14}CO_2$, and it was concluded from these results that the inhibitory effect of the drug must be located at some step in the conversion of glucose into lactate.

Effect of mepacrine on the metabolism of glucose by suspensions of free parasites. Suspensions of free parasites were incubated in the presence of [U-^{14}C]glucose and the individual products of metabolism were determined by the technique of isotope-dilution. The parasite does not contain endogenous carbohydrate and lactate is not produced in the absence of glucose (Bowman *et al.* 1960), so that it can be assumed that the radioactivity (μc/mg. of carbon) of the product will be the same as that of the [U-^{14}C]glucose added.

It has been found that preparations of parasites differed in their ability to utilize added glucose, although samples of the same suspension were relatively constant in this respect. This lack of constancy in different preparations was probably due to the difficulty of ensuring that the same number of parasites was present in each preparation. In addition, this is an asynchronous infection and the proportions of the various growth stages may vary from preparation to preparation. Nevertheless, the percentage of radioactivity in [U-^{14}C]-glucose utilized which was converted into ether-soluble products (Table 5) was reasonably constant. Lactate was the major product identified and together with minor amounts of other organic acids (Table 6) can account for the radioactivity observed in the ether extract. Only small amounts of ^{14}C were detected in citrate, fumarate, malate, oxaloacetate and α-oxoglutarate. Other products of catabolism were glucose 6-phosphate and fructose 6-phosphate (Table 6), and these together with the organic acids and respiratory carbon dioxide accounted for more than 90% of the [U-^{14}C]glucose utilized.

Mepacrine caused a marked redistribution of ^{14}C from that normally found in the products of [U-^{14}C]glucose catabolism. In relation to untreated suspensions, there was a marked decrease in the amount of glucose catabolized which can be

Table 3. *Production of $^{14}CO_2$ by normal and infected reticulocytes from [^{14}C]glucose*

Suspensions of cells (3 ml.) containing 6 mg. of [1-^{14}C]glucose (0·083 μc/mg.), [6-^{14}C]glucose (0·162 μc/mg.) or [U-^{14}C]glucose (0·175 μc/mg.) were incubated at 37° for 1 hr. Glucose utilization was measured concurrently in separate but otherwise identical suspensions. Neither type of cell contained significant amounts of endogenous reducing sugar and it was assumed that the specific radioactivity of the glucose utilized was the same as that of the glucose added. Reticulocyte suspensions contained 1·3 × 10^9 cells, of which 75% were reticulocytes. Infected reticulocyte suspensions contained 1·5 × 10^9 cells, except in the experiment with [U-^{14}C]glucose, in which 1·0 × 10^8 cells were present; in both cases the suspension contained 72% of infected cells.

Cell suspension	Glucose added	Concn. of mepacrine	Glucose utilized (mg.)	Radioactivity (μmc) In glucose utilized (*A*)	Radioactivity (μmc) In $BaCO_3$ (*B*)	100*B*/*A*
Parasitized reticulocytes	[1-^{14}C]	—	1·84	152·7	2·9	1·9
		35 μM	0·83	70·9	1·7	2·4
	[6-^{14}C]	—	1·92	311·0	0·9	0·3
		35 μM	0·82	132·8	0·7	0·5
	[U-^{14}C]	—	1·31	229·2	5·5	2·4
		35 μM	0·43	75·2	2·0	2·7
Reticulocytes	[U-^{14}C]	—	0·54	94·5	1·6	1·7
		1 mM	0·52	91·0	1·8	2·0

Table 4. *Production of $^{14}CO_2$ by free parasites from [^{14}C]glucose*

Suspensions of free parasites (3 ml.) were incubated with [^{14}C]glucose (6 mg.) as described in Table 3. These suspensions contained parasites obtained from about 2 × 10^9 infected reticulocytes. The parasites did not contain detectable amounts of reducing sugar (see text) and it was assumed that the specific radioactivity of the glucose utilized was the same as that of the glucose added.

Glucose added	Concn. of mepacrine (μM)	Glucose utilized (mg.)	Radioactivity (μmc) In glucose utilized (*A*)	Radioactivity (μmc) In $BaCO_3$ (*B*)	100*B*/*A*
[1-^{14}C]	—	2·34	194·2	2·9	1·5
	12·5	1·16	96·3	1·5	1·6
[6-^{14}C]	—	2·20	356·4	1·4	0·4
	12·5	1·24	200·9	1·0	0·5
[U-^{14}C]	—	2·26	395·5	8·3	2·1
	12·5	1·12	196·0	4·7	2·4

accounted for as ether-soluble products (Table 5) and an increase in the hexose 6-phosphate fraction (Table 6). Moreover, the ether extract and the hexose 6-phosphates together account almost completely for the glucose utilized (Expt. 4, Tables 5 and 6).

DISCUSSION

The results indicate that *P. berghei* separated from its host cell, the reticulocyte, catabolizes glucose almost entirely by the Embden–Meyerhof glycolytic pathway. Other malaria parasites such as *P. gallinaceum* have been shown to possess glycolytic enzymes (Speck & Evans, 1945*a*, *b*), and phosphorylated intermediates of this pathway are present in chicken red cells infected with the same parasite (Marshall, 1948). Some evidence that enzymes of alternative pathways may be present in *P. berghei* is given in Tables 3 and 4, although the contribution of these pathways to the amount of glucose catabolized was small.

Lactate was the major product of glucose catabolism by both the free parasite (Table 6) and the

Table 5. *Conversion of* [U-^{14}C]*glucose into ether-soluble products by suspensions of free parasites*

'Standard suspensions' of free parasites (10 ml.) containing [U-^{14}C]glucose (20 mg.; 0·037 μc/mg. in Expts. 1–3; 0·088 μc/mg. in Expts. 4 and 5) were incubated for 1 hr. at 37°. The amount and radioactivity of the glucose utilized was determined as described in Table 4. In each experiment, a number of suspensions (10 ml.) of the same preparation were incubated simultaneously to determine the products listed in this and the subsequent table. To one suspension (10 ml.) from each experiment was added metaphosphoric acid containing unlabelled lactic acid (about 20 mg.) and the suspension was extracted continuously with diethyl ether. Solvent was removed and the extract was oxidized to CO_2 for radioassay as $BaCO_3$.

Expt. no.	Concn. of mepacrine (μM)	Glucose utilized (mg.)	Glucose utilized (μmc) (*A*)	Radioactivity in ether extract (μmc) (*B*)	$\frac{100B}{A}$
1	—	4·00	148·0	117·1	79
2	—	4·44	164·3	137·8	84
3	—	3·60	133·2	113·3	85
4	—	3·55	312·4	265·5	85
	12·5	2·33	205·0	135·3	66
5	—	4·71	414·5	343·3	83
	20·0	1·41	124·1	62·0	50

Table 6. *Effect of mepacrine on the metabolism of* [U-^{14}C]*glucose by suspensions of free parasites*

Incubation procedures and other details are given in Table 5 and in the Experimental section. Each radioactive product was isolated after dilution with a known amount (M^*) of the unlabelled compound. On the assumption that the specific activity of the product (x) is the same as that of the [U-^{14}C]glucose added, the mass (M) of the radioactive product present before dilution can be calculated by the equation $M = M^*y/x-y$, where y is the observed specific activity of the diluted product.

Expt. no.	Compound isolated	Concn of. mepacrine (μM)	Mass (M^*) of unlabelled compound added (mg. of C)	Specific activity (y) of diluted product (μmc/mg. of C)	Isotope in [^{14}C]glucose utilized present in product (%)
1	Lactate	—	20·0	4·9	70·0
2	Lactate	—	16·0	6·9	73·0
	Acetate	—	15·7	0·2	2·0
	Pyruvate	—	18·9	0·3	3·4
	Succinate	—	16·6	0·2	2·0
4	Lactate	—	16·0	13·5	72·8
		12·5	16·0	7·4	60·0
	Glucose 6-phosphate	—	7·2	2·4	5·6
		12·5	7·2	9·6	35·4
	Fructose 6-phosphate	—	4·9	0·8	1·2
		12·5	4·9	0·9	2·1
5	Glucose 6-phosphate	—	7·2	3·6	6·3
		20·0	7·2	7·0	40·8
	Fructose 6-phosphate	—	4·9	0·9	1·1
		20·0	4·9	0·7	2·8

infected reticulocyte (Bowman *et al.* 1960), and the small conversion of [U-^{14}C]glucose into $^{14}CO_2$ by both types of cell suspension (Tables 3 and 4) also indicates that only minor amounts of glucose are oxidized by the tricarboxylic acid cycle. In this respect *P. berghei* differs from the parasites of avian and simian malaria, both of which readily oxidize lactate or intermediates of the tricarboxylic acid cycle (McKee, 1951).

The rate-limiting step of glycolysis by the free parasite is concerned with the further metabolism of hexose 6-phosphate (Table 6). The enzymes involved are 6-phosphohexose isomerase and 6-phosphofructokinase. The equilibrium between glucose 6-phosphate and fructose 6-phosphate, catalysed by 6-phosphoglucose isomerase, is greatly in favour of the former (Lohmann, 1933), so that if the rate-limiting enzyme was 6-phosphofructokinase, a mixture of these hexose 6-phosphates should result in the approximate proportions which were actually observed. For these reasons, it will be assumed for the purposes of subsequent discussion that the rate-limiting enzyme is 6-phosphofructokinase.

Various glycolytic enzymes of *Plasmodium* have been reported to be inhibited by mepacrine, such as hexokinase (Speck & Evans, 1945*a*; Marshall 1948; Fraser & Kermack, 1957), 6-phosphofructokinase and triose phosphate dehydrogenase (Marshall, 1948) and lactic dehydrogenase (Speck & Evans, 1945*a*). The present results show that in *P. berghei* the enzyme most sensitive to mepacrine is 6-phosphofructokinase, hexokinase being inhibited to a lesser extent. This can be shown by the following calculations from the data given for Expt. 4 in Tables 5 and 6. The free parasite suspension utilized 19·7 μmoles of glucose, and 1·3 μ-moles of hexose 6-phosphate accumulated. Thus the overall rate of the hexokinase reaction was 19·7 μ-moles/hr. and that of 6-phosphofructokinase was 18·4 μmoles/hr. Similarly, for the mepacrine-treated suspension, the overall rates of the hexokinase and 6-phosphofructokinase reactions were 12·9 μmoles/hr. and 8·1 μmoles/hr. respectively. Thus the inhibition of hexokinase and 6-phosphofructokinase by mepacrine was 35 and 50% respectively. Fraser & Kermack (1957) showed that the hexokinase in cell-free preparations of *P. berghei* was inhibited by about 25% in the presence of concentrations of mepacrine more than 200 times that used in present experiments, so that, although the possibility cannot be completely excluded, a direct inhibition of hexokinase by the drug seems unlikely to account for the present results. However, another explanation is probable and is based on the fact that the hexokinase of the parasite, like that of rat brain (Weil-Malherbe & Bone, 1951) and other mammalian tissues (Crane & Sols, 1953), is inhibited in a non-competitive manner by glucose 6-phosphate (W. J. Campbell, P. T. Grant & W. O. Kermack, unpublished work). For this reason, the rate of glucose utilization by the parasite would be governed by the 6-phosphofructokinase reaction, and the concentration of glucose 6-phosphate observed would be that which reduced hexokinase activity to equal that of 6-phosphofructokinase. Any decrease in the activity of the latter enzyme would increase the concentration of glucose 6-phosphate and result in a decrease in the hexokinase activity. This product-inhibition of a reaction before the rate-limiting step could thus account for both the accumulation of glucose 6-phosphate and the decrease of hexokinase activity as a consequence of mepacrine inhibition of 6-phosphofructokinase.

This effect of mepacrine would appear to be a selective inhibition since appreciably greater concentrations of the drug have no significant effect on either the utilization of glucose or its conversion into carbon dioxide by normal reticulocytes. This would imply that the 6-phosphofructokinase of the parasite differs from that of the host cell in relative amount or in sensitivity to the drug. This latter possibility cannot be excluded since it has been shown (Mansour & Bueding, 1954; Bueding, 1959) that 6-phosphofructokinase of the parasite worm, *Schistomonas mansoni*, is about 80 times more sensitive to tervalent antimonial drugs than is the enzyme present in the tissues of its mammalian host.

SUMMARY

1. Approximately therapeutic concentrations of mepacrine caused a marked decrease in the utilization of glucose by suspensions of both rodent reticulocytes infected with *Plasmodium berghei* (35 μM) and parasites separated from the host cell (12·5 μM). In contrast, glucose utilization by normal reticulocytes was unaffected by higher concentrations (1 mM) of the antimalarial drug.

2. At these concentrations, mepacrine had no significant effect on the minor amounts of $^{14}CO_2$ produced by suspensions of infected reticulocytes or free parasites from [^{14}C]glucose.

3. Lactic acid and hexose 6-phosphates, mainly glucose 6-phosphate, were the major products of glucose catabolism by the free parasites. The amounts of $^{14}CO_2$ and the distribution of isotope in lactate formed from [1-^{14}C]- and [6-^{14}C]-glucose indicated that almost all the glucose was metabolized by the Embden–Meyerhof glycolytic pathway. Mepacrine caused a decrease in the amount of lactate formed from [U-^{14}C]glucose, with a concomitant increase in hexose 6-phosphate.

4. It is concluded that mepacrine inhibits an enzyme concerned with the further catabolism of

hexose 6-phosphate. This enzyme catalyses the rate-limiting step of glycolysis by the free parasite and may be 6-phosphofructokinase.

One of us (I. B. R. B.) is indebted to the Medical Research Council for a Scholarship.

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Influence of Exercise and Restricted Activity on the Protein Composition of Skeletal Muscle

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(*Received* 20 *June* 1960)

It is well known that when a muscle is exercised it will increase its strength by undergoing hypertrophy. In other words, its volume rises, and this might be accompanied by thickening of individual myofibrils (Mölbert & Jijima, 1959). Conversely, inactivity gives rise to muscular hypotrophy. As the myofilaments are alone responsible for the contraction, whereas the sarcoplasm has the more passive function of supplying nutrients, it would be interesting to establish whether the ratio of myofilaments to sarcoplasm undergoes any changes during exercise and inactivity, or whether these remain constant so that contractile strength and muscular diameter would be directly proportional (Pratt & Eisenberger, 1919; Sassa & Sherrington, 1921; Spector, 1956).

It would be feasible to carry out such an investigation with the aid of histological methods, but they have two major disadvantages. First, fixation and staining are accompanied by shrinkage of sections, rendering impossible sufficiently precise determinations of the proportions of sarcoplasm and of myofilaments in the total cell volume. Secondly, the myofilaments are forming myofibrils which are not clearly separated from the sarcoplasm in sections studied by histological methods. Electron microscopical examination has disclosed that myofibrils have no envelope; moreover, it is