

Fig. 1 Effect of alkaline extract of mucosa from human appendices on the melanophores of frog skin. *a*, Untreated skin; *b*, 5 h after administration of extract into the lymphatic sac.

melatonin (0.006%) in ethanol (30%) produced an effect comparable with the mucosal extract.

Thus an extract of the mucosal lining of human appendices contains a substance with a melatonin-like action. Since no direct demonstration of melatonin synthesis in human mucosa was produced, we investigated an intestinal extract of the rabbit, an animal known to contain large numbers of enterochromaffin cells. Thin-layer chromatography showed the presence of melatonin (R_f 0.73), together with 5-hydroxytryptophan (R_f 0.07), 5-HT (R_f 0.11) and 5-methoxytryptamine (R_f 0.32) (Fig. 2). Paper chromatography, using Filtrak F No. 1 paper with butyl alcohol-acetic acid-water, (12:3:5) or isopropyl alcohol-ammonia-water (8:1:1) as solvents, confirmed the thin-layer chromatography data, and also revealed traces of tryptamine (R_f 0.79 in the butyl alcohol-based solvent system).

The presence of melatonin precursors in the extract suggests that melatonin is synthesised in the intestinal mucosa rather than being transported there from some other site. Since the 5-HT is synthesised in the enterochromaffin cells² it seems likely that it is these cells which contain the melatonin and that it is synthesised there. Evidence to support this was obtained by a comparison of the melanophore-clarifying effects of mucosa, containing varying numbers of enterochromaffin cells, obtained from different cases of appendicitis (Table 1).

Our findings may be interpreted as supporting the suggestion⁶ that the enterochromaffin cells, or the endocrine cells of the appendix which are producing melatonin, are derived from the neuroectoderm of the neural crest. The enterochromaffin cells

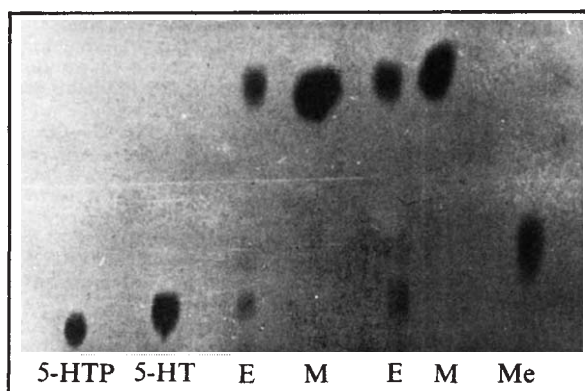


Fig. 2 Thin-layer chromatography of an alkaline extract of the intestinal mucosa of the rabbit. Chromatography was performed on Silufol UV 254 (Kavalier, Czechoslovakia) using isopropyl alcohol-benzyl alcohol-ammonia (10:5:1) as the solvent system. 5-HTP, 5-hydroxytryptophan; 5-HT, 5-hydroxytryptamine; E, extract; M, melatonin; Me, 5-methoxytryptamine.

Table 1 Time changes in clarification effect depending on the number of enterochromaffin cells in mucosal lining of appendices used in preparation of alkaline extracts

State of appendix	No. of appendices	Average number of enterochromaffin cell per crypt*	Time of clarification
Acute catarrhal appendicitis	61	4-5	25-30 min
Acute phlegmonous appendicitis	95	16-30	15-20 min
Acute gangrenous appendicitis	17	0-2	No effect

*The number of enterochromaffin cells was counted in ten sections, taken from different regions of each appendix; in each section the number of enterochromaffin cells were counted in ten crypts—thus in each appendix we counted cells in 100 crypts. The number of cells thus counted was divided by 100 to obtain the number of enterochromaffin cells per crypt.

belong to the amine precursor uptake and decarboxylation series of endocrine peptide-producing cells⁷ which have been described as constituting a peripheral neuroendocrine system, analogous to the central neuroendocrine system of the hypothalamus⁸.

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Pyruvate kinase in malaria host-parasite interaction

THE mammalian malaria host-parasite systems seem particularly useful for studies aimed at elucidating the biochemical mechanisms of such interactions. During the vertebrate phase of their life cycles the malaria organisms are intracellular parasites of the red cell. The mature mammalian host red cell is also relatively simple metabolically; its energy metabolism is solely that of Embden-Meyerhof glycolysis and it also lacks the capacity for protein synthesis. We therefore studied several aspects of red cell glycolysis in monkeys heavily infected with *Plasmodium knowlesi* and mice heavily infected with *P. berghei*. In both these systems we have found an increase in red blood cell adenosine triphosphate (ATP) and a decrease in red cell 2,3-diphosphoglycerate (DPG). We also have evidence that these malaria parasites introduce a pyruvate kinase isozyme into their host red cells in amounts sufficient to alter red cell glycolysis. First, glycolytic intermediate data demonstrate an *in vivo* increase in pyruvate kinase activity in infected red cells; second, there is an increase in pyruvate kinase V_{max} activity in infected cells; and third, gel electrophoretic patterns show a new pyruvate kinase isozyme in infected cells. We suggest that this alteration in red cell glycolysis is in a direction favourable

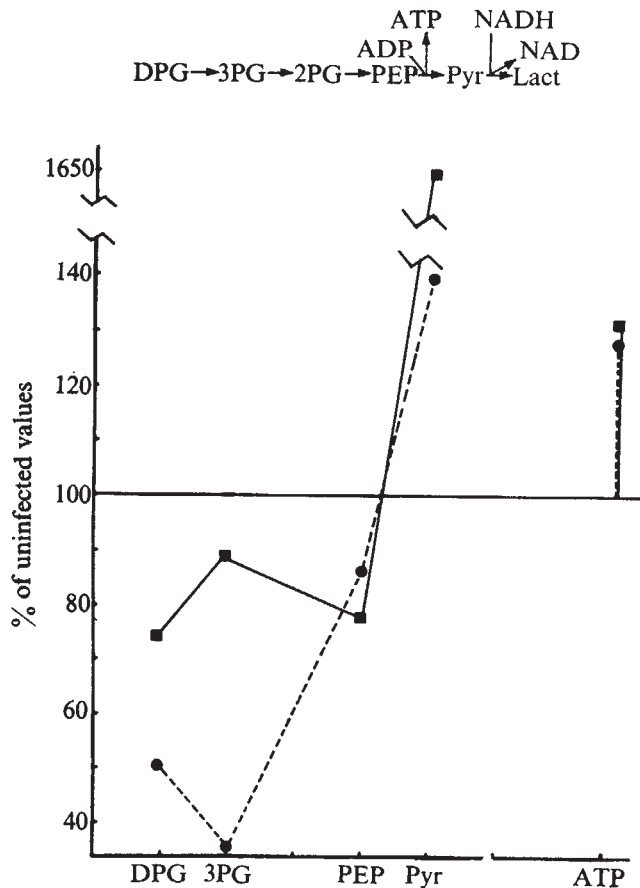


Fig. 1 Glycolytic plots of malaria-infected red cells. —, Glycolytic plot obtained using values on four rhesus monkeys heavily infected with *P. knowlesi* (an average of 40% of red cells infected). The glycolytic intermediates are listed on the horizontal axis in their order of appearance in the glycolytic pathway. For each monkey the level of each intermediate is expressed as a ratio (%) of the monkey's preinfected level. The average percentage for all four monkeys is then plotted to give the solid line in the figure. Control monkeys, similarly bled and treated (including one bled to the same degree of anaemia as his counterpart)—but not infected—do not give this pattern. - - - - -, Comparison of the intermediate levels obtained from the pooled blood of three *P. berghei* infected mice (64% of red cells infected) with the pooled blood obtained from three healthy mice. The small blood volumes obtainable did not enable us to assay the same mice both before and after infection as was done with the monkey. The extraction and assay procedures have been described previously¹.

to variable interpretations and in considering such plots it is useful to know whether glucose consumption has increased or decreased. We have found a 7.5-fold and a 12-fold increase in glucose consumption in *P. knowlesi*-infected monkey red cells (34% infected) and in *P. berghei*-infected mouse red cells (38% infected) respectively. (Similar glucose consumption data abounds in the malaria literature.) With these considerations the simplest interpretation of the glycolytic plots of Fig. 1 is that malaria infection leads to an increase in the *in vivo* activity of pyruvate kinase. Such an increase would lead to a decreased steady-state level of phospho-enol pyruvate (PEP) and preceding intermediates (Fig. 1). The increase in pyruvate and lactate seen in the plots would also be consistent with increased pyruvate kinase activity but since these two intermediates can pass through the red cell membrane (unlike PEP and preceding intermediates) and can arise from other sources (such as muscle), the levels of these two intermediates may not be as directly associated with red cell glycolysis. We and others have previously found that an increase in *in vivo* pyruvate kinase activity does lead to patterns similar to those seen in Fig. 1 including the decreased 2,3-DPG and increased ATP^{2,3}. The mechanism by which these changes occur has been discussed previously³.

Table 1 summarises the increases in pyruvate kinase activity (V_{max}) we have seen in malaria infected blood haemolysates. Clearly, enzyme activity is increased in infected blood. The

to the parasite because it will increase red cell ATP at the expense of red cell DPG.

Figure 1 summarises the glycolytic intermediate data in the form of glycolytic plots for the monkey-*P. knowlesi* and the mouse-*P. berghei* systems. The assay and extraction procedures have been described previously¹. Glycolytic plots are subject

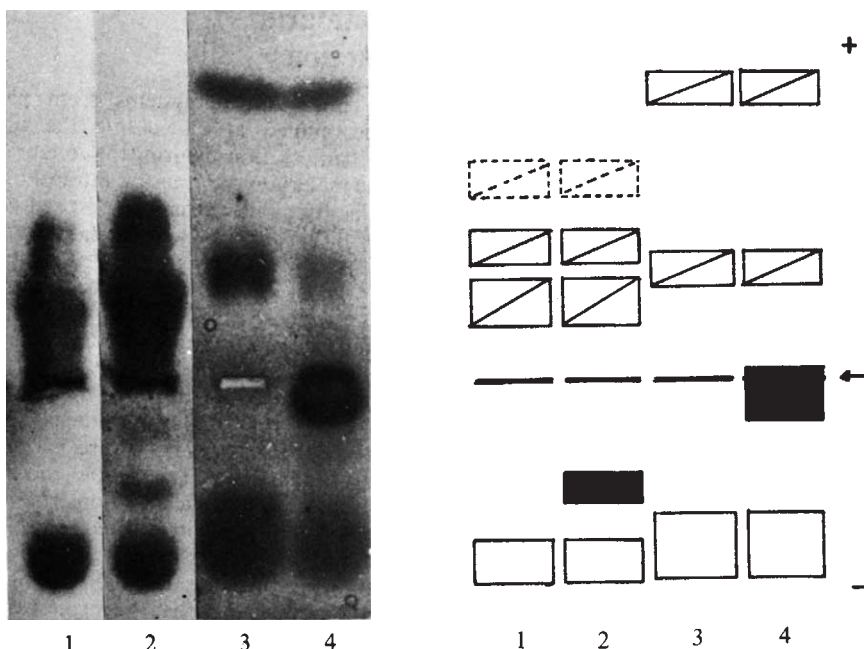


Fig. 2 Starch gel electrophoretic study of pyruvate kinase isozymes found in malaria-infected and uninfected blood samples. The adenylate kinase bands were discerned by staining the opposite face of the gel. This is necessary since the pyruvate kinase stain also stains for adenylate kinase⁵. In the diagram the dark bands are those which stained for pyruvate kinase activity only, the lined bands stained for adenylate kinase, whereas the open bands are haemoglobin bands. The dashed lines represent faint bands which are difficult to see in the photograph. Slot 1, uninfected monkey haemolysate; Slot 2, infected monkey haemolysate; Slot 3, uninfected mouse haemolysate; Slot 4, infected mouse haemolysate. In both the malaria infected mouse and monkey bloods a new pyruvate kinase isozyme can be seen. Presumably, as with human haemolysates, the uninfected mouse and uninfected monkey red cell pyruvate kinase activities are buried within the adenylate kinase area⁵.

Table 1 Increase in pyruvate kinase V_{\max} activity in malaria-infected red cells

Monkey- <i>P. knowlesi</i> system	% Parasitaemia*	% of Uninfected†
I	54	192
II	46	130
III	34	122
IV	26	119
Mouse- <i>P. berghei</i> system		
I	> 80	244
II	> 80	480
III	51	236

*% red cells containing parasite(s).

†Calculated as; $100 \times (\text{Infected } V_{\max}) / (\text{Uninfected } V_{\max})$.

Uninfected values are the same types as discussed in Fig. 1. The four control monkeys of the above experiments showed no or little increase (103%) in pyruvate kinase activity.

pyruvate kinase assay was performed on lysates of red cells washed three times in saline with the buffy coat removed at each washing⁴. Activity was calculated on a per gram haemoglobin basis.

Figure 2 illustrates banding patterns obtained when the above infected and uninfected red cell haemolysates were subjected to starch gel electrophoresis and stained for pyruvate kinase⁵. It can be seen that in both *P. knowlesi* and *P. berghei*-infected blood cells a new band of pyruvate kinase activity is present which was not present in uninfected blood. Presumably these new enzyme bands are of parasitic origin. (Indeed, mature mammalian red cells lack the metabolic machinery to synthesise new enzymes.)

There has been much interest in the role of ATP in malaria infections. In human volunteers inoculated with *P. falciparum* the rate of parasitaemia was directly correlated with the individual's red cell ATP value⁶. Also, in monkeys infected with *P. cynomolgi* the maximum parasitaemia correlated with red cell ATP level⁷. In *in vitro* culture ATP favours the growth of the parasite⁸. Red cell ATP levels have been shown to vary during the course of infection with the magnitude and direction of change depending, perhaps, on the host-parasite system under study and stage of parasitic development during sampling⁹⁻¹². Our studies have demonstrated an increase in ATP levels in heavily infected red blood cells for the mouse-*P. berghei* system and the monkey-*P. knowlesi* system. Pyruvate kinase is also implicated as a major determinant of this increase.

The source of ATP in the mature mammalian red cells is anaerobic glycolysis (such red cells cannot carry out oxidative phosphorylation). From a teleologic (and evolutionary) viewpoint the introduction of pyruvate kinase into the red cell by the parasite is biochemically sound. Pyruvate kinase is a rate-limiting step of red cell glycolysis and a relative increase in pyruvate kinase activity is known to increase red cell ATP at the expense of DPG^{2,3}. ATP is needed and/or used by the parasite, while DPG is important for red cell oxygen transport function but presumably is of no value to the parasite (ATP and DPG are the two major intermediates in monkey and mouse red cells.)

Although our study demonstrates a role for pyruvate kinase in at least two mammalian malaria host-parasite systems, it certainly does not exclude other concurrent enzyme interactions. Certainly, the large increases in glucose consumption cannot be explained simply on the basis of a pyruvate kinase increase. Other factors most certainly must be operative. It would be interesting, however, to see if similar pyruvate kinase data exist for other host-parasite systems. One species of avian malaria also seems to have its own pyruvate kinase isozyme¹⁰. Perhaps *in vivo* pyruvate kinase perturbations are a general host-parasite phenomenon.

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NMR evidence for common tertiary structure base pairs in yeast and *E. coli* tRNA

ABOUT ten years ago Holley¹ and coworkers sequenced the first tRNA and proposed the now famous cloverleaf secondary structure. Since that time about sixty additional tRNAs have been sequenced and all fit the cloverleaf model². This homology constitutes a strong case for the cloverleaf model and there is now compelling evidence from nuclear magnetic resonance (NMR) studies³ that the cloverleaf model does provide a correct description of the secondary structure of tRNA molecules in solution.

In addition to secondary structure homologies, tRNAs also contain constant bases in single stranded regions of the cloverleaf model. The first of these homologies was pointed out by Levitt who noted that residues in positions 15 and 48 are either G₁₅-C₄₈ or A₁₅-U₄₈ (ref. 4). On this basis Levitt suggested that bases in positions 15 and 48 form a tertiary structure base pair. The accumulation of tRNA sequences since Levitt first made his proposal has shown that other homologies exist in Class I (ref. 4) (D4V5) (ref. 5) tRNA, including A₁₅-U₈ (S'U₈), G₁₉-C₅₆ and A₅₈-T₅₄ (refs 4-6) and hence these too may be involved in tertiary structure base pairs.

In the light of these observations we are interested in the possibility of detecting the presence of additional base pairs beyond those predicted by the cloverleaf model and have recently shown that an anomalous 14.8 p.p.m. resonance which occurs in the low field NMR spectra of many *Escherichia coli* tRNAs is a result of a common homologous tertiary structure base between A₁₄ and S'U₈ (refs 7 and 8). The fact that we were able to identify unequivocally a resonance from one tertiary structure base pair suggested that it might be possible to observe resonances from other tertiary structure base pairs.

To test the proposal that most Class I tRNAs contain an identical set of tertiary structure base pairs, we have examined the low field NMR spectra of unfractionated *E. coli* and yeast tRNA at several different temperatures with and without magnesium. The idea behind these experiments was quite simple. Since the proposed tertiary base pairs are the same or nearly the same in all Class I tRNA they should give rise to relatively sharp resonances and thus should stand out above the broad background of unresolved overlapping resonances from secondary structure base pairs. Furthermore, resonances from tertiary structure base pairs should be more temperature sensitive than secondary structure base pairs. The NMR spectra of unfractionated *E. coli* and yeast tRNA are shown in Figs 1 and 2 and they do exhibit several sharp resonances in the low field region. To determine whether or not these resonances correspond to tertiary structure base pairs, the effect of salt and tem-