# Respiratory Systems in the Trypanosomidae

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At least two different types of hydrogen-transport system must be present in flagellates of the family Trypanosomidae (for a classification, see Hoare, 1957) since some species do not contain detectable cytochrome pigments and their respiration is insensitive to cyanide, azide (Brand, 1951; Ryley, 1956) and carbon monoxide (Fulton & Spooner, 1959). These differences may be observed not only among different species which infect the blood of vertebrates, but also between different forms of the same strain, whose life cycle involves both invertebrate (insect) and vertebrate stages. The present work is concerned with the nature and distribution of respiratory enzymes and enzyme systems present in subcellular fractions obtained from some of these protozoa.

## MATERIALS AND METHODS

*Blood-stream forms.* The 'L' strain of *Trypanosoma rhodesiense* (Grant & Fulton, 1957) was isolated from a patient in 1923 and has subsequently been maintained in rodents by blood inoculation. *Trypanosoma vivax* was the strain described by Desowitz (1956), which had been adapted to the rat as experimental host. *Trypanosoma lewisi* was isolated in 1956 from a natural infection of a wild rat. *Trypanosoma congolense* (Busimbi strain) was isolated from cattle in Buganda, East Africa, in 1945, and subsequently maintained in rodents. The following strains were received from the Wellcome Laboratories of Tropical Medicine through the kindness of Dr R. A. Neal: *Trypanosoma gambiense* (Wellcome C.L.) isolated from a patient in Liberia in 1939 and subsequently maintained in rodents; *Trypanosoma evansi* (Wellcome N.S.) isolated from a camel in the Sudan in 1938 and subsequently maintained in mice; *Trypanosoma equinum* (Wellcome Z.U.) obtained from the Argentine Public Health Authorities in 1948 and subsequently maintained in mice.

Groups of infected rats were killed at the height of infection and trypanosomes isolated from the blood by the procedures detailed by Fulton & Spooner (1959). Microscopic examination of each suspension was made on smears treated with Giemsa stain, and the suspension was only retained for experimental use if the content of platelets and white and red blood cells was negligible.

*Culture forms.* Details of the strain of *Strigomonas oncopelti* and its cultivation were given by Ryley (1955). Cultures of *T. rhodesiense* (Wellcome C.T.) and *T. gambiense* (Cheick) were obtained from Dr T. von Brand; they were cultivated in the medium described by Tobie, Brand & Mehlman (1950) except that citrated bovine blood was used for large-scale culture. Usually 36 or 72 conical flasks of 250 ml. capacity, each containing 25 ml. of blood–agar base and 15 ml. overlay, provided sufficient material for any one experiment; cultures were harvested after 6 days' growth at 24°. *Trypanosoma cruzi* and *Leishmania donovani* were culture strains of unknown origin; they were cultivated for 14 days at 24° in the diphasic medium described by Johnson (1947), by using citrated bovine blood. In all cases, the flagellates were isolated from the liquid phase of the medium by centrifuging at 1000*g* for 10 min., and the sedimented cells were washed twice in 0·9% NaCl.

*Cell-counting procedure.* In some experiments, a portion of the suspension of trypanosomes was diluted with a solution containing formaldehyde and crystal violet (Voegtlin & Smith, 1920), and the stained flagellates were counted in a haemocytometer chamber with improved Neubauer ruling.

*Preparation of cell-free extracts.* Blood-stream forms were lysed by suspension in water as described by Grant & Sargent (1960). The suspension was then centrifuged at 1000*g* for 10 min. and the clear supernatant was removed. The residue, which contained about 90% of the total N present in the starting material, was resuspended in water. This suspension was centrifuged, the supernatant discarded, and the washed residue resuspended in water as before. All these procedures were carried out at 2°. The final suspension was used experimentally within 30 min. of its preparation.

Culture forms became swollen but did not lyse when suspended in distilled water (4 vol.). Cell-free preparations were, however, obtained by cooling these suspensions to −70° with subsequent thawing in a water bath at 30°. This freezing and thawing procedure was repeated, and the suspension of completely ruptured cells was then diluted with distilled water (6 vol.) and centrifuged at 1000*g* for 10 min. The supernatant was discarded and the residue resuspended in distilled water. This final suspension (2–5 mg. of N/ml.) was used experimentally within 30 min. of the initial cooling of the intact cell suspension to −70°.

In some experiments (Table 6), enzyme preparations were obtained by freeze-drying suspensions of intact cells of either blood-stream or culture forms. The freeze-dried material was subsequently suspended in distilled water (1–6 mg. dry wt./ml.) with the aid of an all-glass homogenizer. The particulate material obtained after centrifuging

at 1000*g* for 10 min. was again resuspended in water. After centrifuging, the supernatant was discarded and the residue resuspended in water as before.

*Oxygen uptake.* Consumption of $O_2$ was measured in conventional Warburg apparatus in air at either 30° or 37°. The fluid volume added to each flask was 3 ml. and this included 0·1 ml. of 40% (w/v) KOH contained in the centre well. In experiments where cyanide was used as an inhibitor, KOH was replaced by the KOH–KCN or $Ca(CN)_2$–$Ca(OH)_2$ mixtures recommended by Robbie (1946).

*Total nitrogen.* Total N was determined by the micro-Kjeldahl method; the $NH_3$ produced was distilled into a boric acid solution containing methyl red–bromocresol green indicator or alternatively estimated directly with Nessler reagent (Conway, 1950).

*Materials.* DL- and L-α-Glycerophosphate were synthesized and isolated as the sodium salts (Grant & Sargent, 1960). DPNH, mammalian cytochrome *c* and tris were obtained from C. F. Boehringer und Soehne GmbH., Mannheim, Germany. Phenazine methosulphate obtained from the Aldrich Chemical Corp. Inc., Milwaukee, Wis., U.S.A., was recrystallized twice from ethanol before use. Dihydroxyacetone phosphate was prepared from a synthetic sample of the *cyclo*hexylamine salt of the dimethyl ketal of dihydroxyacetone phosphate (Nutritional Biochemicals Corp., Cleveland 28, Ohio, U.S.A.) as described by Ballou & Fischer (1956). Antimycin A was a gift from Dr E. F. Hartree.

## RESULTS

### *Respiratory systems in blood-stream forms*

In previous experiments with *T. rhodesiense* (Grant & Sargent, 1960), the most active cell-free preparations were obtained by lysis of the cells with distilled water. In these suspensions the particulate material contained the substrate-specific L-α-glycerophosphate-oxidase system and this mode of preparation was accordingly used with other strains of the *brucei* group (Table 1). The term *brucei* is used to denote a group of organisms which on morphological and phylogenetic evidence are closely related to *Trypanosoma brucei*. Under optimum conditions of pH and substrate concentration, the initial rate of oxygen uptake by these particulate preparations in the presence of either succinate or DPNH was less than 5% of that found with L-α-glycerophosphate; the addition of catalase (0·5 μM) did not decrease the rate of oxygen uptake. It was concluded that all strains of the *brucei* group contained a hydrogen transport system of similar activity which had a marked specificity for L-α-glycerophosphate as substrate.

The supernatants obtained by centrifuging lysed cell suspensions had no oxidase activity towards either L-α-glycerophosphate or DPNH. However, the addition of both the particulate fraction and DPNH to the corresponding supernatant resulted in an appreciable uptake of oxygen (Table 2). This effect was abolished by prior dialysis of the supernatant, but the uptake of oxygen was largely restored by the addition of trace amounts of either dihydroxyacetone phosphate or L-α-glycerophosphate. Dihydroxyacetone phosphate and a DPN-linked L-α-glycerophosphate dehydrogenase have both been shown to be present in the cytoplasm of *T. rhodesiense* (Grant & Sargent, 1960). It is therefore probable that the oxidation of DPNH by the combined cytoplasmic and particulate fractions of these cells can be attributed to the coupled functioning of the reactions catalysed by the particulate L-α-glycerophosphate oxidase and the cytoplasmic DPN-linked dehydrogenase. The oxidation of DPNH would thus involve indirect hydrogen transfer to the respiratory chain via L-α-glycerophosphate.

The oxygen uptake of particulate preparations obtained in a similar manner from blood-stream

Table 1. *Effect of substrates on the oxygen uptake of cell-free preparations of blood-stream forms*

Reaction mixtures contained 'water-lysed' preparation (approx. 0·5 mg. of N), tris buffer, pH 7·6 (0·14 M), and substrate in a final volume of 3·0 ml. The concentrations of substrates, added either singly or together, were L-α-glycerophosphate (5·5 mM), succinate (6·2 mM), cytochrome *c* (1·0 mg./flask) and DPNH (7·6 mM). Uptake of $O_2$ was measured at 37° over a 45 min. period except with L-α-glycerophosphate when readings were discontinued after 25 min. Negligible $O_2$ uptake was observed in the absence of substrate. – Signifies value not determined.

| | | Uptake of $O_2$ | | | | |
|---|---|---|---|---|---|---|
| | | (μl./hr./$10^8$ cells) | (μl./10 min./mg. of N) | | | |
| Group | Species | L-α-Glycero-phosphate | L-α-Glycero-phosphate | Succinate | Succinate + cytochrome *c* | DPNH |
| *brucei* | *T. rhodesiense* | 89 | 118 | <5 | <5 | <5 |
| | *T. gambiense* | 69 | 111 | | | |
| | *T. evansi* | 63 | 99 | | | |
| | *T. equinum* | – | 101 | | | |
| *vivax* | *T. vivax* | – | 35 | 10 | 12 | 8 |
| *congolense* | *T. congolense* | – | 19 | 9 | 9 | 22 |
| *lewisi* | *T. lewisi* | – | 20 | 17 | 28 | 19 |

forms of other groups (Table 1) showed that there was an appreciable direct oxidation of DPNH and succinate as well as of DL-α-glycerophosphate.

*Effect of inhibitors.* The strain of *T. lewisi* was the only blood-stream form used in these experiments which contained detectable cytochrome pigments (Ryley, 1951; Fulton & Spooner, 1959); cell-free preparations contained succinoxidase activity which was enhanced by the addition of cytochrome *c* (Table 1) and was inhibited by both cyanide and antimycin A. The degree of inhibition was largely independent of the substrate used (Table 3). In contrast, the oxidation of L-α-glycerophosphate by particulate preparations obtained from all strains of the *brucei* group was unaffected by cyanide and antimycin A at the concentrations of these reagents given in Table 3.

*Respiratory systems in culture forms*

Unlike blood-stream forms, cells cultivated *in vitro* did not lyse when suspended in water. Cell-free preparations were, however, obtained by freezing and thawing these latter suspensions. Oxidase activity was confined to the particulate material and the initial rates of oxygen uptake at 30° in the presence of various substrates are given in Table 4. DL-α-Glycerophosphate and succinate were both oxidized, but the highest rate was observed in the presence of DPNH, the ratio of these rates being about 1:2:5 respectively.

Included in Table 4 as a direct comparison with these results are corresponding data obtained with particulate preparations of blood-stream forms obtained by the same freezing and thawing procedure. At 30°, the rate of oxygen uptake in the presence of DL-α-glycerophosphate was appreciably greater than that found with either succinate or DPNH. This result is in general agreement with those given in Table 1, although the freezing and thawing procedure clearly had an adverse effect on the activity of these enzyme systems, the effect on L-α-glycerophosphate oxidase being particularly marked. Under the conditions given in Table 1, oxidase activity at 30° was only about 5–10% lower than that measured at 37°. For these reasons it seemed probable that oxidase activity in preparations of the culture forms was also partly inactivated by the freezing and thawing procedure. This possibility has been further studied by the measurement of α-glycerophosphate and succinic-dehydrogenase activity in freeze-dried preparations of these cells (Table 6).

Particulate preparations of the culture form of *T. cruzi* oxidized DPNH at an appreciable rate; this rate was not influenced by the addition of the cytoplasmic fraction of the cell homogenate as was found with the blood-stream form of *T. rhodesiense* (Table 2).

*Effect of inhibitors.* With particulate preparations obtained from *T. rhodesiense*, *T. gambiense* and *S. oncopelti*, both cyanide and antimycin A had a marked inhibitory effect on the rate of oxygen uptake when DL-α-glycerophosphate or succinate were added as substrates. In contrast, the rate of oxidation of DPNH was not inhibited by cyanide or by antimycin A (Table 5); rather stimulation of the rate of oxygen uptake was observed and this effect was particularly marked with cyanide.

Table 2. *Oxidation of reduced diphosphopyridine nucleotide by cell-free fractions from blood-stream forms of* Trypanosoma rhodesiense

Intact cells (6 mg. of N) were lysed in distilled water (20 ml.) at 2°. The suspension was centrifuged and a portion of the supernatant was dialysed against distilled water for 4 hr. at 2°. The particulate material was washed and resuspended in distilled water (18 ml.). Reaction mixtures contained tris buffer, pH 7·6 (0·14M), particulate suspension (1 ml.) and the additions as shown below in a total volume of 3 ml.

| Addition | Uptake of $O_2$ (μl./flask/ 25 min.) |
|---|---|
| DPNH (22 μmoles) | <5 |
| Supernatant (1 ml.) | <5 |
| Supernatant (1 ml.) and DPNH (22 μmoles) | 58 |
| Dialysed supernatant (1 ml.) and DPNH (22 μmoles) | <5 |
| Dialysed supernatant (1 ml.), DPNH (22 μmoles) and dihydroxyacetone phosphate (2 μmoles) | 47 |
| Dialysed supernatant (1 ml.), DPNH (22 μmoles) and L-α-glycerophosphate (2 μmoles) | 50 |

Table 3. *Effect of inhibitors on the oxygen uptake of cell-free preparations of* Trypanosoma lewisi

Experimental details were as described in Table 1. Enzyme (0·9 mg. of N) was equilibrated for 10 min. with either antimycin A (5 μg./flask) or cyanide (0·5 mM) before the reaction was started by tipping substrate from the side arm. Antimycin A was added as a solution (0·1 ml.) in aqueous 50% (v/v) ethanol.

| Substrate | Uptake of $O_2$ (μl./30 min./flask) | | |
|---|---|---|---|
| | Control | Cyanide | Antimycin A |
| L-α-Glycerophosphate | 53 | 24 | 43 |
| Succinate and cytochrome *c* | 71 | 27 | 48 |
| DPNH | 49 | 16 | 35 |

*Dehydrogenase activity of particulate preparations*

Although a cytoplasmic L-α-glycerophosphate dehydrogenase was present in blood-stream forms of the *brucei* group, this enzyme was DPN-linked and was distinct from the particulate L-α-glycerophosphate dehydrogenase whose activity could be determined with phenazine methosulphate as electron acceptor. Optimum conditions for these assay procedures were determined as described by Grant & Sargent (1961).

Suspensions of the particulate material obtained in an identical manner from freeze-dried samples of either blood-stream or culture forms could be repeatedly frozen and thawed or stored for several days at 2° without significant loss in L-α-glycerophosphate- or succinic-dehydrogenase activity. In the assay of these enzymes with phenazine methosulphate as electron acceptor, 1 mole of oxygen was taken up/mole of substrate utilized (Kearney & Singer, 1956; Grant & Sargent, 1961), so that the rates of oxygen uptake given in Table 6 should be halved to make them comparable with those of the corresponding oxidase systems (Tables 1 and 4). In this way it can be seen that the ratio of dehydrogenase to oxidase activity in blood-stream forms (Table 1) was about 2:1, whereas the ratio was about 3:1 for the culture forms of *T. rhodesiense* and *S. oncopelti* and about 5:1 for the culture form of *T. gambiense* (Table 4). These results suggest that about half of the succinic- and L-α-glycerophosphate-oxidase activity of blood-stream forms had been inactivated during the isolation procedure and that this effect was even greater during the freezing and thawing procedure used to obtain cell-free preparations of the culture forms. Nevertheless, the ratio of activities of succinic to α-glycerophosphate dehydrogenase in preparations of any one strain (Table 6) was similar to that of oxidase activities in preparations of the same blood-stream (Table 1) or culture strain (Table 4). This indicated that inactivation of oxidase activity during the isolation procedure was a general rather than a selective effect on one particular oxidase system.

Table 4. *Effect of substrates on the oxygen uptake of 'frozen and thawed' cell-free preparations*

Reaction mixture contained 'frozen and thawed' preparations (2–5 mg. of N), tris buffer, pH 7·3 (0·14 M), and substrate in a final volume of 3 ml. The concentrations of substrates added either singly or together were DL-α-glycerophosphate (10·0 mM), succinate (5·3 mM), cytochrome *c* (1 mg./flask) or DPNH (5·3 mM). Initial rate of $O_2$ uptake was measured at 30° for 30 min. Negligible $O_2$ uptake was observed in the absence of substrate. (*B*) and (*C*) indicate blood-stream and culture forms respectively.

| | Uptake of $O_2$ (μl./10 min./mg. of N) | | | |
|---|---|---|---|---|
| Species | DL-α-Glycerophosphate | Succinate | Succinate + cytochrome *c* | DPNH |
| *T. rhodesiense* (*C*) | 3·4 | 6·1 | 6·6 | 17·4 |
| *T. gambiense* (*C*) | 2·3 | 3·4 | 4·0 | 14·4 |
| *T. cruzi* (*C*) | 1·2 | 2·8 | 3·6 | 7·2 |
| *L. donovani* (*C*) | <1·0 | 4·5 | 6·0 | 7·2 |
| *S. oncopelti* (*C*) | 1·9 | 3·2 | 3·4 | 12·2 |
| *T. rhodesiense* (*B*) | 17·9 | 1·2 | 1·6 | 3·5 |
| *T. evansi* (*B*) | 16·8 | 1·4 | 1·6 | 3·2 |
| *T. equinum* (*B*) | 15·3 | <1·0 | <1·0 | 2·1 |
| *T. vivax* (*B*) | 5·5 | <1·0 | 1·6 | 2·9 |
| *T. congolense* (*B*) | 3·4 | <1·0 | <1·0 | 2·5 |

Table 5. *Effect of inhibitors on the oxygen uptake of cell-free preparations of culture forms*

Reaction mixtures contained 'frozen and thawed' preparations (2·5, 2·2 and 6·9 mg. of N/flask for *T. rhodesiense*, *T. gambiense* and *S. oncopelti* respectively) and the other components detailed in Table 4. Enzyme was equilibrated for 10 min. with either antimycin A (5 μg./flask) or cyanide (0·5 mM) before the reaction was started by tipping substrate from the side arm.

| | | Uptake of $O_2$ (μl./30 min./flask) | | |
|---|---|---|---|---|
| Substrate | Inhibitor added | *T. rhodesiense* | *T. gambiense* | *S. oncopelti* |
| DL-α-Glycerophosphate | Nil | 20 | 32 | 22 |
| | Cyanide | 6 | 10 | 7 |
| | Antimycin A | 14 | 19 | 6 |
| Succinate | Nil | 37 | 27 | 51 |
| | Cyanide | 7 | 5 | 14 |
| | Antimycin A | 12 | 8 | 15 |
| DPNH | Nil | 87 | 55 | 109 |
| | Cyanide | 87 | 73 | 135 |
| | Antimycin A | 95 | 58 | 106 |

Table 6. *Dehydrogenase activity in freeze-dried preparations*

Reaction mixtures contained freeze-dried preparation (about 200 $\mu$g. of N for blood-stream forms and about 3 mg. of N for culture forms), tris buffer, pH 7·6 (0·14 M), phenazine methosulphate (4·6 mM), 8-hydroxyquinoline (mM) and either DL-$\alpha$-glycerophosphate or succinate (33 mM) in a total volume of 3 ml. Preparations of culture forms (*C*) and blood-stream forms (*B*) were assayed at 30° and 37° respectively. The reaction was started by tipping phenazine methosulphate from the side arm and the oxygen uptake was recorded over 15 min. All results are corrected for an endogenous activity which in no case exceeded 2 $\mu$l. of $O_2$/10 min./mg. of N. – Signifies value not determined.

| | Uptake of $O_2$ ($\mu$l./10 min./mg. of N) | | |
|---|---|---|---|
| Species | (*a*) DL-$\alpha$-Glycero-phosphate | (*b*) Succinate | Ratio (*b*):(*a*) |
| *T. rhodesiense* (*B*) | 410 | 15 | 1:27 |
| *T. gambiense* (*B*) | 430 | – | – |
| *T. evansi* (*B*) | 414 | – | – |
| *T. rhodesiense* (*C*) | 21 | 39 | 1:0·5 |
| *T. gambiense* (*C*) | 18 | 32 | 1:0·5 |
| *S. oncopelti* (*C*) | 10 | 19 | 1:0·5 |

## DISCUSSION

The present experiments establish that trypanosomes and other related protozoa contain different types of respiratory systems in particulate subcellular fractions, a conclusion in agreement with previous studies (Brand, 1951; Ryley, 1956; Fulton & Spooner, 1959), which have been mainly concerned with the spectroscopic examination of intact cell suspensions and with the effects of inhibitors on their respiration.

The high activity and substrate specificity of the L-$\alpha$-glycerophosphate-oxidase system (1) appears to be a characteristic property of the blood-stream forms of the *brucei* group, which includes many trypanosomes of veterinary and medical importance. A cytoplasmic DPN-linked L-$\alpha$-glycerophosphate dehydrogenase (2) is also present in these cells and the results given in Table 2 show that the coupled functioning of the reactions catalysed by these enzymes results in the oxidation of DPNH by indirect hydrogen transfer to the respiratory chain via L-$\alpha$-glycerophosphate. In this manner,

$$\text{L-}\alpha\text{-Glycerophosphate} + \tfrac{1}{2}O_2 \rightarrow \text{dihydroxyacetone phosphate} + H_2O \quad (1)$$

$$\text{Dihydroxyacetone phosphate} + DPNH + H^+ \rightarrow \text{L-}\alpha\text{-glycerophosphate} + DPN^+ \quad (2)$$

$$\text{Sum:} \quad DPNH + H^+ + \tfrac{1}{2}O_2 \rightarrow DPN^+ + H_2O$$

it is possible to account for the uptake of oxygen observed when DPNH was added to crushed cell-free suspensions of *T. rhodesiense* (Fulton & Spooner, 1959).

The significance of these coupled reactions to the oxidative catabolism of glucose by *T. rhodesiense* has been discussed by Grant & Sargent (1960). The other species of the *brucei* group used in the present experiments catabolize glucose by the Embden–Meyerhof glycolytic pathway to pyruvate and a trace of glycerol (Ryley, 1956; Grant & Fulton, 1957), the rates of respiration being similar and in the range 166–194 $\mu$l. of oxygen/hr./$10^8$ cells (Brand, Tobie & Mehlman, 1950). These rates are about double those observed for the isolated L-$\alpha$-glycerophosphate oxidase, and, although the former were calculated from studies involving both normal and infected blood, it seems probable that the oxidase was partially inactivated during the isolation procedure. In agreement with this view, it has been found that the activity of the particulate L-$\alpha$-glycerophosphate dehydrogenase is about double that of the corresponding oxidase. Previous experiments indicated that the particulate dehydrogenase is a component of the oxidase system (Grant & Sargent, 1961) and for these reasons it is suggested that the major part of the respiration of this group of trypanosomes can be accounted for by the L-$\alpha$-glycerophosphate-oxidase system.

Particulate preparations obtained from all strains were capable of oxidizing DL-$\alpha$-glycerophosphate to a greater or less extent and two different respiratory systems could be distinguished. The L-$\alpha$-glycerophosphate oxidase present in blood-stream forms of the *brucei* group was insensitive to both cyanide and antimycin A, a result in agreement with the repeated observation that trypanosomes of this group do not contain detectable cytochrome pigments (Brand, 1951; Ryley, 1956; Fulton & Spooner, 1959). In contrast, succinoxidase and $\alpha$-glycerophosphate-oxidase activities in the culture forms of *T. rhodesiense*, *T. gambiense* and *S. oncopelti*, and in the blood-stream form of *T. lewisi* were inhibited by cyanide and to a lesser

extent by antimycin A. These inhibitors are believed to function specifically at single loci in complex respiratory systems; cyanide on cytochrome oxidase (Keilin & Hartree, 1939) and antimycin A at a locus between cytochrome *b* and *c* (Thorn, 1956). In these experiments, the degree of inhibition caused by each reagent was independent of the substrate used, so that the individual dehydrogenases may be structurally and functionally linked to a common cytochrome chain as has been shown in brain mitochondria by Ringler & Singer (1959).

In addition to the indirect system present in blood-stream forms of the *brucei* group, two other respiratory systems may also be distinguished for hydrogen transfer from DPNH to oxygen as the final acceptor. Thus, DPNH-oxidase activity in preparations of *T. lewisi* was sensitive to cyanide and antimycin A, whereas the system in preparations obtained from the culture forms of *T. rhodesiense*, *T. gambiense* and *S. oncopelti* was insensitive to these reagents. This latter result was unexpected since the intact cells contain cytochrome pigments and their respiration is readily inhibited by cyanide (Ryley, 1955; Fulton & Spooner, 1959). A functional cytochrome system need not, however, be precluded since suprarenal microsomes (Kersten, Kersten & Staudinger, 1958) and mitochondria of either the mid-gut of the silkworm (Pappenheimer & Williams, 1954), or the spadix of *Arum maculatum* (Bendall, 1958) can oxidize DPNH by a system in which a member of the *b* group of cytochromes can act as a terminal oxidase which is insensitive to both cyanide and antimycin A. Alternatively, the oxidation of DPNH by preparations of these culture forms may be effected by an aerobic dehydrogenase similar to that present in *Streptococcus faecalis* (Dolin, 1956).

Among the differences which have been observed between the respiratory systems of the various trypanosomes, perhaps the most interesting is the difference between the type of oxidase and the magnitude of the dehydrogenase activity in the two forms of *T. rhodesiense*. It is usually accepted that the culture form of this trypanosome corresponds to the mid-gut form which is the first of the three known forms to develop in the vector tsetse fly (Trager, 1959). The transition from a warm-blooded mammalian host to an invertebrate involves marked changes in the environment of the flagellate and would include that of temperature and oxygen tension. The latter change might induce an alteration in the type of respiratory system being utilized by the parasite, and an induction of this type has, in fact, been shown to occur in cultures of *Pseudomonas fluorescens* grown under conditions of varying oxygen concentration (Lenhoff, Nicholas & Kaplan, 1956).

## SUMMARY

1. Cell-free preparations from blood-stream trypanosomes of the *brucei* group contain a cytoplasmic L-α-glycerophosphate dehydrogenase and a particulate hydrogen-transport system whose rate of reaction with L-α-glycerophosphate was about 20 times that of reduced pyridine nucleotide or succinate. The reaction of the L-α-glycerophosphate-oxidase system with oxygen was insensitive to both cyanide (0·5 mM) and antimycin A (5 μg./ml.).

2. Cell-free preparations from blood-stream forms of *Trypanosoma congolense*, *Trypanosoma lewisi* and *Trypanosoma vivax* oxidize α-glycerophosphate, succinate and reduced diphosphopyridine nucleotide at about the same rate. The oxidation of these substrates by preparations of *Trypanosoma lewisi* was inhibited to about the same extent by cyanide (0·5 mM). Antimycin A (5 μg./ml.) was also an inhibitor.

3. The ratio of the rates of oxygen uptake by cell-free preparations of the culture forms of *Trypanosoma rhodesiense*, *Trypanosoma gambiense*, *Trypanosoma cruzi*, *Strigomonas oncopelti* and *Leishmania donovani* was about 1:2:5 for α-glycerophosphate, succinate and reduced diphosphopyridine nucleotide respectively. α-Glycerophosphate- and succinic-oxidase activity in the first three of these species was inhibited by cyanide and antimycin A. In contrast, the oxidation of reduced diphosphopyridine nucleotide was slightly stimulated by these reagents.

4. L-α-Glycerophosphate- and succinic-dehydrogenase activities were measured with phenazine methosulphate as electron acceptor in particulate preparations obtained from freeze-dried samples of both blood-stream and culture forms.

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# L-α-Glycerophosphate Dehydrogenase, a Component of an Oxidase System in *Trypanosoma rhodesiense*

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The oxidation of α-glycerophosphate to dihydroxyacetone phosphate with concomitant hydrogen transfer to molecular oxygen may be catalysed by various enzymes or enzyme systems which do not contain pyridine nucleotides. An aerobic α-glycerophosphate dehydrogenase from *Streptococcus faecalis* has been identified as an autoxidizable flavoprotein (Jacobs & Van Demark, 1960) and an enzyme of similar properties has also been found in *Escherichia coli* (Strittmatter, 1960). Rabbit muscle (Green, 1936), mitochondria of pig brain (Ringler & Singer, 1959), sarcosomes of insect flight muscle (Estabrook & Sacktor, 1958; Klingenberg & Bücher, 1959) and mitochondria of the mid-gut of the silkworm (Ito & Horie, 1959) all contain an anaerobic α-glycerophosphate dehydrogenase that is structurally and functionally linked to a respiratory chain containing cytochrome pigments. The complete system catalyses the reaction:

$$\text{α-Glycerophosphate} + \tfrac{1}{2}O_2 \rightarrow \text{dihydroxyacetone phosphate} + H_2O.$$

Another oxidase system which catalyses the same reaction is present in cell-free preparations of blood-stream trypanosomes of the *brucei* group (Grant & Sargent, 1960*a*; Grant, Sargent & Ryley, 1961) but differs from that of mammalian and insect tissue in that the reaction with oxygen is not mediated by cytochrome pigments. Moreover, preparations of these protozoa exhibit no appreciable oxidase activity towards either succinate or reduced pyridine nucleotide as substrate.

The present work is concerned with some properties of an anaerobic L-α-glycerophosphate dehydrogenase, which is the first component of the trypanosomal oxidase system to be identified.

Some of these results have been reported by Grant & Sargent (1960*b*).

## EXPERIMENTAL

*Organism.* The Liverpool strain of *Trypanosoma rhodesiense*, its maintenance in rats and the isolation of these cells from infected rat blood have been described by Grant & Fulton (1957). Microscopic examination was made on smears treated with Giemsa stain and the preparation was retained for experimental use if the content of platelets and red and white cells was negligible.

*Preparation of cell-free extracts.* Cell-free particulate preparations of *T. rhodesiense* containing L-α-glycerophosphate oxidase activity were obtained as described by Grant & Sargent (1960*a*).

In most experiments, the particulate material was dried from the frozen state immediately after preparation in a centrifugal freeze-drier (Edwards High Vacuum Ltd., Sussex) with $P_2O_5$ as desiccant. The dried material was stored *in vacuo* at 2°. Immediately before use, it was resuspended in distilled water (2 mg./ml.) with the aid of either an all-glass homogenizer of the Potter–Elvehjem type (Loughborough Glass Co., Loughborough, Leics.), or a 60 w ultrasonic vibrator of the magnetostrictor type with a