muscle, which is particularly great with DNP. Further experiments on these problems are in progress.

SUMMARY

1. The influence of 2:4-dinitrophenol, methylene blue, brilliant cresyl blue, thionine, phenosafranine and Janus Green B on the oxidative phosphorylation of mitochondria isolated from liver, kidney, brain, heart and skeletal muscle of rats has been studied *in vitro* as well as *in vivo*.

2. All the substances used produced a high degree of inhibition of oxidative phosphorylation both *in vitro* and *in vivo*. In every case, uncoupling of oxidative phosphorylation was accompanied by a change of form of the mitochondria from rod-like to spherical and by swelling.

3. 2:4-Dinitrophenol and brilliant cresyl blue produce activation of adenosinetriphosphatase of liver mitochondria *in vitro*. 5′-Nucleotidase and both acid and alkaline phosphatases are also stimulated. All the substances used stimulated adenosinetriphosphatase, 5′-nucleotidase and both acid and alkaline phosphatases *in vivo*.

4. 2:4-Dinitrophenol and brilliant cresyl blue stimulate adenosinetriphosphatase activity of fresh as well as of aged mitochondria. Also soluble adenosinetriphosphatase is activated by these substances.

5. Phenosafranine strongly inhibits myokinase of liver mitochondria, both *in vitro* and *in vivo*.

6. Prolonged treatment of rats with the substances produces fatty infiltration of the liver. 2:4-Dinitrophenol produces glycogen accumulation instead of fatty infiltration.

7. The significance of the phenomena is discussed.

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Studies on the Metabolism of the Protozoa

7. COMPARATIVE CARBOHYDRATE METABOLISM OF ELEVEN SPECIES OF TRYPANOSOME*

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From the numerous papers on the carbohydrate metabolism of trypanosomes which have appeared during the past twenty years, it has become obvious that, with the possible exception of *Trypanosoma cruzi* (Brand, Tobie, Kissling & Adams, 1949) and the plant trypanosome *Strigomonas oncopelti* (Ryley, 1955), cellular motility depends on a supply of extracellular monosaccharide, which is incompletely broken down to a mixture of organic acids; much of this work is admirably summarized by Brand (1951). Trypanosomes of the *brucei-evansi* group [for classification

* Part 6: Manners & Ryley (1955).

of trypanosomes see Hoare (1949)] seem to carry glucose breakdown no further than the pyruvic acid stage, and *T. lewisi* and *S. oncopelti* (Ryley, 1951, 1955) are able to carboxylate and decarboxylate pyruvic acid with the eventual production of succinic acid and acetic acid or ethanol. There is likewise a gradation in cyanide sensitivity between the *brucei-evansi* group and other species (Brand & Johnson, 1947). In the present study an opportunity has been taken to compare the extent of glucose breakdown under both aerobic and anaerobic conditions by eleven species of trypanosome, and to supplement observations on respiratory sensitivity to cyanide in a number of these species by investigations of the cytochrome spectra and the presence in homogenates of oxidative enzymes.

MATERIALS AND METHODS

Organisms and methods of culture. The trypanosomes used in the present studies were strains maintained in this Laboratory for chemotherapeutic work. The cultural forms of *T. cruzi* were grown in 250 ml. flasks, using Chang's (1947) diphasic medium; organisms were harvested after 14 days' growth at 26°, six flasks usually providing sufficient material for one experiment. The blood-stream forms of *T. cruzi* were obtained from batches of about 200 infected mice (20–30 g.) which were bled when the trypanosome population in the peripheral blood was at its maximum, usually 8–10 days after inoculation. *T. gambiense* was obtained from batches of 6–10 fully grown guinea pigs which were examined daily until the animals seemed heavily infected, a variable period of about 2–4 weeks after inoculation. *T. vivax* and *T. congolense* were grown in batches of 30–50 fully grown rats (discarded breeding stock), which were bled 4–5 days after inoculation; in these infections the trypanosome population increases to a maximum at about this time, after which either the animal dies or the trypanosomes gradually disappear from the blood, the infection relapsing at a later date. *T. rhodesiense*, *T. brucei*, *T. equinum*, *T. equiperdum* and *T. evansi* were obtained from batches of 10–15 fully grown rats, which were bled 3 days after infection, at a time when the trypanosome population was near the maximum compatible with the life of the host. *T. lewisi* was obtained from batches of about 30 rats (150 g.), which were bled 10 days after infection when the parasites were in the 'adult phase'.

Antisera to mouse and rat red cells. These were prepared in rabbits as described by Moulder (1948), freeze-dried, and stored at room temperature for periods up to 1 year; they were reconstituted with distilled water immediately before use.

Harvesting of organisms and preparation of suspensions. Infected animals were chloroformed and bled from the heart by means of a syringe, the blood being mixed with an equal volume of a solution containing 0·8% (w/v) of NaCl and 1% (w/v) of sodium citrate. In experiments in which citric acid determinations were made, infected blood was mixed with a little heparin instead of citrated saline. Trypanosomes were separated from the blood by differential centrifuging at room temperature, using an International SB size 1 centrifuge. The blood was centrifuged for 5 min. at 3000 rev./min., the supernatant was discarded and the trypanosomes which separated as a white layer above the red cells were removed as completely as possible by means of a Pasteur pipette into Ringer-bicarbonate or Ringer-phosphate solution (Ryley, 1955) containing 0·015M-glucose. The trypanosome suspension was again centrifuged, the upper layer being collected and the small lower layer of contaminating red cells rejected. This process was repeated until the preparation was free from blood elements. In very heavy blood infections, the initial trypanosome layer was thick, and two centrifugings sufficed to obtain a clean preparation of parasites. With *T. lewisi*, *T. cruzi* and *T. congolense*, the infections never became very heavy, and the trypanosome layer on centrifuging was quite thin. In order to facilitate removal of red cells in these cases, the preparation after two centrifugings was diluted to give an approximately 10% (v/v) suspension with respect to the contaminating red cells, and excess of antiserum (having a titre usually of 1/20) was added, the mixture being incubated for 5 min. at 37°. The agglutinated red cells were removed by a brief (30 sec.; 500 rev./min.) centrifuging, after which the trypanosomes were spun down (5 min.; 3000 rev./min.) and washed free of serum, using Ringer-bicarbonate or Ringer-phosphate solution as required in the final preparation.

Metabolic experiments. Spectroscopic observations, manometric experiments and the subsequent estimation of metabolites were carried out as described for *S. oncopelti* by Ryley (1955); in addition, citric acid was estimated by the method of Ettinger, Goldbaum & Smith (1952).

RESULTS

The motility of most species of trypanosome depends on a supply of extracellular glucose or other utilizable carbohydrate. With the *brucei–evansi* group of trypanosomes, loss of motility on the removal of substrate coincides with a loss of respiratory activity, and is soon followed by cellular disintegration; an intact cellular structure in these organisms necessitates, among other things, a constant supply of utilizable carbohydrate. When isolated in inorganic media and supplied with glucose, these trypanosomes respire and maintain motility for relatively short periods of time. Such processes can be somewhat prolonged by the addition of serum or other protein to the medium, but, even so, conditions are far from ideal. In the present studies, protein-free suspending media have been used, combined with short-term experiments.

Other trypanosomes are not so dependent on a supply of extracellular glucose, and are more resistant to experimental manipulation *in vitro*. Thus *T. lewisi* (Ryley, 1953) can survive for 1–2 hr. in the absence of glucose, respiring with a Q_{O_2} (μl. of O_2 consumed/mg. dry wt./hr.) of 4·5 and R.Q. of 0·80; in the presence of glucose, the Q_{O_2} is increased to about 68, and the flagellates will respire at a fairly constant rate over a period of several hours. *S. oncopelti* (Ryley, 1955) has an

endogenous Q_{O_2} of 17·7 (30°) and R.Q. of 0·9; this respiratory activity is sufficient to support full motility over a period of many hours. Brand *et al.* (1949), in experiments using whole blood containing *T. cruzi*, were unable to detect any glucose utilization attributable to the parasites. In the present paper, some preliminary experiments carried out with *T. cruzi* will be reported before the carbohydrate metabolism is compared with that of the other trypanosomes.

Experiments with Trypanosoma cruzi

Cultural forms. These experiments were carried out at 30°. When suspended in a Ringer-bicarbonate medium in the presence of a gas phase of air containing 5% (v/v) of CO_2 and studied by the second method of Dickens & Šimer (1931), the flagellates were found to respire with a Q_{O_2} of 14·5 and an R.Q. of 0·77. The addition of glucose (0·015 M) caused an increase in respiratory rate (Q_{O_2} 21) and in R.Q. (1·00); respiration under these conditions continues for many hours at a steady rate. Respiration leads to an accumulation of organic acids in the presence, but not in the absence, of glucose.

One preparation of flagellates (45 mg. dry wt. in vol. of 6 ml.) was allowed to respire in the absence of glucose for a period of 210 min. During this time, 47·8 μmoles of oxygen were utilized (Q_{O_2} 7·0; R.Q. 0·77). There was no net acid production as estimated by bicarbonate disappearance, but analysis of the metabolic solution showed the formation of 7·1 μmoles of volatile acid and 19·8 μmoles of ammonia (by nesslerization); no glycogen could be detected in the cells.

Blood-stream forms. These experiments were carried out at 37°. A preparation of flagellates in Ringer phosphate (4·7 mg. dry wt. in 2·0 ml.) respired at a steady rate over a period of 4 hr. with a Q_{O_2} of 15·0; the addition of glucose (0·015 M) increased the Q_{O_2} to 31·6. Experiments in a Ringer-bicarbonate medium, using the Dickens & Šimer technique, gave R.Q. values of 0·77 in the absence and 0·92 in the presence of glucose. In the absence of glucose, respiration was not accompanied by bicarbonate breakdown, although acid was produced when glucose was present.

Glucose degradation by various trypanosomes

A series of experiments have been carried out in which different species of trypanosome, suspended in a Ringer-bicarbonate medium in the presence of 5% of CO_2 (v/v) in the gas phase, were allowed to degrade glucose under both aerobic and anaerobic conditions. Glucose was present in most cases all the time during preparation of the trypanosome suspensions. Glucose and its various degradation products were estimated before and after a period of incubation, which for the less robust organisms was kept as short as possible. Representative experiments of this series are summarized in Table 1. The table, besides including new data on nine species of trypanosome, includes for the purpose of comparison some previously published data on *T. lewisi* and *S. oncopelti* (Ryley, 1951, 1955). Some of the metabolites were determined at the lower limit of sensitivity of the methods employed, and are possibly of doubtful significance; those which are considered to be the main end-products of glucose degradation are indicated in heavier type. Citric acid estimations have been carried out in the case of six species; although only very small amounts have been detected, sufficient was produced to be readily measurable by the sensitive method of estimation used. Surprisingly enough, the greatest yields of citric acid were found with *T. rhodesiense*; substantially the same yields were obtained from trypanosomes suspended in plasma as in Ringer-bicarbonate solution. Apart from the two forms of *T. cruzi*, the estimated metabolites account for the major part of the glucose degraded. In addition to these results, no evidence has been found for the production of formic acid by *T. lewisi* or *T. cruzi*, of hydrogen by *T. lewisi*, *T. cruzi* or *S. oncopelti*, or of oxalic acid by *T. lewisi*.

Respiratory inhibition by cyanide and iodoacetate

The sensitivity of the respiration of six species of trypanosome to cyanide and iodoacetate has been determined in a limited number of experiments; the results are summarized in Table 2. For these determinations, trypanosomes have been used as suspensions in Ringer-phosphate–glucose or in whole blood. From the table it can be seen that the degree of inhibitions observed are similar for the two suspending media used apart from the case of *T. lewisi*, where respiration, although it is quite sensitive to iodoacetate in inorganic media, is very little affected in whole blood.

Cytochrome systems

From the cyanide-inhibition studies reported in Table 2, it would be expected that *T. lewisi*, *T. cruzi* and *S. oncopelti* should contain cytochrome pigments, whereas *T. vivax* and *T. rhodesiense* might not have these systems. The cytochrome systems of *T. lewisi* and *S. oncopelti* have already been described (Ryley, 1951, 1955). Spectroscopic examination of preparations of the cultural forms of *T. cruzi* reduced with sodium dithionite ($Na_2S_2O_4$) showed: (*a*) an intense band at 553–565 mμ.; (*b*) very faint β-bands at 525–535 mμ.; (*c*) a feeble band at 608 mμ. due to cytochrome *a*.

Table 1. *Metabolic products formed during aerobic and anaerobic glucose breakdown by eleven species of trypanosomes*

Aerobic experiments carried out in Dickens & Šimer manometer flasks with a Ringer-bicarbonate–glucose medium and a gas phase of CO_2 + air (5:95); anaerobic experiments carried out in Warburg flasks using a Ringer-bicarbonate–glucose medium with a gas phase of $CO_2 + N_2$ (5:95). Fermentations carried out at 37° unless otherwise indicated, being stopped after time shown by tipping in acid from a side bulb. Table indicates the total amount of glucose disappearing during the course of the fermentation and the amounts of metabolites recovered expressed in terms of moles of metabolite/mole of glucose disappearing. From this, carbon, acid and redox balances have been calculated, the last by the method of Johnson, Peterson & Fred (1931); the substrate glucose has a redox value of 0. Total cellular dry weight and volume of fermentation systems as indicated. Most of the values for *T. lewisi* taken from Ryley (1951) and for *S. oncopelti* from Ryley (1955).

	T. cruzi		*T. cruzi* (cultural; 30°)		*T. lewisi*		*S. oncopelti* (30°)		*T. congolense*		*T. vivax*	
	CO_2/air	CO_2/N_2	CO_2/air	CO_2/N_2	CO_2/O_2	CO_2/N_2	CO_2/air	CO_2/N_2	CO_2/air	CO_2/N_2	CO_2/air	CO_2/N_2
Duration of experiment (min.)	145	135	116	116	180	180	20	20	90	90	33	33
Dry wt./vol. (mg./ml.)	10·2/4·0	11·5/4·0	41·1/6·0	41·1/6·0	29·9/15·0	29·9/15·0	42·6/6·0	42·6/6·0	11·5/6·0	11·5/6·0	28·8/6·0	28·8/6·0
Glucose used (μmoles)	15·40	27·35	19·35	26·00	81·70	72·20	39·35	47·60	27·20	30·55	21·75	15·50
R.Q.	0·92	—	1·05	—	0·97*	—	2·50	—	1·01	—	0·72	—
Acid (bicarbonate)	1·40	2·76	1·17	2·79	1·19	2·50	1·01	1·08	2·22	2·34	2·53	1·61
O_2	3·60	—	2·49	—	3·17*	—	0·38	—	0·50	—	1·52	—
CO_2	**3·31**	**−0·53**	**2·61**	**−0·46**	**3·06***	**−0·74**	**0·95**	**0·46**	**0·50**	−0·09	**1·10**	**0·11**
Glycerol	0·00	0·03	0·00	0·00	0·00*	0·00*	**0·32**	**0·16**	**0·36**	**0·63**	**0·18**	**0·63**
Ethanol	—	—	0·00*	0·00*	0·05	0·02	**0·91**	**0·58**	0·00*	0·00*	0·00*	0·00*
Lactic acid	**0·12**	**0·22**	0·00*	0·00*	**0·12**	**0·39**	0·01	0·02	0·04	0·03	**0·16**	**0·30**
Pyruvic acid	0·02	0·08	0·00*	0·00*	0·03	**0·19**	**0·28**	**0·21**	0·00	**0·11**	**0·95**	**0·40**
Acetic acid	**0·34***	**0·23***	**0·62**	**0·61**	**0·47**	**0·18**	0·05*	0·09*	**1·00**	**0·46**	**0·80**	0·07*
Succinic acid	**0·23**	**0·47**	**0·14**	**0·67**	**0·18**	**0·89**	**0·22**	**0·43**	**0·49**	**0·70**	0·00	0·00
Citric acid	—	—	0·007*	0·000*	0·000*	0·000*	0·005*	0·000*	0·000*	0·000*	0·008*	0·002*
Carbon balance (recovered/supplied)	5·33/6·00	3·33/6·53	4·45/6·00	3·90/6·46	5·27/6·00	5·70/6·74	5·61/6·00	4·69/6·00	5·66/6·00	5·94/6·09	6·62/6·00	4·25/6·00
Redox balance (−/+)	—	1·02/1·09	—	0·67/0·92	—	1·08/1·52	—	1·56/1·32	—	0·81/0·81	—	0·63/0·63
Acid balance (estimated/bicarbonate)	0·94/1·40	1·47/2·76	0·90/1·17	1·95/2·79	0·98/1·19	2·54/2·50	0·78/1·01	1·18/1·08	2·02/2·22	2·00/2·34	1·91/2·53	0·77/1·61

	T. rhodesiense		*T. gambiense*		*T. brucei*		*T. evansi*		*T. equinum*		*T. equiperdum*	
	CO_2/air	CO_2/N_2	CO_2/air	CO_2/N_2	CO_2/air	CO_2/N_2	CO_2/air	CO_2/N_2	CO_2/air	CO_2/N_2	CO_2/air	CO_2/N_2
Duration of experiment (min.)	60	60	75	75	25	35	30	30	30	30	37	37
Dry wt./vol. (mg./ml.)	14·6/4·0	14·6/4·0	18·2/4·0	18·2/4·0	24·6/4·0	27·7/4·5	14·9/4·0	14·9/4·0	16·9/4·0	16·9/4·0	21·7/4·0	21·7/4·0
Glucose used (μmoles)	21·82	24·40	18·15	30·75	38·15	22·50	29·90	23·05	27·22	23·85	22·85	27·45
R.Q.	0·11	—	0·03	—	0·09	—	0·09	—	0·09	—	0·10	—
Acid (bicarbonate)	1·78	1·19	2·20	1·15	1·87	1·33	1·85	1·15	1·83	1·04	1·72	1·11
O_2	1·18	—	1·07	—	0·65	—	0·67	—	0·66	—	0·59	—
CO_2	0·13	0·05	0·03	0·00	0·06	0·03	0·06	0·04	0·06	0·03	0·06	0·03
Glycerol	**0·38**	**1·03**	0·00	**0·92**	**0·58**	**1·13**	**0·47**	**1·00**	**0·28**	**1·01**	**0·60**	**1·10**
Ethanol	0·00	0·00	—	—	0·00	0·00	0·00*	0·00*	0·00	0·00	0·00	0·00
Lactic acid	0·01	0·05	0·00	0·02	0·00	0·04	0·05	0·08	0·02	0·07	0·02	0·03
Pyruvic acid	**1·56**	**0·83**	**1·72**	**0·84**	**1·43**	**0·87**	**1·37**	**0·85**	**1·50**	**0·77**	**1·30**	**0·88**
Acetic acid	0·00*	0·00*	0·03*	—	0·00*	0·00*	0·01	0·07	0·07*	0·10*	0·03	0·03
Succinic acid	0·02	0·04	0·00	0·00	0·00	0·00	0·00*	0·00*	0·00	0·00	0·06	0·03
Citric acid	0·025*	0·010*	—	—	—	—	—	—	—	—	—	—
Carbon balance (recovered/supplied)	6·21/6·00	6·00/6·00	5·25/6·00	5·34/6·00	6·09/6·00	6·15/6·00	5·75/6·00	5·93/6·00	5·60/6·00	5·78/6·00	6·12/6·00	6·24/6·00
Redox balance (−/+)	—	1·00/1·03	—	0·84/0·92	—	0·93/1·13	—	0·93/1·00	—	0·83/1·01	—	0·97/1·10
Acid balance (estimated/bicarbonate)	1·61/1·78	0·96/1·19	1·75/2·20	0·86/1·15	1·43/1·87	0·91/1·33	1·43/1·85	1·00/1·15	1·59/1·88	0·94/1·04	1·47/1·72	1·00/1·10

* Metabolite determined in a separate but similar experiment.

Baernstein (1953) reports bands at 532, 556 and 604 mμ. No trace of a cytochrome *c* band could be detected, even on examination by using liquid nitrogen.

Spectrosopic examination of preparations of *T. rhodesiense*, *T. equiperdum* or *T. congolense* failed to reveal any cytochrome bands; occasional shadows observed in the spectra were probably due to traces of blood cells which are difficult to remove completely, even with extensive washing. Such results do not mean that no cytochrome pigments are present in these three species, but indicate that, if present, they can occur only in traces.

Table 2. *Effect of cyanide and iodoacetate on the respiration of six species of trypanosomes*

Trypanosomes in Warburg manometers in vol. of 1·2 ml. of Ringer-phosphate–glucose or heparinized whole blood; gas phase air; temp. 37° unless otherwise indicated. In experiments with cyanide, balanced KOH–KCN mixtures used in centre wells as recommended by Robbie (1946). Inhibitor (0·3 ml.) added from side bulb and respiratory inhibition estimated over period of usually 1 hr. Results are expressed as percentage inhibition of normal respiration and are the average of three to five determinations. Data for *S. oncopelti* from Ryley (1955).

	Iodoacetate					Cyanide			
Concn. (M) ...	$3{\cdot}3\times10^{-3}$	10^{-3}	$3{\cdot}3\times10^{-4}$	10^{-4}	$3{\cdot}3\times10^{-5}$	$0{\cdot}46\times10^{-3}$	10^{-4}	$0{\cdot}46\times10^{-4}$	10^{-5}
				(*a*) Ringer-phosphate–glucose					
T. cruzi (culture; 30°)	45	30	22	19	2	85	80	71	52
S. oncopelti (30°)	—	55	31	8	—	88	65	37	—
T. lewisi	93	90	83	58	—	96	92	81	67
T. congolense	72	67	62	38	7	10	11	10	9
T. vivax	75	77	63	57	29	5	−7	−3	−1
T. rhodesiense	76	73	67	33	0	−14	−4	4	−9
				(*b*) Whole blood					
T. cruzi	32	16	2	0	0	70	62	64	56
T. lewisi	18	10	2	10	2	74	64	69	62
T. congolense	63	62	50	18	1	20	4	3	2
T. vivax	76	50	—	39	10	9	2	6	7
T. rhodesiense	84	65	61	52	26	3	2	4	1

Table 3. *Cytochrome and succinic oxidase activity in six species of trypanosome*

Trypanosome preparations well washed in Ringer-phosphate solution, suspended in water and shaken with grade 11 Ballotini glass beads in a disintegrator (Mickle, 1948) at room temperature. Strong phosphate buffer (pH 7·3) added to give a final concentration of 0·025 M. Dry wt. of homogenate and final volume of system in manometer as indicated; *p*-phenylenediamine (PPD) (13 mg./cup), succinate (0·03 M final) and cytochrome *c* ($6{\cdot}5\times10^{-5}$ M final) added from side bulb. Figures give oxygen uptake (μl.) observed over the first hour of incubation, and, in brackets, the corresponding Q_{O_2}; figures for systems containing *p*-phenylenediamine + cytochrome *c* have been corrected for O_2 uptake observed in the absence of homogenate.

		Oxygen uptake (μl.)					
Trypanosome	Dry wt./ml.	Blank	Blank + cytochrome *c*	PPD	PPD + cytochrome *c*	Succinate	Succinate + cytochrome *c*
T. cruzi (culture; 30°)	33·8/2·1	0 (0)	0 (0)	46 (1·36)	41 (1·21)	24 (0·71)	30 (0·89)
T. lewisi	44·0/3·1	—	—	91 (2·07)	225 (5·12)	—	—
	6·2/2·3	7 (1·13)	—	—	—	65 (10·48)	128 (20·65)
S. oncopelti (30°)	52·8/2·5	20 (0·38)	—	377 (7·15)	356 (6·75)	158 (3·00)	207 (3·92)
T. congolense	34·8/2·1	56 (1·61)	58 (1·66)	89 (2·56)	236 (6·79)	73 (2·10)	103 (2·96)
T. vivax	41·2/2·1	22 (0·53)	18 (0·44)	24 (0·58)	42 (1·02)	113 (2·74)	132 (3·20)
T. rhodesiense	31·7/2·1	53 (1·67)	58 (1·83)	84 (2·65)	160 (5·05)	60 (1·89)	78 (2·46)

Oxidase systems

The ability of homogenates to oxidize *p*-phenylenediamine and succinate, and the effect of added cytochrome *c* on any such oxidation, have been determined; results from typical experiments are given in Table 3. With *T. cruzi*, *T. lewisi* and *S. oncopelti*, oxygen uptake in such systems continues at a constant rate for at least 2 hr. With the other three species, the rate of oxygen uptake gradually decreases, being about one-third the initial rate after 2 hr. (see Figs. 1 and 2); the respiration of such homogenates is more stable than that of intact cells, where respiration has usually completely ceased by this time.

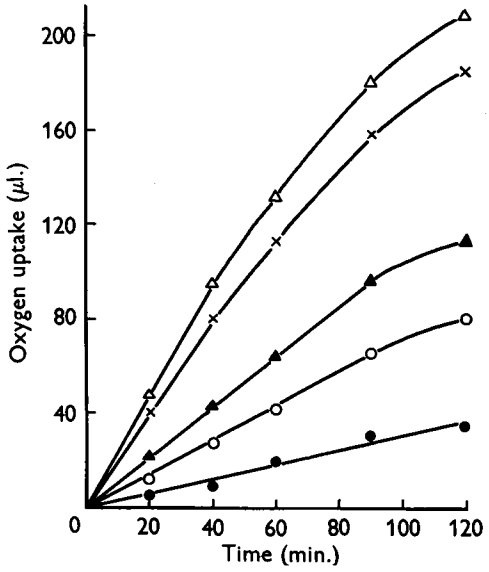

Fig. 1. Oxidase activity in *T. vivax* homogenate. System as described in Table 3. ●, Homogenate with or without cytochrome *c*; ○, homogenate + *p*-phenylenediamine; ▲, homogenate + *p*-phenylenediamine + cytochrome *c*; ×, homogenate + succinate; △, homogenate + succinate + cytochrome *c*.

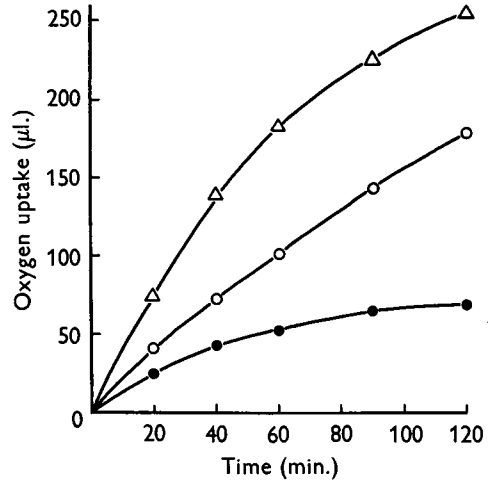

Fig. 2. Oxidase activity in *T. rhodesiense* homogenate. System as described in Table 3. ●, Homogenate with or without cytochrome *c*; ○, homogenate + *p*-phenylenediamine; △, homogenate + *p*-phenylenediamine + cytochrome *c*.

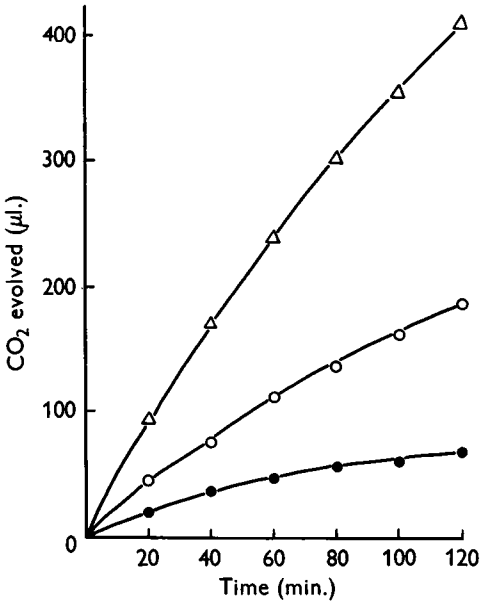

Fig. 3. Dehydrogenase activity in *T. cruzi* homogenate. Each manometer cup contained 0·02 M-$NaHCO_3$, 0·2 ml. of 11% (w/v) $K_3Fe(CN)_6$, 0·08 M succinate or lactate and homogenate (24·6 mg. dry wt.) in total vol. of 2·5 ml.; gas phase $CO_2 + N_2$ (5:95). Temp. 30°. ●, Blank; ○, lactate; △, succinate.

Although the succinic-oxidase activity of homogenates of *T. cruzi* is very low, and the oxygen uptake in the presence of succinate is not stimulated by $3·2 \times 10^{-6}$ M methylene blue, such homogenates show high succinic-dehydrogenase activity as determined by the method of Quastel & Wheatley (1938); this is illustrated by Fig. 3, which also indicates the presence of lactic dehydrogenase.

DISCUSSION

The carbohydrate metabolism of all trypanosomes so far studied is characterized by incomplete oxidation of the substrate; this is most marked with the

brucei–evansi group. Thus Reiner, Smythe & Pedlow (1936) found that *T. equiperdum* converted glucose into pyruvic acid under aerobic conditions, or into a mixture of pyruvic acid and glycerol under anaerobic conditions, and Harvey (1949) obtained similar results with *T. hippicum*. Marshall (1948), using *T. evansi*, and Brand, Tobie, Mehlman & Weinbach (1953), using *T. gambiense*, found that pyruvic acid accounted for most of the glucose degraded under aerobic conditions. Fulton & Stevens (1945) claimed that *T. rhodesiense* produced lactic, formic, acetic and succinic acids and ethanol, besides pyruvic acid and glycerol; their data, however, do not provide a ready evaluation of the amounts of these metabolites produced in terms of glucose utilized. Glowazky (1937), using spot tests, found that lactic and oxalic acids, as well as pyruvic acid, were produced by *T. brucei*. The present results indicate that, in the six species of this group studied, pyruvic acid and glycerol account for almost all the glucose disappearing, and the other metabolites can be produced in only insignificant quantities; the R.Q. in all these cases is very small, but it does seem that a little carbon dioxide is produced. With *T. rhodesiense*, no acetic acid or ethanol, and only traces of lactic and succinic acids, could be detected.

In contrast to this, *T. cruzi*, *T. lewisi*, *T. congolense*, *T. vivax* and *S. oncopelti* degrade glucose beyond the pyruvic acid stage, and produce appreciable quantities of carbon dioxide. *T. cruzi* and *T. lewisi* convert about half the glucose molecule into carbon dioxide, the rest appearing as succinic, lactic and acetic acids; glycerol, ethanol and formic acid production in these species is negligible. The detection of small amounts of citric acid in metabolic solutions of *T. cruzi*, *T. vivax*, *T. rhodesiense* and *S. oncopelti* suggests that the tricarboxylic acid cycle is operating in these organisms, and that traces of this key metabolite leak out of the cell; with *T. rhodesiense*, however, the cycle cannot be very important, as the total yield of carbon dioxide is very small. *T. congolense* is similar to *T. lewisi* and *T. cruzi*, except that smaller amounts of carbon dioxide and larger amounts of acid are produced.

In a recent study of the metabolism of *T. congolense*, Agosin & Brand (1954) found that succinic acid was produced only in traces; their experiments, however, were carried out in the absence of carbon dioxide. In the case of *S. oncopelti* it has been shown (Ryley, 1955) that the amount of succinic acid formed depends on the carbon dioxide tension, and this probably explains the different results obtained with *T. congolense*. Agosin & Brand also found an oxygen/glucose ratio of 2·40, their determinations being carried out in whole-rat blood or rabbit serum. We obtained the much lower value of 0·50, using trypanosomes in Ringer-bicarbonate medium, but in plasma a value of 2·11 was obtained; with *T. lewisi* a ratio of 3·59 was found in plasma, as compared with 3·17 in an inorganic medium. Possibly, in the more fragile trypanosomes metabolizing in inorganic media, aerobic processes decay before anaerobic metabolism, so that the oxygen/glucose ratio decreases.

It is interesting to compare the different hydrogen-accepting systems coupled with anaerobic oxidations. In the *T. rhodesiense* group, oxidation–reduction results in the production of glycerol, while with *T. cruzi* and *T. lewisi* no glycerol is formed, but instead two alternative pathways lead to the production of lactic and succinic acids. *T. congolense* and *T. vivax* are intermediate between these groups, producing glycerol and succinic or lactic acids. *S. oncopelti* is unique among the protozoa in that it is able to form ethanol.

From the cyanide-inhibition experiments reported, it can be seen that *T. rhodesiense* and *T. vivax* are insensitive to the inhibitor, *T. congolense* shows a slight sensitivity, and the other three species are very sensitive to cyanide; cytochrome pigments have been detected only in these last three trypanosomes. The cytochrome system of *T. cruzi* is unusual in that cytochrome *c* seems to be absent. Baernstein & Tobie (1951) first noticed this, and reported that respiration, although sensitive to cyanide, was not affected by carbon monoxide.

Homogenates of *T. lewisi* and *S. oncopelti* have been found to show succinic-oxidase and cytochrome-oxidase activity, which can be stimulated by added cytochrome *c*. In *T. cruzi* oxidase activity in the experimental systems used is very low, and is not affected by exogenous cytochrome *c*. The results obtained with *T. vivax* and *T. rhodesiense* are very puzzling; respiration is not affected by cyanide, succinate does not seem to be involved in their metabolism, and *T. rhodesiense* at least contains no detectable cytochrome pigments. In the experimental systems used, however, activity is marked, particularly the succinic oxidase of *T. vivax* and the cytochrome oxidase of *T. rhodesiense*.

One of the most interesting problems in the trypanosome field concerns the difference between the blood stream and the invertebrate or cultural forms of the parasite. For *T. cruzi*, the results presented here do not reveal any marked difference in the two forms. With *T. gambiense*, Brand & Johnson (1947) found the cultural form was slightly sensitive to cyanide, but the blood-stream form was not (Brand & Tobie, 1948). Although the blood-stream form of this parasite degrades glucose only as far as the pyruvic acid stage, Brand, Weinbach & Tobie (1954) found that the cultural

form, which corresponds to the stage found in the insect vector, respired with an R.Q. of unity and produced large amounts of succinic and acetic acids, as well as smaller amounts of other metabolites; it seems that adaptation to life in the animal has resulted in the loss of much catabolic activity.

SUMMARY

1. *Trypanosoma rhodesiense*, *T. gambiense*, *T. brucei*, *T. evansi*, *T. equinum* and *T. equiperdum* degrade glucose to a mixture of pyruvic acid and glycerol; glycerol production is favoured by anaerobic conditions. The R.Q. is small, usually less than 0·10.

2. *T. cruzi* and *T. lewisi* convert glucose into a mixture of lactic, acetic and succinic acids; *T. congolense* and *T. vivax* produce glycerol, acetic acid and succinic or lactic and pyruvic acids; and *Stringomonas oncopelti* forms glycerol, ethanol, pyruvic and succinic acids. In all cases the R.Q. is high, about unity.

3. The respiration of *T. cruzi*, *T. lewisi* and *S. oncopelti* is very sensitive to cyanide, that of *T. congolense* is slightly sensitive, and the respiration of *T. vivax* and *T. rhodesiense* is insensitive to the inhibitor; cytochrome pigments have been detected in *T. cruzi*, *T. lewisi* and *S. oncopelti*, but not in *T. congolense*, *T. rhodesiense* or *T. equiperdum*.

4. Homogenates of *T. cruzi*, *T. lewisi*, *S. oncopelti*, *T. congolense*, *T. vivax* and *T. rhodesiense* have been examined for cytochrome and succinic-oxidase activity; such activity has been found in all preparations to a varying extent.

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The Component Fatty Acids of *Penicillium lilacinum* Fat

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During study in this Laboratory of the fat-forming potentialities of a selection of mould fungi it was found by one of us (S.S.) that, under certain cultural conditions, *Penicillium lilacinum* Thom can give a high yield of a fat which is solid at ordinary temperature and which is of a very low free acidity—characteristics that are very desirable in edible fats. It was considered of interest to investigate the component fatty acids and glycerides of this fat.

EXPERIMENTAL

The fat was obtained by cultivating *P. lilacinum* Thom as surface cultures for 14 days at 25° on the following medium (g./100 ml.): K_2SO_4, 0·011; $NaH_2PO_4,2H_2O$, 0·730; $MgSO_4,7H_2O$, 0·500; $ZnSO_4,7H_2O$, 0·005; $FeCl_3,6H_2O$, 0·016; sucrose, 17; $NaNO_3$, 0·640; made up with distilled water and the pH adjusted to 6·8. The felts were removed and treated as described previously (Singh, Walker & Meara, 1955) for the extraction of the fat. The product thus obtained was solid at room temperature and had a slight