The nutrition of an intracellular parasite (avian malaria)

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The Nutrition of an Intracellular Parasite (Avian Malaria).

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The obligate intracellular parasites represent an extreme of nutritional specialization. Their study may be expected to lead to knowledge concerning particular substances essential for cellular metabolism which most cells can make for themselves but which intracellular parasites must obtain as nutrients from their host cell. It is interesting to reflect how incomplete would be our knowledge of growth factors and the coenzymes derived from them if study had been confined to organisms capable of growth on simple diets. The simplicity of *Escherichia coli* is deceptive. It grows nicely on a little ammonium salt and a carbon source, but it has within itself the means for synthesis of nearly all the vitamins required by mammals in their diet. Mammalian cells have lost these synthetic mechanisms, and it was the study of the nutritional needs of mammals that revealed the existence of some of the key substances in cellular metabolism.

Intracellular parasites have lost still more synthetic mechanisms. Indeed, they seem to have lost so many that up to the present time it has not been possible to obtain continuous growth, apart from a living host cell, of any microorganism which is in nature an obligate intracellular parasite. Consequently, the study of the nutrition and metabolism of intracellular parasites is fraught with difficulty. Some have approached the problem with the assumption that the metabolism of the parasite could be inferred by subtracting the metabolism of the uninfected host cell from that of the host cell plus parasite combination. But in biological systems the whole is nearly always more than the sum of its parts. Numerous examples are known where the penetration of a host cell by an intracellular parasite is followed by grossly visible morphological changes in the host cell. The only safe assumption, therefore, is that all cells containing intracellular parasites are altered cells, physiologically if not morphologically.

Two other ways of studying the metabolism of intracellular parasites have been more fruitful. In both, the parasites are removed from their host cells. One method then makes use of short-term observations, lasting a few hours, of various *in vitro* activities of the isolated parasites, such as their oxygen consumption. The other approach attempts to get *in vitro* growth and multiplication of the parasite — to substitute a non-living culture medium for the living host cell.

My own work has been concerned largely with this last type of experiment. It seemed that malaria parasites might be particularly suitable material for attempting the extracellular cultivation of an intracellular parasite. They may be expected to have more enzyme systems of their own than rickettsiae, for example. Moreover, their host cell is the erythrocyte, a type of cell readily obtained in large amounts and about which a good deal is known. Accordingly, *Plasmodium lophurae* (COGGESHALL 1938), a bird malaria parasite which produces heavy infections in ducks (WOLFSON 1941) and young chickens, was selected as the experimental material.

It was found that this organism could indeed be removed from its host erythrocyte and kept alive *in vitro* for several days. For the first time it be-

came possible to study some of the nutritional requirements of an intracellular parasite itself, as distinguished from the host cell-parasite complex. The methods used and the results obtained so far in such a study will be reviewed in the present paper and discussed in relation to other work on the physiology of malaria parasites.

Methods.

The ways of freeing the parasites from their host blood cells, of preparing the culture media, of setting up the culture experiments, and of following the survival and development of the organisms have been described in detail (TRAGER, 1950, 1952, 1955a). The procedures may be briefly summarized as follows.

Sterile defibrinated duck blood was centrifuged and the serum and much of the buffy coat removed. The cells were frozen by immersion of the centrifuge tubes in a dry ice-alcohol mixture. They were then thawed in ice water and resuspended in $1\frac{1}{3}$ times their volume of a special nutrient solution. This solution was prepared by mixing aseptically appropriate sterile stock solutions. It contained: salts with a high potassium content; sodium acetate, glycerol and glucose; casein hydrolysate supplemented with glycine, histidine, tryptophane, cystine and asparagine; glutathione; bovine albumin (plasma fraction V); gelatin; purines and pyrimidines; the B vitamins (except B_{12}); ascorbic acid; hexose diphosphate (magnesium salt); a high concentration of nicotinamide. Its freezing point depression was the same as that of a 0.18 M solution of sodium chloride. The suspension of hemolyzed red cells was centrifuged at room temperature for 45 to 50 minutes at 3,000 RPM. A clear very deep red supernatant extract was obtained. It had a pH of 7.1 to 7.2, which was brought to 7.0 by the addition of 0.1 N HCl. To this extract was added, at the rate of 0.2 ml. per 10 ml. of extract, a solution that gave final concentrations of L-malic acid 6.0 mM, yeast adenylic acid 1.4 mM, and cozymase 0.15 mM. The pH of the mixture was now 6.8-6.9. This constituted the standard red cell extract (Std. RCE).

Sterile 50 ml. Erlenmeyer flasks (usually 8 to 12 for a single experiment) were each equipped with a rubber stopper bearing cotton-plugged tubes for the ingress and egress of gas. 0.25 ml. of normal duckling plasma was placed in each flask and to it was added 0.02 ml. of a chick embryo extract (TRAGER 1952). The flask was twirled rapidly so as to form a thin plasma clot lining the bottom 1 cm. of its wall. Three ml. of Std. RCE (or desired modifications of it) were then added. Further supplements to be used or tested (as adenosinetriphosphate [ATP], sodium pyruvate, and coenzyme A [Co A]) were not added until just before inoculation of the parasites. They were contained in small volumes, so that the total final volume of medium was about 3.3 ml.

For the preparation of the parasites, heparinized blood (3 mg. heparin per 100 ml. blood) from a duckling heavily infected with *P. lophurae* (with about 90% of the parasites in the uni-nucleate trophozoite stage) was centrifuged briefly and the cells were resuspended in Std. RCE. To 4 ml. of such a 10% cell suspension, held in a stoppered 50 ml. Erlenmeyer flask, were added 0.07 ml. of guinea pig serum and 0.4 ml. of rabbit anti-duck erythrocyte serum. The mixture was incubated with agitation for one-half hour at 37 or 40° C. 0.3 ml. of the whole hemolyzed suspension so obtained, or 0.5-0.6 ml. of a purified suspension of free parasites made from it, were inoculated to each culture flask. Usually about one hour elapsed from the time the infected blood was taken until the flasks were inoculated. Stained films were made from the suspension of blood in Std. RCE (before hemolysis) and from the suspension used for inoculation.

The culture flasks were placed on a rocker (16 cycles per minute) in an incubator at 40-40.5°C. A slow current of moist air with 5% CO_2 was passed through them. After the first 18 hours of incubation most of the free parasites had adhered to the plasma clot to form a scum which outlined the margin of the fluid as it rocked back and forth. Each flask was removed individually from the incubator and the culture fluid was drawn off and rapidly replaced with 3.8 ml. of fresh warm medium, to which the special supplements (ATP, etc.) were then added separately. With a fine capillary-tipped pipette bits of scum were sucked up and used for the preparation of a wet mount and a stained film. After the first change, the changing of the culture medium was repeated at intervals of approximately 12 hours. Samples of the scum were taken once daily.

The survival and development of the parasites were followed by morphological methods (see TRAGER 1950 for detailed discussion). The wet mounts were examined immediately under phase contrast, a procedure which permitted a rough but very useful estimate of the general condition of the organisms. The stained films were used to determine the proportions of organisms in the different stages of development, and the proportion of degenerate organisms.

Results.

Before considering the results obtained, it must be pointed out that the composition of the nutrient solution used in making the red cell extract was not entirely arbitrary. Earlier work had shown the favorable effect, on malaria parasites developing *within* erythrocytes, of the presence in the medium of a high potassium content, of glucose, of glutathione, of red cell extract (TRAGER 1941, ANDERSON 1953), of the B vitamins (TRAGER 1943, 1947 a, ANFINSEN et al. 1946) and of purines and pyrimidines (ANFINSEN et al. 1946). The favorable effects of gelatin were observed with the free *P. lophurae* (TRAGER 1950). Other constituents of the medium were based on the composition of erythrocytes and in part on general nutritional considerations. The favorable effects of rocking and aeration had been observed with parasites developing intracellularly in suspensions of erythrocytes *in vitro* (TRAGER 1943, ANFINSEN et al. 1946) and were confirmed with the extracellular *P. lophurae*. Changing of the medium in the manner described was necessary for the best results. The free parasites were damaged if they were packed by centrifugation and resuspended (TRAGER 1952).

The prolongation of survival in vitro by supplements of known chemical nature. The Std. RCE (as used originally without malate) gave good survival of the extracellular parasites for 1 day but not for 2 days. It was then found that the addition of both ATP (2 mM per l.) and sodium pyruvate (5 mM per l.), initially and with each change of culture medium, carried virtually all of the parasites through to the second but not to the third day (TRAGER 1950)

(Table I). Survival of 90-95% of the parasites to the third day was obtained when the culture flasks were further supplemented with malate (6 mM per l.) and coenzyme A (0.02 mM per l.) (TRAGER 1952, 1954) (Table II). The deleterious effects of omitting these supplements became apparent even after only 1 day of incubation if a purified suspension of free parasites was used to inoculate the culture flasks (Table III). The effect here was noticeable mainly in the proportions of multinucleate forms, rather than in the per cent of degenerate parasites (Table III).

TABLE I.

The effect of adenosinetriphosphate (ATP) and sodium pyruvate on the extracellular development of *P. lophurae* as seen in stained films [1].

Flasks	Supplements [2]	% degenerate parasites after	
		1 day	2 days
1	None	3	12
2		0.5	18
3	ATP plus pyruvate	0.5	3
4		0.5	3

[1] Data from TRAGER (1950).
[2] To Std. RCE but without malate.

TABLE II.

The effect of two preparations of coenzyme A (No. 1 about 3% pure, No. 2 about 75% pure) in the presence and absence of added pantothenate on the extracellular survival of *P. lophurae* as seen in stained films [1].

Exp.	Flasks	Pantothenate added	Coenzyme A added [2]		% degenerate parasites after
		μg. per ml.	Prep. No.	Units per ml.	3 days
A	1, 2	0	—	0	19, 12
	3, 4	0	1	8	7, 7
	5, 6	4.9	—	0	14, 8
	7, 8	4.9	1	8	10, 5
B	1, 2	0	—	0	11, 18
	3, 4	0	1	8	5, 9
	5, 6	4.9	—	0	19, 17
	7, 8	4.9	1	8	17, 6
C	1, 2	0	—	0	7, 20
	3, 4	0	1	8	13, 7
	5, 6	0	2	8	9, 4
D	1, 2	0	—	0	7, 8
	3, 4	0	2	8	3, 5
	5, 6	4.9	—	0	14, 12
	8	4.9	2	8	3

[1] Data from TRAGER (1954).
[2] To Std. RCE also supplemented with ATP and pyruvate.

TABLE III.

The effect of coenzyme supplements on the development of purified suspensions of extracellular *P. lophurae* after 1 day, as seen in stained films [1].

Exp.	Flasks	Supplements [2]	% parasites	
			With 2 to 4 nuclei	Degenerate
A	Inoculum	——	6	0.5
	1		7	3
	2		9	2
	3	None	7	5
	4		7	4
	5		14	3
	6	ATP, pyruvate, Co A	14	2
	7		16	3
	8		13	1
B	Inoculum	——	5	0
	1		17	4
	2	ATP, pyruvate, Co A	21	2
	3		12	5
	4	None	11	6

[1] Data from TRAGER (1955).
[2] To Std. RCE.

In flasks containing the complete medium of Std. RCE (with malate) plus ATP, pyruvate and Co A, inoculated with the free parasite-hemolyzed red cell mixture and kept under the conditions outlined under Methods, the 3-day period of extracellular survival *in vitro* was accompanied by development of the parasites, which have in the host a 36 to 40 hour cycle (TERZIAN 1941). The cultures were begun with 90% or more of the parasites in the stage of uni-nucleate trophozoites. After 18 hours the proportion of multi-nucleate forms had increased, and this increase usually continued to the 2nd day. On the 3rd day there was a decrease in the proportion of multinucleate forms accompanied by the presence of young developing parasites. Some parasites of normal appearance and in all stages of development were present on the 4th day, but a majority were degenerating.

More recently it has been found that improved survival on the 4th day (about 75% in morphologically good condition) could be obtained if folinic acid was added initially and with each change of culture medium (TRAGER 1955 b). Synthetic leucovorin (Lederle) was used at a concentration of 0.013 mM. Parasites in a stained film from such a 4-day-old culture are shown in Fig. 1. Such cultures were infective to ducklings even after 5 days *in vitro*. So far, however, it has been impossible to keep a majority of the extra-

TABLE IV.

The effect of dilution of the red cell extract on the extracellular survival of
P. lophurae as seen in stained films [1].

Flasks	Concentration of Std. RCE	% parasites degenerate after 2 days
1	Full strength (1 ×) [2]	4
2		6
3	0.5 × [2]	10
4		16
5	0.1 × [2]	85
6	0.5 × [3]	39

[1] Data from TRAGER (1953).
[2] Supplemented with ATP, pyruvate and Co A.
[3] Not supplemented with ATP, pyruvate and Co A.

TABLE V.

Effect of concentration of the red cell extract on the development of purified
suspensions of extracellular *P. lophurae* after 1 day, as seen in stained films [1].

Flasks	Concentration of Std. RCE [2]	% parasites	
		With 2.4 nuclei	Degenerate
Inoculum	——	2	0
1	Full strength (1 ×)	14	1
2		12	1
3	0.3 ×	7	3
4		8	2
5	2 ×	11	9
6		12	9

[1] Data from TRAGER (1955).
[2] Concentration after addition of the inoculum, which was contained in
full strength extract. All flasks received ATP, pyruvate, and Co A.
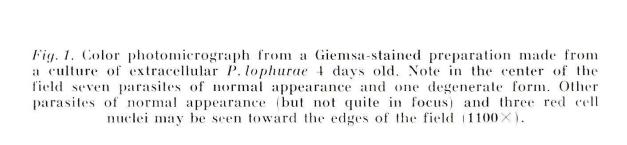
cellular parasites alive in culture after the 4th day. Lipoic acid,
triphosphopyridine nucleotide, and flavin-adenine dinucleotide
were among the coenzymes and other materials tested which had
no effect. Various liver extracts and preparations of duck liver
mitochondria had either no effect or a deleterious effect.

The red cell extract. In the complete nutrient solution plus all
the supplements known to be favorable, but with a minimal
amount of red cell extract (introduced with the inoculum), the
parasites did not do well. In experiments of 2 days' duration or
longer it could be shown that even a half-strength red cell extract
was not as favorable as the full strength Std. RCE (Table IV). In
1-day experiments with purified parasites it was found that ⅓

Fig. 1. Color photomicrograph from a Giemsa-stained preparation made from a culture of extracellular *P. lophurae* 4 days old. Note in the center of the field seven parasites of normal appearance and one degenerate form. Other parasites of normal appearance (but not quite in focus) and three red cell nuclei may be seen toward the edges of the field (1100×).

strength and more dilute extracts supported progressively less development of the organisms (Table V). Extracts more concentrated than Std. RCE were likewise relatively unfavorable (Table V), but this may have been a result of the methemoglobin which formed when red cell extract was concentrated by lyophilization or by dialysis in the cold against 20% polyvinylpyrrolidone. If the stroma of the red cells was left in the hemolysate the resulting medium supported only very poor survival even for 1 day. Perhaps the ATP-ase and DPN-ase known to be associated with red cell stroma (CLARKSON and MAIZELS 1952, GARZÓ et al. 1952, ALIVASATOS et al. 1956) destroyed the ATP and diphosphopyridine nucleotide too rapidly to permit their utilization by the parasites.

The materials in duck erythrocyte extract responsible for its activity in the development of extracellular *P. lophurae* were nondialyzable (TRAGER 1957). This was also true of the activity of chicken erythrocyte extract for extracellular *P. gallinaceum* (CLARKE 1952). By means of zone electrophoresis of duck erythrocyte extract on starch a fraction was obtained free from hemoglobin which had ¼ to ½ of the activity of the original extract (in promoting the development of multi-nucleate parasites of *P. lophurae*) but only 1% of the protein (TRAGER 1957). The activity of this fraction was demonstrated in the presence of a low concentration of whole red cell extract introduced with the inoculum of free parasites. Similar activity was found in certain partially purified hexokinase preparations from yeast (TRAGER, unpublished).

Discussion.

The ability of extracellular *P. lophurae* to survive and develop *in vitro* up through but not beyond 4 days suggests that the parasites may contain initially some essential factor which becomes depleted by about the 4th day and which is not supplied by the culture medium. The culture medium does, however, supply other essential materials which become depleted even sooner—among them pyruvate, malate, the coenzymes cozymase, ATP, and Co A, and the substances of unknown nature present in red cell extract. All of these materials must be considered as essential nutrients of the parasites. What additional nutrients or what special conditions they may require to permit continuous extracellular development *in vitro* remain to be discovered. Enough is known now to indicate the general pattern of the nutritional requirements of malaria parasites.

It has long been apparent that erythrocytic malaria parasites digest hemoglobin, leaving a hematin-like pigment (DEEGAN and

MAEGRAITH 1956) as a residue, and it has seemed likely that the parasites obtain their bulk nitrogen in this way (BLACK 1947, MORRISON and JESKEY 1948, MOULDER 1948, 1955). FULTON and GRANT (1956) have shown that *P. knowlesi* obtains 80% of its methionine from the globin of the host cell. The other 20% presumably comes from the plasma, and it is of interest in this connection that *P. knowlesi* maintained in red cell suspensions *in vitro* needs methionine in the medium (McKEE 1951). Recent electron microscope studies show that *P. lophurae* engulfs into food vacuoles portions of the cytoplasm of its host erythrocyte; it is an intracellular phagotroph (RUDZINSKA and TRAGER 1957). Within these food vacuoles the digestion of hemoglobin and concomitant appearance of pigment granules proceed. These observations suggest a possible explanation for the favorable effects on extracellular parasites *in vitro* of gelatin in the medium, and of the scum formation. Both of these conditions might be expected to be more conducive to phagotrophy than if the parasites were suspended in a non-viscous liquid medium. It may well be that the culture medium should be modified further in these directions.

Glucose seems to be the chief energy source for malaria parasites, whether they are in erythrocytes or maintained extracellularly (FULTON 1939, 1951, TRAGER 1953). Pyruvate and malate, however, are also essential for extracellular *P. lophurae,* and malate has been found to have a particularly striking effect on extracellular *P. gallinaceum* kept *in vitro* for 1 day (CLARKE 1952). These substances also promote the respiration of isolated parasites (BOVARNICK et al. 1946, SPECK et al. 1946).

In any discussion of the further nutritional needs of the parasites it becomes necessary to distinguish between experiments concerned with the host cell-parasite complex and those concerned with the parasite itself. This is especially true with respect to the growth factors, and is well illustrated by studies with pantothenic acid and Co A.

Pantothenic acid was the first growth factor found to favor the development of malaria parasites. The survival of *P. lophurae* developing intracellularly in dilute suspensions of duck or chicken erythrocytes was materially prolonged in the presence of added calcium pantothenate (TRAGER 1943). Further evidence for a rôle of pantothenic acid in the development of malaria parasites was soon available. Certain analogs of the vitamin were effective antimalarial agents (MARSHALL 1946). *P. gallinaceum* could not develop in chickens rendered deficient in pantothenate (BRACKETT et al. 1946). Nonetheless, it has not been possible to demonstrate any effect of pantothenic acid on extracellular *P. lophurae in vitro.*

The coenzyme derived from pantothenic acid, Co A, has however a marked favorable effect on the extracellular survival of these organisms in culture (TRAGER 1952, 1954). Although the host cell-parasite complex requires the vitamin pantothenic acid for the development of the parasite, the parasite itself requires the complete Co A. The Co A may be synthesized from pantothenate by the host erythrocyte, a hypothesis supported by the finding that uninfected erythrocytes contain enzymes capable of cleaving Co A.

One may assume that a somewhat parallel situation exists with respect to the folic acid group of compounds. Here certain sulfa drugs were observed to act as anti-malarial agents (COGGESHALL 1940, WALKER and VAN DYKE 1941), and this action was antagonized by para-aminobenzoic acid (MAIER and RILEY 1942). Then it was shown that para-aminobenzoic acid had a marked effect on *P. knowlesi* developing intracellularly in suspensions of monkey erythrocytes maintained *in vitro* (ANFINSEN et al. 1946), and quite recently para-aminobenzoic acid in the diet of the host has been found essential for the development of *P. berghei* in intact rats (HAWKING 1954). Drugs of the pyrimethamine group, among the most active antimalarials, are thought to be effective by way of antagonism to folic acid and related compounds (HITCHINGS 1952). Their effectiveness is counteracted in a most irregular manner by para-aminobenzoic acid, folic acid, and folinic acid (BISHOP 1954, GREENBERG 1949, 1953, ROLLO 1955, THURSTON 1954). The presence of folic acid in the medium has been found to favor the intra-erythrocytic development of an avian malaria (GLENN and MAN-WELL 1956) and of the human *P. falciparum* (TRAGER, unpublished) in erythrocyte suspensions *in vitro*. Para-aminobenzoic acid and folic acid, however, do not appear to influence the survival of extracellular *P. lophurae* whereas folinic acid does. Although the precise coenzyme form of folic acid remains unknown, folinic acid is considered to represent a step in this direction (PFIFFNER and BIRD 1956). Perhaps that is why folinic acid has some favorable, though irregular, effects on *P. lophurae* developing extracellularly.

Sufficient depletion of the host's diet in ascorbic acid (McKEE and GEIMAN 1946), thiamin (RAMA RAO and SIRSI 1956), riboflavin (SEELER and OTT 1946) or biotin (TRAGER 1947 b), as well as in pantothenate, has inhibitory effects on the development within the host of different species of malaria parasites. Clearly these substances are required by the host-parasite complex, but in what form they must be supplied to the parasite or in what way they exert their effect remains unknown.

P. lophurae when maintained extracellularly *in vitro* requires

two additional coenzymes, adenosinetriphosphate and diphospho-pyridine nucleotide. Both of these are synthesized within red blood cells, and the amount of ATP particularly seems to be associated with the viability of erythrocytes upon incubation or storage (GABRIO et al. 1954). The need of *P. lophurae* for external sources of coenzymes ordinarily found within but not outside of cells provides at least a partial explanation of their obligate intracellular parasitism. Certain vital activities of rickettsiae, a different kind of intracellular parasite, likewise require external sources of cozymase and of Co A (BOVARNICK and ALLEN 1954, GILFORD and PRICE 1955).

Although malaria parasites contain a few mitochondria (SEN GUPTA et al. 1955, RUDZINSKA and TRAGER 1957) and although a whole array of glycolytic enzymes (BOVARNICK et al. 1946) and respiratory enzymes, including those of the Krebs cycle (SPECK et al. 1946), have been demonstrated in parasites freshly removed from their host cells, a possibility remains that the parasites may depend on certain host-cell enzymes. Indeed the enzymes shown to be present in the freshly isolated parasites are for the most part the same as those present in uninfected erythrocytes (RUBENSTEIN and DENSTEDT 1953), but they are present in greater amounts. It would be most interesting to attempt to differentiate, perhaps by immunological means, between, for example, the hexokinase of isolated *P. lophurae* and the hexokinase of uninfected duck or chicken erythrocytes.

The possibility that the parasites may in part depend on enzymes of the host cell is strengthened by the finding that they require non-dialyzable materials other than hemoglobin present in red cell extract. The discovery of the nature of these materials is one of the most interesting and significant problems awaiting solution. Perhaps they are enzymes which the parasites themselves lack.

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Résumé.

Dans les expériences décrites, le parasite de la malaria aviaire *Plasmodium lophurae* a été isolé des érythrocytes de leur hôte et maintenu in vitro pendant 4 jours, viable et capable de se développer. Le meilleur milieu artificiel, employé jusqu'à présent, était un extrait concentré d'érythrocytes de canard dans une solution nutritive adéquate, à laquelle on a ajouté du malate, du pyruvate, du triphosphate d'adénosine, du coenzyme A, du coenzyme I et de l'acide folinique. Les substances favorables au développement extracellulaire de *Pl. lophurae* n'étaient pas dialysables et ont été obtenues, relativement purifiées, dans une fraction de l'extrait érythrocytaire, libre d'hémoglobine.

L'énergie principale leur est fournie par le glucose, mais elle doit être complétée par un supplément de malate et de pyruvate. Normalement, la plasmodie reçoit probablement des érythrocytes les coenzymes nécessaires à son développement. Reste à savoir si les plasmodies ont besoin et utilisent également les enzymes de leur hôte.

Zusammenfassung.

Es werden Versuche besprochen, bei welchen der Parasit der Vogelmalaria, *Plasmodium lophurae,* aus den Erythrocyten seines Wirtes befreit wurde und während 4 Tagen in vitro lebend und entwicklungsfähig gehalten werden konnte. Als bestes bis jetzt geprüftes Kulturmedium erwies sich ein konzentriertes Extrakt aus Enten-Erythrocyten in einer geeigneten Nährlösung, die zusätzlich Apfelsäureester, Brenztraubensäureester, Adenosintriphosphat, Coenzym A, Coenzym I und Folinsäure enthielt. Die im Erythrocytenextrakt enthaltenen Substanzen, welche sich für die extracelluläre Entwicklung von *Pl. lophurae* als fördernd erwiesen, waren nicht dialysierbar; sie wurden erhalten in einer teilweise gereinigten haemoglobinfreien Fraktion des Extraktes.

Die Malariaparasiten beziehen den größten Teil ihres Stickstoffes aus dem Haemoglobin der Wirtsblutkörperchen. Als Hauptenergielieferant dient ihnen Glukose, doch muß diese zumindest mit Apfelsäureester und Brenztraubensäureester ergänzt werden. Die Parasiten benötigen externe Coenzymquellen, die sehr wahrscheinlich unter natürlichen Bedingungen aus der Wirtszelle gewonnen werden. Ob sie außerdem auch Enzyme aus der Wirtszelle brauchen und verwenden, bleibt noch offen.