tion and to "plasma skimming," and the rapid return of hematocrit to normal in the next 24 to 48 hr is thought to be due to a return of these red blood cells to the active circulation.

The two viscosity measurements and the corresponding hematocrit determinations indicated by the arrows in Fig. 1 were high, and the general physical state of the two animals concerned was very poor. These values were therefore not used in the determination of the average hematocrit and viscosity values in the figure.

Gross inspection of the viscera of the hamsters revealed that the hamsters with elevated blood viscosity exhibited (i) much more than the usual degree of cyanosis of the venous blood, (ii) patchy or generalized hyperemia of the viscera, (iii) engorged prominent arteries along the curvature of the stomach and in the omentum, and (iv) a greatly reduced tendency to bleed from the vena cava puncture wound.

The addition of heparin *in vitro* in amounts sufficient to render the blood incoagulable (100 units/2 ml blood) reduced an elevated blood viscosity due to fat feeding to, or near to, normal (Table 1). Also, the injection of heparin intravenously in doses of about 400 units per hamster reduced an elevated blood viscosity after fat feeding and, at the same time, significantly increased the bleeding tendency from the puncture wound in the vena cava. In no instance was the viscosity reduced to normal and in all instances the effect of heparin *in vivo* was transient.

The maximum adhesiveness and aggregation of the red blood cells in hamsters occurs 2 to 4 hr after the peak of the plasma lipemia and lasts for several hours after the lipemia clears (*1–3*). It would appear that the maximum increase in the viscosity also occurs after the peak of the lipemia. The possible mechanism by which high fat meals cause aggregation and adhesiveness of the red blood cells has already been discussed (*1–3*).

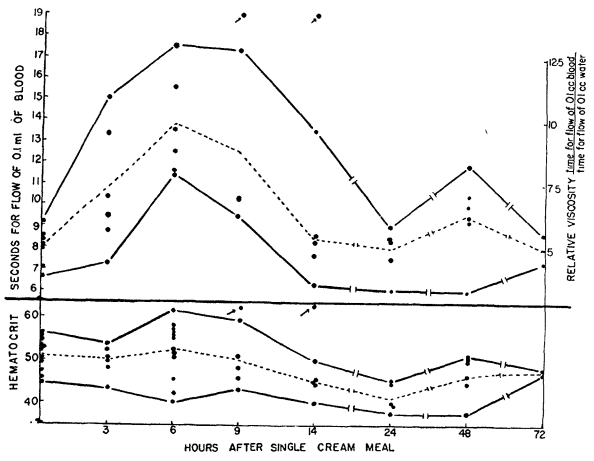

Fig. 1. Changes that occur in the viscosity and hematocrit after a standard fat meal. Each dot for viscosity represents the average of two to three viscosity determinations in the same hamster. Solid lines: maximum and minimum values for viscosity and hematocrit; dashed line: average of all determinations for each time interval.

Table 1. Effect of *in vitro* heparin upon viscosity of the blood. Time in seconds for flow of 0.1 ml of blood.

From inferior vena cava	From syringe containing heparin		
	Immediately after withdrawal	5 min after withdrawal	After additional heparin
15.4	15.0	9.2	
16.0	15.0	9.0	
9.0	9.6	9.0	7.0
10.5	7.7	5.0	

If it is shown in human beings that large fat meals may be followed by aggregation and adhesiveness of the red blood cells with slowing of the circulation and an increase in the relative viscosity of the blood, it will be necessary to consider seriously whether this mechanism is a factor in human disease, particularly in chronic vascular and thrombotic diseases and in multiple sclerosis (*9*).

References and Notes

1. R. L. Swank, *Am. J. Physiol.* **164**, 798 (1951).
2. R. L. Swank and C. Cullen, *Proc. Soc. Exptl. Biol. Med.* **82**, 381 (1953).
3. C. Cullen and R. L. Swank, *Circulation* **9**, 335 (1954).
4. G. P. Fulton, R. G. Jackson, and B. R. Lutz, *Science* **105**, 361 (1947).

5. This study was started at the Montreal Neurological Institute, Montreal, Canada. It was supported jointly by grants from the Multiple Sclerosis Society of Canada and from the Department of Health and Welfare, Ottawa, Canada.

6. R. Fahreus, *Physiol. Revs.* **9**, 241 (1929).
7. R. L. Swank and J. Roth, *Rev. Sci. Instr.*, in press.
8. The high-fat diet was made from purina, whole-wheat flour, butter, and a mixed vitamin supplement.
9. R. L. Swank, *Arch. Neurol. Psychiat.* **69**, 91 (1951).

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New Method for Study of Intracellular Parasites with the Electron Microscope

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Electron microscopic examination of parasites contained within an erythrocyte is difficult, because the red blood corpuscle itself is too thick for the penetration of the 50-kv electron beam. In the electronic image, the infected blood cell appears uniformly dense, within which the parasites cannot be identified. Several methods have been proposed for overcoming this difficulty. The first is to subject unfixed cells to osmotic hemolysis in distilled water (*1, 2*). Another method is to make ultra-thin sections suitable for electron microscopy, either by means of special microtomes (*3*) or by splitting of specimens (*4, 5*).

We wish to report a method (*6*) that is suitable for routine use and is much easier to use than the method of ultra-thin sectioning. This method has been used for the electron microscopic study of malarial parasites contained within the red blood corpuscle.

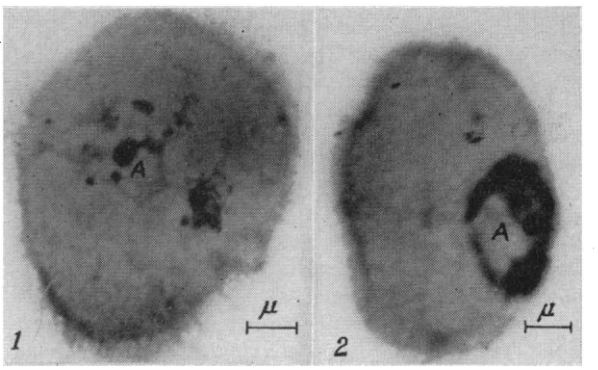
In this method, infected blood is first smeared on collodion-coated glass slides which are fixed subsequently by exposure to the vapor of 2 percent OsO_4 solution for 3 min. The slides, after a careful wash, are hydrolyzed in normal HCl at 60°C for 10 min and then again washed in distilled water. After the slides are thoroughly dry, the collodion film with the hydrolyzed smear of blood is stripped from the glass and mounted on steel mesh for examination under the electron microscope.

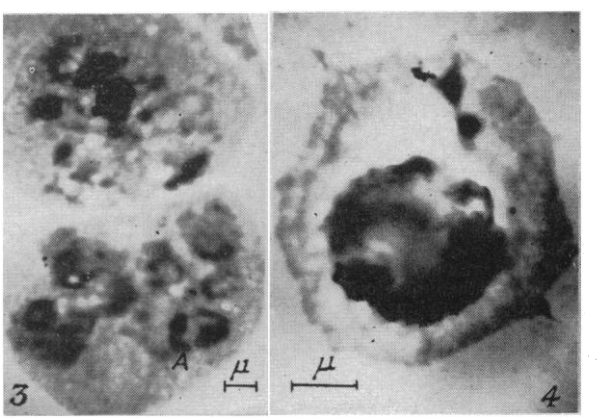
Some of typical electron micrographs of *Plasmodium berghei* obtained by this procedure are reproduced in Figs. 1 to 7. They were made with a 50-kv electron microscope assembled in the Institute of Nuclear Physics, Calcutta (*7*).

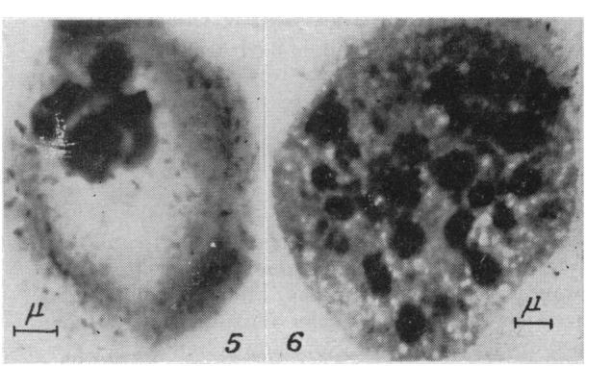
Figure 1 shows an early trophozoite stage with double infection. One of the parasites, *A*, is of ring form with a dense chromatin bead situated on a faint ring of cytoplasm, measuring 1.2 μ in diameter. A faint dot, caused possibly by the fragmentation of the chromatin, is visible at the other end of the incomplete ring. The mass density of chromatin is considerably greater than that of the cytoplasm. A late trophozoite stage is shown in Fig. 2, where a large fleshy form has developed. In many cases at this stage, the infected corpuscles are found to be multiply infected with a few parasites showing double chromatin (Fig. 3*A*).

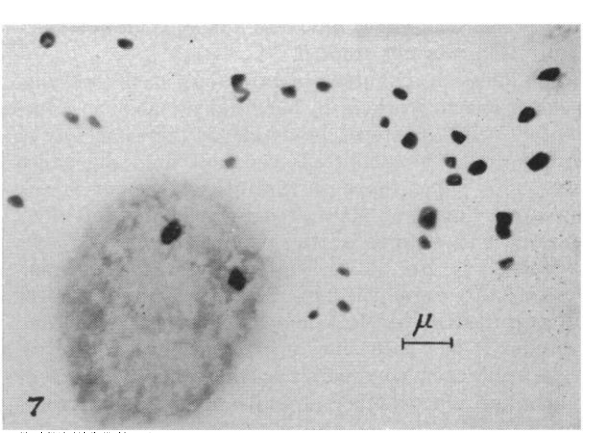
The schizont stages are shown in Figs. 4 to 6. In Fig. 4, the chromatin has begun to divide, but the cytoplasm still envelops it as an irregular covering. Segmentation is more advanced in Fig. 5, where discrete granules scattered throughout the erythrocyte are also visible. Chromatin division is complete in Fig. 6, in which at least 18 discrete and comparatively dense merozoites of varying sizes may be counted. Most of these bodies are ovoid and of average size 1.0 by 0.8 μ. Owing to the associated cytoplasm, a slight variation in density within the bodies of some of the merozoites can be noticed clearly.

At a later stage (Fig. 7), the majority of the merozoites have come out of the host cell, while a few are still inside. Although the size and shape of individual merozoites vary a great deal, the average dimension of the merozoites outside the host cell, is about 0.42 by 0.32 μ, which is smaller than that obtained for the bodies within the fully developed schizont (Fig. 6). On a light print many merozoites show the presence of a distinct vacuole inside. The size of the infected









Figs. 1 and 2. *Plasmodium berghei* in trophozoite stage. Fig. 3. A multiply infected cell. Fig. 4. Early schizont stage. Figs. 5 and 6. Late schizont stage showing progressive segmentation and merozoite formation. Fig. 7. Merozoites scattered around the host cell.

red blood corpuscle is also found to vary with the life-cycle of the parasite. The average size of a noninfected cell is about 5 μ, that of the infected ones is usually 6 to 7 μ. The maximum size is reached in the mature schizont stage (Fig. 6), when the red blood cell measures about 9 to 10 μ.

This brief note describes the results of a preliminary attempt to make malaria parasites visible under the electron microscope while they are still within the erythrocyte. The electron micrographs reproduced here show that the hydrolysis of the fixed cells as described in a foregoing paragraph reduces the masking effect of the cytoplasm to a marked extent. The intracellular parasites are then clearly visible. Such treatment has little effect on the parasites themselves, since mild acid hydrolysis with HCl is a standard cytologic procedure for the study of internal details of cells.

References and Notes

1. C. Wolpers, *Klin. Wochschr.* **21**, 1049 (1942).
2. E. G. Nauck, D. Peters, and R. Wigand, *Z. Naturforsch.* **5b**, 259 (1950).
3. G. B. Chapman and J. Hillier, *J. Bacteriol.* **66**, 362 (1953).
4. A. H. F. Laurell, *Nature* **163**, 282 (1949).
5. R. C. Backus, *J. Appl. Phys.* **24**, 1415 (1953).

6. This research was supported by grants from the Ministry of Education, Government of India. We are indebted to M. N. Saha, director, Institute of Nuclear Physics, and to R. N. Chaudhury, director, School of Tropical Medicine, for their interest in this work.

7. N. N. Das Gupta *et al., Indian J. Phys.* **22**, 497 (1948).

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Effect of Azide and Cyanide on the Respiration of a Species of *Mycobacterium*

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Clifton and coworkers (*1–3*) and Winzler (*4*) have shown that sodium azide acts like dinitrophenol in preventing assimilation of added metabolites by certain microorganisms. Instead, the metabolites are oxidized completely to carbon dioxide and water. According to Clifton (*1*) cyanide inhibits the rate of oxidation, although a slight increase in oxygen uptake may occur. The effect of azide and cyanide on autorespiration was not studied.

The question of autorespiration has been somewhat troublesome to workers in bacterial metabolism. Does the cell utilize its stored foodstuffs in the same way as it does added metabolites? And how does the added metabolite affect the autorespiration? An answer to these questions was attempted by studying the effect of azide and cyanide on the respiration of *M. tuberculosis* ATC. No. 8420. Thoroughly washed suspensions of this organism have a relatively high rate of autorespiration, which is increased by both azide and cyanide. It has been shown (*5*) that the addition of certain nitrogen compounds, such as methylamine or ammonium ion, whether assimilated or not, increases the autorespiration. In order to differentiate this effect from that of the drugs, it was shown that the azide and ammonium ion effects were additive (the assimilation of the latter is completely inhibited by azide) and that azide and cyanide allow for the more complete oxidation of added metabolites, such as acetate, caproate, pyruvate, and trehalose, whereas the ammonium ion does not. This increase in the oxidation of added metabolites occurs over and above that caused by the drugs on autorespiration. It therefore appears that these so-called "resting cells" are continuously breaking down, oxidizing, and resynthesizing their stored foodstuff and that these processes are not materially affected by the addition of metabolites.

The organism was grown in 20 ml of Long's synthetic medium for 4 to 6 days. The cells were harvested, and the masses were thoroughly broken up and washed with water by two centrifugations in Hopkins tubes. Seven-tenths milliliter of packed cells was suspended in 7.0 ml of 0.05*M* Na-K-phosphate buffer *p*H 6.0, and 0.5 ml of the suspension was used in each Warburg vessel which had a final fluid volume of 2.0 ml. The effect of azide and cyanide was greater at *p*H 6.0 than at 7.8.

Cyanide concentrations are difficult to keep constant, and it is possible to state only that amounts of KCN varying from 6.0 to 12.0 μg/ml increased the autorespiration on the average 10 percent, and 25 to 37 μg/ml increased it 20 percent after a slight initial depression that lasted 20 to 40 min. Larger amounts depressed for a much longer period of time. Azide was more effective. It increased the autorespiration 25 to 100 percent when 0.1 to 0.5 mg/ml were added, and

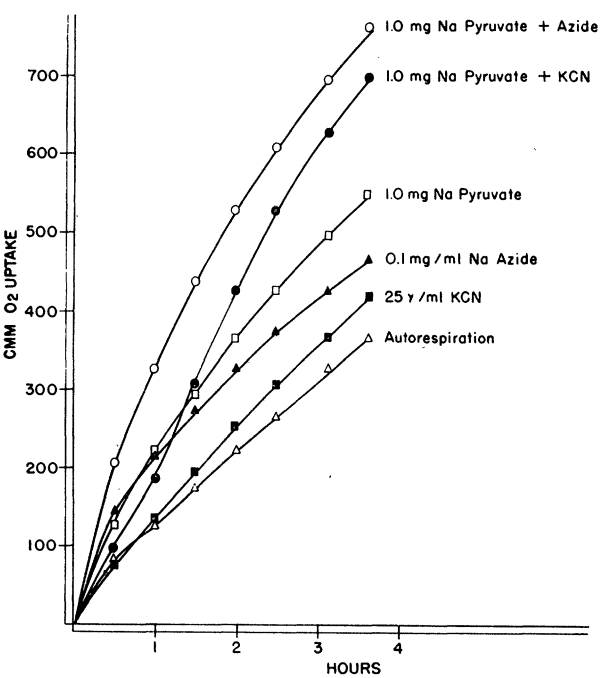

Fig. 1. Effect of cyanide and azide on the autorespiration and on the oxidation of pyruvate.