Barrenscheen and Lang (*1*) found a specific ATP-ase in guinea pig and rabbit liver with pH optima of 8.2 and 9, while Satoh (*7*) reported that ATP was dephosphorylated by the combined action of phosphomonoesterase and pyrophosphatase. DuBois and Potter (*2*) report an optimum of pH 9 for rat liver ATP-ase. Studies of two specimens of pooled rat serum have failed to reveal an acid optimum for ATP-ase activity. It is possible that such species differences as have been found for prostatic phosphatase (*5*) exist with regard to "acid" ATP-ase.

References

1. Barrenscheen, H. K., and Lang, S. *Biochem. Z.*, 1932, **253**, 395.
2. DuBois, K. P., and Potter, V. R. *J. biol. Chem.*, 1943, **150**, 185.
3. Englehardt, V. A., *et al. C. R. Acad. Sci. U. R. S. S.*, 1941, **30**, 644.
4. Fiske, C. H., and Subbarow, Y. *J. biol. Chem.*, 1925, **66**, 375.
5. Gutman, A. B., *et al. J. Amer. med. Ass.*, 1942, **120**, 1112.
6. Perlman, G. E., and Ferry, R. M. *J. biol. Chem.*, 1942, **142**, 923.
7. Satoh, T. *J. Biochem.* (Japan), 1935, **21**, 19.
8. Singher, H. O., and Meister, A. *J. biol. Chem.*, 1945, **159**, 491.

The Mode of Action of 7-Methyl Folic Acid

Gustav J. Martin, Leo Tolman, and Jack Moss

Research Laboratories, National Drug Company, Philadelphia, Pennsylvania

The primary action of sulfonamides (*1*) has been found to be directed against the incorporation of p-aminobenzoic acid into pteroylglutamic acid. 7-Methyl folic acid has been reported (*2*) as an effective folic acid displacing agent. The 7-methyl folic acid used in these experiments is N-(4-(((2-amino-4-hydroxy-7 - methyl - 6 - pteridyl) - methyl) - amino) benzoyl) - 1(+) - glutamic acid. To determine the mode of action of folic acid displacers, attempts were made to counteract the growth-inhibiting action of sulfonamides and folic acid displacers by various agents. *Staphylococcus aureus* # 209 grown in a bouillon medium was used as the test organism. Table 1 gives the results obtained.

TABLE 1

Counteraction of 7-Methyl Folic Acid and Sulfathiazole

Compound	p-Aminobenzoic acid	Pteroylglutamic acid	Pteroic acid	Glutamic acid	p-Aminobenzoyl 1(+) glutamic	Sulfathiazole
7-Methyl folic acid...	+	+	+	−	−	+
Sulfathiazole.....	+	−	+	−	−	−

+ = counteracts
− = no effect

The concentrations per 10 ml. of chemicals used were: 7-methyl folic acid, 1–10 mg.; sulfathiazole, 0.05–1.0 mg.; p-aminobenzoic acid, 1–5 mg.; pteroylglutamic acid, 1–5 mg.; pteroic acid, 1–10 mg.; glutamic acid, 1–5 mg.; and p-aminobenzoyl-1(+)-glutamic acid, 1–5 mg.

When *Staph. aureus* is the test organism, it seems that the action of the sulfonamide is to prevent the incorporation of p-aminobenzoic acid into pteroic acid. Pteroylglutamic acid does not counteract the sulfonamide, which indicates that pteroic acid and not pteroylglutamic acid is involved in *Staphylococcus* metabolism. The fact that p-aminobenzoylglutamic acid does not counteract the sulfonamide would be anticipated if pteroic acid is the vital factor in growth. Pteroic acid is more effective than p-aminobenzoic acid in counteracting sulfathiazole.

With the same test organism, methyl folic acid is counteracted by pteroylglutamic acid, pteroic acid, and p-aminobenzoic acid but not by p-aminobenzoyl-1(+)-glutamic acid, indicating the synthesis of pteroic acid as the first step in the formation of pteroylglutamic acid. Pteroic acid, p-aminobenzoic acid, and pteroylglutamic acid are equally effective on a molar basis in counteracting methyl folic acid. It seems probable that methyl folic acid interferes with the synthesis of pteroylglutamic acid at the stage of pteroic acid formation and at the stage of union with glutamic acid. It has the further capacity to displace formed pteroylglutamic acid. Thus, methyl folic acid represents another displacement compound incorporating into one molecule the capacity to inhibit the synthesis of and displace a given metabolite. As with a sulfonamide, methyl folic acid interferes with the incorporation of p-aminobenzoic acid into pteroylglutamic acid. The counteraction of methyl folic acid by sulfathiazole indicates similarity of structure resulting in mutual interference superseding that of interference with the metabolism of pteroylglutamic acid.

References

1. Lampen, J. O., and Jones, M. J. *J. biol. Chem.*, 1946, **166**, 435.
2. Martin, G. J., Tolman, Leo, and Moss, Jack. *Arch. Biochem.*, 1947, **12**, 318.

Effect of Age of Infection Upon the Oxidative Metabolism of *Trypanosoma lewisi*[1]

James W. Moulder[2]

Departments of Bacteriology and Parasitology and *Biochemistry, University of Chicago*

When the nonlethal protozoan parasite, *Trypanosoma lewisi*, is inoculated into its natural host, the rat, reproduction of the trypanosomes occurs only during the first few days of the infection. Thereafter, reproduction is inhibited, and the population consists entirely of adult, nonreproducing organisms (*2*). Taliaferro and his associates (*5*) have shown that cessation of parasite reproduction is caused by an antibody which specifically inhibits the reproduction of the trypanosomes, and in 1932 Taliaferro termed this antibody ablastin.

In an investigation of the mechanism of action of ablastin upon *T. lewisi*, the oxidative metabolism of trypanosomes taken from rats at different ages of infection has been compared. The study was limited to infections 2-10 days of age in which no number crisis had been brought about by the appearance of trypanolysins (*1*). Reproduction of the trypanosomes ceased on the fourth or fifth day of infection. Table 1

[1] Supported by grants from the Dr. Wallace C. and Clara A. Abbott Memorial Fund and the John Rockefeller McCormick Memorial Fund of the University of Chicago.

[2] Mr. and Mrs. Frank G. Logan Research Fellow.

shows that, compared with young reproducing organisms, the adult nonreproducing trypanosomes respiring on glucose have a higher rate of oxygen uptake, a higher respiratory quotient, and an increased sensitivity to malonate inhibition of oxygen uptake. These differences are all statistically significant. The increased rate of oxygen consumption per 10^9 organisms may be due to the fact that the mean size of trypanosomes in young infections is less than that in old ones (*4*),

TABLE 1

Day of infection	Oxygen uptake		Respiratory quotient		Inhibition of oxygen uptake by 0.01 M Na malonate	
	No. observations	Mean oxygen uptake (micromoles/10^9 organisms)	No. observations	Mean R.Q.	No. observations	Mean inhibition (%)
2	4	46	3	0.76	3	13
3	7	49	2	0.75	2	10
4	6	42	1	0.95	1	15
5	5	63	1	0.92	1	23
6	3	54	1	0.75	1	22
7	1	79	1	0.99	1	11
8	5	63	1	0.94	1	18
9	2	65	1	0.91	1	28
10	3	56	2	0.98	2	27
Arrangement of four-fold tables	Under 5 days—5 days and over Under 55 micromoles—55 micromoles and over		Under 4 days—4 days and over Under 0.85—0.85 and over		Under 5 days—5 days and over Under 18 per cent—18 per cent and over	
P	0.015		0.012		0.011	

Each Warburg vessel contained 0.75–1.50 × 10^8 trypanosomes in 1.0 cc. Ca-free phosphate saline (pH 7.6, 0.130 M total cation concentration) containing 0.01 M glucose and 2.0 per cent bovine serum albumin. Oxygen uptake and R.Q. were determined by the direct method of Warburg. Observations were started after equilibration for 15 minutes in an atmosphere of air at 37.5° and were continued for a period of 120 minutes. To test the statistical significance of the differences between young and adult trypansomes, χ^2 and P were calculated from four-fold tables, constructed as shown above after application of the Yates correction for continuity.

but the changes in respiratory quotient and malonate sensitivity are independent of variations in the mean size of trypanosomes. The malonate inhibition of respiration is interesting, because it is believed that succinate is not oxidized by *T. lewisi* (*3*). Added fumarate does not relieve the malonate inhibition.

The possible relation of the changes in oxidative metabolism to the inhibition of the reproduction of the parasites by the specific antibody, ablastin, is now being investigated.

References

1. Coventry, F. A. *Amer. J. Hyg.*, 1930, **12**, 366.
2. Rabinowitsch, L., and Kempner, W. *Z. Hyg. Infektionskr.*, 1899, **30**, 251.
3. Reiner, L., Smythe, C. V., and Pedlow, J. T. *J. biol. Chem.*, 1936, **113**, 75; Evans, E. A., Jr. Personal communication.
4. Taliaferro, W. H. *J. exp. Zool.*, 1925, **37**, 127.
5. Taliaferro, W. H., and Taliaferro, L. G. *Amer. J. Hyg.*, 1922, **2**, 264; Taliaferro, W. H. *J. exp. Med.*, 1924, **39**, 171; Coventry, F. A. *Amer. J. Hyg.*, 1925, **5**, 127; Taliaferro, W. H. *Amer. J. Hyg.*, 1932, **16**, 32.

Fluorescence of Red Blood Cells

D. H. Hamly

Department of Botany, University of Toronto

George J. Hummer[1] and Charles Sheard

Division of Physics and Biophysical Research, Mayo Foundation, Rochester, Minnesota

Keller and Seggel and also Seggel (*3*) reported on the presence of red fluorescing reticulocytes in the blood of man and of animals. The significance of these fluorocytes in relation to the problems of anemia aroused the interest of many investigators, and by 1940 there had appeared an extensive series of German and French contributions on this subject. The articles by Seggel, Ungricht, Chytrek, and Watson, Grinstein, and Hawkinson (*4*) are of particular interest because of their views on the relationship between the number of fluorocytes and other clinical findings.

After the appearance of certain peculiar cases of anemia at the Mayo Clinic it was thought that Seggel's method of fluorescent examination might be of assistance. Accordingly, there was set up a fluorescence microscopic ensemble which permitted the satisfactory examination of fluorescing coproporphyrin mounted on a quartz slide. When, however, no fluorocytes were observed in the blood specimens of clinically selected cases, in newly-born guinea pigs, or in rabbits poisoned with phenylhydrazine, it seemed possible that this was due to inadequacy or insufficiency in one or both of the following: (1) absolute brightness of the fluorescence microscopic image, and (2) image contrast, brightness level, and the resolving power of the eye under very low illumination. Although one of us had considered similar problems with the ordinary light microscope (*1*), it was thought that these problems in the case of the fluorescence microscope were peculiar and required careful experimental analysis. An analysis of the factors involved in fluorescence microscopy is reported in another communication (*2*).

During the course of our investigations, in which we were able to increase the intensity of the ultraviolet illumination through the selection of better optical components, sources of radiation, and instrumental arrangement, various examinations were made of blood samples clinically selected as being anemic. Each sample of fresh blood was examined after dilution with physiologically isotonic saline solution. Sufficient dilution was required to permit the cells to lie flat. No other method of dilution was used, since Seggel had found that any kind of isotonic saline solution could be used if sublimate was absent.

Examinations of the fluorescence of samples of blood from a newborn guinea pig, from a rabbit poisoned with phenylhydrazine, and from a patient suffering from pernicious anemia gave negative results until an adequate intensity of illumination was obtained. Under favorable conditions, brightly fluorescing reticulocytes were observed. In the case of samples of blood drawn from a rabbit to which phenylhydrazine had been administered on three separate occasions during the course of four days, it was estimated that the fluorocytes were about 1 per cent of the erythrocytes observed. High intensity of illumination, accurate focusing, and proper dark adaptation of the eye must be obtained and maintained in order that fluorocytes of low order of fluorescence may be observed.

[1] Formerly a Fellow in Surgery, Mayo Foundation. Present address: Beverly Hills, California.