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.

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Fluorescence of Red Blood Cells

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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.

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As already reported by Seggel, fluorescence of blood cells is an evanescent phenomenon, and its duration is related to the original brightness. In the case of the longest period of fluorescence of a fluorocyte observed by us, it was found that 120 seconds elapsed before the fluorocyte was too faint to be seen. In contrast, about the shortest time involved in a low-order fluorescence was only 4 seconds. While these data are considerably more extreme or of a wider time range than those reported by Seggel in the case of a rabbit to which phenylhydrazine had been administered, it cannot be concluded that, in our experiments, more coproporphyrin was present in the fluorocytes. It is probable that the conditions of observation were different in two particulars from those under which Seggel worked: (1) intensity of illumination and (2) level of dark adaptation.

Samples of blood from a patient having pernicious anemia were examined under optimal conditions and with the benefit of previous experiences in this type of investigation. Although fluorescence was observed, it was of a yellowish-green hue similar to that of willemite and not the red fluorescence characteristic of coproporphyrin. Although the significance of this yellowish-green fluorescence is not known to us, the presence of a brightly fluorescing yellowish-green pigment in a reticulocyte undoubtedly indicates the presence of some other modification of the normal hemoglobin of red blood cells.

The number of fluorocytes per unit volume of blood cannot be correlated with the amount of fluorescing pigment in the blood. This statement is deduced from the probability that the total time of visibility for fluorescence per unit area under constant intensity of illumination is roughly proportional to the fluorescing pigment present. This being the case, if the population is one of fluorocytes of uniform size, the total statistical time of fluorescence should be proportional to the amount of pigment per unit volume. These considerations would explain the lack of correlation between the fluorocyte count and other clinical findings in the evidence submitted by Seggel and others and would offer a basis for the criticism of Seggel's work by Chytrek. These considerations are especially pertinent, since in no case is it possible to determine the correct total time of fluorescence. The number of reticulocytes carrying the reddish fluorescent pigment must be in excess of those observed, since some cells will contain so little of this pigment that fluorescence will be below the level of visibility or will last for too short a period to permit recognition. In general, however, the number of fluorocytes which can be observed may be taken to indicate the severity or the state of the disease.

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IN THE LABORATORY

Improved and Improvised Sterilizing Containers for Bacteriological Petri Dishes and Pipettes

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In spite of the close of the war it is still difficult to obtain such simple and relatively inexpensive items as sterilizing containers for bacteriological Petri dishes and pipettes, and some of those available have a common defect which this writer has observed for several years and now wishes to record so that manufacturers may correct it if they wish to do so.

For example, one type of round Petri dish container, 10 inches tall, has a lid 2½ inches high which slides over 1½ inches of the container. The lid fits so closely that its removal is often extremely difficult. A lid ½ inch high would be just as effective and would save much effort in handling. There is on the market an aluminum container, without a rack, with a shallow lid for four dishes, which is quite ideal. It is cheap, durable, and altogether satisfactory.

Some commercial containers for pipettes are open to the same kind of criticism as that just mentioned for Petri dishes. One sample has a cover 6 inches long, of which 2 inches fit over the container. Both container and cover are finely made of copper, are seamless, and are almost as closely fitted as a piston in a cylinder. It generally requires considerable effort to close or open one of these, and the upper third of the pipettes is exposed to aerial contamination when the lid is off, if they do not actually fall out.

Another container, made of stainless steel, not seamless, is 17 inches long, with a lid 1½ inches long and a sliding area of 1 inch. This is much easier to handle and exposes only ½ inch of the pipettes to the air. A loosely fitting lid ½ inch long with a short screw would be even better.

The writer has often used as containers for pipettes the discarded cartons in which adhesive tape or surgical plasters have been purchased. Some of these have shallow screw tops, and they will stand repeated sterilization in the dry air sterilizer very well, but not in the autoclave.

They are particularly useful for sterilizing Pasteur pipettes, but are too short for standard commercial pipettes. This difficulty has been met by lengthening them with an additional section fastened with Scotch tape, which also withstands repeated dry air sterilization remarkably well.

Coffee cans make very satisfactory substitutes for the commercial containers for Petri dishes, but they should first be boiled in a strong solution of lye to remove the lacquer.