ous spheres agree well with each other and are equal to the value of concentration of the solution before it is suspended, as shown in Table 1, provided the size of the illuminated area is smaller than one third the diameter of the sphere. The result offers proof of the correctness of our theory.

TABLE 1

Diam of sphere (μ)*	Transmittance (%)	Calc conc (*M*)†
2.2	95.4	0.009
3.0	86.6	.020
5.2	71.6	.028
6.0	65.7	.030
7.5	56.6	.031
9.7	52.2	.029
13.5	38.9	.031
22.4	21.1	0.030

* The diameter of the reduced image of the light source formed at the center of a sphere, 2 μ.

† Concentration of the solution before suspension, 0.030 *M*.

The circumstances are not so favorable in the case of nuclei of living cells, as their forms are not always spherical, nor are their contents always uniform. Nevertheless, it can be expected that our method would give better results than those hitherto reported if the specimens be suitably treated so that the nuclei approximate spherical forms.

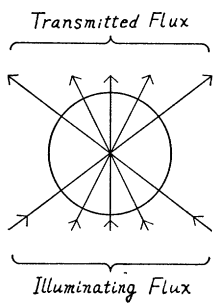

FIG. 2.

An experiment has been performed with this objective in mind. Nuclei from the liver cells of 4 rats were separated by citric acid, as described by Mirsky (*8*), and washed thoroughly with 30% sucrose solution. The nuclei were thus made approximately homogeneous and were then fixed in formalin and stained by the Feulgen reaction. Their DNA content was determined indirectly by measuring the total amount of the regenerated fuchsin—that is, by the measurement of spectral transmittance of each nucleus using our optical system. The result is shown in Fig. 3, in which the amount of DNA in arbitrary units is taken as the abscissa, and the numbers of nuclei containing DNA in the amount of 8.0–8.5, 8.5–9.0, 9.0–9.5 etc., in our unit are plotted as the ordinate.

It can be concluded from Fig. 3 that nuclei of the liver cells of rats are classifiable into at least three groups definitely distinguished from one another by the amount of DNA. The amount for each group shows an arithmetical progression, whereas the

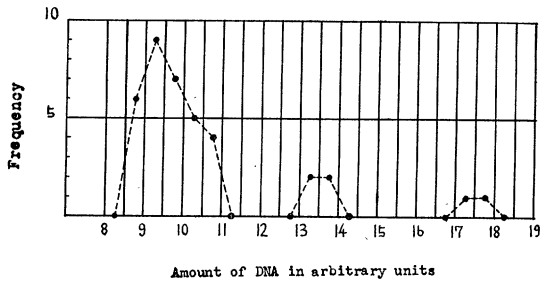

FIG. 3.

amounts found by Swift (*3*), and Lison and Pasteels (*5*) showed a geometrical progression. The relative error of our measurements is 2–3% (number of measurements: 20–32), which is to be compared with the error of measurements by Swift (*3*) and Lison and Pasteels (*5*)—i.e. 7–24% (number of measurements: 36–97). The improvement in accuracy may be attributed to our revised optical system in microspectrophotometry.

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A Medium for the Study of the Bacterial Oxidation of Ferrous Iron[1]

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The study of the bacterial oxidation of ferrous iron in acid mine waters has long been retarded for the want of a suitable synthetic medium.

Prior to the formulation of such an inorganic medium, acid mine waters were used as natural media for the cultivation of the autotrophic bacteria reported by Colmer and Hinkle (*1*), in 1947, and by Leathen and Madison (*2*), in 1949, to be responsible for the rapid oxidation of ferrous iron to the ferric state under acid conditions. Such "natural" media were prepared for use by sterilization, either by autoclaving or by filtration, dependent upon the chemical characteristics of the particular mine effluent.

There were many objections to using such media. The most outstanding were variability of chemical constituents and pH, difficulties of collection and transportation, and sterilization. The medium described here eliminates all these objections and has

[1] This contribution is one of a series of papers by the Multiple Fellowship on Mine Acid Control sustained at Mellon Institute by the Sanitary Water Board, Department of Health, Commonwealth of Pennsylvania.

many distinct advantages, such as uniformity of inorganic constituents, stability during sterilization, excellent keeping qualities, and easily observable growth. To a degree, it is a differential medium. Above all, the medium can be altered to facilitate biochemical and physiological studies of the microorganism. Such studies are now in progress.

The basic medium, the composition of which is as follows, compares favorably with the major chemical composition of acid mine effluents:

Ammonium sulfate [$(NH_4)_2SO_4$]	0.15 g
Potassium chloride [KCl]	.05 "
Magnesium sulfate [$MgSO_4 \cdot 7H_2O$]	.50 "
Dipotassium phosphate [K_2HPO_4]	.05 "
Calcium nitrate [$Ca(NO_3)_2$]	0.01 "
Distilled water	1,000 ml

Sterilization is accomplished by autoclaving for 15 min at 15 psi.

A stock solution of ferrous iron is prepared as follows:

Ferrous sulfate [$FeSO_4 \cdot 7H_2O$]	10 g
Distilled water	100 ml

This solution is sterilized by filtration, using either Berkefeld or Fisher-Jenkins filters. If refrigerated, the solution will remain sterile and without appreciable oxidation for several weeks.

After the autoclaved basic medium cools, 10 ml of the 10% ferrous sulfate solution, per liter of base, is added aseptically. Most often, 100 ml aliquots of the base are placed in 250-ml Erlenmeyer flasks and sterilized. Then 1.0 ml of the ferrous sulfate solution is added aseptically to each flask after cooling. The resultant medium is opalescent and has a pH of about 3.50. Appreciable oxidation does not occur as long as the medium remains sterile.

This medium has been used for over two years in our study of the rapid bacterial oxidation of ferrous iron to the ferric state in acid mine water. Stock cultures of the iron-oxidizing bacteria have been maintained in the medium, without change, throughout the same period.

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Lactic Dehydrogenase and DPN-ase Activity of Blood[1]

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In the course of our studies on the enzymes of the erythrocyte we found that by simply removing the stroma material from the hemolysate from washed red cells the lactic dehydrogenase activity may be increased to about five times that of the stroma-containing hemolysate. This increase in activity was observed both in aerobic experiments with methylene blue, and in anaerobic experiments with ferricyanide, using the method of Quastel and Wheatley (*1*).

These findings have suggested a means of establishing the distribution of the enzymes lactic dehydrogenase and DPN-ase in the blood.

Preparation of stroma-free hemolysate. The red cells are washed four times with a saline-bicarbonate solution and then hemolyzed by freezing and thawing three times. After centrifuging the hemolyzed sample for 20 min at 2,500 rpm (radius of rotor, 13 cm), the sparkling clear supernatant is separated from the precipitated stroma. Dialysis is not necessary if additional nicotinamide is not present during the process of hemolysis. Rabbit blood was used in all the experiments reported here, but the general results have been duplicated with human blood.

Estimation of the dehydrogenase and DPN-ase activity. Into the side bulb of the Warburg reaction flask is introduced 0.2 ml ferricyanide-bicarbonate reagent (*1*). In the main compartment of the flask the following reagents are placed:

1) Sodium cyanide, previously neutralized with HCl, to give a final concentration of 0.05 *M* of CN^- in the reaction medium (*2*).
2) Sodium DL-lactate, to give a final concentration of 0.13 *M* in the reaction mixture.

Other substances may be added as required. In the experiments to be reported here the additional materials indicated in Table 1 were dissolved in the saline-bicarbonate solution. When DPN was added, its final concentration in the reaction medium was 0.865×10^{-4} *M* (determined spectrophotometrically [*3*]). Nicotinamide was added in certain experiments to protect DPN (*4, 5*). The quantity of the liquid in the flask finally was made up to a volume of 2.6 ml with the $NaCl$-$NaHCO_3$ mixture. The final concentrations of NaCl and $NaHCO_3$ were 0.127 *M* and 0.025 *M*, respectively. Each experimental run included a control vessel from which lactate was omitted. The reaction medium in the flasks was equilibrated at 37° C with a gas mixture containing 95% nitrogen and 5% CO_2. The experiments were run at 37° C and with agitation of the flasks at a rate of 108 oscillations/min. The gas output during the first 5 min was disregarded.

The Q values were calculated as follows:

$$Q^{N_2+CO_2}_{\text{lactate}} = \frac{\mu l\ CO_2 \text{ evolved in experimental flask} - \mu l\ CO_2 \text{ in corresponding control flask}}{\text{mg dry-cell residue}}$$

The conditions of the various experiments and the results are indicated in Table 1.

On testing the DPN-ase activity of the fresh stroma-free hemolysate, after the method of Mann and Quastel (*4*) and with added DPN (Expts 4*e* and 4*f*, Table 1), a relatively enormous evolution of CO_2 was obtained whether or not nicotinamide was added (*6*). The volume of gas evolved was many times that obtained by other workers with hemolysates in the presence of

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