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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TABLE 1

LACTIC DEHYDROGENASE AND DPN-ASE ACTIVITY IN THE COMPONENTS OF THE BLOOD

Expt No.	Preparation (0.2 ml/flask)	DPN ($0.865 \times 10^{-4} M$)	Nicotinamide (M)	$Q_{lactate}^{N_2+CO_2}$ (μl CO_2 evolved/mg dry-cell residue/hr)
1*a*	Whole blood (fresh)	DPN added	0.01	1.25
2*a*	Washed red blood cells	" "	0.01	2.60
b	Washed red blood cells	Without DPN	0.01	2.60
3*a*	Serum	DPN added	0.01	5.20
b	"	" "	Omitted	5.30
c	"	Without DPN	0.01	2.70
d	"	Without DPN	Omitted	3.00
4*a*	Hemolysate	DPN added	0.02	21.00
b	"	" "	Omitted	5.40
c	"	Without DPN	0.02	4.05
d	"	Without DPN	Omitted	3.29
e	Hemolysate freed from stroma	DPN added	0.02	20.20
f	Hemolysate freed from stroma	" "	Omitted	21.20
g	Hemolysate freed from stroma	Without DPN	0.02	2.14
h	Hemolysate freed from stroma	Without DPN	Omitted	1.95
5*a*	Hemolysate	DPN added	0.02	20.00
b	"	" "	0.01	18.20
c	"	" "	0.05	13.80
d	"	" "	Omitted	5.50

stroma and the absence of nicotinamide. Thus, a recalculation of some of the data in the literature for the dehydrogenase activity of hemolysates (in the absence of nicotinamide), on the weight basis, gives activity values, Q, not greater than 9 μl CO_2/mg dry-cell residue/hr. The majority of values are between 2.5 and 4 μl (*1*). The stroma-free hemolysate, therefore, is practically devoid of DPN-ase. After further study, it was found that rabbit serum itself possesses a marked lactic dehydrogenase activity (Expts 3*a–d*). Every precaution was taken to obtain clear serum free from hemolysate. At the time the authors were not aware that Warburg and Christian (*7*) had already demonstrated the presence of lactic apo-dehydrogenase in the serum of the rat. It was evident, also, from Expts 3*a* and 3*b*, with and without added nicotinamide, that serum has practically no DPN-ase activity.

The dehydrogenase activity of the stroma-containing hemolysates, on the other hand, compared with that of the stroma-free preparation, was found to be very low (Expt 4*b*), but the activity could be restored in such specimens by the simple addition of nicotinamide to give a final concentration of 1 to 2×10^{-2} M (Expt 4*a*). It is apparent from Expts 4*a* and 4*c* that the nicotinamide acts by inhibiting the DPN-ase, and not by participating in the main reaction. That the inhibition is of the competitive type is indicated further in Expts 5*a–d*, in which the amount of nicotinamide was varied. Thus, nicotinamide, in 1×10^{-2} M concentration, produced an almost complete inhibition of the DPN-ase.

It was of interest to investigate also the permeability of the red cells to DPN. As may be observed in Table 1 from the results of Expts 2*a* and 2*b*, with washed red cells, the dehydrogenase activity was not increased by the addition of DPN to the medium. To test whether the failure to activate the dehydrogenase was due to impermeability of the cell membrane to DPN, or to destruction of the coenzyme by contact with, or during passage through, the membrane, another series of experiments was devised as follows:

The lactic dehydrogenase activity of the stroma-free hemolysate (with added DPN, 0.865×10^{-4} M) was measured, (*a*) in the hemolysate alone, (*b*) in the presence of washed red cells, (*c*) in the presence of stroma, and, in each case, with or without the addition of nicotinamide.

Typical results of such experiments are given in Table 2.

Again, the volume of CO_2 evolved by the stroma-free hemolysate (+DPN) alone was very large, amounting to 27 μl/mg dry residue/hr (Expt 6*a*). The addition of nicotinamide caused a slight decrease in the activity of the DPN-ase–free preparation (Expt 6*b*) (*8, 9*). On addition of washed red cells, however, the Q-value was six times greater in the presence of nicotinamide than in its absence (Expts 6*c* and 6*d*). To test whether any hemolysis was likely to occur during the experimental period, a control sample was included in the run, containing the same number of red cells, but no added hemolysate (Expt 6*g*). The same control served also to establish that the formation of pyruvate from lactate by the added red cells during the experiment was negligible ($Q + 0.05$).

The results in the experiments with cell stroma were as anticipated, namely, that the CO_2 production was greatly increased (by more than ten times) in the presence of nicotinamide (Expts 6*e* and 6*f*). It is evident from Expts 6*c* and 6*d* that the DPN is destroyed when in contact with the red cells. In other words, the DPN-ase of the red cell is distributed on the cell surface. Any DPN that may be liberated from the interior on breakdown of the cell likewise is rapidly destroyed on contact with the stroma.

It is probable that DPN-ase is present on the surface of the cells in all tissues, since it is common knowledge that the breakdown of cells by grinding or mincing the tissue leads to complete destruction of

TABLE 2

EVIDENCE FOR THE PRESENCE OF DPN-ASE ON THE SURFACE OF THE RED CELL

Expt No.	Preparation (0.2 ml/flask)	Added materials	Nicotinamide (M)	$Q^{N_2+CO_2}_{lactate}$
6*a*	Stroma-free hemolysate + DPN $0.865 \times 10^{-4}M$	—	—	27.20
b	Stroma-free hemolysate + DPN $0.865 \times 10^{-4}M$	—	0.03	26.00
c	Stroma-free hemolysate + DPN $0.865 \times 10^{-4}M$	Red cell suspension,* 0.05 ml	—	4.25
d	Stroma-free hemolysate + DPN $0.865 \times 10^{-4}M$	Red cell suspension, 0.05 ml	0.03	24.20
e	Stroma-free hemolysate + DPN $0.865 \times 10^{-4}M$	Unwashed stroma,† 0.05 ml	—	1.71
f	Stroma-free hemolysate + DPN $0.865 \times 10^{-4}M$	Unwashed stroma, 0.05 ml	0.03	19.50
g	DPN alone $0.865 \times 10^{-4}M$	Red cell suspension, 0.05 ml	0.03	0.05

* Corresponding to 2.34 mg dry-cell residue per flask.

† Corresponding to 2.67 mg dry-cell residue per flask. The quantity of stroma used was about 10 times that represented by the quantity of cells in *.

the DPN except when an excess of nicotinamide is present (*4, 10–12*).

The evidence for the presence of DPN-ase on the cell surface is not inconsistent with the recent findings of McIlwain (*13*) that the DPN-ase in minced neural tissue preparations is associated with the cell debris.

One important implication of these observations is that DPN as such, cannot exist in the circulating plasma. Other workers (*14*), and the authors, have demonstrated that the coenzyme is not present in the plasma. Even though, as McIlwain (*15*) has shown, the reduced form of the coenzyme ($DPN \cdot H_2$) is not a substrate for DPN-ase, the existence of $DPN \cdot H_2$ in the plasma also is not possible since, in the presence of the plasma lactic dehydrogenase and pyruvate, it would be oxidized and thus be liable to rapid destruction by the red cells.

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Low-Temperature Sterilization of Organic Tissue by High-Voltage Cathode-Ray Irradiation[1, 2, 3]

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Recently a limited number of human tissue banks have been established to preserve blood vessels (*1*), bone (*2*), and cartilage (*3*), since in this way these substances can be made available for transplantation into humans whenever needed. It has been rather difficult, however, to keep these banks supplied with adequate amounts of sterile material since the tissues may often be contaminated before, during, or after removal from the body, at operation or autopsy, and as a result are not safe for transplantation. It is obvious that if a method could be found for sterilizing human tissue without denaturing it, this would be of great value.

The Surgical Research Laboratory of the Children's Medical Center became interested in this broad problem of organic tissue sterilization while attempting to sterilize blood vessels to insure a more constant supply of sterile vascular grafts for human use. In initial experiments attempts were made to decontaminate blood vessel segments with chemical antiseptics (*4*) and complex antibiotic combinations (*5*), but consistently satisfactory or adequate results were not obtained. In 1948 the Department of Food Technology at the Massachusetts Institute of Technology reported the marked bactericidal action of high-voltage cathode-ray irradiation in the sterilization of food (*6*). With the cooperation of John Trump at that institution, irradiation of intentionally contaminated blood vessel segments was carried out using a compact 3-mev electrostatic generator he designed (*7*), which produces high-voltage cathode rays that can penetrate organic material to a depth of 1.5 cm (*8*).

Initially, 125 blood vessel segments that had been

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