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### ANAEROBIC METABOLISM IN LAKE WASHINGTON SEDIMENTS

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#### ABSTRACT

A method of measuring the dehydrogenase activity of sediments at 10C was calibrated by direct microcalorimetry at the same temperature. Field measurements of dehydrogenase activity of sediments in Lake Washington were converted to rates of metabolic heat release by means of the significant regression of dehydrogenase activity on metabolic heat release. Simultaneous field measurements at 10C of oxygen uptake by undisturbed sediment cores were converted to rates of metabolic heat release by the factor 4.8 cal liberated per milliliter of oxygen consumed.

Dehydrogenase activity varies from place to place and differs between layers of the same core. Although there is a general trend of decreasing activity with sediment depth, some subsurface layers have relatively high activity. Dehydrogenase activity, which was detected down to the deepest layers of the sediment cores, 21 to 31 cm, appears to be more a function of plankton remains than of total organic carbon in the sediment. The integrated metabolic heat release based on dehydrogenase activity of the entire core was invariably greater than the metabolic heat release calculated from the rates of oxygen uptake. Thus, it appears that the rate of total oxygen uptake by the sediment surface underestimates benthic community metabolism in the sediment column.

#### INTRODUCTION

A common measure of benthic community metabolism is the rate of uptake by the sediment surface of dissolved oxygen from the overlying water. The rate of respiratory uptake of oxygen is estimated by determining the difference between the rates of total oxygen consumption and residual oxygen uptake after poisoning the overlying water. If total oxygen uptake by the sediment surface is an integrated measure of metabolism in the sediment column (Teal and Kanwisher 1961), oxygen consumption due to the abiotic oxidation of reduced metal ions, sulfides, and other reduced substances in the sediment should represent anaerobic metabolism taking place in the sediment column (Pamatmat 1971a). Pamatmat has pointed out that, although inorganic reduced substances in the sediment are end products of anaerobic metabolism, we are uncertain about the bioenergetic equivalence between the ongoing rate of anaerobic metabolism and the measured rate of oxygen uptake by abiotic chemical oxidation. In Puget Sound, rates of chemical oxidation are often greater than respiration (Pamatmat 1971a, 1971b), although in sandy bottoms exposed to surf and currents aerobic respiration predominates (Smith 1971, Smith et al. 1972; M. M. Pamatmat, R. S. Jones, H. Sanborn, and A. M. Bhagwat, EPA report in preparation).

Determining how accurately total anaerobic metabolism in the sediment is estimated by the rate of chemical oxidation requires direct measurement of anaerobic metabolism. An imposing difficulty in measuring anaerobic benthic metabolism is the diversity of metabolic types of heterotrophic bacteria,

which could include various fermenters, nitrate reducers, denitrifiers, sulfate reducers, and methane bacteria, in addition to anaerobic macrofauna, meiofauna, and microfauna. Quantitative chemical analysis of metabolic end products would not be practicable.

The measurement of metabolism by direct calorimetry circumvents the complexities of dealing with mixed metabolic types (Forrest et al. 1961). ZoBell et al. (1953) observed that sediment bacteria liberated enough heat to raise the temperature of organically rich sediment. Ordinarily, however, very sensitive and expensive microcalorimeters (Calvet and Prat 1963) would be required to detect the heat output by sediment bacteria. Extremely sensitive microcalorimeters, unfortunately, exhibit base-line instability caused by small fluctuations in ambient temperature; thus, increasing sensitivity becomes self-defeating. For this reason, Doyle (1963) concluded that the calorimeter would not be a practical instrument for ecological studies of sediment bacterial metabolism. Our present experience with a microcalorimeter leads us to the same conclusion as Doyle's. Hence, the development of a chemical method for measuring microbial community metabolism appears to be necessary. The microcalorimeter is indispensable for calibrating such a chemical method, as Doyle has suggested.

A product of intermediary metabolic reactions is ionic hydrogen ( $H^+$ ), which is passed along in a chain of enzyme-catalyzed reactions to an ultimate hydrogen acceptor such as oxygen. The measurement of metabolism in terms of oxygen consumption is a measure of hydrogen produced. In anaerobic metabolism, substances other than oxygen are the hydrogen acceptors. Methylene blue, in the absence of oxygen, is reduced by  $H^+$  to a colorless compound and this reaction was much used by bacteriologists as a qualitative test for anaerobic dehydrogenase activity (Thimann 1963). Since reduced substances in sediments likewise reduce methylene blue (ZoBell 1946), this dye cannot be used to measure anaerobic metabolism in sediments.

Various tetrazolium salts (Ninehan 1955) are superior to methylene blue for quantitative assay of respiratory enzyme activity. Triphenyltetrazolium chloride (TTC), in the absence of oxygen, reacts with hydrogen to form a red-colored compound, triphenylformazan (TPF), whose amount can be measured colorimetrically. Another salt, 2-(*p*-iodophenyl)-3-(*p*-nitrophenyl)-5-phenyl-tetrazolium chloride (INT), can be reduced by  $H^+$  even in the presence of some oxygen to produce a similarly colored formazan compound (Nachlas et al. 1960).

Lenhard (1956) first developed the use of TTC for the quantitative determination of dehydrogenase activity of soil microorganisms. Subsequently, Stevenson (1959), Lenhard et al. (1962, 1965), Bucksteeg (1966), Ford et al. (1966), Edward and Rolley (1965), and Casida et al. (1964) used the method for assaying dehydrogenase activity as a measure of metabolic processes taking place in soils, sediments, and activated sludge. These workers have demonstrated that total dehydrogenase activity as measured by the amount of formazan produced from TTC is an easily measured indication of oxidative capacity in anaerobic systems. The method, unfortunately, gives only a relative measure of metabolic activity. The concentration of formazan produced during incubation of a sample is a function of incubation time, pH,

temperature, kind of substrate added if any, substrate concentration, TTC concentration, plus the population density of microorganisms. Until a standard procedure is calibrated in terms of naturally occurring metabolic rates at different temperatures, the use of TTC will remain of limited value in ecological research, e.g., in the study of energy flow through ecosystems.

We have adapted a method of measuring dehydrogenase activity with the use of TTC and have compared the results with those of direct microcalorimetry. The results show that TTC can be used for estimates of the naturally occurring rates of anaerobic metabolism, at least by bacteria, in subsurface sediments. Whether a similar method can be developed for metazoans remains to be seen.

The method was used aboard ship in Lake Washington at the same time that the oxygen uptake by replicate sediment cores was being measured.

## METHODS

### *Dehydrogenase Activity of Sediments*

We considered the following three methods, all involving the reduction of TTC to formazan. Lenhard et al. (1962) and Bucksteeg (1966) incubated their samples without additional substrate for 19 hr at 30C and for 1 hr at 20C, respectively. Lenhard et al. (1965) incubated samples with glucose for 15 min at 37C. In all experiments the reaction was stopped with absolute ethanol and the absorbance of the centrifugate was measured at 480 or 483 nm, although Lenhard et al. (1962) read the optical density at 546 nm to avoid interference by chlorophylls. Obviously, positive results depend upon the level of metabolic activity of the sample: if it is low one may incubate longer at a higher temperature, with or without additional substrate; if activity is relatively high, no substrate is necessary and a shorter time and lower temperature will suffice. As long as one is interested only in relative activities and the natural population is not drastically affected by the conditions of the experiment, then it appears that the determination of dehydrogenase activity could be performed at conditions different from the source of the samples.

The amount of formazan produced is dependent on the concentration of TTC (Lenhard et al. 1965). As little as 0.01% may be sufficient when the natural metabolic activity of the sample is low, but to ensure a large excess of TTC, up to 1% concentration has been used (Stevenson 1959). If TTC is present in large excess, that rate of formazan production is constant from the initial time of the experiment (Lenhard et al. 1965). Also, when TTC is reduced biologically, the amount of formazan produced is proportional to the hydrogen produced when the incubation period does not exceed 2-3 hrs (Farkas 1966).

Our initial tests were done with Puget Sound samples taken in summer when bottom temperatures were 10-13C. Positive results were obtained when samples were incubated with glucose for 3 hrs at 37C, but no activity was detected when samples were incubated with glucose at 10C. It remains to be seen whether incubation at 37C for 3 hrs will give results that correlate

with the natural activity at much lower temperatures, i.e., whether the enhancement of activity at 37C is in direct proportion to the natural activity.

We believe there is a need for a method to be used at low temperatures, because much higher temperatures could favor only a few species and could be detrimental to psychrophiles. The thermal shock of a sudden temperature change from about 10C to 37C is difficult to assess. We decided on 10C as a reasonably low temperature and found a way to increase the sensitivity of the method at this temperature.

#### *Selection of substrate.*

Besides glucose, we tried citrate, malate, lactate, succinate, and pyruvate. Sodium citrate gave the highest activity during a 3-hr incubation at 10C (Table 1); glucose yielded on the average only 39% of the activity due to citrate. This is in contrast to the findings of Lenhard et al. (1965) that glucose was more effective than the sodium salts of lactic, citric, succinic, and glutamic acids. Evidently there is a question of dehydrogenase specificity, which in Lake Washington sediments differs from that in activated sludge. This could be a critical problem and will be discussed later. We did not test the different substrates with samples from different parts of Lake Washington, but used sodium citrate throughout the study of six stations.

Dehydrogenase activity increases with increasing concentration of sodium citrate, reaching a peak at 0.15 M and declining at a higher concentration (Figure 1). The effect of additional substrate appears to be to enhance or amplify the activity in direct proportion to the natural dehydrogenase activity. Figure 2 shows that at a final concentration of 0.04 M the measured activity is on the average 5.3 times that of replicate samples without any substrate, while at 0.20 M it is 1.4 times the activity at 0.04 M.

Sodium citrate has an unexplained effect on the absorbance of the HgCl<sub>2</sub>-treated blanks (Figure 3). The blanks with 0.04 M citrate have about 43% higher absorbance than replicate blanks with 0.20 M citrate or without substrate, and there appears to be no difference between the two latter treatment replicates.

One might think that the preferable procedure would be to leave out any substrate because it could stimulate the activity of some species and not others; but our results show that the increase in activity is proportional to natural activity without added substrate. We decided to compromise with the use of 3 ml 0.25 M sodium citrate in a final volume of 20 ml for a nominal final concentration of 0.04 M.

#### *Amount of sediment.*

The measured activity increases but the increment of activity decreases with increasing amount of sediment (Figure 4), which may be an indication of nutrient limitation with increasing amount of sediment. Therefore, normalizing the result to unit weight of dry sediment is computationally inadequate. To minimize error, we have tried using as close to 0.3 g dry sediment as possible but the final weights still varied from 0.1 to 0.5 g; this wide range may be a source of variability in the final results. Obviously, the amount of sediment should be limited to a quantity within which the activity

is proportional to quantity. If the problem is caused by nutrient limitation, it could be solved by periodic stirring of the sediment during incubation, provided that this does not oxygenate the sample.

*Exclusion of oxygen.*

The presence of oxygen causes an underestimate of dehydrogenase activity. We have tried guarding against the presence of oxygen during incubation by flushing and sealing the incubation flask with nitrogen. We have not detected a significant difference between nitrogen-flushed and unflushed replicates. Evidently the concentration of reduced substances in the sediment is enough to consume oxygen in solution and keep the settled sediment anoxic during the course of the reaction. The formation of formazan itself is prima facie evidence of anaerobic metabolism; no dehydrogenase activity is detected when the sample is aerated.

*Blanks.*

Lenhard et al. (1965) and others have used as their blanks the reaction mixture minus TTC. We were concerned, however, with the possibility of a chemical reduction of TTC since sometimes we are dealing with highly reduced sediments. Although Casida et al. (1964) have shown that chemical reduction of TTC appears to occur only at much higher temperatures (above 65C), we think that an appropriate blank is one that contains TTC but has been poisoned to kill any dehydrogenase activity. Mercuric chloride has been shown to be the most effective agent in stopping dehydrogenase activity (Lenhard 1965). We obtained the same blanks with HgCl<sub>2</sub>-treated and autoclaved samples.

*Procedure.*

On the basis of the above findings, we have tentatively established the following concentrations of reagents and procedure for the preliminary study of anaerobic metabolism of sediments in Lake Washington: (1) TTC solution--1 g of 2,3-5 triphenyltetrazolium chloride in 100 ml of distilled water; (2) Tris buffer--6.037 g of tris(hydroxymethyl)aminomethane plus 20 ml of 1.0 N HCl in 1 liter of distilled water, pH adjusted to 8.4; (3) sodium citrate solution--74 g of sodium citrate in 1.0 liter of distilled water; (4) saturated mercuric chloride (HgCl<sub>2</sub>) solution; (5) absolute ethyl alcohol.

To each graduated 50-ml Erlenmeyer flask are added 3 ml of Tris buffer, 3 ml of sodium citrate solution, 2 ml of TTC, and 2 ml of sediment. The total volume is made up to 20 ml with distilled water, the flask is swirled a few times, and then allowed to stand undisturbed in the dark for 3 hrs at 10C. Triplicate samples are run. For blanks, duplicate samples are prepared similarly except for the addition of 1 ml saturated HgCl<sub>2</sub>. After 3 hrs the activity is stopped with 20 cc of absolute alcohol, which also extracts the formazan. The mixture is shaken every 15 minutes for 1 hr to complete the formazan extraction. The mixture is centrifuged and the clear supernatant is read at 483 nm in a spectrophotometer. The sediment is dried overnight at 90C and the optical density of each sample is normalized to 1 g of dried sediment. The difference between the untreated and the HgCl<sub>2</sub>-treated samples represents the dehydrogenase activity of the sediment.

Optical density is proportional to the concentration of formazan produced. Lenhard et al. (1965) and other workers have expressed the final result in terms of micromoles of formazan produced. We consider this an unnecessary step, and converted optical density per gram of dry sediment directly into rate of heat output.

#### *Direct Microcalorimetry*

The calorimeter used is a gradient type having 12,000 copper-constantan junctions. It is buried in sand in a stainless steel vacuum Dewar vessel; the sand serves as the heat sink. The temperature control system consists of a closed 0.11-m<sup>3</sup> double-walled tank (which surrounds the vacuum Dewar vessel with an airspace between the walls), connected to a 0.19-m<sup>3</sup>, floor-model, externally circulating temperature bath with a temperature constancy of  $\pm 0.02\text{C}$ . During the present experiments the water bath was set at 10C. The calorimeter, electrically calibrated by means of a resistance heater, has a sensitivity of 1 microvolt for a steady heat flow of 0.62  $\mu\text{cal/sec}$ . A signal of 1 microvolt represents a temperature difference between the inner and outer walls of the calorimeter of  $5 \times 10^{-6}$  degree Celsius. The details of this method will be described in a separate paper (Pamatmat, MS in preparation).

The sediment sample is packed inside a 60-ml, screw-capped culture tube, and placed in the water bath. After temperature equilibration, the sample is quickly transferred to the calorimeter. After the calorimeter restabilizes (at least 12 hrs) the sample is removed. The empty calorimeter restabilizes much more rapidly (4 hrs) and the decrease in thermal emf following removal of the sample represents the rate of metabolic heat release. The sample is assayed for dehydrogenase activity immediately or within a few hours. The sediment sample in each tube weighed 9-15 g when dried.

#### *Microcalorimetric Calibration of Dehydrogenase Activity*

The significant regression of dehydrogenase activity on the actual rate of metabolic heat release (Figure 5) signifies a fairly high degree of functional relationship between the two measures of community metabolism. The sediment samples from Lake Washington had metabolic rates of 3-17  $\text{mcal g}^{-1}\text{hr}^{-1}$ , corresponding with relative dehydrogenase activities giving absorbances of 0.27-0.86  $\text{g}^{-1}$ . In this range, the regression of dehydrogenase activity on metabolic heat release is

$$Y = 0.037 + 0.046X$$

where  $Y$  is dehydrogenase activity in absorbance units per gram and  $X$  is metabolic heat release in millicalories per gram per hour. The regression coefficient is significant at  $P < 0.001$  with 95% confidence limits of 0.039-0.053. The  $Y$ -intercept is not significantly different from zero; this may signify that purely chemical exothermic side reactions in the sediment, e.g., neutralization of organic acids (Forrest et al. 1961) are negligible.

To obtain samples with even higher rates of metabolic heat release, two sediment samples from Lake Washington were treated with dried ground zooplankton and glucose and allowed to ferment for two days. These samples were then placed in culture tubes for calorimetry measurements. After their removal from the calorimeter, their dehydrogenase activity was immediately

assayed. The regression line with these samples included with the untreated samples is given by the equation

$$Y = 0.166 + 0.030X$$

The regression is significant at  $P < 0.001$  with 95% confidence limits of 0.025-0.035. The  $Y$ -intercept is significantly greater than zero. Furthermore, there is a significant difference in the slopes of the two regressions. The difference between the two regressions may be attributed to the effect of the added substrate and consequent increase in exogenous metabolism. When removed from the calorimeter the culture tubes containing the treated samples were under internal pressure due to the liberated gaseous fermentation products. The bacterial populations in the treated samples may have been in a logarithmic phase of growth whereas those in the untreated samples may have been closer to steady state. Although both regressions indicate that the enzyme activity measured in 3 hrs is proportional to the undisturbed rate of anaerobic heat release by the microbial community, it appears that the relationship between dehydrogenase activity and metabolic heat release may vary according to the microbial population's growth phase.

The first regression equation was used to estimate the natural anaerobic metabolism of sediments in Lake Washington.

#### *Field Methods and Procedures*

Samples were taken from 19 stations (Figure 6) with a multiple corer that takes undisturbed sediment cores (Pamatmat 1971a). Oxygen uptake by the intact cores from all stations was measured immediately at 10C while replicate cores from five stations were sectioned and some layers were assayed for dehydrogenase activity on board ship. The core from a sixth station (Station 19) was stored at 10C in the dark for one week before it was assayed for dehydrogenase activity. Each layer, except the already liquid 0- to 1-cm layer, was mixed with a small amount of distilled water to facilitate homogenization and measurement of replicate samples. These layers also were analyzed for concentration of reduced substances by the method of Pamatmat (in press).

The procedure for analysis of reduced substances consists of adding 1 ml of 10 N  $H_2SO_4$  and 10 ml of 0.01 N (0.02 N with highly reduced sediments)  $K_2Cr_2O_7$  to 50 ml of distilled water. The solution, in a bottle train, is stripped of dissolved oxygen by bubbling with nitrogen gas for about 10 minutes. Sediment samples are added to the solution with a special sampler (Pamatmat 1971b) to avoid exposure to air; the mixture is stirred continuously with a magnetic stirrer for 5 minutes and allowed to settle for about 15 minutes. To a 20 ml aliquot of the supernatant, 1 ml saturated KI is added. After the mixture has been allowed to stand for 5 minutes the liberated iodine is titrated with thiosulfate. The total reduced substances determined by this method apparently do not include organic carbon as highly organic sediment samples that had been oven-dried at 90C and finely ground did not reduce dichromate. It is possible, however, that in the drying

process volatile organics that would have reduced the dichromate had been lost; in any case, their amount is considered negligible in comparison with the total inorganic reduced substances.

## RESULTS

### *Reduced Substances in the Sediment*

The concentration of reduced substances in the sediment at the six stations increases and then decreases with depth (Figure 7). With respect to the increase with depth, Lake Washington sediments appear to be similar to sediments in Puget Sound and the continental terrace off Washington and Oregon (Pamatmat, in press), but subsurface sediments in Lake Washington have higher concentrations of total reduced substances. Sediment below 11 cm which contains lower concentrations may have been deposited before the onset of the period of eutrophication of the lake. It is interesting that the concentration of organic matter, although quite variable, has a decreasing trend with sediment depth (Shapiro et al. 1971). The decrease may be attributed to long-term mineralization through anaerobic metabolism, which in turn produced the accumulated reduced end products.

Pamatmat (1971b) has shown that the concentration of reduced substances is proportional to the oxygen depth of the sediment. Furthermore, there is a significant correlation between inorganic chemical oxygen uptake by the intact core and the concentration of reduced substances in the upper 4-cm layer of the sediment. The concentration increases with depth but the concentrations of deeper layers do not correlate with the oxygen uptake by the sediment. It appears that reduced substances below about 4 cm are accumulating while in the upper 4 cm they may be approximating steady-state condition except when the flux of fresh, more readily oxidizable organic matter increases during the summer; then, anaerobic metabolism in the immediate subsurface layers increases, resulting in higher concentrations of reduced substances, and therefore in higher rates of chemical oxygen consumption.

### *Dehydrogenase Activity Versus Reduced Substances*

The absorbance of duplicate  $\text{HgCl}_2$ -treated blanks shows little variability. The coefficient of variation is usually less than 5% but occasional large coefficients of up to 20% raised the average coefficient to 5.2%. There is no correlation between the absorbance of the blanks and the concentration of reduced substances ( $r = 0.043$ , 23 df); hence reduced substances apparently did not reduce TTC to formazan. The absorbance of the blank is probably largely due to phytoplankton pigments and their degradation products.

The absorbance of triplicate reaction mixtures shows greater variability than that of the blanks, with an average coefficient of variation of 19%. As explained previously, part of the variability may have been due to differences in sediment weights in replicate samples, although occasional large variations within replicates could not be explained by this cause. There is a significant increase in the variance with increasing level of dehydrogenase activity ( $r = 0.52$ , 31 df).



There is no correlation between dehydrogenase activity and concentration of reduced substances ( $r = 0.024$ , 23 df). Effenberger (1966), however, noted a negative correlation between dehydrogenase activity and the redox potential of activated sludge. As the  $E_h$  decreased from about +190 to +50 mv the dehydrogenase activity (expressed as relative units) increased from about 120 to 280. Since the effect of anaerobic metabolism is known to be a decrease in  $E_h$  of the medium, the lower the  $E_h$  (in this experiment approaching some steady-state condition) the greater the metabolic activity and hence the higher the measured dehydrogenase activity. This may seem to contradict our result, indicating no significant correlation between dehydrogenase activity and concentration of reduced substances in Lake Washington. The discrepancy is explained by the fact that the concentration of reduced substances in the sediment is a function of time as well. Deeper sediments have been anaerobic for a longer time than surface sediments and are no longer being oxidized by dissolved oxygen diffusing into the sediment; while surface sediments, especially when being turned over by macrofauna, are still periodically aerobic. Under steady-state conditions, and where no other factors such as differences in sediment compaction and oxygen tension of the overlying water prevail, one might expect a correlation between the concentration of reduced substances in the surface layer and its dehydrogenase activity.

#### *Distribution of Dehydrogenase Activity*

The relative measures of dehydrogenase activity at the six stations are shown in Figure 8. The core from station 2 was as long as those from the other stations but was assayed to 5 cm only. There is a trend of decreasing activity per unit weight of sediment. There are significant differences between stations and between layers at each station. There is only a poor correlation between dehydrogenase activity and percentage carbon of the sediment ( $r = 0.42$ , 16 df). It is interesting that when the sediment samples to which zooplankton and glucose had been added are included in the correlation test,  $r$  worsened to -0.21 with 18 df. This shows that when an amount of readily oxidizable organic matter (although insignificant relative to the large amount of old organic matter) becomes available there is a rapid increase in dehydrogenase activity.

There is a highly significant correlation between dehydrogenase activity and absorbance of the blanks ( $r = 0.80$ , 95 df). The results of these correlation tests signify that anaerobic dehydrogenase activity in Lake Washington is more a function of planktonic remains in each layer than of total organic matter, much of which originated from sewage that was discharged into the lake until 1968, when the last sewage plant stopped discharging effluents into the lake (Edmondson, 1970). There is a heterogeneous spatial distribution of total organic matter in the lake sediments (Shapiro et al. 1971) although total organic matter does not reflect the amount of metabolizable compounds. The benthic macrofauna of the lake, especially of the deeper waters, evidently do not turn over and mix the sediments appreciably (Kleckner 1967); X-radiography of sediment cores reveals undisturbed laminations (Edmondson and Allison 1970). In general, the available information on Lake Washington sediments agrees with the present picture of variable rates of anaerobic metabolism from place to place and from one layer to the next.

Other investigators have found significant correlations between dehydrogenase activity and carbon content of sediments (Lenhard et al. 1962), between dehydrogenase activity and ultimate oxygen demand of the sediments (Lenhard et al. 1962; Edwards and Rolley 1965). Ford et al. (1966) and Stevenson (1959) observed good correlations between dehydrogenase activity and the oxygen consumption of replicate samples, which indicate that the organisms were able to use either oxygen or TTC as hydrogen acceptors, though not necessarily with equal efficiency. On the other hand, Edwards and Rolley (1965) found no correlation between the rate of total oxygen consumption by intact sediment cores and dehydrogenase activity; it is not clear how the sediment samples were taken for dehydrogenase assay, but presumably they were from the top 2-cm layer of the sediment cores. This may be a spurious discrepancy between the results of Edwards and Rolley and those of Ford et al. and Stevenson. The oxygen uptake by intact cores may be diffusion limited. It is possible too, however, that a real discrepancy exists, because the organisms could be facultative anaerobes on the one hand and strictly anaerobic on the other.

In experiments with decomposition of flax residues in soils, Stevenson (1959) found that during the first three days there was an increase in enzyme activity that was not correlated with an increase in bacterial numbers; however, over the long run the change in enzyme activity paralleled the change in bacterial numbers. Casida et al. (1964) also noted that dehydrogenase activity increases with increase in total bacterial numbers in organically treated soils. When dehydrogenase activities of different untreated soils were analyzed, however, Stevenson found no relationship with total number of bacteria, and Casida et al. found no relationship with Gram-negative bacteria, fungi, or actinomycetes. Evidently different soils harbor microbial species of different metabolic types in sufficient variety to explain Stevenson's findings. The lack of correlation may have resulted more from differential success with different species in plate cultures than from inherent differences in dehydrogenase activities in different species.

#### *Metabolic Heat Release Versus Oxygen Uptake*

The relative measures of dehydrogenase activity at the six stations were converted to rates of metabolic heat release (in millicalories per gram per hour) and, from the measured dry weight of each layer, these were expressed as millicalories per layer per hour (Figure 9). The rate of total heat production by each core was obtained by integrating each curve.

The rates of total oxygen consumption by the intact cores and the residual oxygen uptake or abiotic chemical oxidation after poisoning of the overlying water were converted to rates of heat release by using the factor of 4.8 cal liberated per milliliter O<sub>2</sub> uptake.

The bottom water temperatures at stations 2, 3, 4, 9, 14, and 19 were 8.2, 8.4, 8.3, 7.9, 8.0, and 13.3C, respectively. Hence, the *in situ* metabolic activity may be expected to be slightly less at the first five stations and more at station 19 than the estimated activity at 10C.

Table 2 shows the results of the two methods of estimating benthic community metabolism. Note that the estimated dehydrogenase activity of the core from station 2 would have been higher if the entire core had been assayed. The estimate by the dehydrogenase method may or may not include the metabolic activity of some aerobes. In the top 1-cm layer, for example, where aerobic microorganisms would be found in nature, their metabolism would be included if they were able to use TTC as hydrogen acceptor in the absence of oxygen. Rarely were *Chironomus* larvae seen in the sediment, but when they were present we do not know whether their metabolism was included in the enzyme assay.

With the exception of station of station 19, the estimate of benthic anaerobic metabolism by the dehydrogenase method greatly exceeds the estimate of benthic aerobic plus anaerobic metabolism by the total oxygen uptake and even more greatly exceeds the estimate of anaerobic metabolism by chemical oxygen consumption. Even if the estimate of anaerobic metabolism by the former method is reduced by the metabolism of the top 1-cm layer, which may have included some aerobic respiration, a large discrepancy would remain. The discrepancy would be larger still if possible anaerobic metabolism by macrofauna is included. It is clear that the rate of total oxygen uptake does not give an accurate estimate of the total benthic metabolism in the sediment column. It is equally clear that the rate of total oxygen uptake by the sediment surface is equivalent to the rate of dehydrogenase activity in a thinner upper layer. We do not know if the week-long storage of the core from station 19 has anything to do with the closer agreement between the two methods for that core.

## DISCUSSION

### *General Applicability of the TTC Method*

A number of questions may be raised about the method of using TTC plus substrate to measure anaerobic metabolism. If TTC is a more efficient hydrogen acceptor than the natural hydrogen acceptors available to the organisms, it may actually stimulate dehydrogenase activity. The added substrate undoubtedly raises the natural level of metabolic activity. Hence, the apparent dehydrogenase activity measured is certainly not the natural level of metabolic activity *in situ*, even if all other physical and chemical conditions in nature are maintained. If TTC alone without additional substrate is used, the measured activity may still be higher than *in situ* rates.

By comparing any dehydrogenase method with direct calorimetry, the above questions become immaterial; the effects of TTC and added substrates are in essence systematic errors that are taken care of by the comparison. If the method is to be of wide applicability, however, it is essential to verify that the magnitude of the systematic error remains the same for different microbial communities. The discrepancy between our results and those of Lenhard et al. (1965) regarding the relative effects of added glucose and sodium citrate presupposes nonuniformity of the systematic error due to added substrate. We have shown, however, a direct proportionality between the results with two concentrations of sodium citrate and with no added

substrate. There should be a significant regression of measured dehydrogenase activity without added substrate and metabolic heat release. If the systematic error due to the effect of TTC alone is the same on all microbial organisms, the only problem would be one of low sensitivity in some if not most places. We have not investigated the effect of much longer incubation time without additional substrate on the rate of formazan production. As mentioned earlier, Farkas (1966) has placed a limit of 3 hrs on incubation time, but it is not clear why he should find this necessary. Obviously, incubation time may be extended for as long as the rate of formazan production remains constant.

In any case, the regression showing a high degree of functional relationship between dehydrogenase activity and metabolic heat release under one set of conditions (10C, 3-hr incubation, 1% TTC, 0.04 M sodium citrate) warrants further investigation of the relationship under a wider range of conditions. It seems particularly imperative to test the relationship at different temperatures and with the dehydrogenase method carried out at the same or higher temperatures than the calorimetry. With this information it may be possible to obtain positive results with any dehydrogenase method and by comparison with a calibrated method determine the equivalent metabolic heat release in nature.

The dehydrogenase activity of some Metazoa has been shown to be correlated with their aerobic metabolism (Curl and Sandberg 1961; Packard and Taylor 1968), but the assay is much more difficult than the measurement of oxygen consumption and therefore the latter remains the preferred method of measuring aerobic metabolism. A dehydrogenase assay, however, could be a very useful measure of anaerobic metabolism by metazoans and deserves to be developed.

The metabolism of heterotrophic sediment bacteria has also been assessed in terms of their uptake of radioactive glucose or acetate (Wood 1970; Harrison et al. 1971; Sorokin and Kadota 1972). Hobbie (1969) discusses the significance and limitations of the method. Such a method also might be compared with direct calorimetry. Since the measurement of dehydrogenase activity requires less expensive equipment and materials, it would seem preferable to the measurement of the rate of uptake of a specific substrate.

#### *Anaerobic Metabolism in Lake Washington Sediments*

The spatial variations in anaerobic metabolism in Lake Washington sediments are explainable in part by the variations in organic carbon and in phytoplankton pigments and their degradation products.

In the absence of comparable data, we cannot say much about the absolute level of anaerobic metabolic activity in Lake Washington sediments. In view of its past history of eutrophication, which has been thoroughly studied and documented by Edmondson (1969a, 1969b, 1970), benthic anaerobic metabolism in this lake may be expected to be relatively high. As sedimentation rate of oxidizable organic matter increases, anaerobic metabolism becomes a relatively more important process in the mineralization of organic matter in sediments. Following pollution abatement anaerobic activity of sediments should be expected to decrease.

Sanitary engineers, however, have shown that dehydrogenase activity is quite sensitive to toxic substances (Lenhard et al. 1965); such substances may have been present in the sewage effluents to Lake Washington. Hence, where there is high organic matter in subsurface sediment but low dehydrogenase activity, the presence of toxic substances may be suspected. Shapiro et al. (1971) have detected a decrease with time in the organic matter content of each dated sediment layer. It would be highly interesting to follow the dehydrogenase activity of those layers.

There is measurable metabolic activity down to at least 30 cm and probably deeper. As a result of compaction, sediment weight per layer increases with depth; hence, the actual contribution of deeper layers to total metabolism is greater than is indicated by the decreasing trend of activity per unit weight. Possibly activity per unit weight of sediment decreases with depth because of decreasing available metabolizable energy, but activity per layer is maintained somewhat because of increasing surface area with depth. Questions may be raised about the chemical nature of metabolism in deeper layers, the possible functional relationships between different metabolic types, and the role of burrowing and deposit-feeding macrofauna. From present knowledge of sedimentation rate in Lake Washington (Stockner and Benson 1967), the sediment layer at 30 cm deep appears to be about 100 years old, and sediment bacteria have been mineralizing the organic matter in that layer for that length of time.

All indications at present are that the rate of abiotic oxygen uptake by intact sediment surface provides an excessively low estimate of anaerobic metabolism in the sediment column. Evidence of this is in the large discrepancy between the rate of heat release by the entire core and the rate of oxygen consumption by the intact core, and in the increasing concentration of reduced by-products of anaerobic metabolism with depth of sediment layer. The latter suggests that the rate of formation of reduced by-products in the sediment column is not in equilibrium with the rate of their oxidation and the concentration of reduced substances is not at steady state. Evidently only reduced substances in the topmost sediment layer are effectively oxidized; deeper in the sediment column reduced by-products are accumulating, except for gaseous products which could diffuse upward into the overlying water.

In ecological investigations, we are interested in the quantitative role of the benthos in the annual cycle of materials and energy flow through the ecosystem, that is, we want to know how much of the annual net primary production settles to the bottom and how much of this is mineralized during that year by the benthos. Obviously, if the benthic community in the deeper sediment layers is metabolizing long-buried organic matter, its metabolic activity is not related to the annual sedimentation of organic matter and the energy flow through the rest of the ecosystem. Naumann (*cf.* Hayes 1964) may have recognized this problem when he talked about the "actual" and the "historical" layers of lake sediments. He thought that the "actual" layer for most northern European lakes was about 10 cm. It would seem from our present data that the "actual" layer in Lake Washington is thinner than 10 cm. It may be that the true measure of the benthic community's impact on the annually sedimented organic matter is reasonably estimated by the rate of total oxygen consumption by the sediment surface. It would be

desirable to obtain direct measurements of sedimentation rates; these plus information on the average leftover organic matter in the bottom should allow us to place an upper limit to the annual extent of degradation of the current year's sedimented organic matter. The limiting values hopefully will agree with the estimated annual oxygen consumption by the sediment.

There are good indications that the total reduced substances in the sediment, measured as the milliequivalents of standard dichromate reduced, is a measure of the accumulated by-products of anaerobic metabolism from the time the sediment layer was effectively cut off from the "actual" layer. Thus, if each layer can be dated as were the cores from Lake Washington, it seems possible to estimate from the concentration of reduced substances the total anaerobic metabolism that has taken place.

As for the total benthic community metabolism in the sediment column, there appears to be no easy single method available. Direct calorimetry may be the only means of measuring aerobic plus anaerobic metabolism of undisturbed sediment cores, but it does not look promising for field studies. At present the practical way to estimate total aerobic and anaerobic metabolism in sediments is to combine the rate of respiratory oxygen uptake by undisturbed sediment cores with estimates of anaerobic metabolism derived from dehydrogenase assay of subsurface sediment layers.

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Table 1. Relative levels of dehydrogenase activity resulting from the use of the same concentration of different substrates added to replicate sediment samples from Lake Washington

Substrate	Dehydrogenase activity (absorbance/gram dry sediment)	
	Replicates	Average
Sodium citrate	1.17, 1.18, 1.23	1.19
Sodium malate	0.89, 0.89, 0.93	0.90
Sodium lactate	0.66, 0.77, 0.70	0.71
Sodium succinate	0.57, 0.57, 0.58	0.57
Sodium pyruvate	0.01, 0.02, 0.01	0.01
Glucose	0.45, 0.46, 0.48	0.46

Table 2. Comparisons of dehydrogenase activity converted to rate of metabolic heat release (in calories per core per hour) with total oxygen uptake and chemical oxidation converted to metabolic heat release (in calories per core per hour) by using the factor  $1 \text{ ml O}_2 = 4.8 \text{ cal}$

Station	Core length (cm)	Total O <sub>2</sub> uptake	Chemical oxidation	Dehydrogenase activity	
				Range	Average
2	6	0.27, 0.34, 0.26, 0.16, 0.16	0.10, 0.11, 0.12	0.37-0.54	0.39
3	32	0.38, 0.42, 0.38, 0.31	0.24, 0.26	0.88-1.6	1.3
4	21	0.41, 0.45, 0.28, 0.33, 0.38	0.18, 0.18, 0.20	2.8 -3.6	3.0
9	21	0.35, 0.28, 0.22, 0.19, 0.17	0.12, 0.13	1.7 -2.8	2.1
14	21	0.45, 0.39, 0.36, 0.36, 0.32, 0.31, 0.27, 0.24, 0.19, 0.19	0.11, 0.08, 0.11, 0.10, 0.11	3.4 -4.7	4.0
19	16	0.36, 0.36, 0.28, 0.25	0.21, 0.23, 0.24	0.39-0.46	0.41

Table 3. Lake Washington Data

	Stn 1		Stn 2		Stn 3	
	Feb	July	Feb	July	Feb	July
DEPTH (m)	59	50	58	58	10	15
TEMPERATURE (°C)	5.6	7.6	5.2	7.5	5.4	17.8
ORGANIC CARBON (% dry wt)	6.8	*	5.3	*	6.7	*
% SAND	15	*	33	*	*	*
% SILT	65	*	43	*	*	*
% CLAY	20	*	24	*	*	*
BOTTOM O <sub>2</sub> concentration (ml/l)	8.4	5.8	8.2	6.2	8.5	7.0
NUTRIENTS (µg-at/l)						
Phosphate						
Bottom water	2.16	*	1.14	0.72	2.68	0.34
Interstitial						
0-5 cm	2.83	6.1	1.11	6.3	0.78	4.5
5-10 cm	22.2		1.24	1.3		
Ammonia						
Bottom	1.78	*	1.48	0.06	0.32	0
0-5 cm	>140	38	>170	>100	24.4	27
5-10 cm			>240	>280		
Silicate						
Bottom	103	*	101	55	81	30.2
0-5 cm	213	210	186	263	104	127
5-10 cm	249		282	117		
Nitrate						
Bottom	15.7	*	20.3	8.5	29.9	0.2
0-5 cm	3.0	0	2.1	0	3.8	0.6
5-10 cm	2.9	2.4	2.4			
RATES OF O <sub>2</sub> UPTAKE (ml O <sub>2</sub> /m <sup>2</sup> /hr)						
Total Uptake						
	46	38	19	29	11	24
	53	32	22	42	10	19
	36	31	25	35	11	26
		25			24	16
Chemical Oxidation						
	25	20	19	17	17	14
		10		15		11
Respiration						
	21	18		25	7	10
		22		20		8

Table 4. Respiratory electron transport activity (ETS) at Lake Washington station 1, 1972 (in milligrams O<sub>2</sub> per cubic meter per hour).

23 May Phytoplankton+ Bacteria			1 June Phytoplankton+ Bacteria			16 June Phytoplankton+ Bacteria			6 July Phytoplankton+ Bacteria			10 July Phytoplankton+ Bacteria		
Light level	Depth (m)	ETS	Light level	Depth (m)	ETS	Light level	Depth (m)	ETS	Light level	Depth (m)	ETS	Light level	Depth (m)	ETS
100%	0	4.630	100%	0	5,560	100%	0	5,665	100%	0	15,022	100%		
50%	1.55	3.910	50%	1.5	4,820	50%	1	5,725						
25%	3.25	4.360	25%	3.0	4.280	25%	2	8,875						
10%	5.25	5.710	10%	4.5	3.395	10%	3.25	7.480						
1%	10.25	4.320	1%	9.0	3.165	1%	6.5	9.680						
	15	2.513		15	.392		10	2.005		5				
	20	1.333		20	.640		15	.621		10				
	25	1.118		25	.475		20	.208		15	3.910			
	30	.875		30	.407		25	.215		20	.654			
	40	.691		40	.299		30	.157		30	.185		30	.330
	50	.493		50	.275		40	.156		40	.139		40	.513
	60	.405					50	.224		50	.037		50	.511
										60	.079		60	.294
Zooplankton			Zooplankton			Zooplankton			Zooplankton					
60-0			60-0			58-0			60-0					
			20-0			20-0								

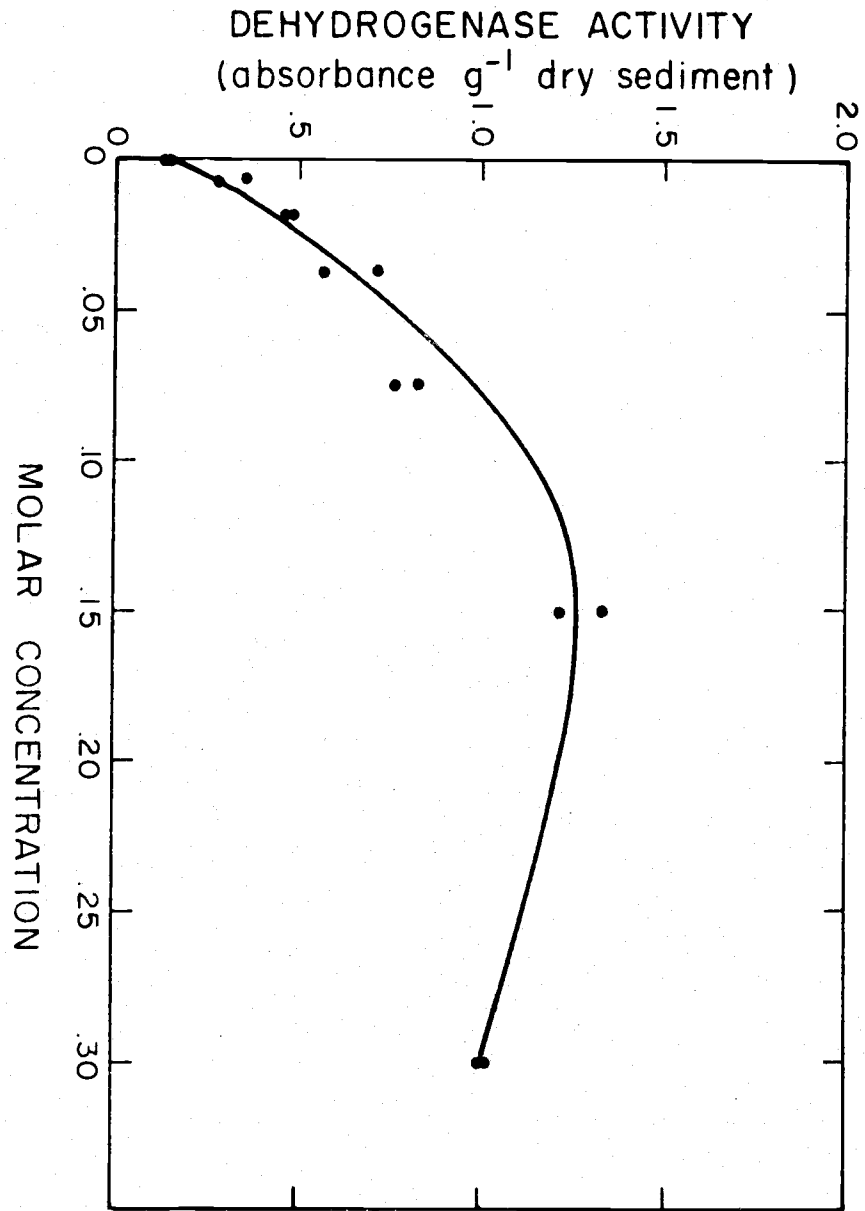


Figure 1. Dehydrogenase activity as a function of sodium citrate concentration.

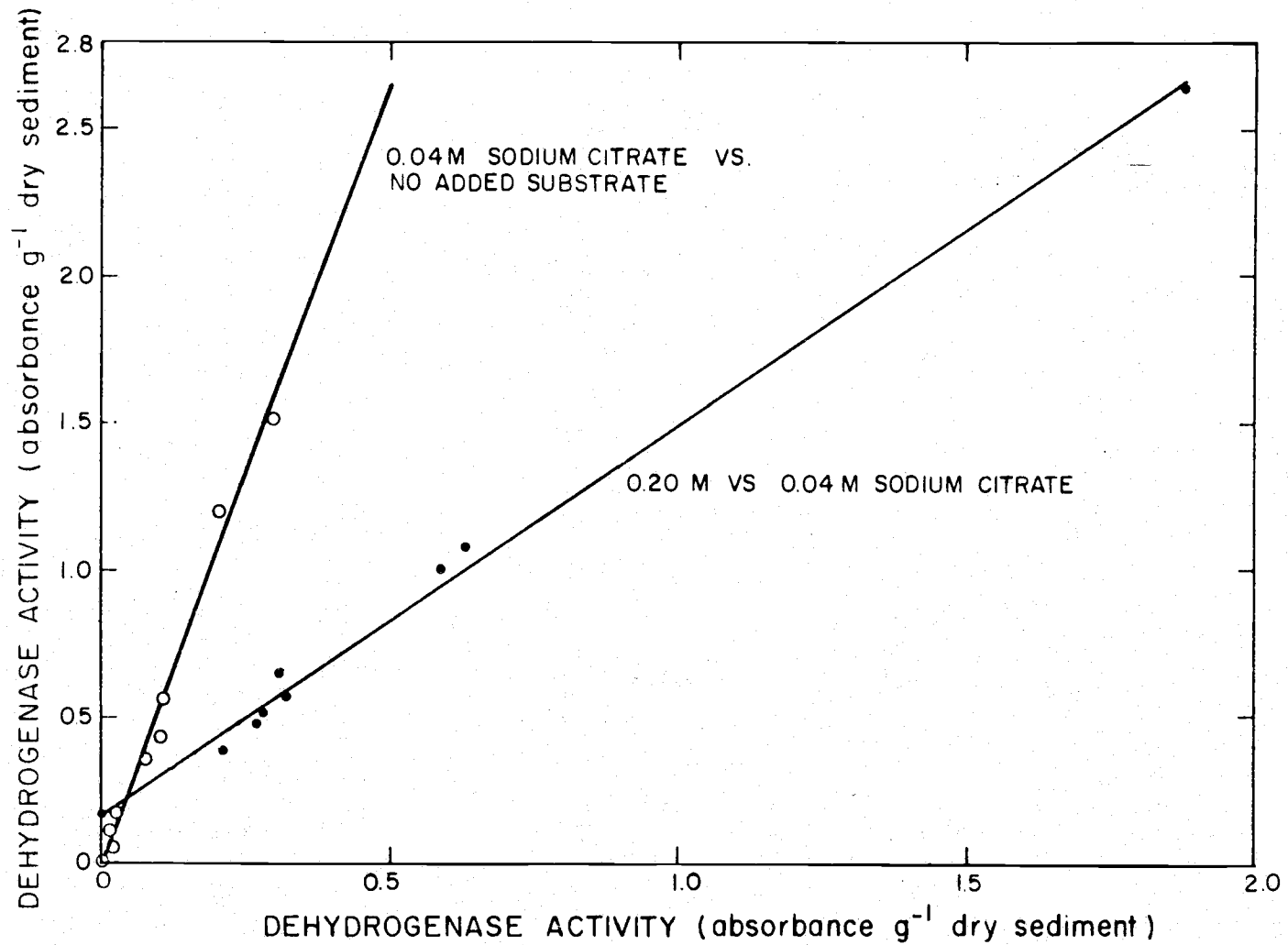


Figure 2. Dehydrogenase activity at 0.04 m sodium citrate as compared to dehydrogenase activities at 0.20 m sodium citrate and without added substrate.

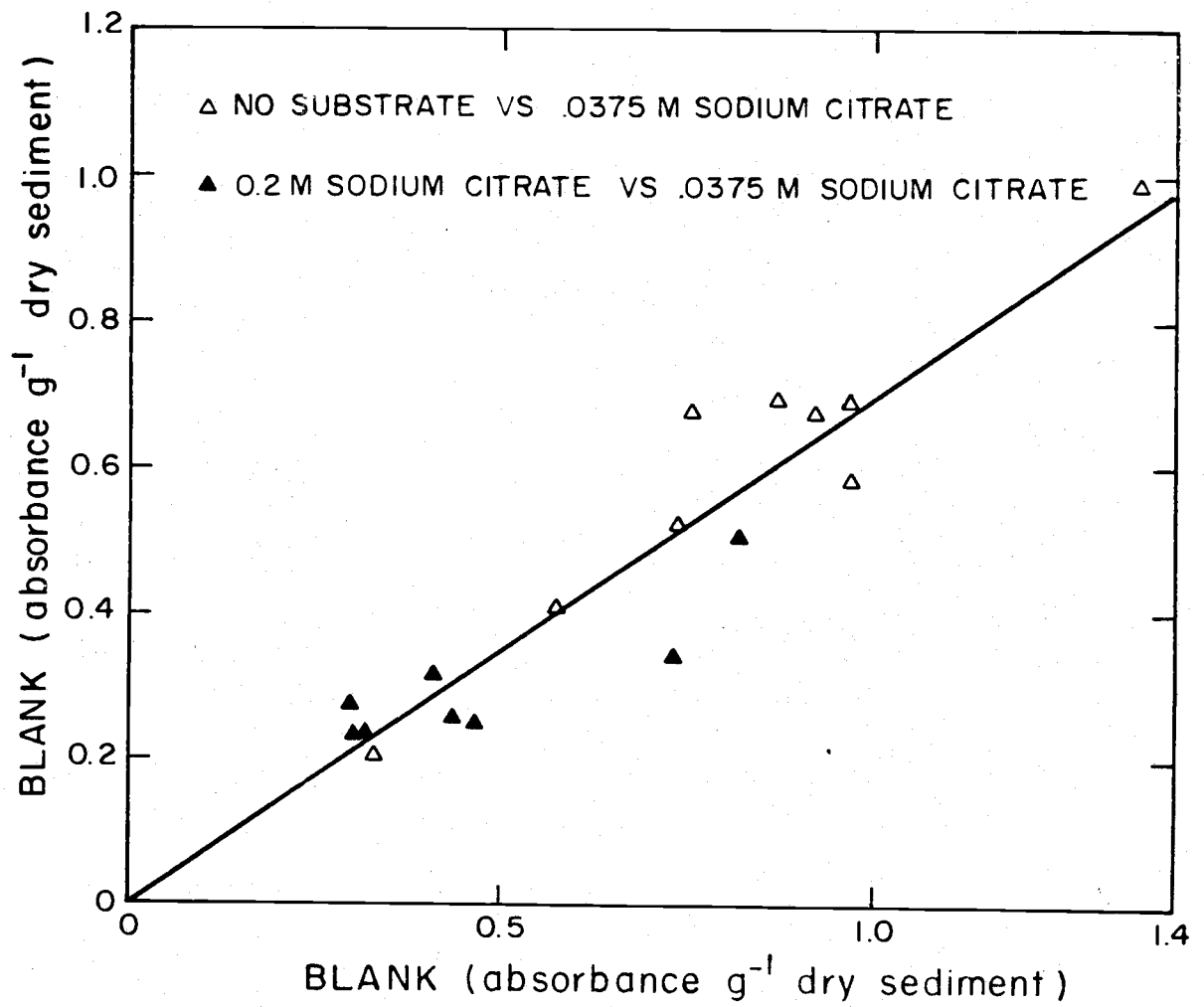


Figure 3. Effect of substrate concentration on the absorbance of mercuric-chloride-treated blanks.



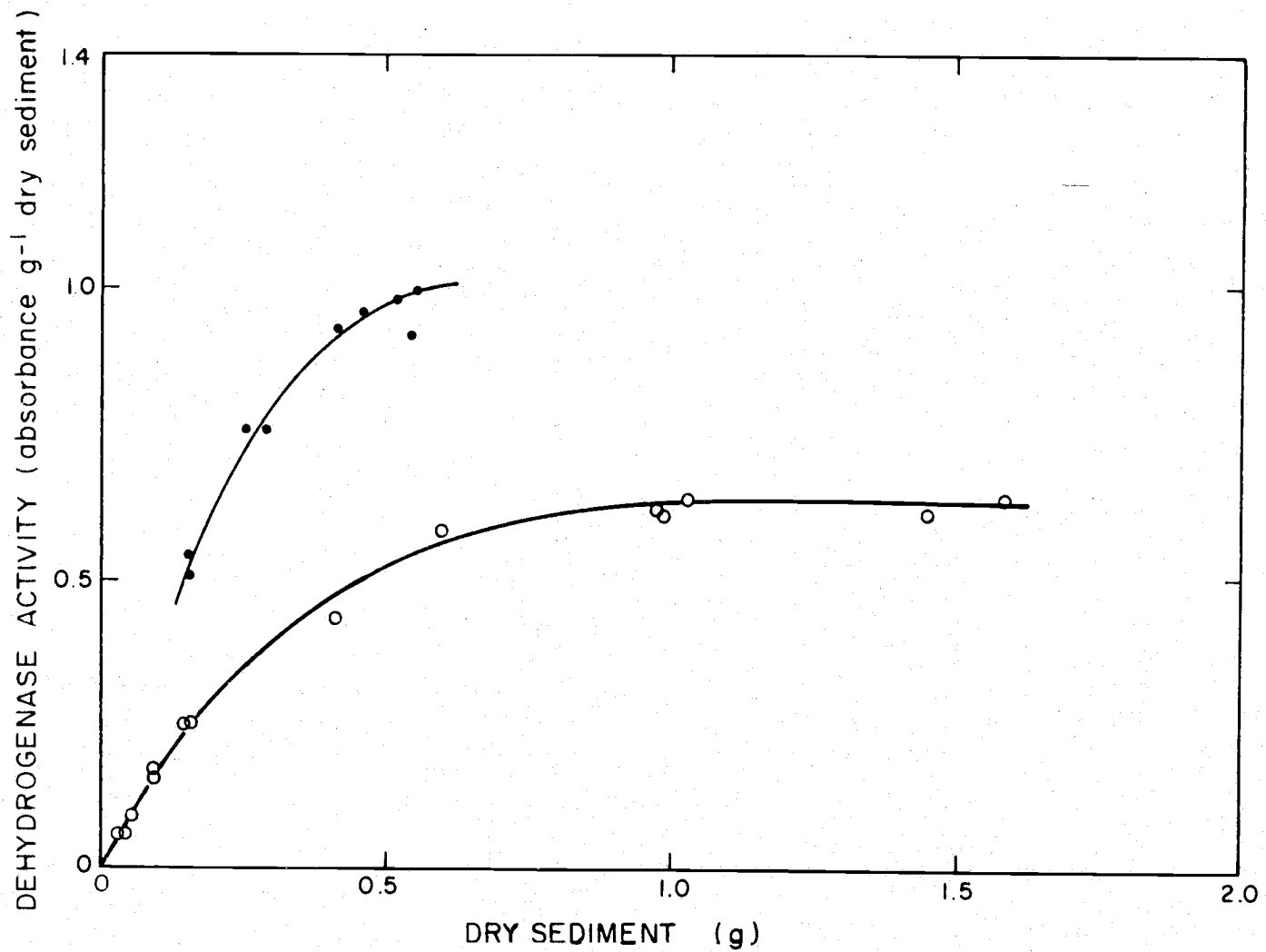


Figure 4. Dehydrogenase activity as a function of amount of sediment of two different levels of metabolic activity.

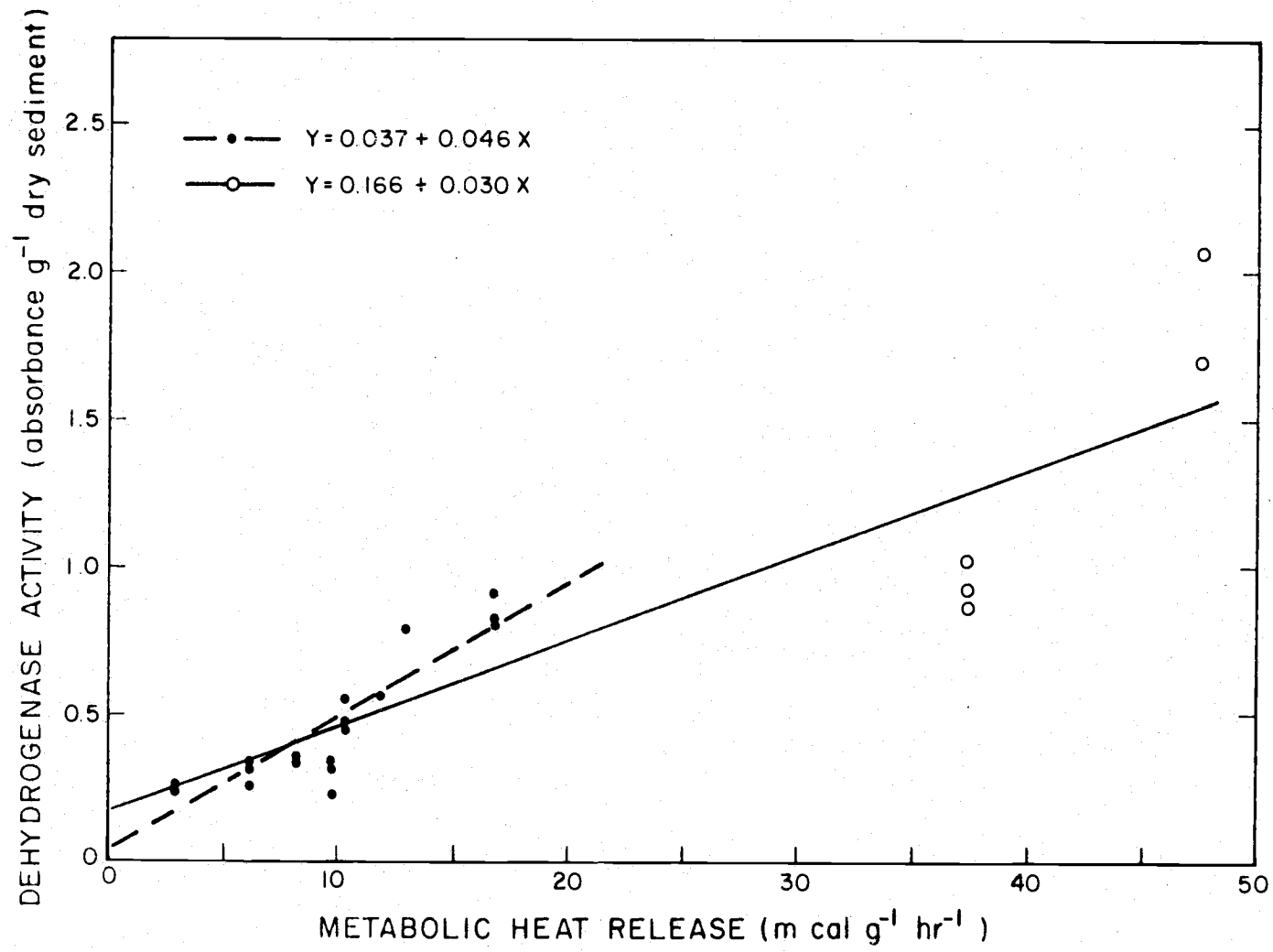


Figure 5. Dehydrogenase activity as a function of the rate of metabolic heat release.

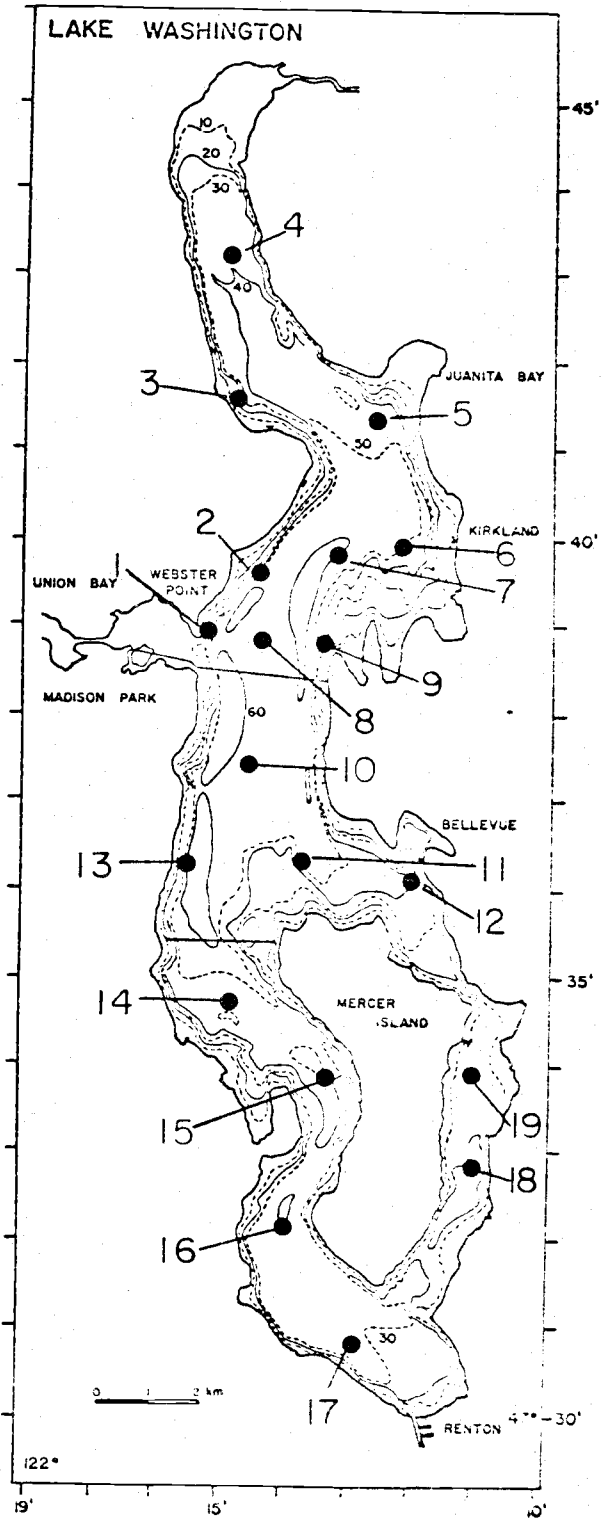


Figure 6. Chart of Lake Washington showing 10-m depth contours and location of stations.

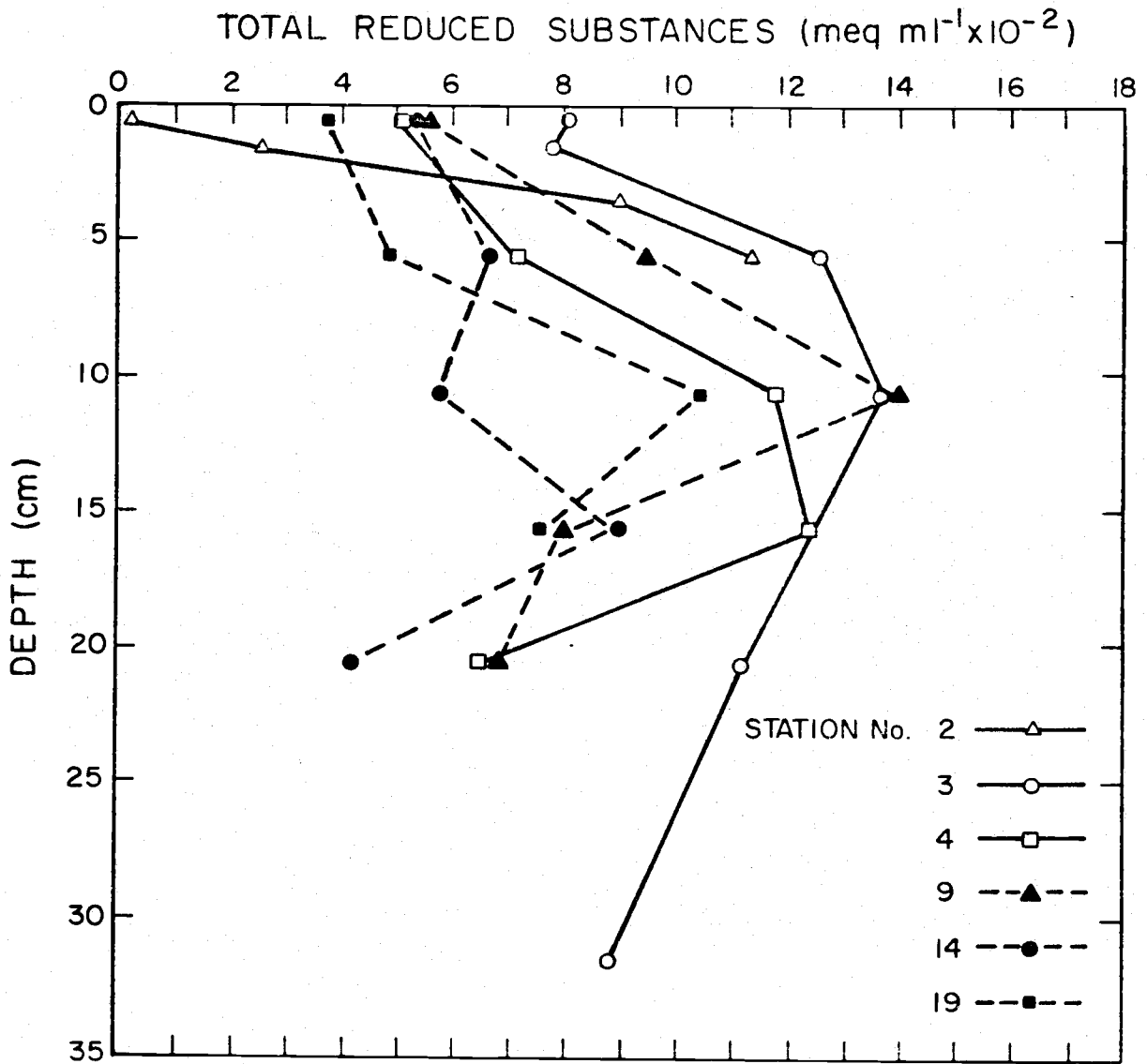


Figure 7. Vertical distribution of the concentration of reduced substances in Lake Washington sediments.

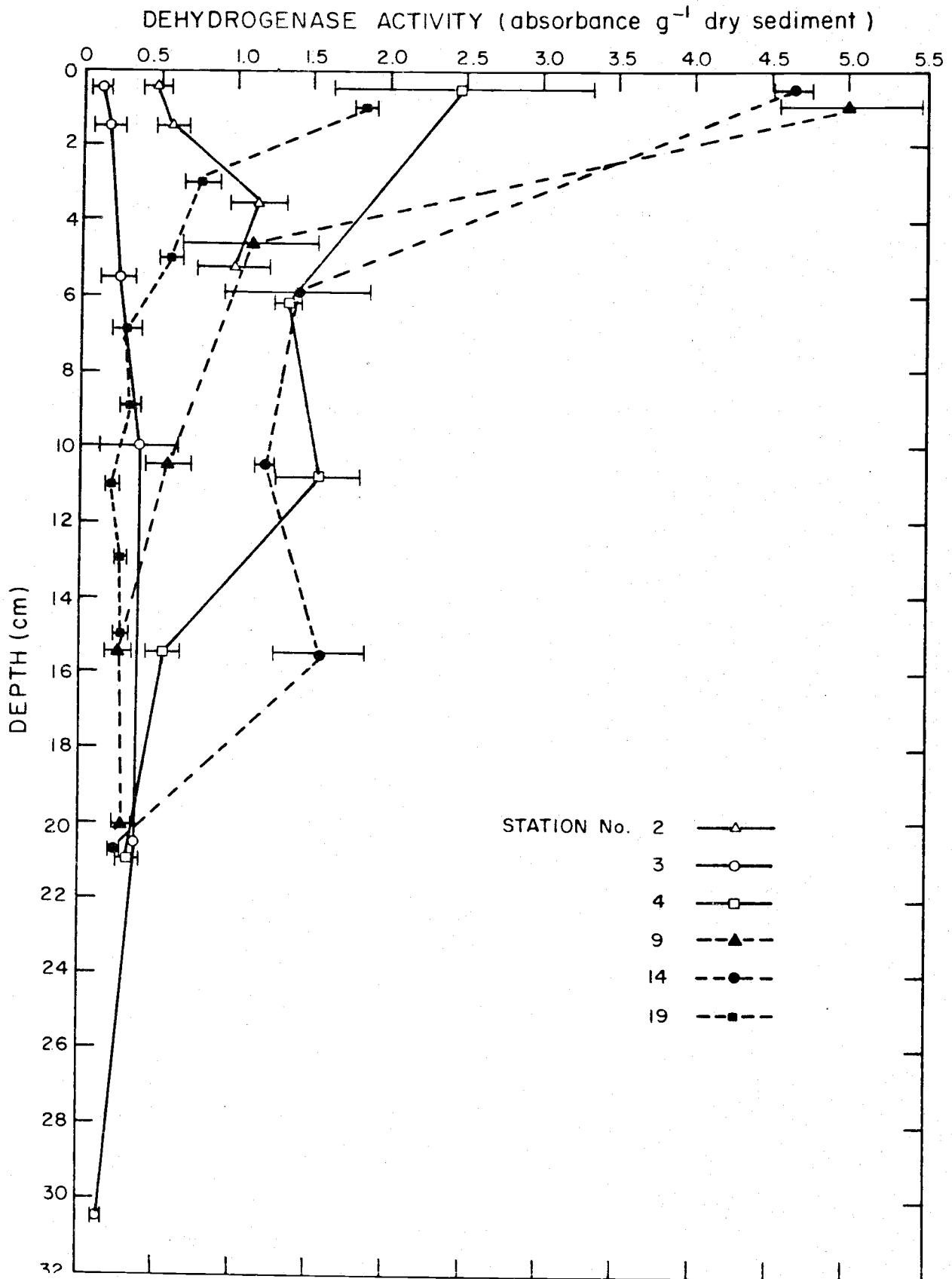


Figure 8. Vertical distribution of dehydrogenase activity at six stations in Lake Washington in October 1972. The horizontal bars denoting  $\pm$  one standard deviation have been displaced where they would have overlapped.

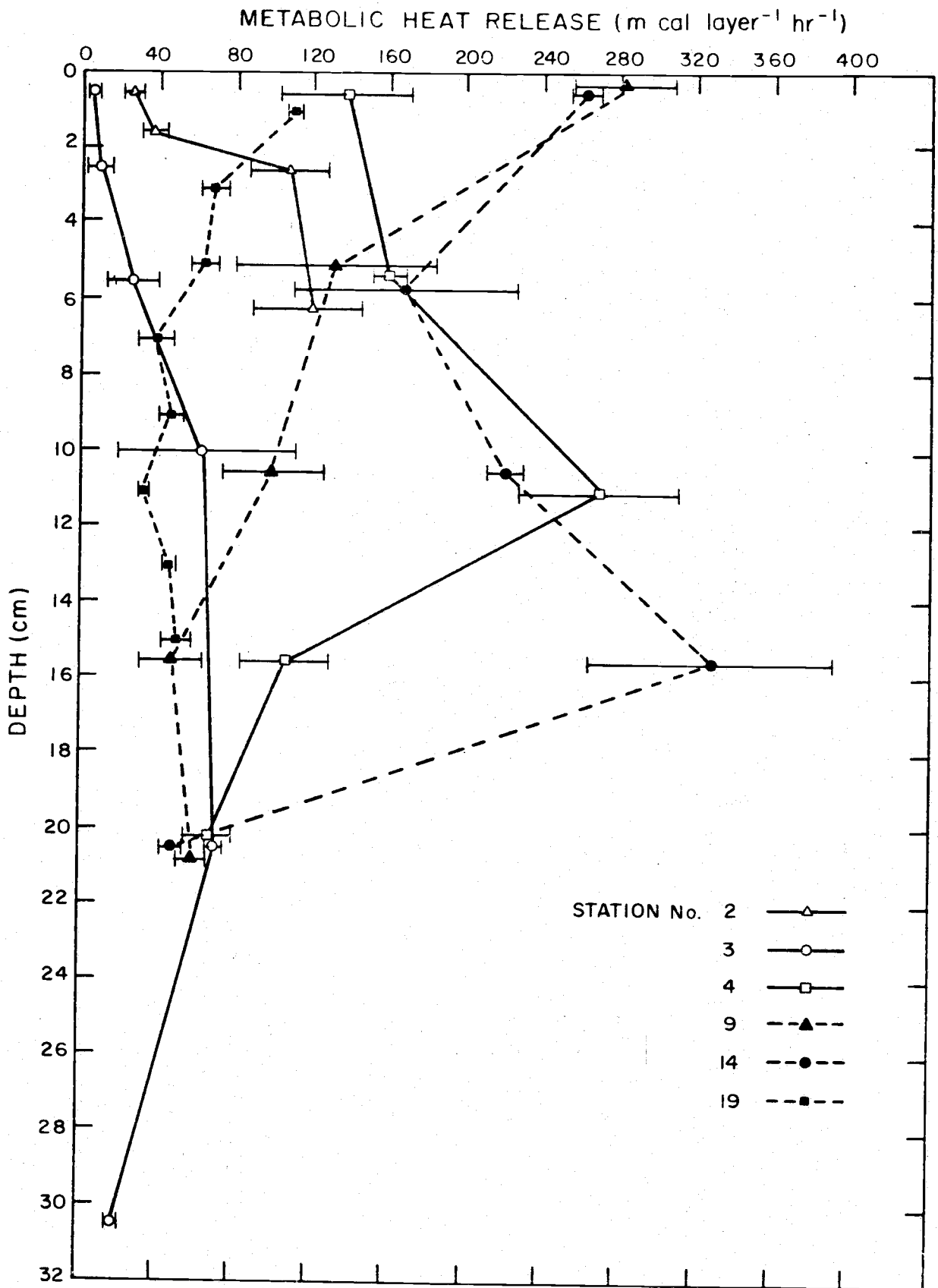


Figure 9. Vertical distribution of metabolic heat release at six stations in Lake Washington in October 1972. The horizontal bars denoting  $\pm$  one standard deviation have been displaced where they would have overlapped.