The Utilization of Organic Acids by Planktonic Heterotrophs in West Blue Lake, Manitoba

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ABSTRACT

The occurrence of several heterotrophic parameters are described with reference to the oligotrophic waters of West Blue Lake, Manitoba. The relationship between uptake velocities and concentration of nine organic acid substrates (lactic, pyruvic, fumaric, malic, acetic, succinic, glycolic, citric and formic acids) upon kinetic analysis produced values (V_{max}, T, K + S and v values) which in most cases were of the same order of magnitude as reported in the literature for bacterial populations. Except for one substrate (formic acid) uptake velocities increased in the light and the dark with time, in most cases with lag in uptake velocity being present during 48 hr experiments. Such lags were felt to indicate periods of increase in bacterial populations within sample bottles. The relationship between uptake velocity and depth for three of the nine substrates (lactic, malic and succinic acids) produced responses which were believed to be associated with the temperature profile and nutrient availability of the water column. Evidence is given for the presence of different bacteria utilizing lactic and succinic acid than malic acid. Relative heterotrophic productivity as estimated for these three substrates was believed to be greater than primary productivity on a day to day basis. "In situ" experiments using NaH $^{14}CO_3$ have shown that initial uptake rates were highest for 12 to 24 hr experiments and that subsequent decreases in uptake rates with time indicated re-assimilation of excreted products. It was felt that an equilibrium response between the organisms and the confines of the samples bottle in addition to a coupled oscillatory response were in operation controlling excretion and re-assimilation. Loss of activity during filtration of samples was investigated but no such response was measured.

INTRODUCTION

Before the 1960's measurements of algal heterotrophy consisted of laboratory culture experiments using various algae and a variety of substrates used at relatively high amounts (Belcher and Fogg, 1958; Belcher and Miller, 1960; Cramer and Myers, 1952; Droop and McGill, 1966; Lewin, 1953; Lewin and Lewin, 1960; Lwoff, 1932; Pringsheim, 1921; Pringsheim and Wiessner, 1960; and Samejima and Myers, 1958). A modification of the Steeman-Nielson technique (1952) for studying primary productivity by Parsons and Strickland (1962) produced a method for measurement of the "relative heterotrophic potential" in a marine environment using radioactively labelled glucose and acetate as substrates. Since their development the method has been used to monitor bacterial heterotrophy using a number of labelled organic substrates for both freshwater and marine environments (Allen, 1969; Andrews and Williams, 1971; Hamilton, Morgan and Strickland, 1966; Hamilton and Austin, 1967; Hamilton and Preslan, 1970; Hobbie and Wright, 1965a, 1965b, 1968; Munro and Brock, 1968; Vaccaro, 1969; Vaccaro, Hicks, Jannasch and Carey, 1968; Vaccaro and Jannasch, 1966, 1967; Williams, 1970; Williams and Gray, 1970; and Wright and Hobbie, 1966, 1967) and for algal heterotrophy in a marine environment (Hellebust, 1971; Hellebust and Guillard, 1967; North and Stephens, 1967; Sloan and Strickland, 1966; and Stephens and North, 1971). For the most part, results of these studies have been described in terms of Michaelis-Menten kinetics and as such have been questioned (Williams, 1970; and Hamilton and Preslan, 1970).

The research undertaken at West Blue Lake during the summer of 1971 in partial fulfillment of this thesis was designed to observe and measure some of the heterotrophic processes which were occurring within the lake, which

until this time had not been examined. This project would then be a part of an overall study of the lakes biological productivity. Experiments were designed to measure saturation responses of natural lake populations for nine labelled organic acids, and applying the results to analysis by Michaelis-Menten enzyme kinetics. This would provide information on the rates of substrate removal and substrate selection by the heterotrophic organisms in the lake water. Secondly, experiments were conducted to measure possible changes in the rate of substrate uptake with time for the nine labelled organic acids, and to determine what length of time incubation periods should be. Thirdly, it was to be determined to what extent would heterotrophic uptake of three labelled substrates change with depth. In addition, rates of excretion and re-assimilation of ^{14}C-labelled products were to be measured to provide some indication as to whether a coupled oscillatory response existed between algal excretion and bacterial assimilation of ^{14}C-labelled substrates. Also, a "filtration effect" (Arthur-Rigler, 1965) would be determined, so as to check if any loss of activity was resulting due to filtration.

LITERATURE REVIEW

The study of algal heterotrophy has not been a topic of only recent investigation. It can be traced back to the late nineteenth century, when Beijernick (1898) discovered that Chlorella could be grown in the dark with glucose as the organic substrate. Much of the subsequent work has dealt with the culturing of many different species of algae, representative of almost all classes on a wide variety of substrates. This method has recently been superceded by the use of radioactively labelled substrates, which represented a much more sensitive method of dealing with the phenomenon both for culture and "in situ" experiments. The method required new techniques and the use of specialized equipment and in turn resulted in the introduction of a number of new terms.

In general, heterotrophy can be defined as the utilization of a soluble organic substrate by an organism for growth and energy, and can be applied to both bacteria and algae. The definition of "heterotrophy" may be broken down further to include two more specific groups of organisms. Firstly, there are those which are capable of synthesizing essential metabolites from an organic substrate by the use of energy obtained from light, and are known as "photo-organotrophic" organisms. Secondly, there are "chemo-organotrophic" organisms which utilize an organic substrate for growth and energy but do not require light. A further modification of this mode of nutrition has been shown by a few flagellates which ingest particulate inorganic or organic matter producing the required metabolites and energy. This process has been termed phagotrophy. Finally, there is a type of nutrition common

to both autotrophs and heterotrophs known as auxotrophy which relates to the organisms requirement of accessory growth factors; such as vitamins, being required for growth and energy. Much of the early literature on algal heterotrophy has shown that algae can be capable of both photo-organotrophy (Lewin, 1953; Samejima and Myers, 1958; Pringsheim and Wiesner, 1960; Droop and McGill, 1966; Sloan and Strickland, 1966; North and Stephens, 1968; and Hellebust, 1971) and chemo-organotrophy (Cramer and Myers, 1952; Lewin, 1953; Belcher and Fogg, 1958; Samejima and Myers, 1958; Belcher and Miller, 1960; Lewin and Lewin, 1960; Pringsheim and Wiesner, 1960; Droop and McGill, 1966; Sloan and Strickland, 1966; Hellebust and Guillard, 1968; and Hellebust, 1971); whereas the heterotrophic bacteria (Wright and Hobbie, 1965, 1966; Hobbie and Wright, 1965a, 1965b, 1968; Hobbie, 1967; Hobbie and Crawford, 1969; Harrison, Wright and Morita, 1971; Jannasch, 1967a, 1967b, 1968; Vaccaro and Jannasch, 1966, 1967; Vaccaro; Hicks, Jannasch and Carey, 1966; Vaccaro, 1969; Hamilton, Morgan and Strickland, 1966; Hamilton and Austin, 1967; and Hamilton and Preslan, 1970), are within a defined group known as chemo-organotrophs. The chemo-organotrophic utilization of a number of labelled substrates at very low levels has recently been illustrated by a number of workers for marine and freshwater environments (Wright and Hobbie, 1965, 1966; Hobbie and Wright, 1965a, 1965b, 1968; Vaccaro and Jannasch, 1966, 1967; Hamilton et al., 1966, 1967, 1970; Jannasch, 1967a, 1967b, 1968; Munro and Brock, 1968; Vaccaro, Hicks, Jannasch and Carey, 1968; Vaccaro, 1969; Le B. Williams and Gray, 1970; Le B. Williams, 1970; and Harrison, Wright and Morita, 1971).

It has been stated by Parsons and Strickland (1962) that the amount of total dissolved carbon in the oceans is always in excess to the amount of particulate carbon. Basically the same was stated by Birge and Juday (1934) for a freshwater situation. They found that for a number of Wisconsin lakes the ratio of dissolved carbon to particulate carbon could vary from 5:0 to 8:1, depending upon the trophic state of the lake. For the oceans of the world, Parsons and Strickland (1962) have calculated an average value of 1000 mg soluble-C/m^3, with this value being higher in surface waters and waters nearer to land. These large amounts of soluble organics could have a number of possible origins:

(a) excretory products of the biota

(b) death and decomposition of plant and animal matter

(c) deposition of allocthanous organic matter

(d) grazing and subsequent release of ingested material by zooplankton.

Of particular significance may be the occurrence of extracellular excretion by algae. To date there has been ample evidence indicating that many algae representing all of the classes are capable of excreting various organic compounds in varying amounts. These extracellular products have been defined as soluble organic substances liberated by healthy algal cells as a consequence of growth, and occur in greater variety and amount than has generally been realized (Fogg, 1966). It has been repeatedly suggested that these extracellular products may be present in sufficient amounts to act as a source of energy in aquatic environments (Lucas, 1946, 1961; Tolbert and Zill, 1956; Fogg, 1958; and Fogg and

Nalewajko, 1964).

The production and excretion of glycolic acid was one of the first processes to gain importance as an extracellular product. Tolbert and Zill (1956) found that the main excretory product of Chlorella was glycolic acid. A year later Tolbert and Zill (1957) reported some of the physiological factors affecting glycolate excretion and hypothesized an intracellular and extracellular equilibrium for glycolate between the cell and the medium. Fogg and Watt (1965); Fogg, Eagle and Kinson (1969); Nalewajko (1966); Miller, Mayer and Tanner (1963); and Whittingham and Pritchard (1963) indicated that the liberation of glycolic acid by Chlorella in addition to a number of other species (Nalewajko, 1966) was affected by a number of physiological factors such as CO_2 tension, population density, light intensity and pH of the medium. Culture and 'in situ' experiments have lead to the estimation that the excretion of soluble organic compounds by algae represent between 2 and 50% of the total carbon fixed photosynthetically (Allen, 1956; Fogg, 1958; Fogg and Nalewajko, 1964; Fogg, Nalewajko and Watt, 1965; Nalewajko, 1966; and Horne, Fogg and Eagle, 1969), with up to 95% having been reported by Fogg, Nalewajko and Watt (1965). Of these amounts of products excreted, Fogg, Nalewajko and Watt (1965) believed that glycolic acid was one of the principal substances. Tolbert and Zill (1956) found that glycolate excretion by photosynthesizing Chlorella cells represented 10% of the total carbon fixed. However, these early data were based on the qualitative and quantitative identification of glycolic acid by the colorimetric method of Calkins (1943) which has since been shown to be equally sensitive to a number of interfering substances

(Tolbert and Zill, 1956; and Fogg, Eagle and Kinson, 1969), therefore possibly overestimating the significance of glycolates role in extracellular production. Fogg, Eagle and Kinson (1969) using ion exchange and a modification of Calkins method quantitatively estimated the amount of glycolic acid in Lake Windemere as ranging from 0.0 mg/l to 0.061 mg/l; which were distinctly lower than the range of 0.045 to 0.29 mg/l reported by Fogg and Nalewajko (1964) using the ordinary Calkins method. In addition to the excretion of glycolic acid, Allen (1956) has shown oxalic acid and pyruvic acid to be excreted by Chlamydomonas.

Carbohydrate excretion by algae has been investigated by Guillard and Wangersky (1958); Marker (1965); and Hellebust (1967). Members of the Chrysophycaeae and Chlorophyceae produced the highest amount of carbohydrates, 123 mg / l and 23 mg / l respectively, with the dinoflagellates and cryptomonads producing the least amounts, less than 3 mg / l (Guillard and Wangersky, 1958). The important carbohydrates were identified as glucose, galactose, arabinose, xylose and ribose (Guillard and Wangersky, 1958; Marker, 1965; and Hellebust, 1967). The importance of nitrogenous substances as being products liberated by various algae has been shown by Fogg (1952); Fogg and Westlake (1955); Stewart (1963); and Hellebust (1965). Some of these compounds which have been identified are alanine, aspartic acid, arginine, leucine, proline, valine, threonine in addition to other unidentified free amino acids, peptides and polypeptides. Comprehensive studies by Merz, Zehnfenning and Klima (1962) and Hellebust (1965, 1967) summarize much of the excretory work by illustrating that a variety of algae of the Chrysophyceae, Bacillariophyceae and Chlorophyceae were capable of excreting different polysaccharides, organic acids, sugar

alcohols, amino acids and peptides. Besides these more common substances, the presence and importance of other organic substances of a more complex nature, such as vitamins, hormones, auxins, enzymes and other growth promoting and inhibiting substances has been shown (Lucas, 1946, 1961; Johnston, 1955; Bentley, 1958; and Fogg, 1966). The inter-relationships between these compounds, the immediate environment and the organisms of the environment have been termed by Lucas (1946) as "non-predatory" inter-relationships, and the mediating substances as "ectocrines". In conclusion, Fogg (1966) has stated that excretion of extracellular products by photosynthesizing algae of between 5 and 35% of the material produced in photosynthesis, must be supposed to constitute a major source of carbon and energy for heterotrophs.

The second possible source of soluble organic matter in natural waters, is bacterial decomposition of particulate organic matter. This process has been discussed by both Hutchinson (1953) and Ruttner (1966) in sections of their texts on limnology. Raymont (1967) in his book has stated that the death and decomposition of phytoplankton and zooplankton contributes to the amount of soluble and particulate organic matter in the water column. In general the literature has dealt mainly with the decomposition processes of bacteria and the production of organic phosphorus and organic nitrogen and the subsequent mineralization of these compounds to inorganic salts. However, there is a general lack of information regarding the intermediate products of bacterial mineralization. Anderson and Zeutchel (1970) have suggested that yet a further possible source of extracellular organic matter might arise from grazing and release of ingested materials by zooplankton on phytoplankton, the relative importance

of this depending upon the exact rate and frequency with which zooplankton graze the phytoplankters.

Much of the early work on measuring the heterotrophic capabilities of algae was done by culture methods using high amounts of added substrate; in most cases between the range of 0.1 to 1.0 grams substrate/l. Early work by Pringsheim (1921) and Lwoff (1932) revealed that there were a number of flagellates which could grow in the dark on acetate and not on glucose. Later, Cramer and Myers (1952) showed that the green flagellate Euglena gracilis var bacillaris could grow in darkness on a number of organic acids and amino acids at a substrate concentration of 5 gm/l whereas the Vischer strain could only use acetate and butyrate. In addition the bacillaris strain was shown to utilize glucose at a concentration of 10 gm/l in the dark, uptake being a function of CO_2 tension, pH of medium, substrate concentration and whether the cultures were pre-conditioned or not. Further work on the flagellates Chamydobotrys, Chlorogonium and Euglena gracilis by Pringsheim and Wiessner (1960) indicated that these species could photoassimilate or grow quite normally on acetate at 1.0 gm/l in a CO_2-free atmosphere. Lewin (1953) showed that seven isolates of Navicula pelliculosa, five other species of Navicula and one of Nitzchia were capable of growth in the dark on glucose at a substrate concentration of 5 gm/l. One isolate of Navicula pelliculosa in addition to utilizing glucose in the dark at a concentration of 73 mg/l without CO_2 could also grow effectively on glycerol or fructose in the absence of CO_2 in the light. Subsequently, Lewin and

Lewin (1960) indicated that out of forty-four axenic cultures of marine littoral diatoms, the majority favoured growth in the dark on glucose, with lactate and acetate being widely used. The concentration of these substrates being glucose--5.0 gm/l; acetate--1.0 gm/l and lactate 2.0 gm/l. Belcher and Fogg (1958) and Belcher and Miller (1960) revealed that a number of selected species of the Xanthophyceae were capable of utilizing a wide range of organic substrates at a concentration of 0.01 µ-moles, some species being more selective than others. The response varied from those showing a facultative chemo-organotrophic response to those that were obligate phototrophs. The most efficient substrate supporting growth was glucose followed by acetate and citrate. Samejima and Myers (1958) studying the heterotrophic capabilities of Chlorella, indicated that out of many monosaccharides only glucose and galactose, used at a concentration of 10 gm/l, clearly supported growth of C. pyrenoidosa and C. ellipsoidea in the dark. Likewise Scenedesmus could grow reasonably well on glucose with slow but continued growth on galactose, mannose, fructose, maltose, sucrose or lactose. The addition of glucose and galactose together resulted in no additive effects. Therefore, for the Chlorella species tested there appeared to be a common rate - limiting reaction saturated by glucose. Of the organic acids tested only acetate used at a concentration of 1.0 gm/l at pH 5.6 - 5.7 supported growth for both Chlorella species. Glycolate used at the same concentration supported slight and apparent growth of C. ellipsoidea but not of C. pyrenoidosa, which differed from the early observations of Tolbert and Zill (1956). The fact that all growth rates observed in darkness were lower than light-saturated rates was believed to suggest the possibility of a growth factor stimulation of heterotrophic

growth for these species (Samejima and Myers, 1958). The authors (Samejima and Myers, 1958) concluded that the apparent inability of an algal cell to take up an organic substrate appears to be a result of permeability restrictions. Droop and McGill (1966) tested thirty-nine strains of supralittoral algae representative of the Chlorophyceae, Bacillariophyceae and Chrysophyceae for their ability to utilize glycolic acid for chemotrophic and phototrophic growth at a pH of 8.0. They indicated that out of the thirty-nine strains tested only nine species showed a positive response to glycolate at a concentration of 1 gram/l in the light. It was doubtful whether this uptake could support growth. In the dark, at the same concentration, it was reported that there was no growth of any of the species on glycolate, whereas acetate at 1 gram/l was shown to be taken-up by a variety of species in the light and the dark.

The amount of soluble organic matter in natural waters which would be available as a substrate for heterotrophic organisms has been reported as being low (Plunkett and Rakestraw, 1955; Menzel, 1964; and Hobbie and Wright, 1965a), even though the total amount of soluble organic matter has been reported as being high (Birge and Juday, 1934; and Parsons and Strickland, 1962). Wright and Hobbie (1966) and Hobbie (1967) have suggested that because of small amounts of rapidly turning over soluble organics, that more important information regarding the significance of dissolved organic matter as an energy source in aquatic ecosystems could be attained by studying actual rates of supply and regeneration rather than determining actual concentrations of dissolved organic matter. Since the average condition for natural waters is low

concentrations of suitable substrates, the development of a new technique for measuring heterotrophy within a natural population was required. Parsons and Strickland (1962) devised a C-14 method analogous in many ways to the C-14 technique outlined by Steeman-Nielson (1952) to study primary productivity. The method of Parsons and Strickland (1962) allowed for the measurement of the "relative heterotrophic potential" of natural waters in an attempt to compare autotrophic and heterotrophic processes. The method consisted of incubating a prefiltered water sample with an added amount of C-14 labelled acetate or glucose (250 μ gC/l) for a short period of time (< 4 hours) in the dark at near "in situ" temperatures, followed by 'measuring' the amount of radioactive substrate taken up. By using a modified form of the primary productivity equation as used by Steeman-Nielson (1952), the velocity of the uptake could be calculated:

$$v \ (\text{mg C m}^{-3} \text{ hr}^{-1}) = \frac{\text{c.f.}\ (Sn + A)}{C\mu t} \qquad (1)$$

where: v is the rate of uptake in mg C m^{-3} hr^{-1}; C is the radioactivity of the filtered organisms (cpm); μ is the micro-curies of C-14 added in the form of labelled substrate; A the added concentration of substrate carbon; Sn the concentration of any of the same substrate carbon already present (mg C /m^3); t the time in hours of the incubation; C the cpm from 1 μCi of C-14 labelled substrate, which should be corrected for geometry and self-adsorption in the counting assembly used; f a factor to correct for possible isotopic discrimination that may occur against the C-14 isotope as compared with C-12. In this method neither the nature nor the amount of the natural substrate is known but this difficulty is

thought to be overcome by adding so much substrate to a sample that the total amount present is practically equal to the added amount. The equation is said to be valid provided that; (1) C-14 excretion is insignificant, (2) C-14 taken up is being incorporated as cell-carbon, (3) isotopic discrimination is minimal, (4) natural substrate concentrations (Sn) are small compared with A. By using this formula they determined that the relative heterotrophic potential of glucose-carbon was about 50 - 100 percent greater than that of acetate carbon by the same population. The apparent rate of uptake increased proportionally with time which varied from 1 to 10 hours, which was suggested to be similar in manner to a population growing exponentially. Parsons and Strickland (1962) furthered the applicability of their method by applying kinetic analysis. By varying the volumes of added active and inactive substrate solutions to a series of water samples, each of the same volume, and by keeping t small so that v remained relatively constant during the incubation period they found that uptake of C-14 acetate and glucose under these conditions was non-linear. The uptake rates could then be kinetically analyzed by Michaelis-Menten enzyme kinetics or the Langmuir adsorption isotherm:

$$v = \frac{k\ (Sn + A)}{K + (Sn + A)}$$

where: v was the velocity of substrate utilization; (Sn + A) the substrate concentration; k a velocity constant; and K a constant (units mg C $/m^3$) of the form of a Langmuir isotherm constant or a Michaelis-Menten constant. If K was less than Sn, then the intercept on the abscissa, allowed one to calculate the amount of substrate carbon initially present in the sample.

If Sn was zero or much less than K, the intercept was then a constant characteristic of some enzyme system in the micro-organisms present in the sample. Therefore, by use of the modified Steeman-Nielson equation plus the Michaelis-Menten equation it was possible to: (1) estimate the rate of removal of specific substrates by a natural population; (2) compare the "heterotrophic potentials" of various aquatic ecosystems.

Wright and Hobbie (1965 also used C-14 labelled glucose and acetate to measure the heterotrophic response of Lake Erkin waters. They found that their data could be analyzed according to Michaelis-Menten enzyme kinetics giving a value for the maximum theoretical velocity of uptake. However, Wright and Hobbie (1965) found that additional information could be obtained if a Lineweaver-Burk plot was used. The original Michaelis-Menten equation has been given as:

$$v = \frac{V\ (Sn)}{Km + Sn} \qquad (2)$$

where: v was the velocity of uptake at a given substrate concentration Sn, V was the maximum velocity and is attained when the uptake sites are continually saturated with substrate, Km the Michaelis constant which by definition was the substrate concentration when the velocity v was one-half the maximum velocity V. By taking the inverse of equation (2) and multiplying both sides by Sn, a linear form Lineweaver-Burk plot was obtained:

$$\frac{Sn}{v} = \frac{Km}{V} + \frac{S}{V} \qquad (3)$$

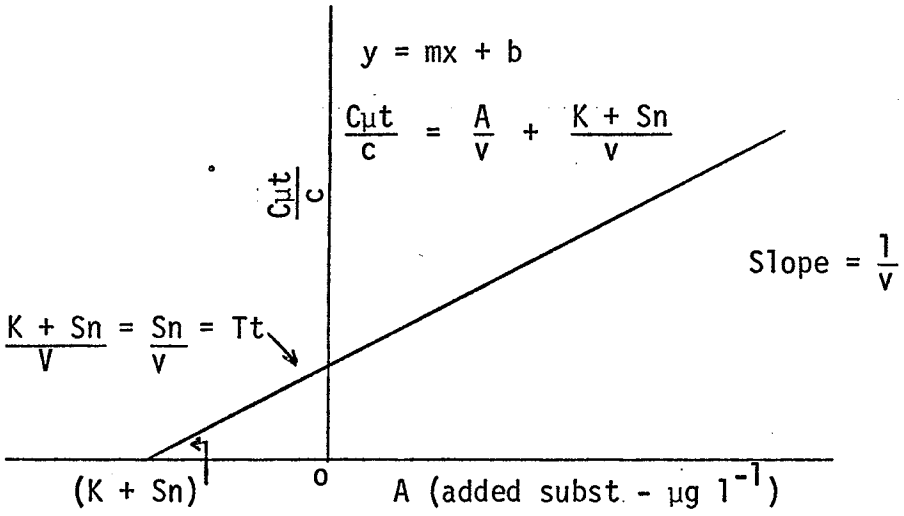
Combining Parsons and Strickland's velocity of uptake equation (1) with equation (3) produced an equation which described the uptake kinetics of natural plankton when the natural substrate was unknown:

$$\frac{(Sn + A)}{V} = \frac{C\mu t}{c} \qquad (4)$$

Substituting equation (4) into equation (3) gave:

$$\frac{C\mu t}{c} = \frac{(K + Sn)}{V} + \frac{A}{V} \qquad (5)$$

From this equation, by plotting $C\mu t/c$ against A from uptake measurements at several substrate concentrations, a number of parameters could be determined from the graphic illustration.



The intercept on the negative absicissa was equal to (K + Sn); the reciprocal of the slope was equal to V, the maximum rate of uptake. The intercept on the ordinate gave the transport time (Tt), which represented the time required for complete removal of the natural substrate (Sn), assuming the rates of removal and replacement remained unchanged (Wright and Hobbie, 1965, 1966; Vaccaro and Jannasch, 1966; and Hobbie, 1967). The sum

(K + Sn) was believed to approximate the "in situ" substrate concentration (Sn) if K was very small. Therefore the sum (K + Sn) indicated maximum "in situ" amounts. The use of equation (5) required that; (1) there was no appreciable reproduction or death of microplankton during the experiment and; (2) substrate removal by the microplankton during the incubation was negligible.

By use of this method Wright and Hobbie (1965, 1966) and Hobbie and Wright (1965a, 1965b) indicated that two different uptake responses could be exhibited by microplankton. An experiment (Wright and Hobbie, 1965), where the amount of added substrate varied in a series of aliquots from 0.08 mg/l to 5.08 mg/l, produced results which according to enzyme analysis indicated the presence of two different responses evidently related to substrate concentration. Uptake at low substrate concentrations (0 to ≃ 500 µg glucose or acetate/l) followed Michaelis-Menten enzyme kinetics and was taken to imply an active transport response. This response was attributed to bacterial uptake. Uptake detected at higher substrate concentrations (0.5 to 4.0 mg glucose or acetate/l) produced an irregular response and was due to another type of uptake mechanism, not capable of being analyzed by enzyme kinetics. Further experiments with large samples containing proportions of algae indicated that the response was associated with the algae. There existed a direct correlation between the rate of uptake and the concentration of added acetate or glucose in the sample. The slope of this linear relationship was shown to be identical with a constant Kd derived from the kinetics of simple diffusion (Wright and Hobbie, 1965; 1966). If Sn was known, uptake due to diffusion (vd) by plytoplankton could be calculated.

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$$vd = (Kd)(Sn) \qquad (6)$$

The Kd could then be used to calculate the turnover time due to diffusion, Td at the natural substrate concentration.

$$Td = \frac{Sn}{vd} = \frac{1}{Kd} \qquad (7)$$

The relative rate of removal of substrate by bacteria and algae could then be compared in terms of the ratio between the two natural uptake velocities.

$$\frac{Tt}{Td} = \frac{vd}{v} \qquad (8)$$

In this way the relative importance of the two mechanisms in removing a substrate could be assessed, even though natural substrate concentrations, composition and individual rates of uptake by the species was not known. Wright and Hobbie (1965b) were able to reproduce this linear relationship for a pure culture of Chlamydomonas on a range of substrates varying from 0 to 2.0 mg - glucose / l , the response following simple diffusion kinetics. Whereas with bacteria using the same range of substrate concentrations it was found that uptake was linear to 0.3 mg - glucose / l, and then the rate levelled off; saturation occurring at a relatively low substrate concentration. These latter results conformed to Michaelis-Menten kinetics and were believed to be systems of permeases. Selective filtering enabled them to separate the bacteria from the larger algae. The relative nature of an uptake response could therefore be controlled by varying the concentration of either the phytoplankton or bacteria in the sample or by varying the amount of added substrate, a low substrate concentration (< 0.5 mg /l) favouring bacterial uptake by an active transport system and a high substrate concentration (> 0.5 mg/l)

favouring algal uptake by simple diffusion.

Vaccaro and Jannasch (1966), working in the Atlantic Ocean and Hamilton, Morgan and Strickland (1966), Vaccaro and Jannasch (1967), Vaccaro (1969) and Hamilton and Preslan (1970) working in the Pacific Ocean all had varying degrees of success in showing uptake at low substrate concentrations of natural populations. Vaccaro and Jannasch (1966) were able to calculate V and K + Sn values for water samples from the Atlantic but had little success with water samples from the Pacific. The sigmoid functions shown by their data were thought to represent multiple uptake by several species or non-linear uptake by a single species. However, by conditioning the water sample, either by letting it stand for 12 - 24 hours or by adding a small amount of glucose and then letting it stand, before making any uptake measurements, resulted in linear responses seemingly by reducing the number of functional species with the subsequent increase in total population. Hamilton and Preslan (1970) attained variable results when measuring the uptake responses of ten labelled organic compounds by natural populations of a dozen stations in the eastern tropical Pacific Ocean. In many cases they found that the uptake data did not lend itself to enzyme analysis. This was believed to have been due to either very low levels of uptake or extreme variability in uptake from one substrate to another. Those stations which produced uptake responses showed higher chlorophyll a, phaeophytin and productivity measurements than those stations which produced no analyzable kinetic results. Their results did not fit the uptake patterns reported by Vaccaro and Jannasch (1967) as being characteristic. The nutrient rich waters of a limited number of stations, as was reflected by high productivity values, was

thought to be responsible for the high heterotrophic activities found at these stations. Williams and Gray (1970) similarly showed that the heterotrophic marine populations of Southampton estuary, (rich in nutrients) compared with the populations of the English Channel (significantly less rich in nutrients) were capable of responding more rapidly to the oxidizing of added amino acids. Similar results of Vaccaro and Jannasch (1966) and Vaccaro (1969) have indicated that heterotrophic activity in the oceans is more intense in estuaries, coastal waters and regions of upwelling, with low and variable responses being characteristic of mid-ocean locations. However, near-shore populations tended to be less efficient with respect to uptake of glucose than species more typical of the open ocean. Jannasch (1967a, 1967b, 1968) has felt that the results obtained from culturing natural populations of marine bacteria on sterile, filtered and supplemented seawater in a chemostat, seemed to suggest that heterotrophy of many marine bacteria could be controlled by the very small amounts of naturally occurring substrate.

Andrews and Williams (1971) have recently shown that there was a pronounced seasonal fluctuation of the rate of substrate utilization in English Channel waters. Glucose and amino acid oxidation rates were reported as being very low during winter (<1%/day) rising to a maximum in the summer occasionally reaching rates of 250%/day for glucose. A quite similar annual cycle has been reported by Allen (1969) for Lake Lötsjön in Sweden. He measured turnover times of glucose and acetate rather than substrate oxidation, and found much longer turnover times in winter than in mid-summer (m) 0.4 - 1.0 hrs versus 300 hrs; for

summer and winter respectively). Allen (1969) stated that seasonal variations of bacterial activity correlated well with observed changes in water temperature and fluctuations in total bacterial number.

The various formulae which have evolved as a result of the C-14 method of measuring heterotrophic responses have made possible the measurement of a number of kinetic parameters, even though the actual amount of naturally occurring substrate was unknown. Since the origin of the method in 1962 a number of techniques have been proposed which would allow for the separation of (K + Sn), thus giving a measure of (Sn). Hobbie and Wright (1965a) outlined a method which employed the kinetics of bacterial transport systems. By using pure cultures of bacteria specifically suited to substrates of glucose or acetate, for which K values were known, and applying these to natural water samples, an estimate of the naturally occurring substrate levels could be measured. Estimates of naturally occurring levels of glucose and acetate obtained by this method correlated favourably with those of Vallentyne and Whittaker (1956). Vaccaro, Hicks, Jannasch and Carey (1968) showed that measurements of (S) by this bacterial bioassay method correlated closely with glucose amounts as determined by the enzymatic method of Carey and Hicks (1968), Hamilton, Morgan and Strickland (1966) and Wright and Hobbie (1966) have suggested than an additional method of assaying for (S) was by diluting natural samples with distilled water and water of similar ionic content, but the method has not been consistently successful. Vaccaro and Jannasch (1966) separated K from S in one experiment by assuming that the K for a test population of bacteria remained constant for each assay sample, and that S=0 in the case of the lowest value recorded.

for (K + S). This provided for a useful upper limit for the value of K and could be used to determine the value of (S) in subsequent samples. The K value could also be determined from the classical definition of K which states that the Michaelis contant (K) is equal to the substrate concentration (A) when the velocity (v) is exactly one-half the maximum velocity (V) (Wright and Hobbie, 1966). Wright and Hobbie (1966) have also shown for determining K that this value is equal to 1/10 the substrate concentration at which 90% of V is achieved. Hobbie and Wright (1965a, 1968); Wright and Hobbie (1966) and Hobbie (1967) have shown from their data that V is a measure of heterotrophic capacity or heterotrophic potential and is a good means of comparing heterotrophic populations because V is directly proportional to biomass. Therefore, these investigators believed that V showed the presence of a functioning uptake system for a substrate and that it represented an upper limit for uptake velocity. Hamilton, Morgan and Strickland (1966) added that because V was dependent on population size it should be converted to a "per cell" basis, facilitating easy cross-comparisons of results between experiments. However, Hamilton, Morgan and Strickland (1966) found that different bacteria under similar test conditions could exhibit different V values; therefore V on a per cell basis would not produce a valid estimate of the population size. Hamilton and Austin (1967) found that by using a number of specifically labelled glucose substrates the values for V could vary. The amount of variation depended upon whether one was measuring utilization or incorporation which in turn depended upon where the label was placed on the glucose molecule. Utilization or incorporation would also vary with the population used. Such variability of the V value was thus believed capable of leading to poor estimates of relative biomass.

Just as V was found to be dependent on specific transport systems so it was found for the (K + Sn) value (Hobbie and Wright, 1965a). Wright and Hobbie (1966) have stated that the (K + Sn) value provided a maximum value for (K) which was believed to give some indication of the efficiency by which the substrate was being taken up. A low (K) value indicated that the organisms involved were very efficient removers of the substrate at low substrate concentrations. Hamilton, Morgan and Strickland (1966) indicated that in theory an organism is more likely to be successful if it exhibited a low (K) and high (V), while the organisms which they tested exhibited high (K) values and low (V) values which indicated that their test bacteria were poorly adapted to survival on the test substrate. On the other hand Hobbie (1967) found Lake Erkin samples to have low (K + Sn) values, which reflected efficient bacterial uptake of glucose and acetate; these substrates never accumulating naturally during the year.

Wright and Hobbie (1966) and Hobbie (1967) believed that important information could be gained from turnover rates of organic compounds. Such a measurement was thought to indicate the efficiency with which a natural population was removing a substrate over a period of time (Hobbie, 1967) and served as a comparison between bacterial uptake and algal diffusion which in turn would reveal the relative importance of the two components in the uptake of a substrate. By measuring turnover times of acetate and glucose by algae and bacteria in Lake Erkin, Hobbie and Wright (1965a) and Wright and Hobbie (1966) found that algal uptake was always less than 10% of bacterial uptake, even though in this case the algal biomass exceeded the bacterial biomass. Turnover times for bacteria in two Swedish lakes have been

shown to vary from 1.8 - 430 hours while those of algae ranged from 1000 - 6000 hours (Wright and Hobbie, 1966 and Allen, 1969). Hobbie (1967) indicated that for Lake Erkin, turnover times can vary from a summer minimum of 10 hours to a winter maximum of 1000 hours for glucose and acetate.

The role of heterotrophic organisms in natural waters involves the respiration of organic compounds with the subsequent release of inorganic elements. Hamilton and Austin (1967) and Williams and Askew (1968) and Harrison, Wright and Morita (1971) each described methods by which a radio-active substrate is incubated with a sample containing heterotrophs, and the amount of respired $^{14}CO_2$ is collected and measured, giving an indication of natural mineralization processes. Williams and Askew (1968) found from their studies that the turnover of glucose in the English Channel varied from 30 - 60 days in winter and spring months to 1 - 6 days in the summer. Harrison, Wright and Morita (1971) using labelled acetate and glucose to measure mineralization rates of lake sediments found that the top square centimeter of sediment was capable of mineralizing much more glucose than the overlying square centimeter column of lake water. The same was found for acetate. Allen (1969) has indicated that rates of uptake of glucose and acetate by bacteria were highest immediately above the sediments by early summer.

Hamilton and Austin (1967) and Hobbie and Crawford (1969) determined that respiration losses of $^{14}CO_2$ could be an important source of error in the calculation of heterotrophic uptake data. Respiration amounts expressed as a percentage of the substrate taken up varied from 8-60% for nineteen different substrates which were tested. The percentage of the total uptake respired as $^{14}CO_2$ was found to vary with the type of substrate, position of the label and the

time of the year. It was found that glucose -U-^{14}C was respired at a greater rate than glucose -6-^{14}C. Hamilton and Austin (1967) have indicated that for a Pseudomonas species which they investigated, utilization of glucose was predominantly by the Entner - Doudoroff pathway and that the amount of glucose incorporated into the organism or the amount which was released depended upon where the label was placed on the glucose molecule. Recently, Williams (1970) determined growth yields (i.e. the relationship between the amount of material incorporated into cellular material and the amount needed for respiration) for Mediterranean, North Atlantic and English Channel waters. His results indicated average growth yields of 67% for glucose and 78% for amino acids. That is, most of the material taken up remained within the cell. Slightly higher results have been reported by Stephens and North (1971). Working with Platymonas and Nitzschia they found that liberation of $^{14}CO_2$ as the respiratory products of oxidation of glycine and alanine was of significance, even for short term experiments, and that this could have considerable effect on estimates of substrate incorporation and other kinetic parameters. Uptake rates in error by 50% were indicated.

In an attempt to separate heterotrophic populations, which were actively taking up glucose and amino acids into size groupings according to their efficiency of removing these labelled substrates, Williams (1970) estimated that 50.5% of the activity was associated with organisms between 1.2 and 3.0 μ in diameter, 32% with organisms between 3 and 8 μ ; and 20% with organisms of 8 μ diameter or larger. He concluded that about 50% of the substrate was probably taken up by bacteria, smaller than 1 μ in diameter. Seventy percent of the labelled organisms passed through a 3 μ filter and it was believed that these organisms were larger bacteria and

small protozoans of about 1 μ in size, while the remaining 30% which was caught probably consisted of protozoa, algae or bacteria which were attached to particles of detritus. From this, Williams (1970) concluded that bacteria were the organisms responsible for the uptake of most of the labelled amino acids and glucose. Munro and Brock (1968) working with naturally occurring microbial populations attached to sand grains, mainly composed of diatoms and bacteria, showed that the assimilation of two organic substrates, acetate and glucose, at high substrate concentrations was by bacteria and not the diatoms which was demonstrated by auto-radiography.

The literature on heterotrophic uptake of labelled substrates by algae at amounts representative of "in situ" levels is limited, however a few recent papers have offered some enlightening results (Sloan and Strickland, 1966; Hellebust and Guillard, 1967; North and Stephens, 1967; Hellebust, 1971; and Stephens and North, 1971). Sloan and Strickland (1966) found dark uptake of glucose by Cyclotella cryptica and of glutamate by Thalassiosira rotula, at a substrate level of 250 μg / l, but concluded that heterotrophic survival of these species and most other algae in the open ocean would be impossible because the substrate levels used were approximately ten times higher than that likely to be encountered in the natural environment. North and Stephens (1967) working with a marine flagellate, Platymonas, showed that this organism was capable of accumulating and assimilating a variety of amino acids at substrate concentrations ranging from 1×10^{-6} to 2×10^{-7} molar. Autoradiography demonstrated that the radioactivity was in fact associated with the Platymonas. Uptake

of glycine, ranging in concentration from 10^{-4} molar to 1.4×10^{-6} molar produced data which could be analyzed by kinetics. A straight-line Lineweaver-Burk plot was obtained and was comparable in form to one showing uptake by an active transport mechanism. Stephens and North (1971) showed that Platymonas and Nitzschia were capable of assimilating glycine, alanine, and arginine at low, natural amino acid concentrations (1.5×10^{-7} molar-4×10^{-7} molar) and that arginine resulted in the liberation of non-volatile compounds by both species, whereas glycine and alanine extruded mainly $^{14}CO_2$. Also Hellebust and Guillard (1968) found that Melosira nummuloides grew well on arginine, glutamine, proline, asparagine and glutamic acid at a concentration of 1×10^{-4} molar, and that uptake of these amino acids for a short period of time (10 minutes) was of the same order of magnitude as that of photoassimilation of $^{14}CO_2$. The addition of 2,4-dinitrophenol, an inhibitor, resulted in practically no uptake of α-aminoisobutyric acid, an amino acid analog. The uptake of amino acids in this case was apparently by an active process. Hellebust (1971) found that Cyclotella cryptica could take up glucose in the dark. Uptake sufficient to support growth was found to be saturated at 1×10^{-4} molar glucose, and the presence of inhibition by a number of inhibitors again suggested that an active system was in operation. The rate of uptake of glucose was shown to increase with increasing temperature and apparent competitive inhibition occurred with addition of other hexoses and pentoses. Wright and Hobbie (1966) noted competitive inhibition of an active transport system, when mannose was added it inhibited the glucose uptake system of a bacterial sample.

METHODS

1. Description of Sampling Area

Research for this thesis was conducted at West Blue Lake during the spring and summer of 1971. West Blue Lake, a lake of pleistocene glacial origin is situated in the Duck Mountain Provincial Park, approximately 300 miles northwest of Winnipeg, Manitoba. The Duck Mountains, at an altitude of 670 meters, represents one in a series of cuestas making up the Manitoba Escarpment, which runs diagonally from south-central Manitoba to west-central Manitoba. West Blue Lake is apparently the remnant of a local meltwater channel which carried water from the disintegrating glaciers in the northwest to glacial Lake Agassiz which at that time lay to the east. Subsequent re-advancement and retreat of the ice front gorged-out the channel producing a lake with three distinct basins in the form of a chain. With the final advance and retreat of the glacier, ice blocks were fractured loose, melted, and produced a varient kettle-type multibasin channel lake. The lake is well protected along its shores by elevated remnant shorelines and elevated bedrock, covered by coarse, unsorted moranic deposits. The north basin is approximately 20 meters deep, the central basin is 30 meters deep and the south basin is 15 meters deep. West Blue Lake is 4.8 kilometers long and 0.52 kilometers wide at the widest point. Mean depth of the lake is 11.3 meters and it is 160 ha. in area.

West Blue Lake stratifies thermally in late May or early June. Complete or partial mixing usually occurs in late May and October, with the period of mixing in May generally being incomplete. The lake is covered with ice from early November until spring breakup in mid to

late May. Two stations chosen for sampling were Station 5, located in the most northerly basin, and Station 2, located in the central basin (Bell and Ward, 1968). Of the four basic experimental procedures used in this study, three were applied at Station 5, on account of its convenience to the laboratory. The remaining procedure was applied at Station 2. Although no comparison was made of the two stations during this research, previous analysis of variance in primary productivity data obtained at both stations had shown them to be similar (Ward, F.J., Pers. Commun.).

2. Experimental Procedures

In situ experiments designed to measure the heterotrophic uptake of selected organic acids by natural lake populations were primarily based on the procedures of Parsons and Strickland (1962) and Wright and Hobbie (1965); except for a few modifications which will be discussed later in this chapter. Basically, the experiments consisted of exposing natural populations "in situ" to a labelled organic acid substrate for a certain length of time and then determining the amount of radioactivity incorporated. Using these data, incorporation rates could be calculated which then could be applied to enzyme kinetics analysis.

(A) Preparation and Standardization of ^{14}C-labelled Organic Acids

Nine different ^{14}C-labelled organic acids were used as substrates for various heterotrophy experiments. These were acetic, citric, formic, fumaric, glycolic, lactic, L-malic, pyruvic and succinic acids. All radioactive chemicals used for this study were supplied by Amersham-

Searle as a lyophilized sodium salt in quantities of 50 or 100 μC_i per glass ampoule. Each ^{14}C-organic acid was diluted with 0.2 μ millipore filtered autoclaved, distilled water to a final approximate activity of 1.0 μCi-substrate / 1.0 milliliter of aqueous solution. The isotope, prepared as such, was further filtered through a 0.2 μ millipore filter to remove any large particulate contaminants which were found to occur in some of the ^{14}C-labelled substrates. Each isotope was prepared in this manner, dispensed into 30 ml screw cap bottles and frozen until required. The actual activity of each isotope was obtained by adding 5 λ aliquots of 0.2 μ membrane filtered isotope to 10.0 mls of Bray's (1960) dioxan based fluor along with one membrane filter (0.45 μ, 25 mm Millipore HA) which had been wetted with distilled water. Six replicates were made for each standardization. Activity of the standards were determined by liquid scintillation (Appendix 1).

(B) Determination of the Saturation Points for the Nine Labelled Substrates

This set of experiments was designed to measure levels at which saturation responses were produced by adding increasing amounts of ^{14}C-labelled isotope to samples containing natural lake water populations.

Lake water samples were collected from the experimental depth (5 m) at Station 5 with a 4 liter PVC Van Dorn sampler. The sample was passed through a 153 μ Nitex screen to remove the majority of zooplankton and then returned to the laboratory in a blackened 4.0 liter plastic jar. Aliquots of 25 mls were dispensed into each of 26 light, 30 ml bottles.

An additional 13-30 ml bottles were used for control determinations. Each control consisted of 25 mls of 0.2 μ millipore filtered, autoclaved lake water (25 mm Millipore HA). The following increasing amounts of standardized isotope were added to replicate pairs of sample bottles and sterile water controls, 0.01, 0.02, 0.05, 0.07, 0.1, 0.2, 0.5, 0.7, 1.0, 1.2, 1.5, 1.7 and 2.0 ml. The activity of the isotope was approximately 1.0 μCi/ml with the exception of succinic acid which was approximately 0.1 μCi/ml. The isotope was dispensed by means of 0.2 ml and 2.0 ml Gilmont micrometer burets. The bottles were then placed in a light-tight box and returned to Station 5 where the bottles were placed on a suspension apparatus and returned to the lake at an "in situ" depth of 5 meters. The samples were incubated for 12 hrs (10:00 a.m. to 10:00 p.m.). Following incubation the samples were removed from the lake, placed in the light-tight box and returned to the laboratory for processing. Each sample was filtered through a 0.45 μ Millipore HA filter (25 mm diameter) under mild vacuum (<20 mm Hg). Following filtration of the sample each filter was washed with 100 mls of distilled water to remove unincorporated and absorbed ^{14}C-organic acid. The filters from each sample were then placed directly into scintillation vials containing 10.0 mls of Bray's (1960) fluor. Activity was measured by liquid scintillation.

(C) The Effect of Time on the Heterotrophic Uptake of Nine Labelled Substrates.

Several experiments were conducted to determine changes in the rate of uptake of organic acids with time, and to establish the validity of the 12 hr incubation time used in the saturation experiments.

Water samples were collected as above and returned to the laboratory in a darkened 4.0 liter plastic jar. Aliquots of 25.0 mls were dispensed into 12 light-30 ml bottles, and 12 dark-30 ml bottles (darkened with black plastic tape). Each bottle was then incubated with 1.0 ml (approximately 1.0 μCi) of labelled isotope (approximately 0.1 μCi in the case of succinate). The 12 light and 12 dark 30 ml bottles were then placed in a light-tight box and returned to Station 5, suspended at an "in situ" depth of 5 meters. The incubation period began at 10:00 a.m. for each experiment and sampling, consisting of removing three light and three dark bottles was performed at 6 hrs, 12 hrs, 24 and 48 hrs. The six sample bottles, after being removed from the lake were placed in a light-tight box and returned to the laboratory for filtering. Filtration and counting procedures were the same as previously described.

Previous experiments had indicated that not all of the ^{14}C-organic acid could be removed from the membrane filters by washing, so control blanks were prepared in order to determine the mean amount of ^{14}C-labelled substrate which was retained after washing with 100 mls of distilled water. Three blanks, each consisting of 25 mls of 0.2 μ millipore filtered lake water (25 mm Millipore HA) and 1.0 ml of ^{14}C-labelled isotope were prepared The blanks were then incubated in a refrigerator for 6 hrs at approximately 5-10^{o}C. Following incubation the blanks were removed, filtered and washed with 100 mls of distilled water. The filters were then removed from the filtering apparatus and each filter added to individually marked scintillation vials containing 10.0 mls of Bray's (1960) fluor. Activity was determined by liquid scintillation.

(D) The Heterotrophic Uptake of Three Labelled Substrates with Depth

A number of experiments were conducted to determine the extent of organic acid uptake throughout a water column.

Water samples were collected at Station 2 with a 2 liter PVC Van Dorn sampler from the following depths-0,3,7,12,17,20,25 and 30 meters which were representative of the entire water column. Each water sample was passed through a 153 μ Nitex screen to remove the majority of the zooplankton. Two 30 ml bottles were filled with 25 ml aliquots from the water sample for each of the mentioned depths. In total there were 16 bottles. Each sample was then inoculated with 1 ml (approximately 1 μCi), of ^{14}C-labelled isotope (approximately 0.1 μCi in the case of succinate). As in other experiments a sterile water control blank was prepared for each set of samples from each of the sampling depths. All sample and control bottles were incubated at "in situ" depths for a period of 4 hrs (11:00 a.m. to 3:00 p.m.). Following incubation all bottles were returned to the laboratory with minimum delay, filtered, washed and prepared for scintillation counting as previously described. Succinic, lactic and malic acids were used in three such experiments.

(E) The Uptake and Excretion of ^{14}C by Natural Populations

On four occasions (19/5/71; 23/6/71; 30/7/71; and 1/9/71) experiments were conducted which were designed to measure uptake of $^{14}CO_2$ by natural lake populations and to measure the relative rates and amounts of labelled excretory products.

For each experiment 4.0 liter lake water samples were collected

from Station 5 at 5 meters with a PVC Van Dorn sampler. The lake water sample was then passed through a 153 μ Nitex screen to remove most of the zooplankton and was returned to the laboratory in an opaque plastic jar. Forty μCi of ^{14}C-Na-bicarbonate was added to a 1000 ml aliquot of the lake water sample yielding a concentration of 5.0 μCi / 125 ml. Aliquots of 25 mls of this labelled sample were immediately dispensed into 16 light and 16 dark - 30 ml bottles. After filling, the glass sample bottles were placed in a light-tight box and returned to Station 5, attached to a suspension apparatus and suspended at 5 meters. The incubation period began at 10:00 a.m. and lasted from 12 to 24 hrs. At 2 hr intervals 2 light and 2 dark bottles were returned to the laboratory in a light-tight box. Once in the laboratory each sample was shaken lightly and exactly 10.0 mls removed by syringe. Each 10.0 ml sub-sample was then filtered through a 0.45 μ membrane filter (Millipore HA) with the filtrate being collected below in a 125 ml vacuum flask. All filtration was done under mild vacuum. The procedure was conducted simultaneously for all four samples (2 light and 2 dark bottles) for each sampling period. Each sample was filtered to dryness, followed by removing the filter from the filtration apparatus and fuming for exactly 1 min over HCl. Upon completion of fuming each filter was placed in the appropriately labelled scintillation vial containing 10.0 mls of Bray's (1960) fluor. The filtrate collected from each sample (10 mls) was transferred to individually marked 30 ml glass bottles. Each filtrate was acidified to a pH of 2.0 with 2 N HCl. Each sample was

then shaken to disperse the acid and then aerated for 10 mins. Subsequently, a 1.0 ml aliquot was removed from each sample by syringe and injected into the appropriately labelled scintillation vial containing 9.0 mls of Bray's (1960) fluor. The above procedure was repeated at the appropriate sampling periods during the experiment.

During each experiment a number of separate factors were measured and recorded. Transparency was measured at Station 5 from 0 to 17 meters using a submarine photometer (T.S. Hardy Luxmeter). Dissolved carbon dioxide content (mg/l) and total alkalinity (mg/l were measured for water samples from 5 meters at Station 5 titrimetrically (Standard Methods of Waste Water Treatment).

(F) Assessment of Filtration Effect

In conjunction with one uptake and excretion experiment (30/7/71) an attempt was made to determine the extent, if any, of a filtration effect (Arthur and Rigler, 1965). The experimental procedure was basically the same as that described above for the uptake and excretion experiments. It differed in that sampling was done at four hr intervals during the first 12 hrs, with the fourth and final sampling taking place 12 hrs after the third sampling. This required a total of 8 light and 8 dark bottles. Additionally, in order to measure any filtration effect 8 further light bottle samples were required, two bottles per each sampling period. The contents of both bottles were combined (50.0 mls) and aliquots of increasing volume (1,3,5,7,10 and 20 mls) were each filtered through 0.45 μ membrane filters (25 mm Millipore

HA) under mild vacuum. The filtrate of each aliquot was collected in separate vacuum flasks. Following filtration, the filters were fumed over HCl for 1 min and then placed in the appropriately labelled scintillation vial containing 10.0 mls of Bray's (1960) fluor. The filtrate of each subsample was then acidified to a pH of 2.0 with 2 N HCl, and bubbled with air for 10 mins. A 1.0 ml aliquot was removed from each of the subsample filtrates and injected into labelled vials containing 9.0 mls of Bray's (1960) fluor. This procedure was repeated for each of the four sampling periods. Activity of all samples was counted by liquid scintillation.

(G) Radioactivity Measurements

As described, all samples were filtered onto membrane filters which were placed in individual scintillation vials containing Bray's (1960) fluor. Radioactivity was measured using a liquid scintillation counter (Picker Liquimat 220), adjusted to a preset statistics of 1.5% $\pm$ 2 σ, which was equivalent to 16,384 counts per min. Each sample was either counted until the statistics level was reached or for a maximum counting time of 20 mins. Counting efficiency, determined by the channels ratio method (Wang and Willis, 1967) varied from 70 to 80%, and all activities were expressed in terms of DPM.

3. Calculation of Uptake Data

(A) Saturation Curve Data

The saturation curve data for each of the nine organic acids

used was treated according to the rate formula of Parsons and Strickland (1962).

$$V\ (\ \mu gC\ l^{-1}hr^{-1}) \quad \frac{c.f.\ (S + A)}{C\ \mu t}$$

where:

V = velocity of uptake ($\mu gC\ l^{-1}hr^{-1}$ at a given substrate concentration

c = radioactivity of sample as collected on membrane filter (DPM/25 ml)

S = concentration of a given substrate present in the natural sample ($\mu gC/l$). In this case, the S value was neglected because the amount of added labelled substrate was always sufficiently greater.

A = the concentration of the added substrate ($\mu gC/l$)

C = the count per min from 1 μCi of C-14 in the counting apparatus used, ie) 2,220,000 DPM

μ = the amount of microcuries of substrate added to the sample bottle (25 ml)

t = incubation time in hours

f = a factor to compensate for the effect of any isotopic discrimination that may occur against the ^{14}C isotope as compared with the normal ^{12}C atom (1.05).

Solving for V, and plotting V on the ordinate versus A on the abscissa, an asymptotic Michaelis-Menten curve was constructed for each of the nine different labelled organic acids which were used as substrates. This enabled one to ascertain the zone of zero-order kinetics or in other words, the substrate concentration at which saturation began. This information, besides being used for further expression of the saturation

type response gave an indication of substrate amounts that should be used for the subsequent uptake with time and uptake with depth experiments where substrate amounts used were in the range which initiated saturation. Analysis of the data is further facilitated if the Michaelis-Menten curve is converted into its linear form using a modified Lineweaver-Burk plot (Wright and Hobbie, 1965) which follows the following equation:

$$\frac{S + A}{v} = \frac{K + S}{V} + \frac{A}{v}$$

where:

v = uptake rate at a defined substrate concentration

V = maximum or saturation rate; and,

K = the transport constant

This equation is arrived at by taking the original Michaelis-Menten equation:

$$v = \frac{V(S)}{K+S}$$

and multiplying the inverse of both sides by S giving:

$$\frac{S}{v} = \frac{K}{V} + \frac{S}{V} \qquad (1)$$

however, in the case of uptake kinetics of natural populations, the amount of natural substrate is not known therefore the substrate concentration (S) is really the added substrate (A) plus the unknown amount of naturally occurring substrate (S), i.e. (S + A)

$$\therefore \quad \frac{S}{v} = \frac{K}{V} + \frac{S}{V} \quad \text{can be written,}$$

$$\frac{S + A}{v} = \frac{K}{V} + \frac{S + A}{V} \qquad (2)$$

but by rearranging the original Parsons-Strickland formula we get:

$$\frac{C\mu t}{c} = \frac{f.\ (S + A)}{v}$$

or if f is disregarded:

$$\frac{C\mu t}{c} = \frac{(S + A)}{v} \qquad (3)$$

therefore by substituting equation (3) into equation (2) we get:

$$\frac{C\mu t}{c} = \frac{K}{V} + \frac{S + A}{V} \qquad (4)$$

by rearranging equation (4) we get:

$$\frac{C\mu t}{c} = \frac{(K + S)}{V} + \frac{A}{V}$$

When $\frac{C\mu t}{c}$, as the ordinate, is plotted against A on the abscissa, a straight-line results with the V_{max} equal to the reciprocal of the slope, the intercept on the abscissa equal to -(K + S), and the point where the line intersects the ordinate equal to the turnover time (T). T is the time (hr) required for the substrate to be entirely removed by the natural population (Wright and Hobbie, 1966).

All Michaelis-Menten plots were converted to linear Lineweaver-Burk plots and analysed in this method. Following such treatment all nine substrates were ranked according to V_{max} values, which reflect substrate preferences by the natural populations. (Robinson, Hendzel and Gillespie, 1972).

(B) The Effect of Time on the Heterotrophic Uptake of Nine Labelled Substrates

This experiment was repeated for all nine of the test substrates. Gross uptake (DPM) was calculated for each sample and means of the triplicates were calculated. The mean activity of the controls was then substracted

from this value yielding net uptake as DPM. Using this data and substituting it in to the velocity formula (Parsons and Strickland, 1962), velocity of uptake ($\mu gCl^{-1}hr^{-1}$)was calculated for the mean values of the light and dark samples for each of the sampling periods.

(C) The Heterotrophic Uptake of Nine Labelled Organic Substrates with Depth

Mean gross uptake (DPM) was calculated from the replicate samples for each of the sampling depths. Net uptake was calculated by substracting the appropriate control blank value. Uptake velocity was then calculated for each of the sample depths by using the uptake velocity formula of Parsons and Strickland (1962). Uptake velocity was then plotted against depth for the three substrates used.

An attempt was made to calculate heterotrophic productivity beneath one square meter of surface area of lake water (Station 2) for each of the three organic acids used. Net uptake values (DPM) were mathematically converted into carbon/volume values (μgC-substrate / M^3). The resulting data were plotted on graph paper, μgC-substrate/M^3 versus depth (meters). In order to determine the total amount of carbon fixed beneath one square meter of lake water surface (Station 2), the area beneath the graphed line was measured with a planimeter and this value was then converted into mgC-substrate fixed / $meter^{-2}/hr^{-1}$.

(D) The Uptake and Excretion of ^{14}C by Natural Populations

For these experiments mean gross uptake of $^{14}CO_2$ and excretion of labelled material was calculated for both light and dark samples.

All gross figures were calculated in terms of a constant volume - 25 mls. For this thesis only the filtrate (excretion) data was considered. All filtrate data (DPM/25 ml) were applied to a primary productivity equation (Strickland and Parsons, 1961).

$$\frac{Y}{Z} \cdot K \cdot W \cdot 1000 = \text{productivity } (mgC/M^3)$$

where:

Y = activity of filtrate in DPM

Z = activity of ampoule in DPM (2,220,000)

K = correction factor for isotopic discrimination (1.05)

W = (a) + (b)

where:

$(a) = \frac{12}{44} \times CO_2$ (mg/l)

$(b) = \frac{12}{100} \times$ alkalinity (mg/l)

In this way relative production of labelled excretory products was computed.

Relative re-assimilation rates of the excreted radioactive products were computed from the excretory data of the four experiments. In all cases, except for part of the dark filtrate data of experiment #3, the initial rates of re-assimilation were the highest and the rates of carbon re-assimilation from the filtrates were computed by taking the difference between the initial excretion rate and each successive excretion rate of the remaining sampling periods. This difference was taken to indicate net re-assimilation of ^{14}C-labelled excretory products in terms of mgC M^{-3} hr^{-1} from the filtrate. The above procedure was followed for all four excretion experiments.

(E) Assessment of Filtration Effect

All activity values of the filters and filtrates were calculated in terms of a constant volume - 25 mls; in all cases. This was repeated for the data collected from the four sampling periods and was expressed in tabular form.

RESULTS

(A) Saturation Curve Responses

The results of the nine organic acids used in determining the saturation responses were graphically expressed as:

(a) Michaelis-Menten plots; and

(b) linear Lineweaver-Burk plots

Asymptotic relationships were attained between substrate added and velocity of uptake for all nine of the test substrates (Figs. 1-9). V_{max} values derived from Lineweaver-Burk conversions (Figs. 10-18) ranged from 142 x 10^{-3} µgC $l^{-1}hr^{-1}$ to 3 x 10^{-3} µgC $l^{-1}hr^{-1}$ (Table 1). Turnover times for the nine substrates as determined from the Lineweaver-Burk conversions ranged from 950 hrs to 0.0 hrs (Table 1). Finally, K+S values, also determined from the Lineweaver-Burk conversions ranged from 42.5 µgC $l^{-1}hr^{-1}$ to 0.0 µgC $l^{-1}hr^{-1}$ (Figs. 10-18). The velocity of uptake (v) for the nine test substrates varied from a range of 3.0 x 10^{-3} - 390.0 x 10^{-3} µg C $l^{-1}hr^{-1}$ for pyruvic acid to a range of 0.1 x 10^{-3} - 2.5 x 10^{-3}µgC $l^{-1}hr^{-1}$ for formic acid (Appendix 2). While velocity, expressed as $\frac{C\mu t}{c}$ varied from a range of 647 - 31,031 µgC $l^{-1}hr^{-1}$ for citric acid to 363 - 549 µgC $l^{-1}hr^{-1}$ for pyruvic acid (Appendix 3).

(B) Effect of Time on the Heterotrophic Uptake of Nine Labelled Organic Acid Substrates

The results for the uptake with time experiments for the nine organic acids used as substrates were variable in nature with no real definite pattern being obvious, however a few general trends could be noted amongst the nine acids which appeared to be worthy of comparison.

Figs. 1 - 9. The relationship between substrate concentration (A - μgC/l) and velocity of uptake (V - μgC $l^{-1}hr^{-1}$ x 10^{-4}) for nine organic acids in West Blue Lake, Manitoba, at a depth of 5 meters; Station 5, during July and August 1971.

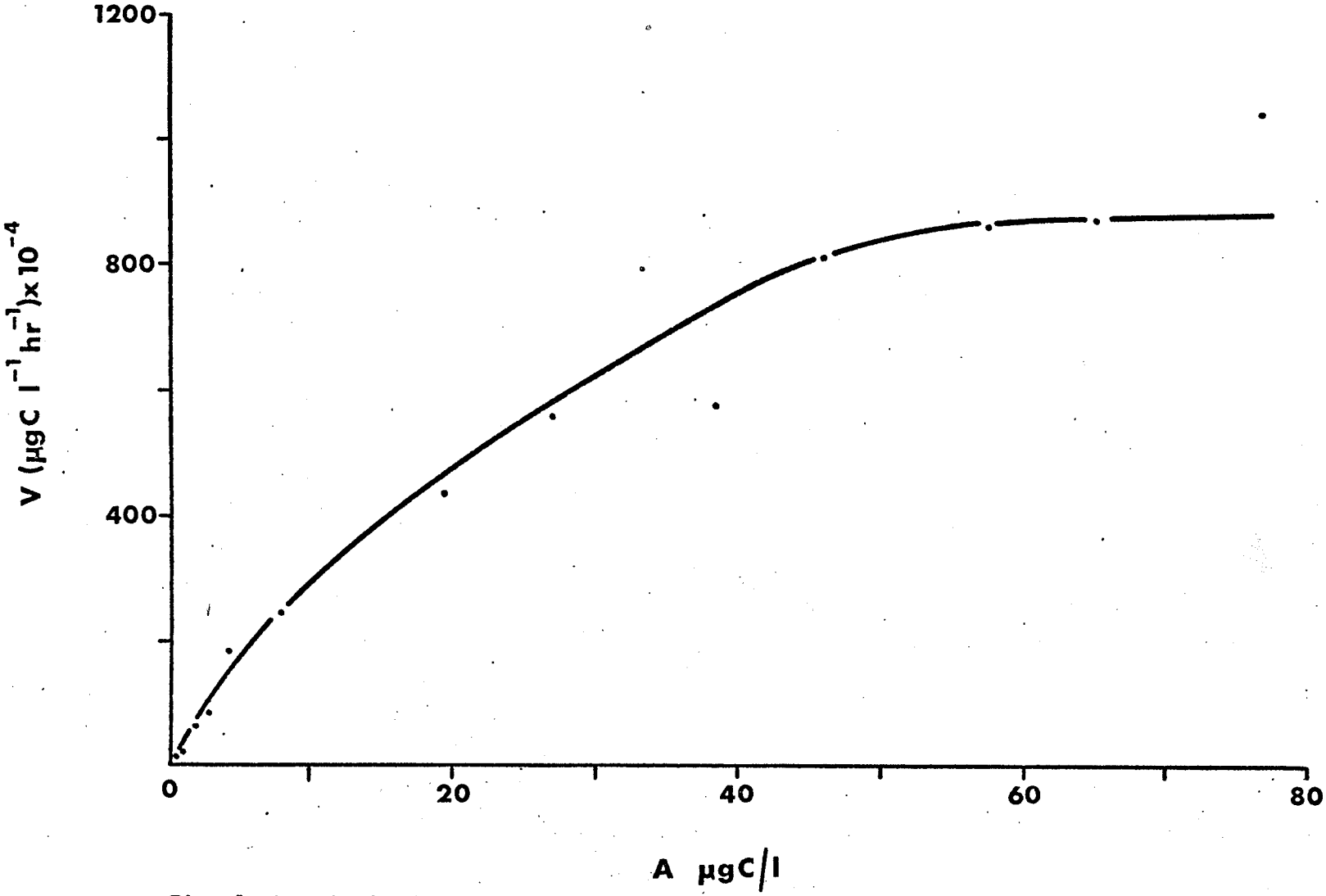


Fig. 1 Lactic Acid (25/8/71)

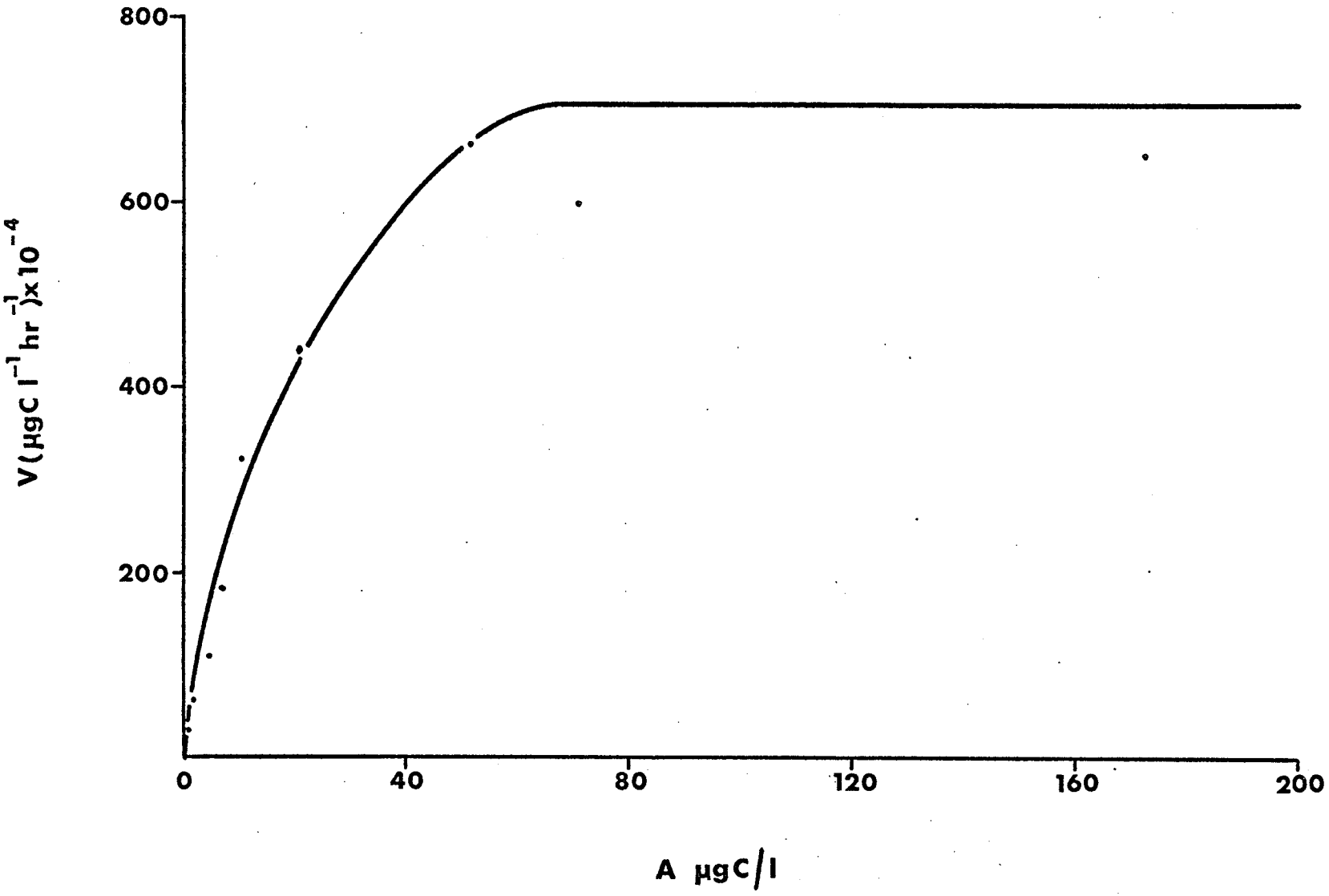


Fig. 2 Pyruvic Acid (31/7/71).

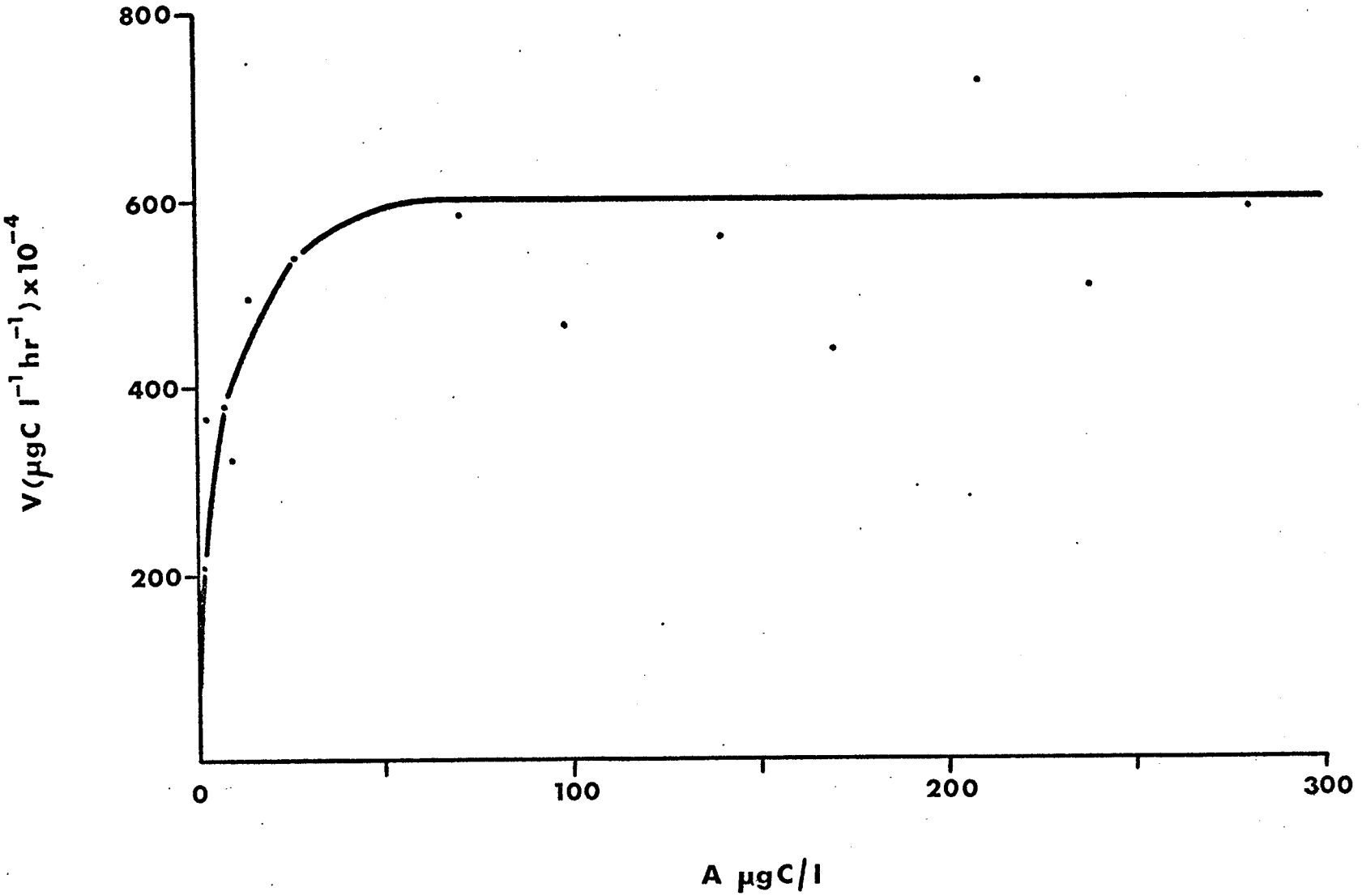


Fig. 3 Fumaric Acid (29/8/71)

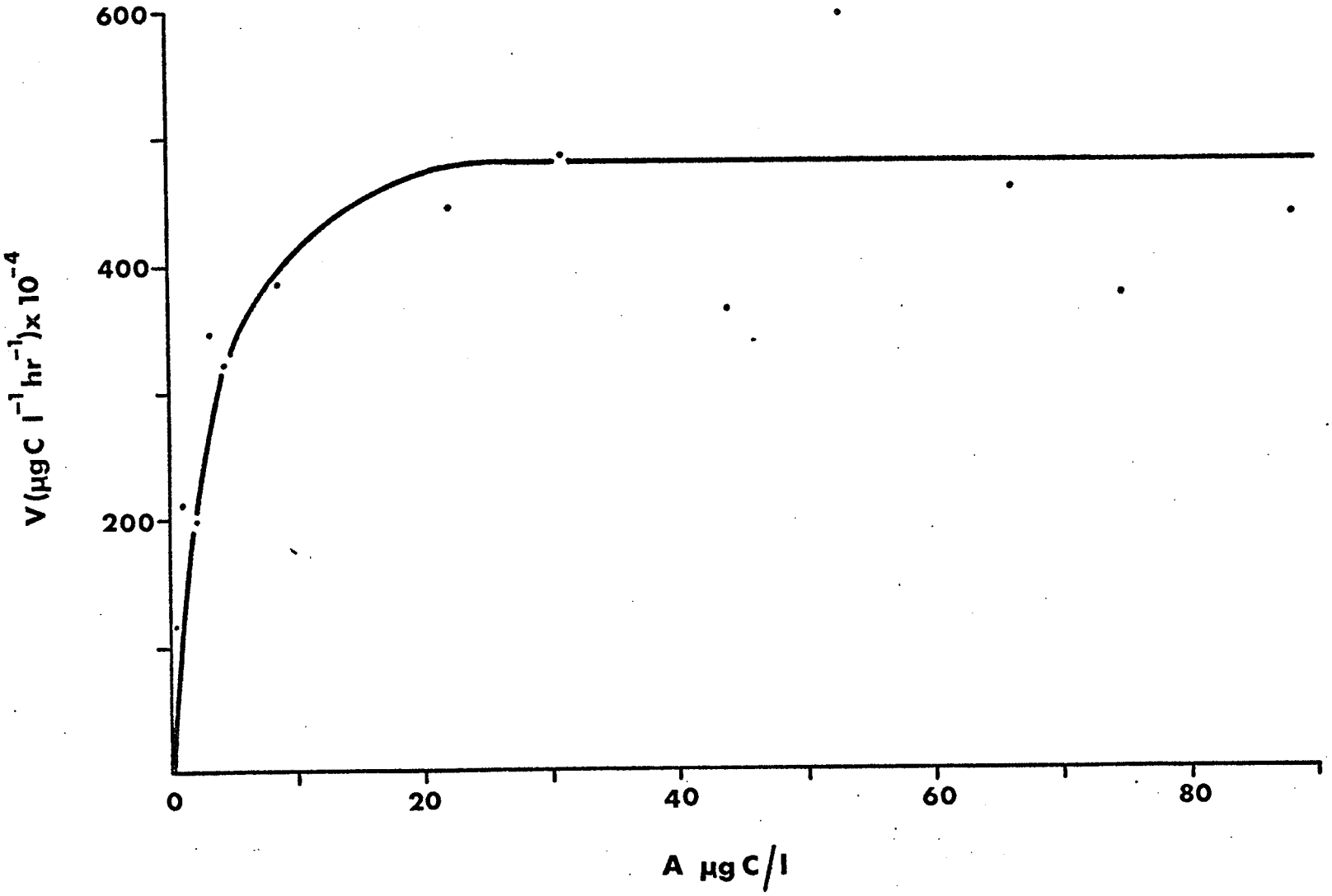


Fig. 4 Malic Acid (5/8/71)

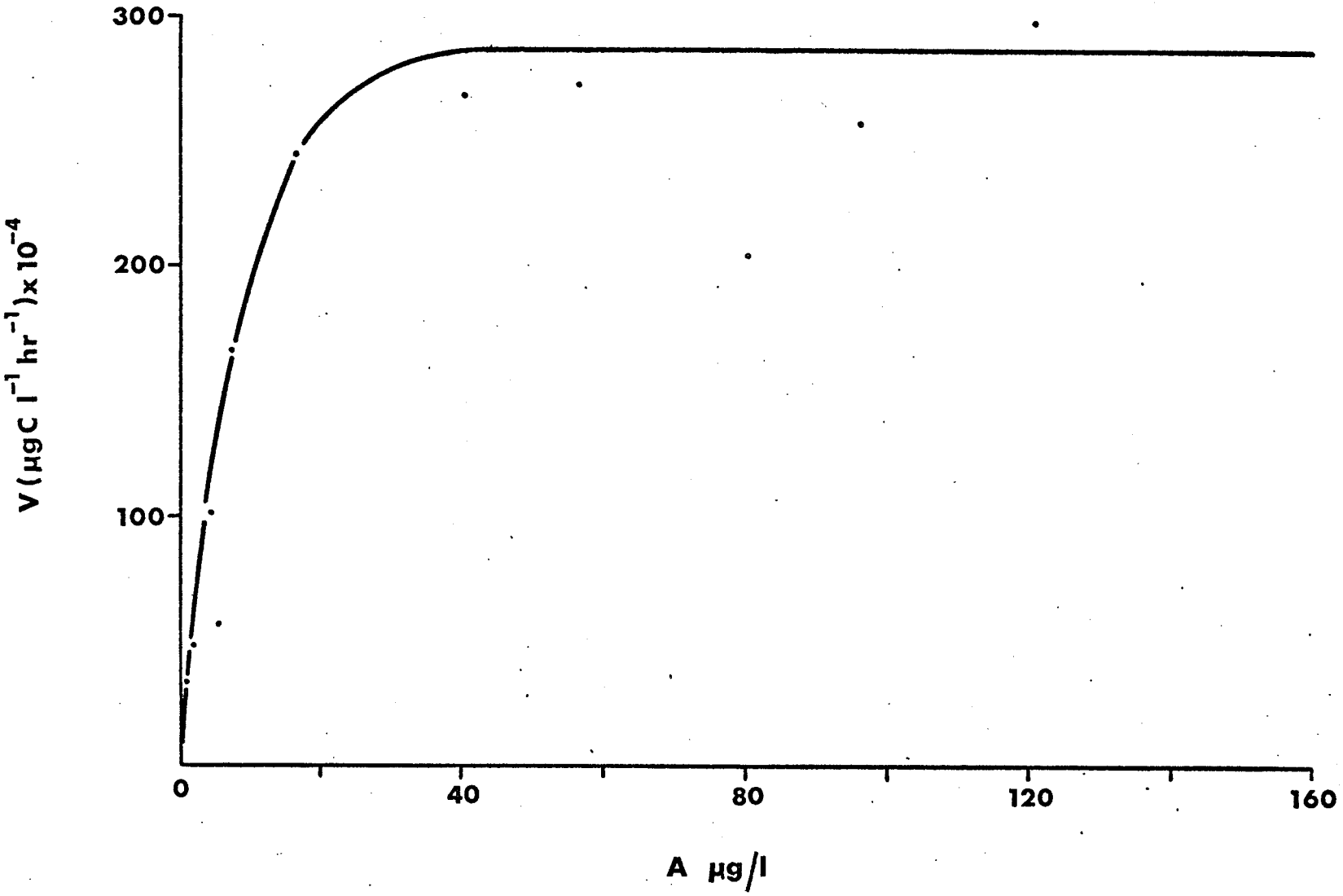


Fig. 5 Succinic Acid (8/7/71)

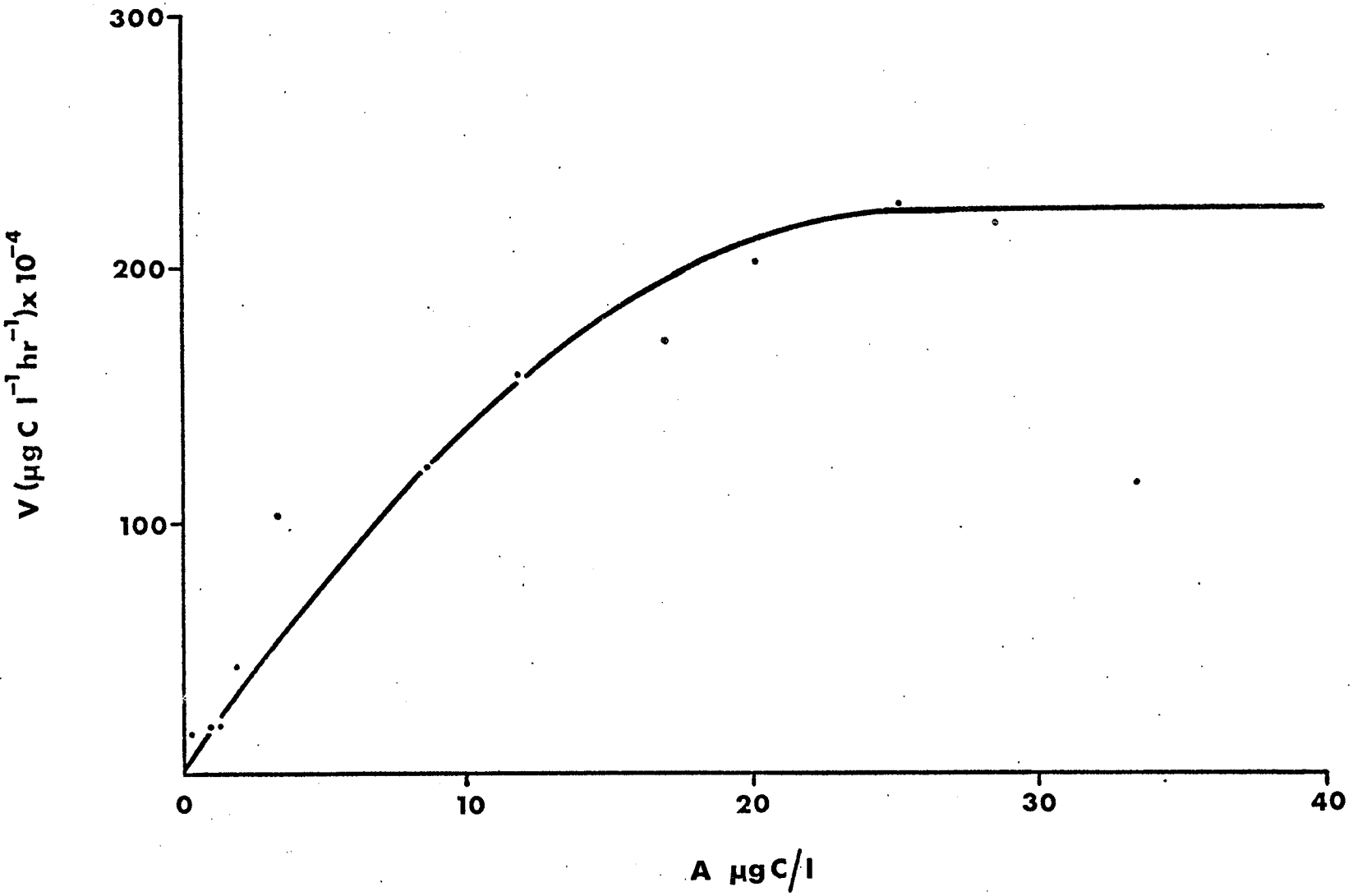


Fig. 6 Acetic Acid (12/8/71)

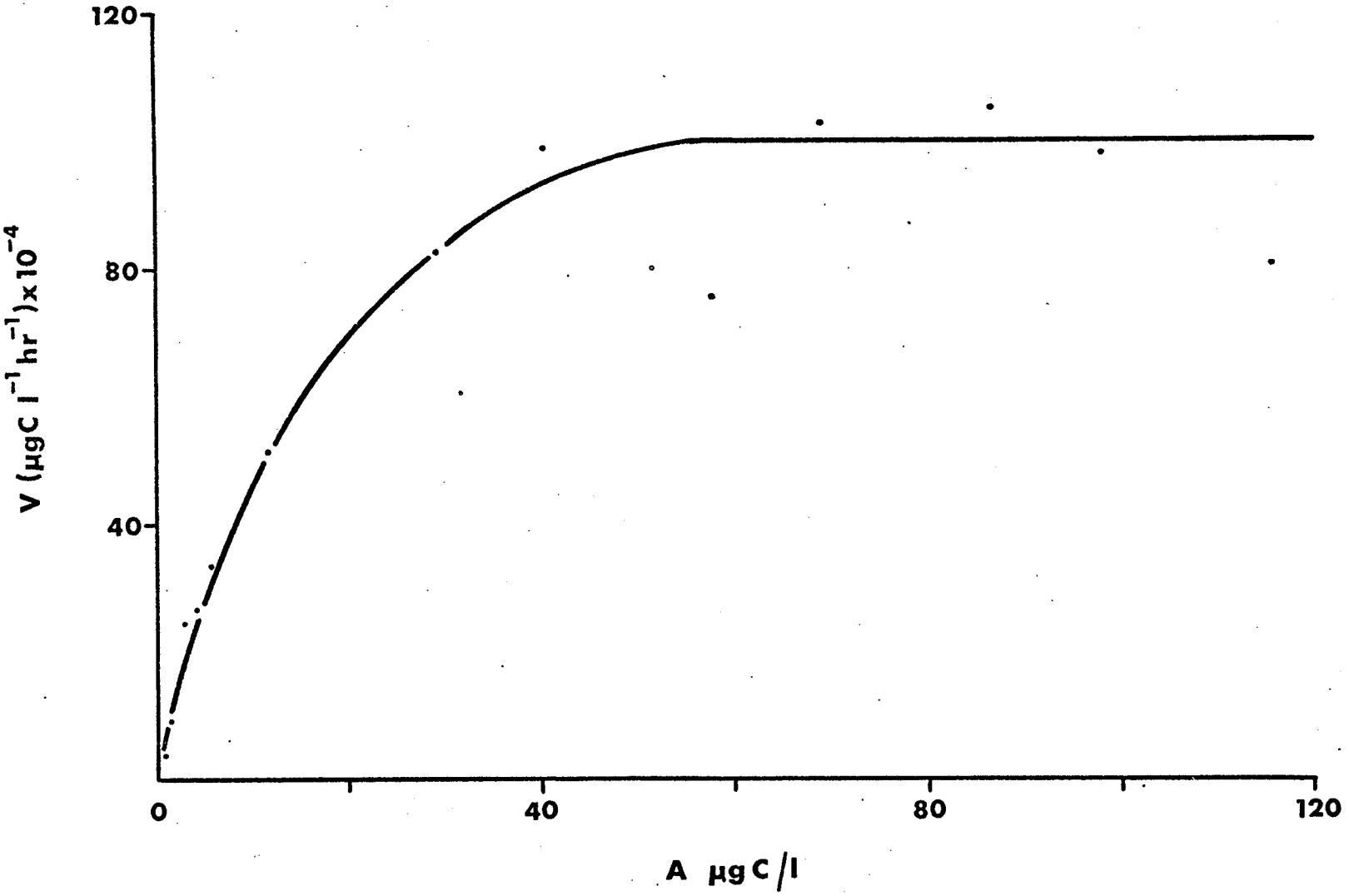


Fig. 7 Glycolic Acid (28/7/71)

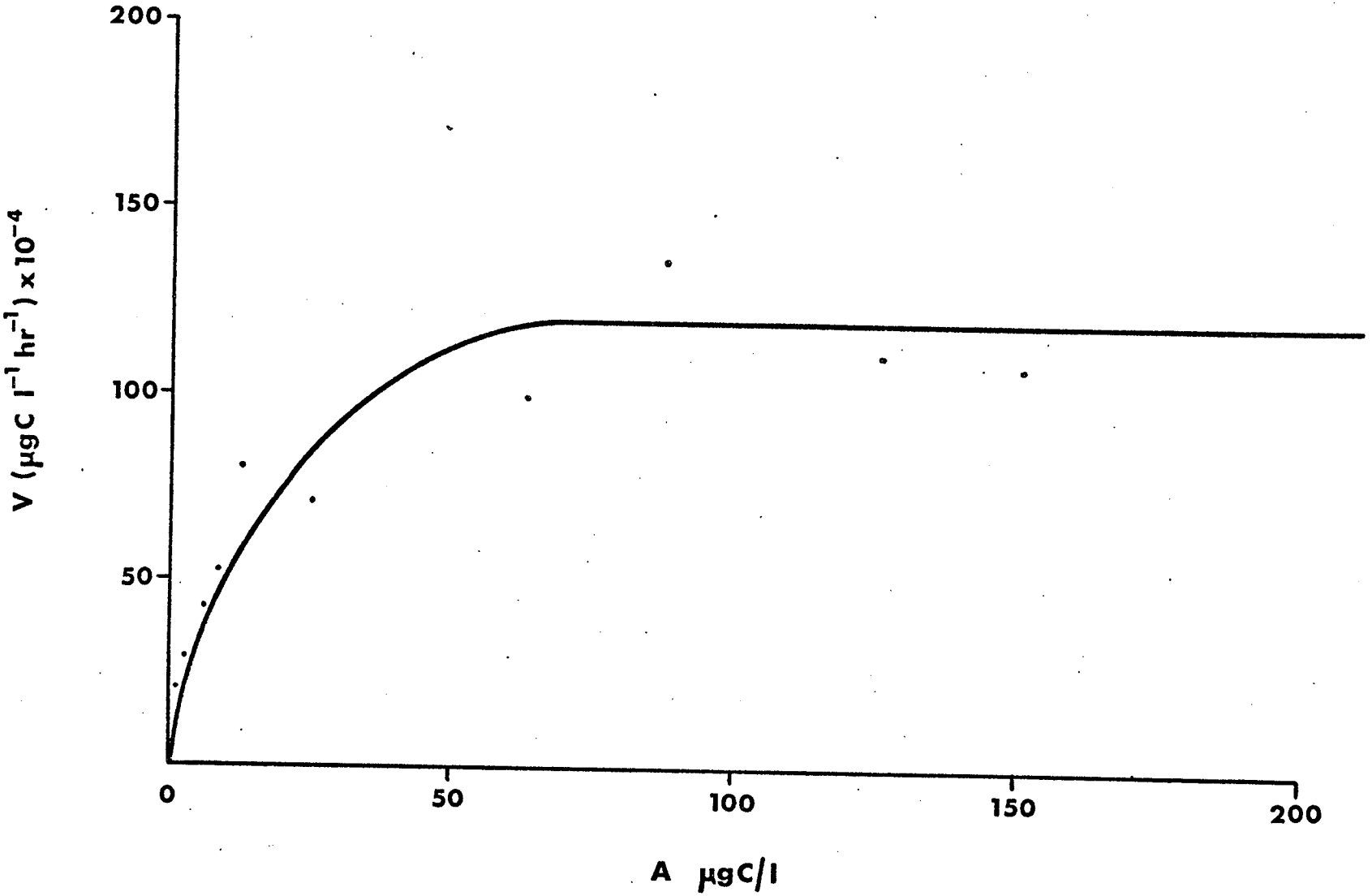


Fig. 8 Citric Acid (2/8/71)

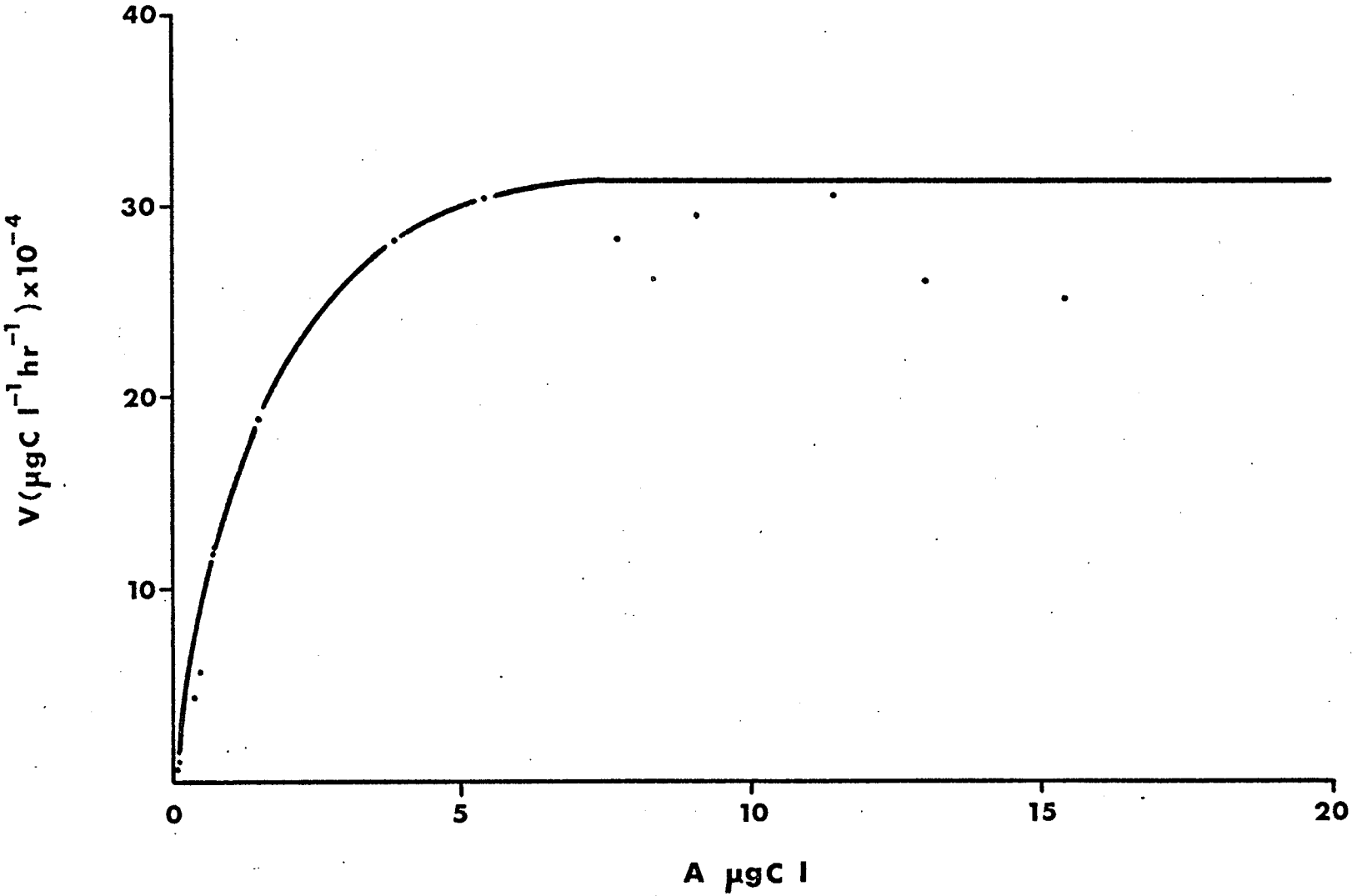


Fig. 9 Formic Acid (29/8/71)

Figs. 10 - 18. Lineweaver-Burk transformations of the Michaelis-Menten relationship between substrate concentration (A - μgC/l) and velocity of uptake ($\frac{C\mu t}{c}$) for nine organic acids in West Blue Lake, Manitoba, at a depth of 5 meters, Station 5, during July and August 1971.

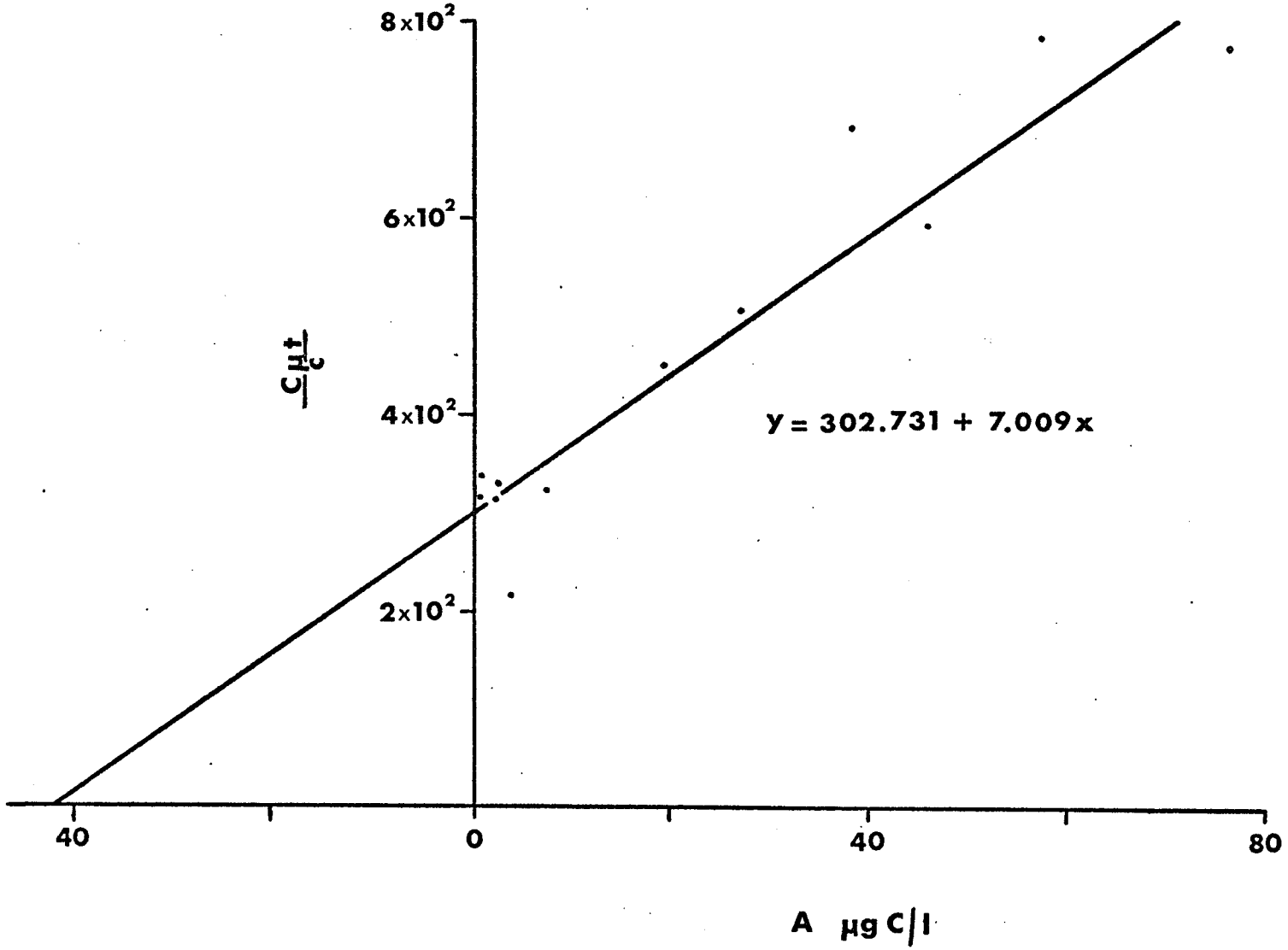


Fig. 10 Lactic Acid (25/8/71)

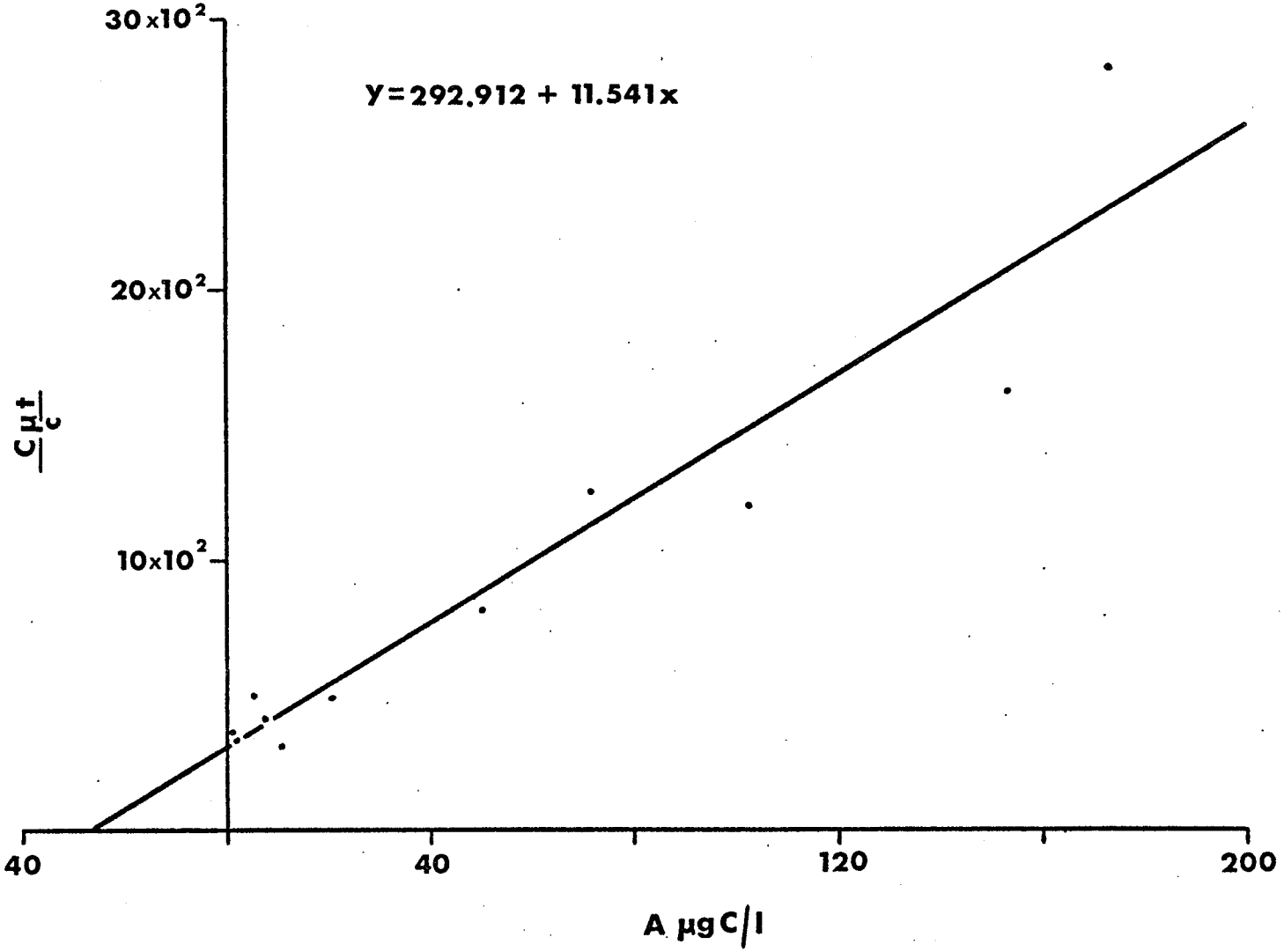


Fig. 11. Pyruvic Acid (31/7/71)

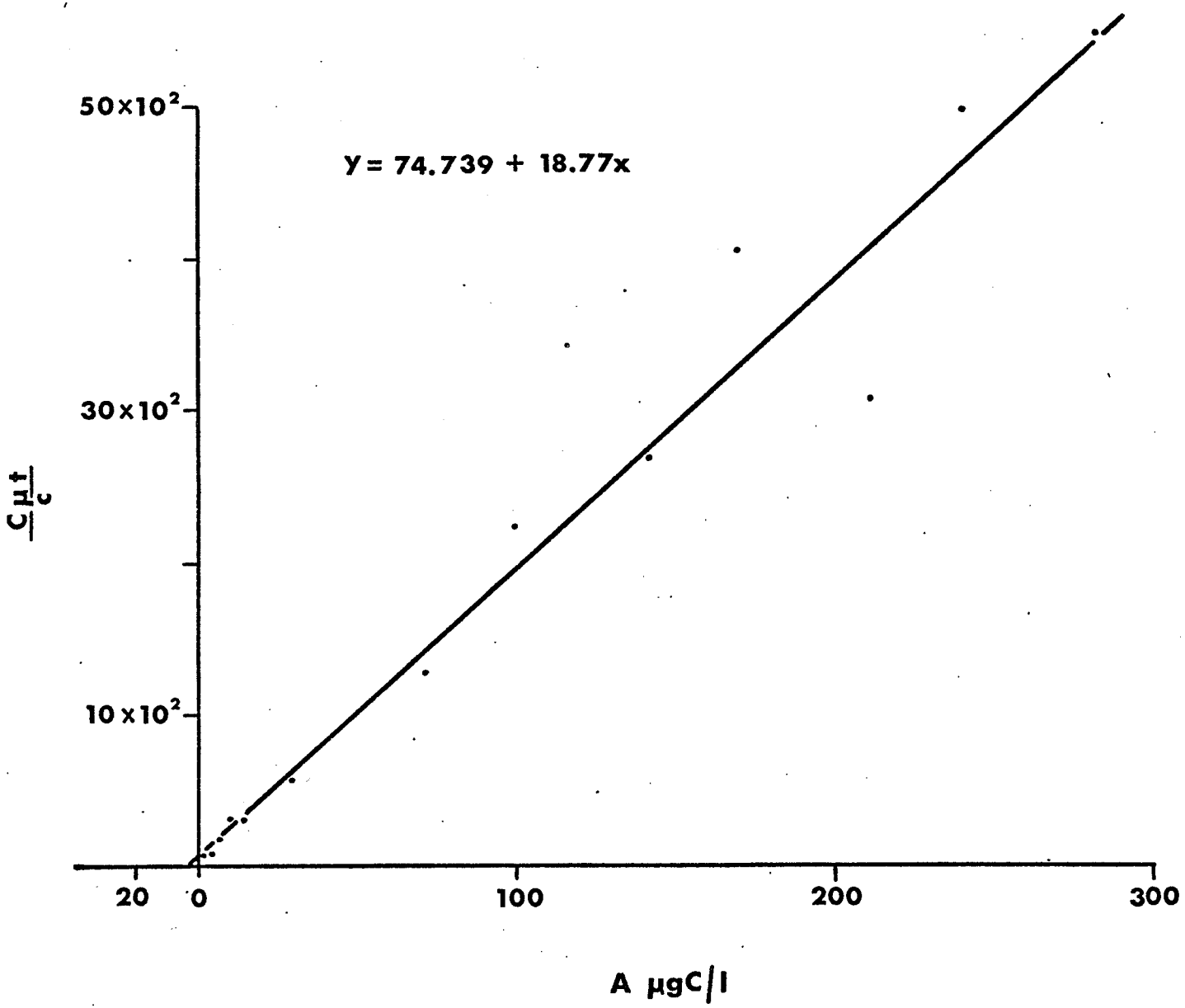


Fig. 12. Fumaric Acid (29/8/71)

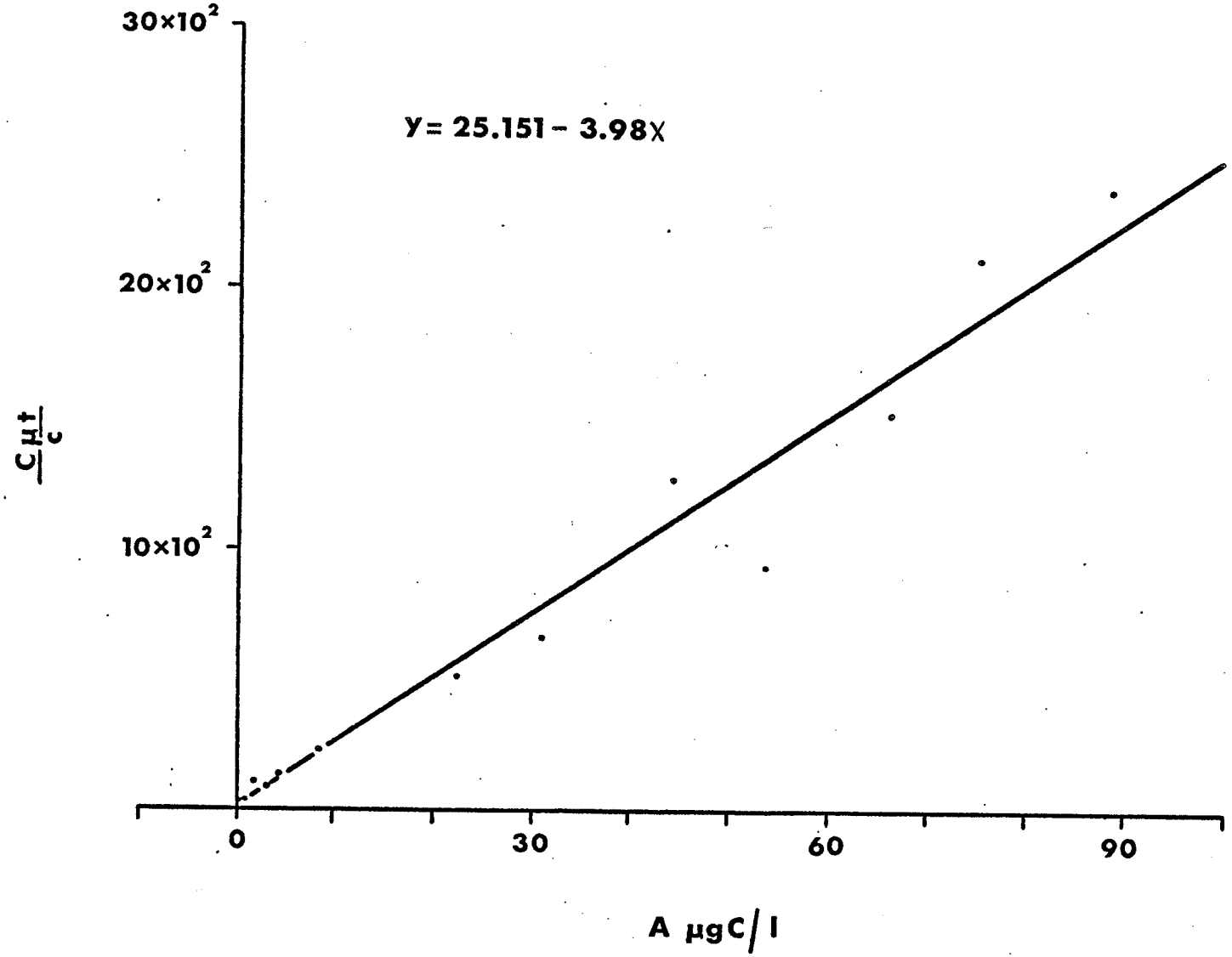


Fig. 13. Malic Acid (5/8/71)

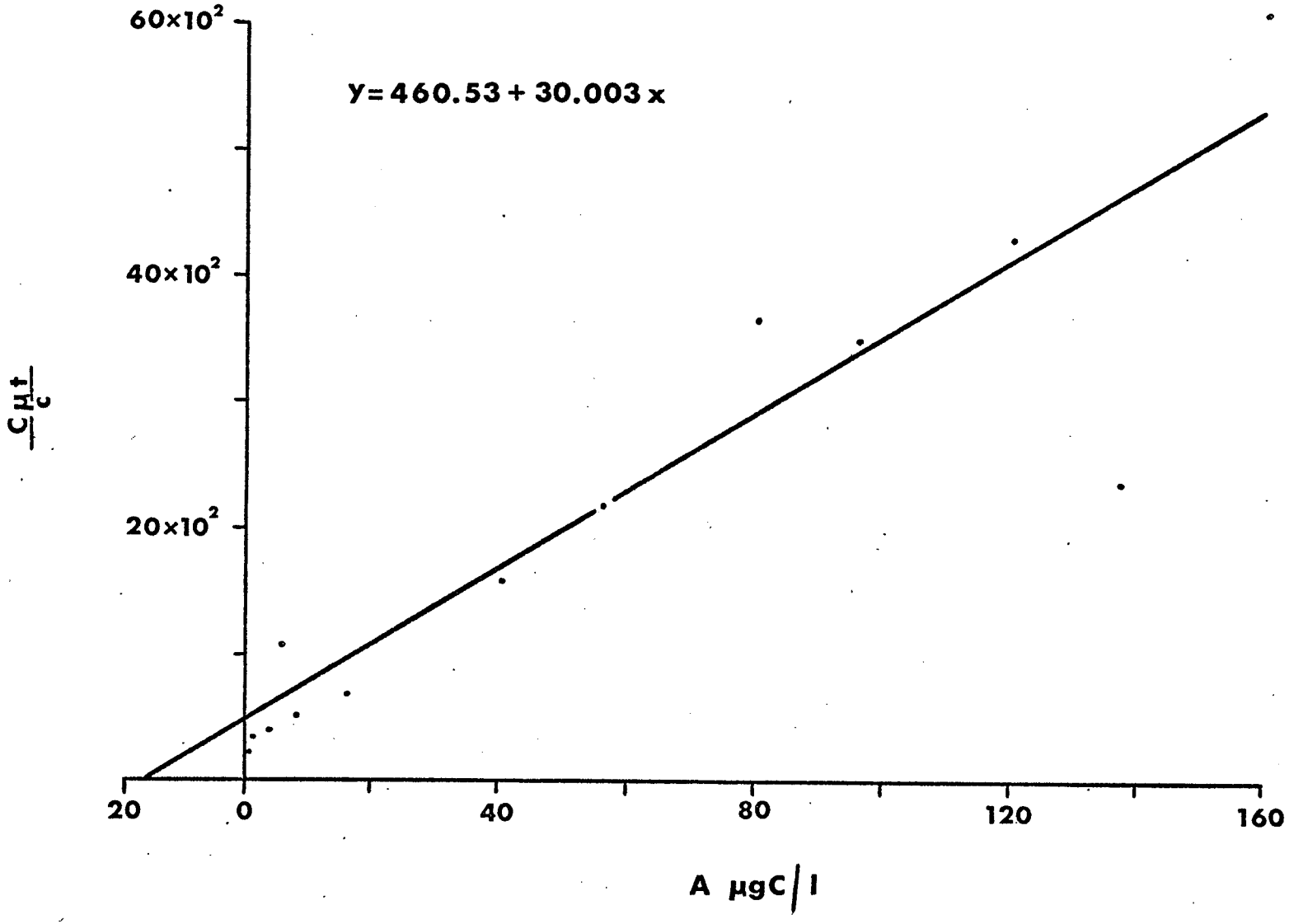


Fig. 14. Succinic Acid (8/7/71)

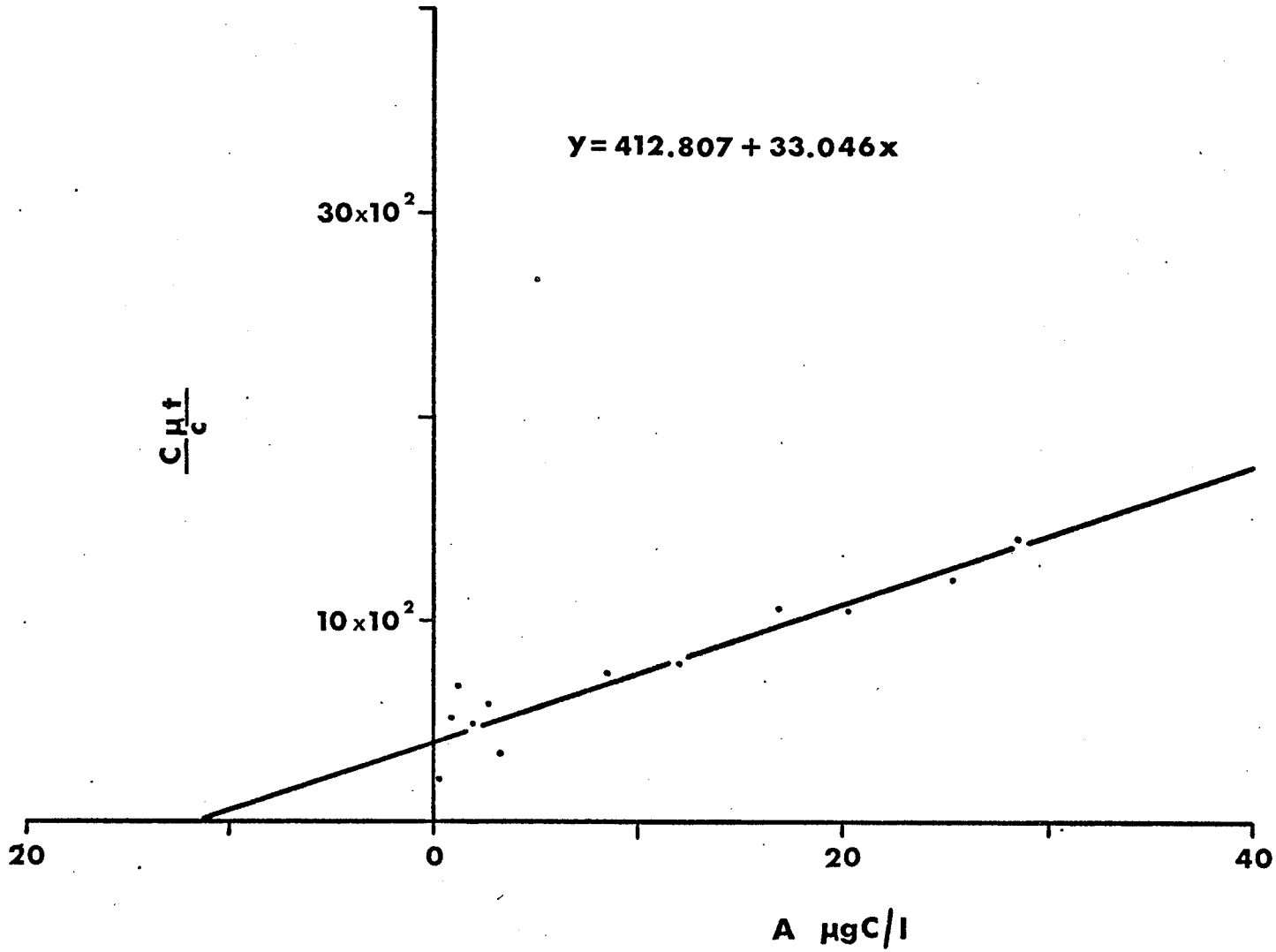


Fig. 15. Acetic Acid (12/8/71)

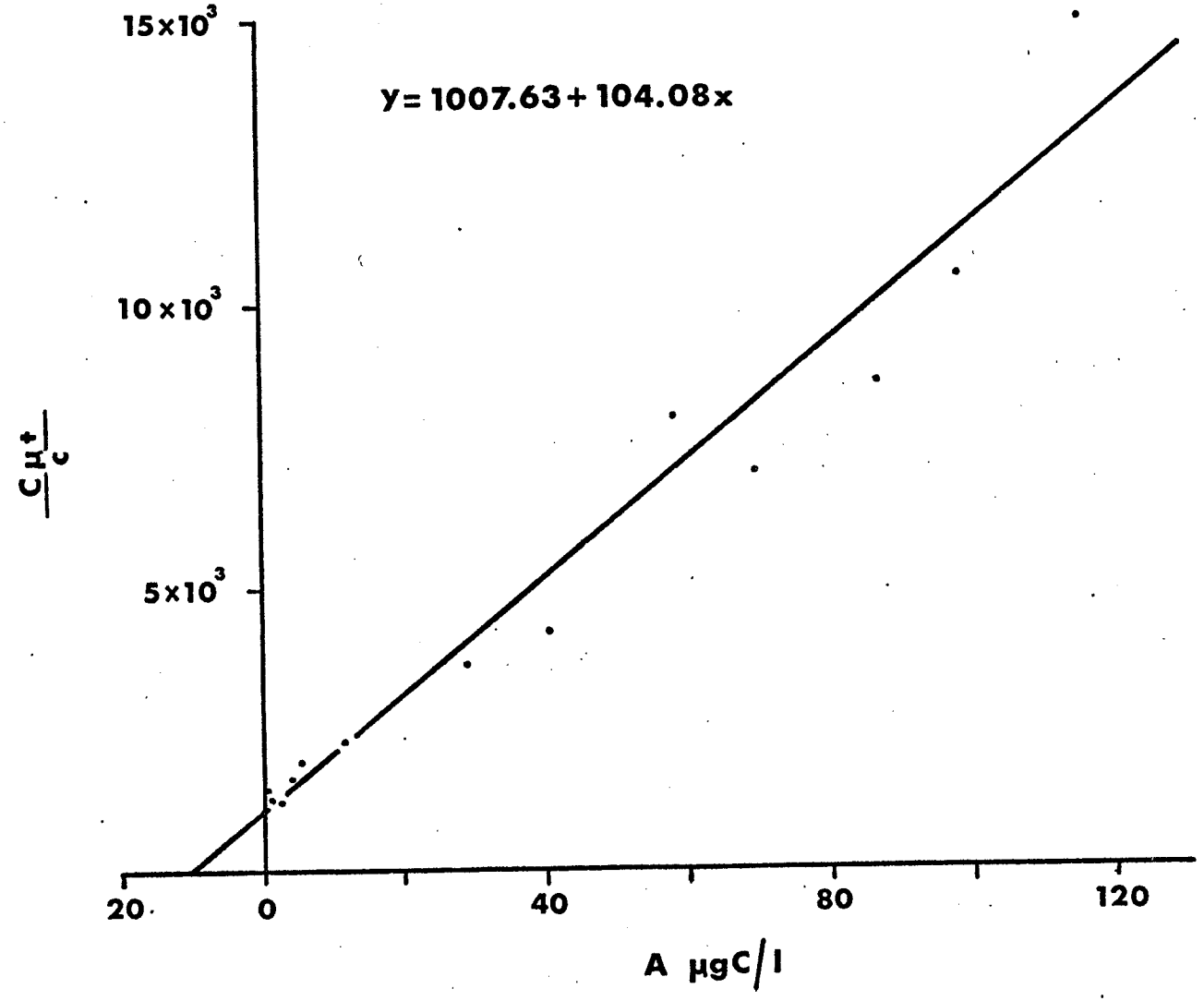


Fig. 16. Glycolic Acid (28/8/71)

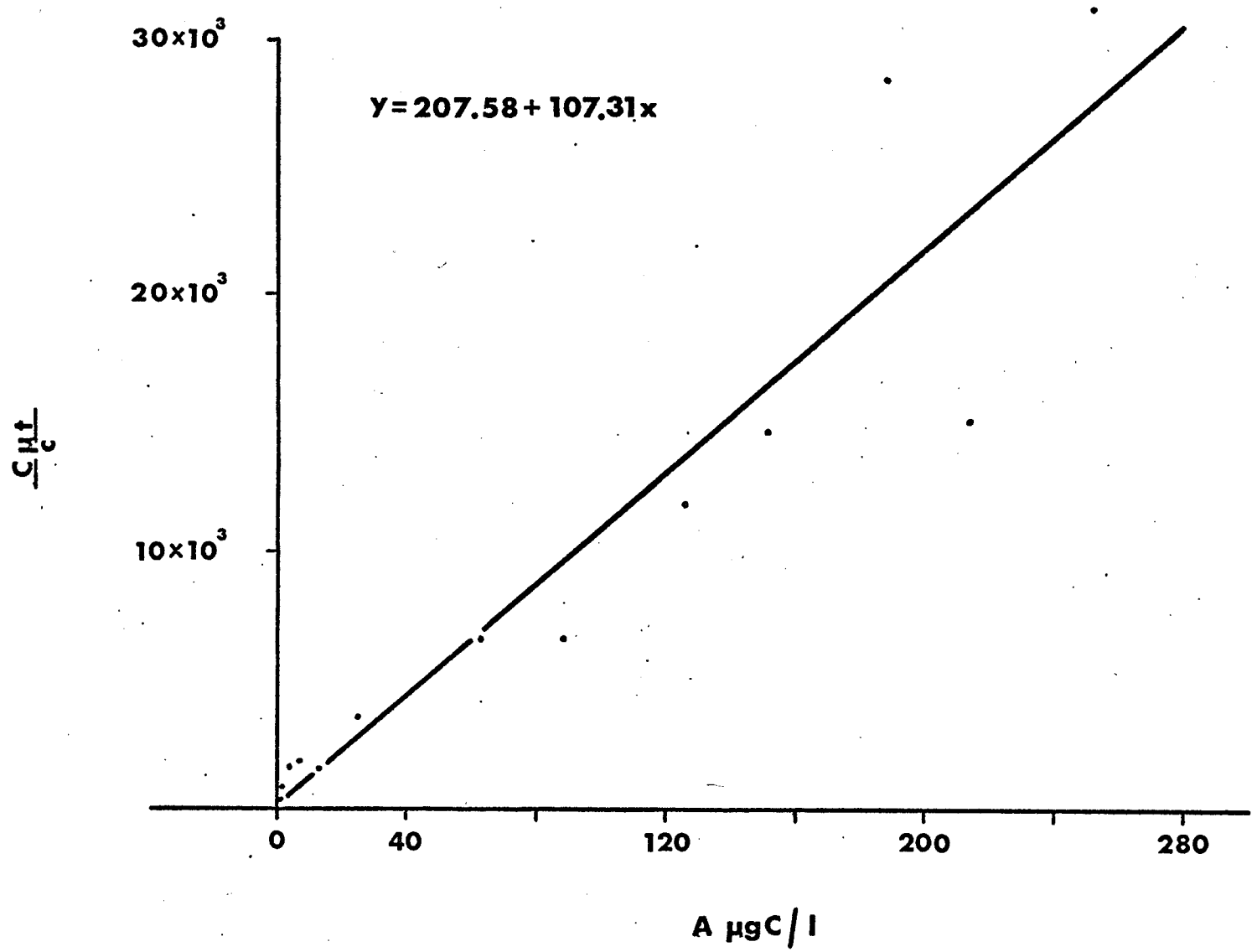


Fig. 17. Citric Acid (2/8/71)

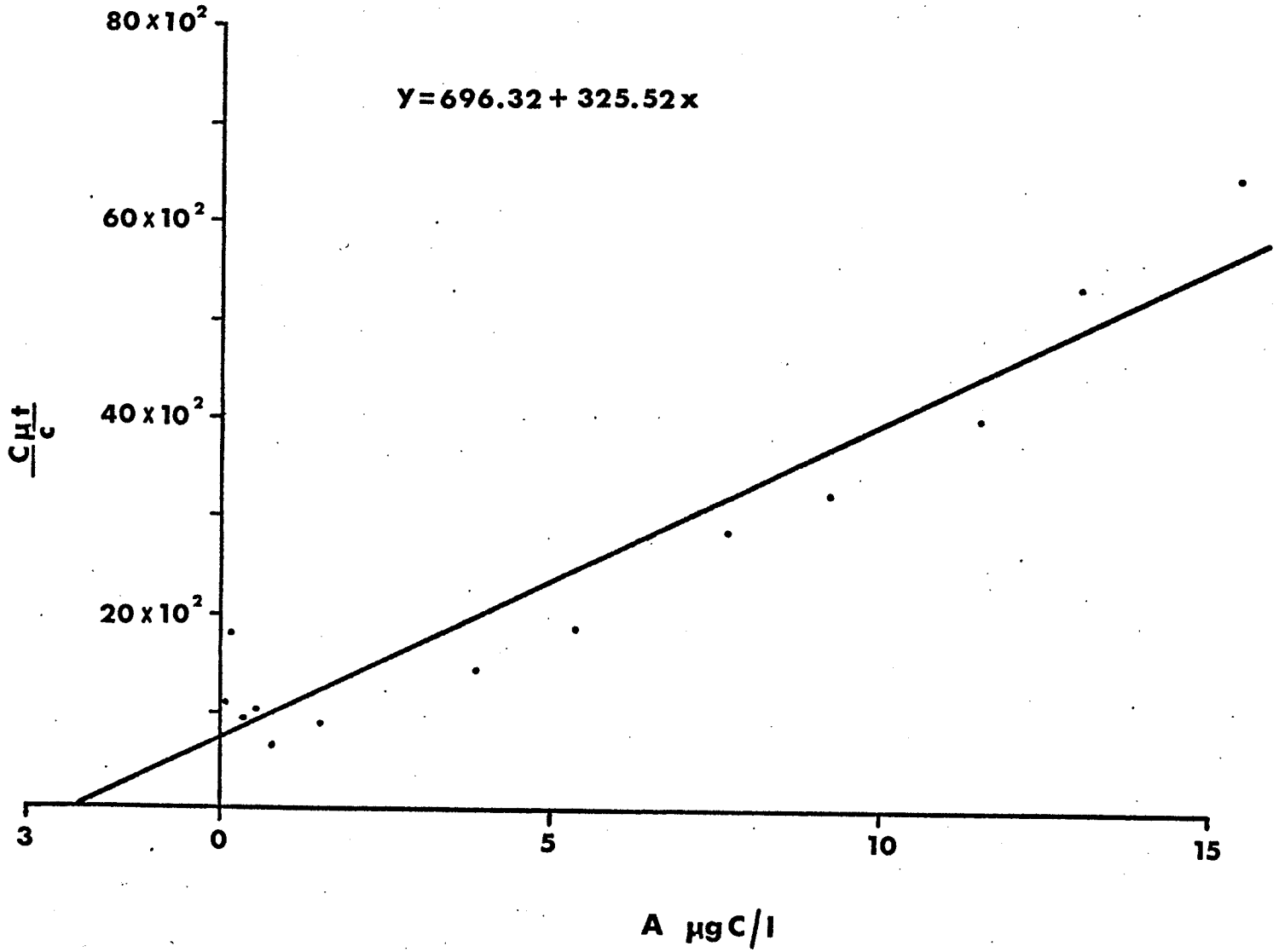


Fig. 18. Formic Acid (29/8/71)

Substrate	V_{max} [i] ($\mu gCl^{-1}hr^{-1}$)	Turnover Time (Hrs) $(T)_{ii}$	T as Determined by $\frac{K+S}{Vmax}$ (Hrs)	K+S [iii] $\mu gC/l$
Lactic Acid	142×10^{-3}	300	303	42.5
Pyruvic Acid	87×10^{-3}	295	295	25.0
Fumaric Acid	53×10^{-3}	75	76	4.0
Malic Acid	39×10^{-3}	0	0	0
Succinic Acid	33×10^{-3}	460	455	15.0
Acetic Acid	30×10^{-3}	375	363	11.0
Glycolic Acid	9×10^{-3}	950	938	9.0
Citric Acid	9×10^{-3}	250	215	2.0
Formic Acid	3×10^{-3}	700	687	2.1

i Maximum velocity of uptake
ii Time of substrate removal
iii Maximum natural substrate concentration

TABLE 1. The kinetics of uptake of nine organic acids in West Blue Lake, Station 5, at a depth of 5 meters, during July and August, 1971.

In most cases, the velocity of uptake ($\mu gC\ l^{-1}hr^{-1}$) for the nine organic acids, measured at 6,12,24 and 48 hrs for light and dark samples increased with each successive sampling period (Table 2). The velocity of uptake with time in the light and the dark varied from a low range of 4×10^{-3} - 2×10^{-3} $\mu gC\ l^{-1}hr^{-1}$ in the light and 221×10^{-3} to 1203×10^{3} $\mu gC\ l^{-1}hr^{-1}$ in the dark (Table 2). In most cases, velocity of uptake of the nine test substrates varied only slightly between the light and the dark samples.

(C) Effect of Depth on the Heterotrophic Uptake of Three Labelled Organic Acid Substrates

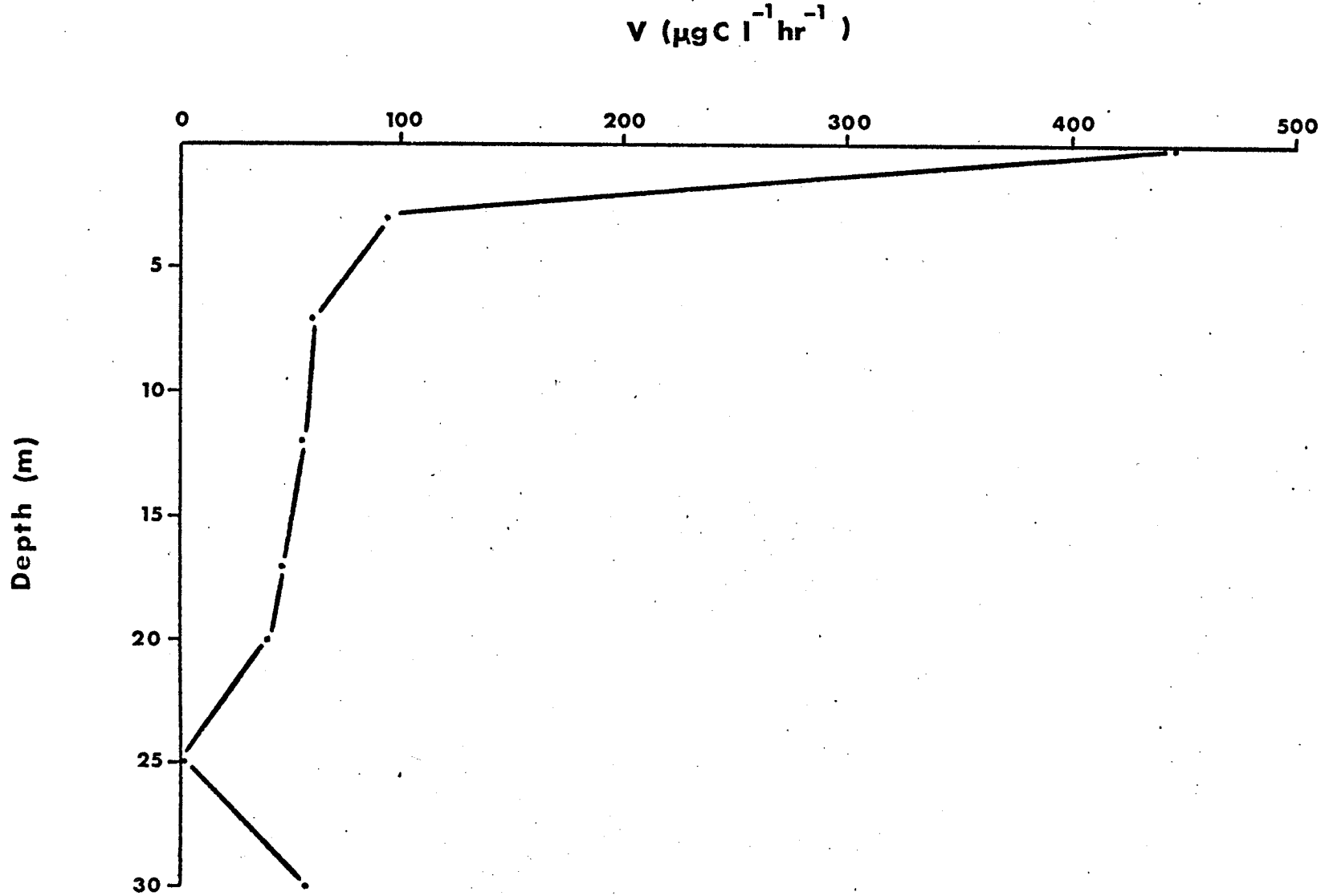
The velocity of uptake of succinic acid by natural populations in the water column decreased from a high value of 45×10^{-3} $\mu gC\ l^{-1}hr^{-1}$ at 0 meters to a low value of 0.0×10^{-3} $\mu gC\ l^{-1}hr^{-1}$ at 25 meters (Fig. 19, Table 3). The uptake of lactic acid ranged from a high of 50×10^{-3} $\mu gC\ l^{-1}hr^{-1}$ at 0 meters down to a low velocity of 5×10^{-3} $\mu gC\ l^{-1}hr^{-1}$ at 20 meters (Fig. 20, Table 3). For the final substrate, malic acid, the uptake velocity ranged from a high of 2580×10^{-3} $\mu gC\ l^{-1}hr^{-1}$ at 0 meters to a low velocity of 371×10^{-3} $\mu gC\ l^{-1}hr^{-1}$ at 30 meters (Fig. 21, Table 3).

Heterotrophic productivity as determined by the planimeter method indicated that beneath one square meter of lake water surface at Station 2, malic acid had an incorporation rate of 2366.4 $mgC\ M^{-2}hr^{-1}$ (Fig. 22, Table 4) lactic acid had an incorporation rate of 24.4 $mgC\ M^{-2}hr^{-1}$ (Fig. 23, Table 4), and that succinic acid had an incorporation rate of 7.1 $mgC\ M^{-2}hr^{-1}$ (Fig. 24, Table 4).

Time (hrs)	Velocity of Uptake ($\mu gC\ l^{-1} hr^{-1}$) per Substrate								
L-Light D-Dark	Glycolic Acid	Malic Acid	Succinic Acid	Pyruvic Acid	Citric Acid	Acetic Acid	Lactic Acid	Formic Acid	Fumaric Acid
6 - L	$9x10^{-3}$	$23x10^{-3}$	$31x10^{-3}$	$111x10^{-3}$	$23x10^{-3}$	$26x10^{-3}$	$29x10^{-3}$	$4x10^{-3}$	$331x10^{-3}$
12 - L	$12x10^{-3}$	$41x10^{-3}$	$39x10^{-3}$	$65x10^{-3}$	$20x10^{-3}$	$20x10^{-3}$	$33x10^{-3}$	$3x10^{-3}$	$54x10^{-3}$
24 - L	$9x10^{-3}$	$72x10^{-3}$	$18x10^{-3}$	$162x10^{-3}$	$37x10^{-3}$	$56x10^{-3}$	$102x10^{-3}$	$2x10^{-3}$	$241x10^{-3}$
48 - L	$21x10^{-3}$	$242x10^{-3}$	$981x10^{-3}$	$445x10^{-3}$	$164x10^{-3}$	$99x10^{-3}$	$386x10^{-3}$	$2x10^{-3}$	$1480x10^{-3}$
6 -D	$915x10^{-3}$	$24x10^{-3}$	$28x10^{-3}$	$63x10^{-3}$	$21x10^{-3}$	$5x10^{-3}$	$26x10^{-3}$	$3x10^{-3}$	$221x10^{-3}$
12 - D	$14x10^{-3}$	$23x10^{-3}$	$31x10^{-3}$	$59x10^{-3}$	$23x10^{-3}$	$11x10^{-3}$	$20x10^{-3}$	$1x10^{-3}$	$50x10^{-3}$
24 - D	$11x10^{-3}$	$74x10^{-3}$	$27x10^{-3}$	$278x10^{-3}$	$46x10^{-3}$	$120x10^{-3}$	$150x10^{-3}$	$2x10^{-3}$	$450x10^{-3}$
48 - D	$32x10^{-3}$	$243x10^{-3}$	$885x10^{-3}$	$709x10^{-3}$	$239x10^{-3}$	$146x10^{-3}$	$389x10^{-3}$	$2x10^{-3}$	$1203x10^{-3}$

Table 2. Effect of time on the heterotrophic uptake of nine organic acids in the light and the dark during a 48 hr incubation period, at a depth of 5 meters; Station 5, during July and August, 1971.

Fig. 19. The relationship between depth of the water column (meters) and the velocity of uptake (V- μgC $l^{-1}hr^{-1}$) of the Succinic Acid in West Blue Lake, Manitoba, (20/8/71). (Amount of added substrate = 80 μgC/l).


V (µg C l⁻¹ hr⁻¹)
0
100
200
300
400
500
Depth (m)
5
10
15
20
25
30

Depth (meters)	Velocity of Uptake ($\mu gC\ l^{-1} hr^{-1}$) per Substrate		
	Succinic Acid (20/8/71)	Lactic Acid (31/8/71)	Malic Acid (31/8/71)
0	45×10^{-3}	50×10^{-3}	2580×10^{-3}
3	9×10^{-3}	46×10^{-3}	2573×10^{-3}
7	6×10^{-3}	58×10^{-3}	2530×10^{-3}
12	6×10^{-3}	15×10^{-3}	2603×10^{-3}
17	5×10^{-3}	7×10^{-3}	2549×10^{-3}
20	4×10^{-3}	5×10^{-3}	2284×10^{-3}
25	0×10^{-3}	5×10^{-3}	1283×10^{-3}
30	6×10^{-3}	19×10^{-3}	371×10^{-3}

Table 3. Uptake of three organic acids at 8 depths throughout the water column, Station 2, West Blue Lake, Manitoba.

Fig. 20. The relationship between depth of the water column (meters) and the velocity of uptake (V-μgC $l^{-1}hr^{-1}$) of Lactic Acid in West Blue Lake, Manitoba, (31/8/71). (Amount of added substrate = 38 μgC/l).

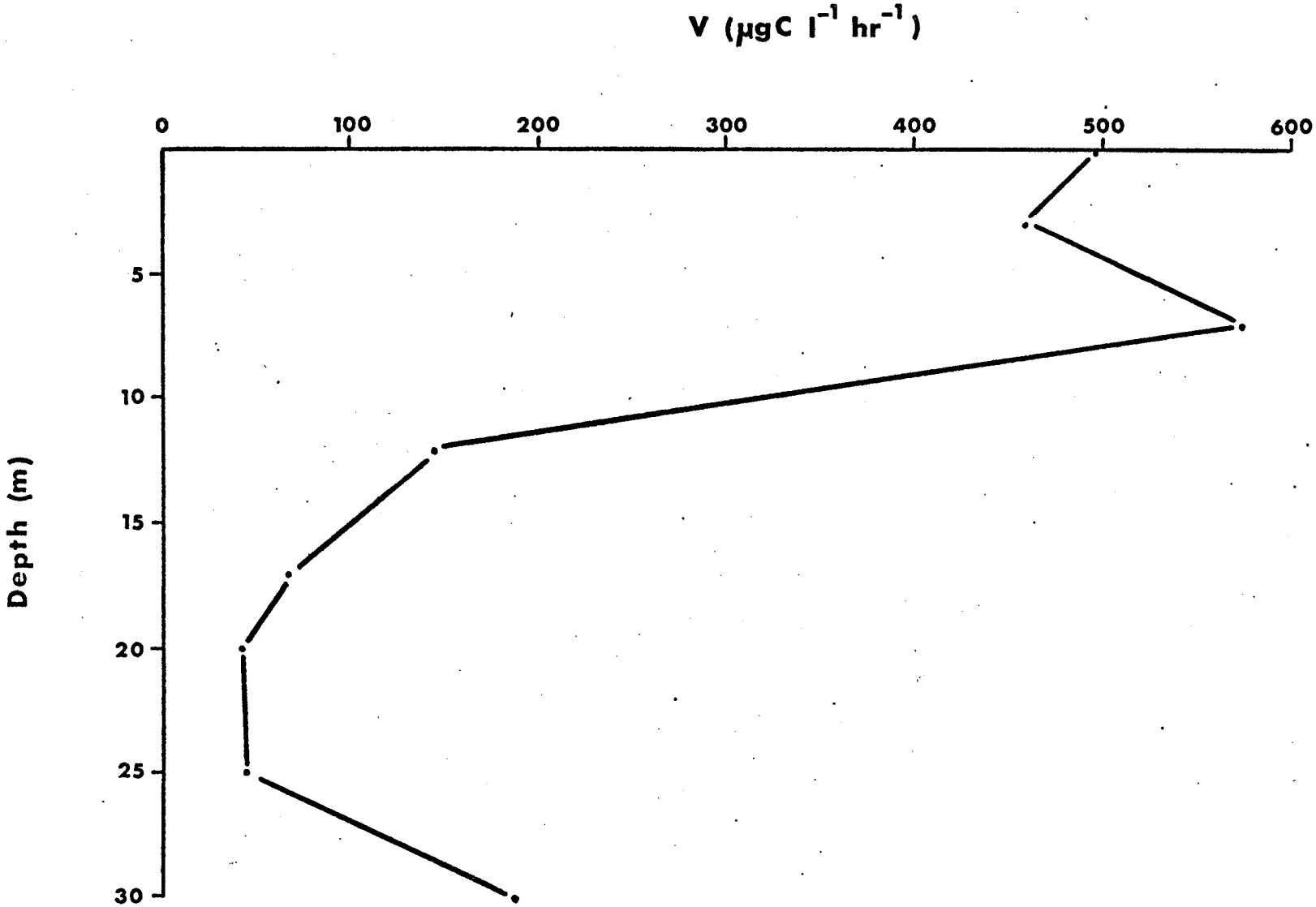

V (µgC l⁻¹ hr⁻¹)
0
100
200
300
400
500
600
Depth (m)
5
10
15
20
25
30

Fig. 21. The relationship between depth of the water column (meters) and the velocity of uptake (V-μgC $l^{-1}hr^{-1}$) of Malic Acid in West Blue Lake, Manitoba, (31/8/71). (Amount of added substrate = 44 μgC/l).

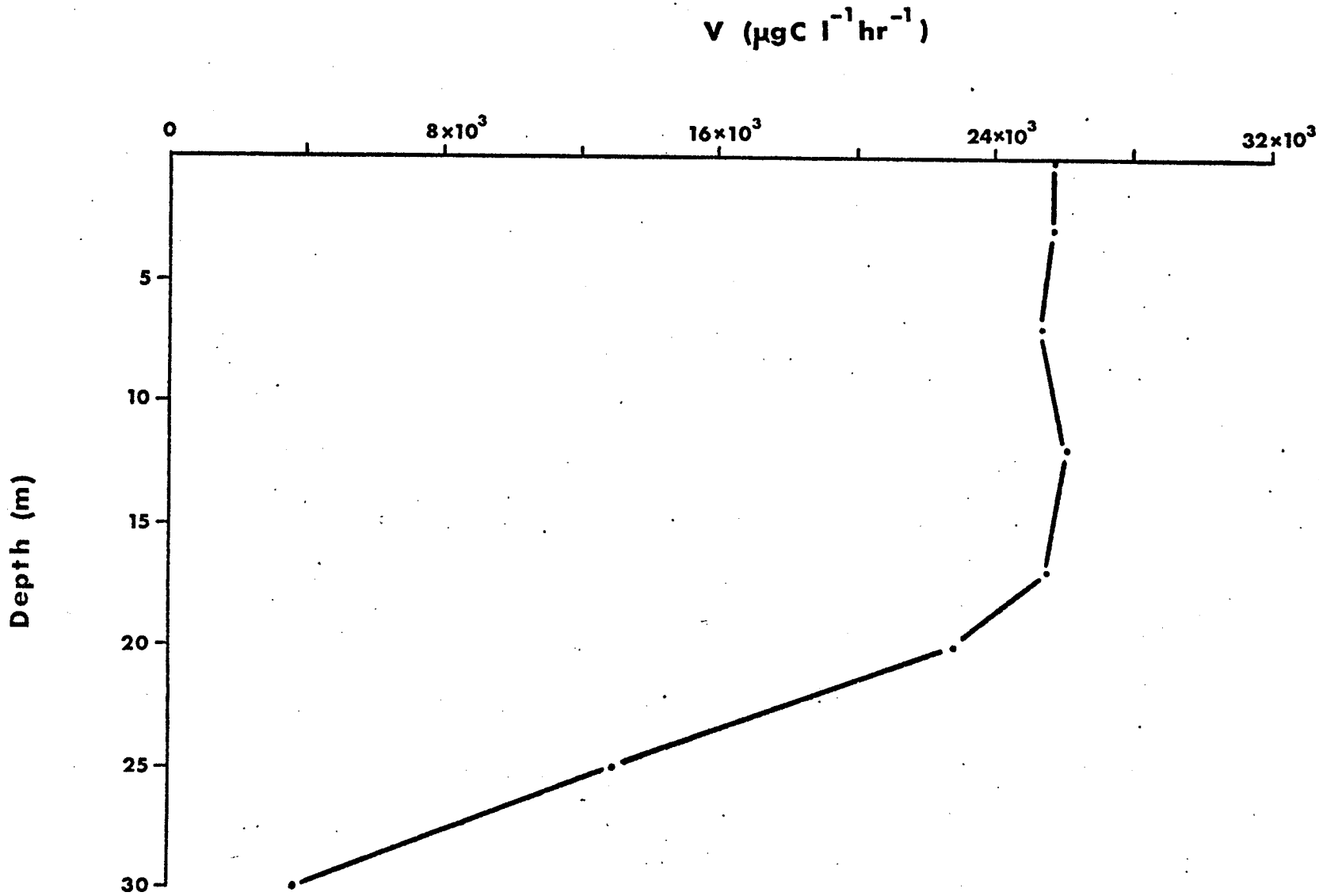
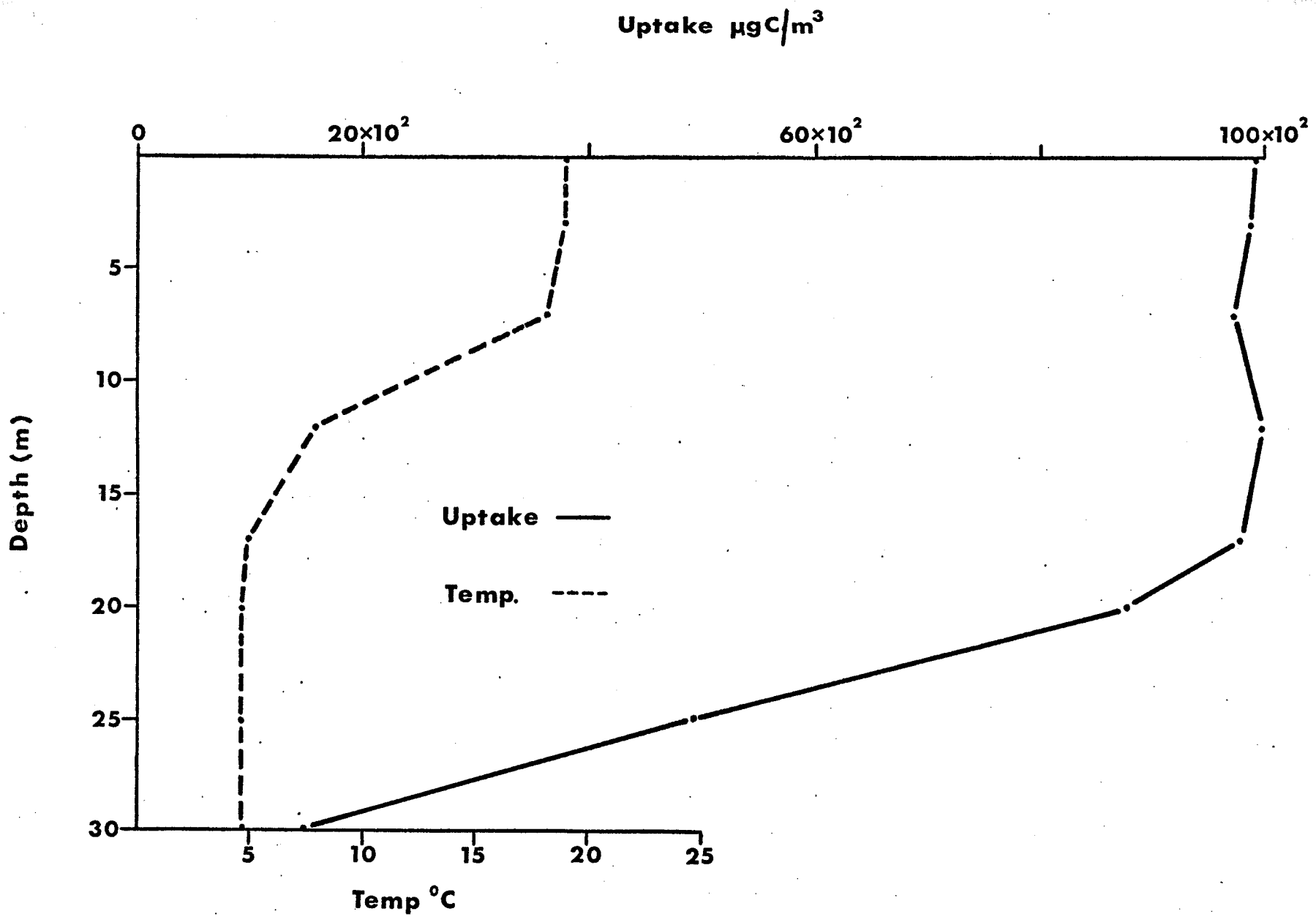

V (µgC l⁻¹hr⁻¹)
0
8×10³
16×10³
24×10³
32×10³
Depth (m)
5
10
15
20
25
30

Fig. 22. The relationship between uptake of Malic Acid ($\mu gC/m^3$) with temperature and depth of the water column for West Blue Lake, Manitoba, (31/8/71). (Amount of added substrate = 44 $\mu gC/l$).


Uptake μgC/m³
0
20×10²
60×10²
100×10²
5
10
15
20
25
30
Depth (m)
Uptake
Temp.
5
10
15
20
25
Temp °C

Substrate	Integrated Productivity Values (mgC $m^{-2}hr^{-1}$)
Succinic Acid	7.1 mgC $m^{-2}hr^{-1}$
Lactic Acid	24.4 mgC $m^{-2}hr^{-1}$
Malic Acid	2366.4 mgC $m^{-2}hr^{-1}$

Table 4. Integrated productivity values for three organic acids.

Fig. 23. The relationship between uptake of Lactic Acid ($\mu gC/m^3$) with temperature and depth of the water column for West Blue Lake, Manitoba, (31/8/71). (Amount of added substrate = 38.0 $\mu gC/l$).

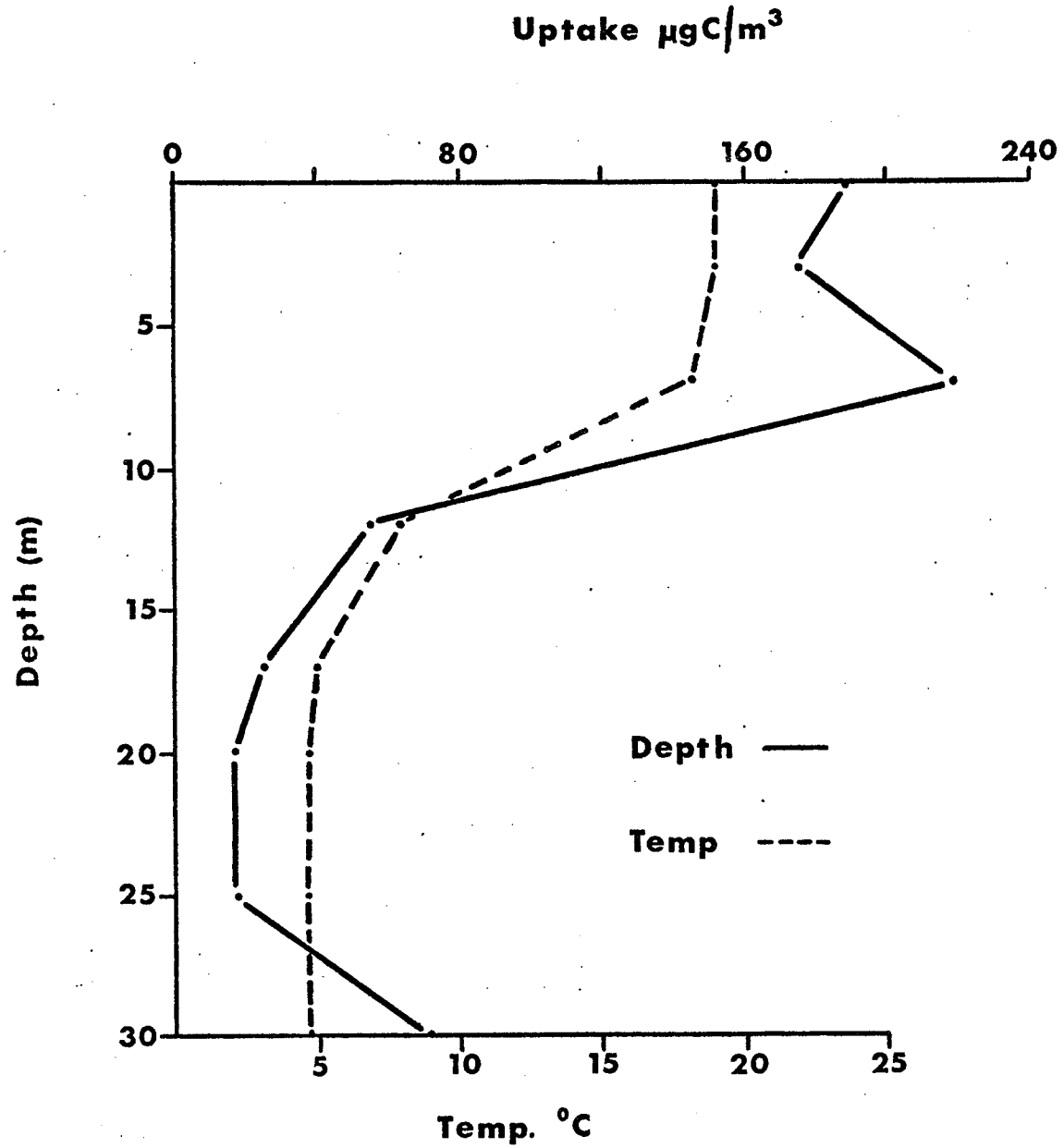
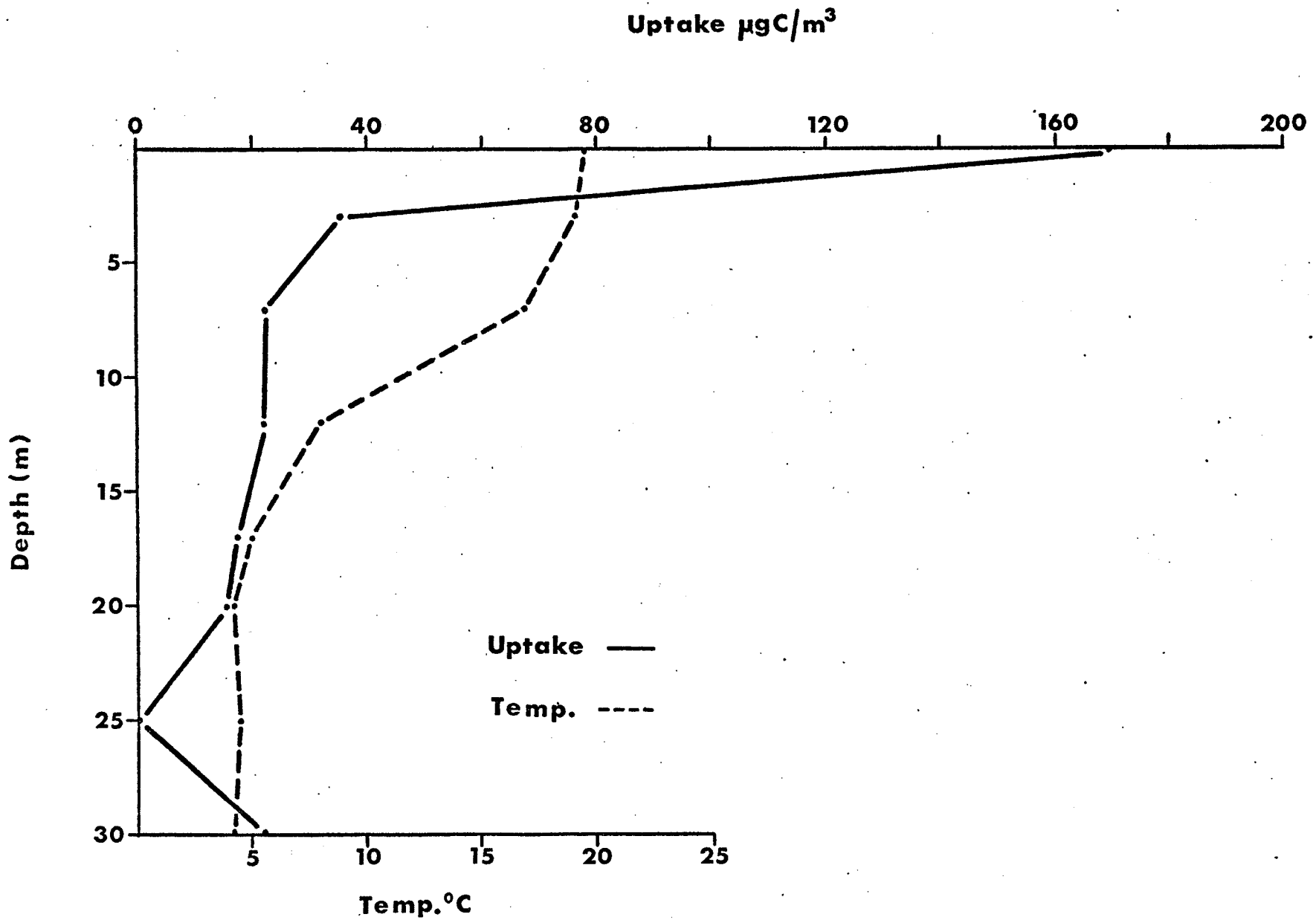

Uptake µgC/m³
0
80
160
240
5
10
15
20
25
30
Depth (m)
Depth
Temp
5
10
15
20
25
Temp. °C

Fig. 24. The relationship between uptake of Succinic Acid ($\mu gC/m^3$) with temperature and depth of the water column at Station 2, West Blue Lake, Manitoba (20/8/71). (Amount of added substrate = 80 $\mu gC/l$).


Uptake µgC/m³
0
40
80
120
160
200
5
10
15
20
25
30
Depth (m)
Uptake
Temp.
5
10
15
20
25
Temp.°C

(D) The Uptake and Excretion of ^{14}C by Natural Populations

Data for excretion of ^{14}C-labelled products and re-assimilation of ^{14}C-labelled products, as it occurred in a series of light and dark bottles were collected from four experiments conducted during the summer of 1971.

Experiment #1 (19/5/71), a 16 hr experiment with a sampling interval of 2 hrs, exhibited excretion rates in the light bottle samples which ranged from 1.987 mgC $M^{-3}hr^{-1}$ at 2 hrs to 0.784 mgC $M^{-3}hr^{-1}$ at 16 hrs, whereas excretory rates in the dark bottle samples were from 2.043 mgC M^{-3} hr^{-1} at 2 hrs to 0.197 mgC $M^{-3}hr^{-1}$ at 16 hrs (Table 5). The rate of re-assimilation of labelled excretory products ranged from an initial rate of approximately 0.7 mgC $M^{-3}hr^{-1}$ to a final rate of 1.203 mgC $M^{-3}hr^{-1}$ at 16 hrs for the light samples and from 1.184 mgC $M^{-3}hr^{-1}$ at 4 hrs to 1.846 mgC $M^{-3}hr^{-1}$ at 16 hrs for the dark samples (Table 6).

Experiment #2 (23/6/71) of 24 hrs duration had a sampling interval of 2 hrs up to 14 hrs with a final sampling 10 hrs later. Excretory rates for the light filtrate samples ranged from 6.742 mgC $M^{-3}hr^{-1}$ at 2 hrs to 0.422 mgC $M^{-3}hr^{-1}$ at 24 hrs. The dark sample filtrate had a highest excretion rate of 69.224 mgC $M^{-3}hr^{-1}$ at 2 hrs decreasing to 0.355 mgC M^{-3} hr^{-1} at 24 hrs (Table 7). Re-assimilation rates for the light filtrate samples ranged from 4.663 mgC $M^{-3}hr^{-1}$ at 4 hrs to 6.320 mgC $M^{-3}hr^{-1}$ at 24 hrs, whereas for the dark filtrate samples the range was from 67.859 mgC $M^{-3}hr^{-1}$ at 4 hrs to 68.802 mgC $M^{-3}hr^{-1}$ at 24 hrs (Table 8).

Experiment #3 (30/7/71) was of 24 hrs duration with a sampling period of 4 hrs up to 12 hrs, with the final sampling period taking place 12 hrs after the proceeding one. Light filtrate excretion rates ranged

Time (hrs)	Filtrate Carbon mgC $m^{-3}hr^{-1}$ Light	Filtrate Carbon mgC $m^{-3}hr^{-1}$ Dark
2	1.987	2.043
4	1.244	0.859
6	1.263	0.552
8	1.027	0.368
10	1.049	0.337
12	0.793	0.293
14	0.858	0.254
16	0.784	0.197

Table 5. Excretion of labelled products (mgC $m^{-3}hr^{-1}$) by natural lake populations during a 16 hr incubation period (19/5/71), at a depth of 5 meters, Station 5.

Time (hrs)	Filtrate Carbon mgC $m^{-3}hr^{-1}$ Light	Filtrate Carbon mgC $m^{-3}hr^{-1}$ Dark
2	--- (1.987)	--- (2.043)
4	0.743	1.184
6	0.724	1.491
8	0.960	1.675
10	0.938	1.706
12	1.194	1.750
14	1.129	1.789
16	1.203	1.846

Table 6. Re-assimilation of labelled excreted products (mgC $m^{-3}hr^{-1}$) by natural lake populations (19/5/71), at a depth of 5 meters, Station 5. Values in brackets represents original amounts of excreted carbon present.

Time (hrs)	Filtrate Carbon mgC $m^{-3}hr^{-1}$ Light	Filtrate Carbon mgC $m^{-3}hr^{-1}$ Dark
2	6.742	69.224
4	2.079	1.385
6	2.473	1.193
8	0.712	1.871
10	2.894	1.281
12	1.030	0.519
14	0.578	0.550
24	0.422	0.355

Table 7. Excretion of labelled products (mgC $m^{-3}hr^{-1}$) by natural lake populations during a 24 hr incubation period (23/6/71); at a depth of 5 meters, Station 5.

Time (hrs)	Filtrate Carbon mgC $m^{-3}hr^{-1}$ Light	Filtrate Carbon mgC $m^{-3}hr^{-1}$ Dark
2	--- (6.742)	--- (69.224)
4	4.663	67.839
6	4.269	68.031
8	6.030	67.353
10	3.848	67.943
12	5.712	68.705
14	6.164	68.674
24	6.320	68.802

Table 8. Re-assimilation of labelled excreted products (mgC $m^{-3}hr^{-1}$) by natural lake populations (23/6/71), at a depth of 5 meters, Station 5. Values in brackets represent original amount of excreted carbon present.

from 0.940 mgC $M^{-3}hr^{-1}$ at 4 hrs to 0.792 mgC $M^{-3}hr^{-1}$ at 24 hrs (Table 9). Re-assimilation rates of the excretory products for the light filtrate samples were 0.091, 0.505 and 0.309 mgC $M^{-3}hr^{-1}$ for 8,12 and 24 hrs respectively. The re-assimilation rates for the dark filtrate samples were 0.262 and 0.175 mgC $M^{-3}hr^{-1}$ for 8 and 24 hrs respectively (Table 10). No re-assimilation rate was calculable for the 12 hr dark filtrate sample.

Experiment #4 (1/9/71) was a 12 hr experiment with a 2 hr sampling interval. Excretion values for the light filtrate samples ranged from 54.226 mgC $M^{-3}hr^{-1}$ at 2 hrs to 5.812 mgC $M^{-3}hr^{-1}$ at 12 hrs with a low value of 4.248 mgC $M^{-3}hr^{-1}$ being recorded at 8 hrs. The excretion rate for the dark filtrate samples ranged from 220.314 mgC $M^{-3}hr^{-1}$ at 2 hrs to 7.249 mgC $M^{-3}hr^{-1}$ at 10 hrs with a low rate of 5.034 mgC $M^{-3}hr^{-1}$ being measured at 8 hrs (Table 11). The re-assimilation rates of the excreted products for the light filtrate samples ranged from 39.498 mgC $M^{-3}hr^{-1}$ at 4 hrs to 48.414 mgC $M^{-3}hr^{-1}$ at 24 hrs. A high rate of 49.978 mgC $M^{-3}hr^{-1}$ was recorded at 8 hrs. The range for the dark filtrate samples was from 143.016 mgC $M^{-3}hr^{-1}$ at 4 hrs to 213.065 mgC $M^{-3}hr^{-1}$ being recorded at 8 hrs (Table 12). No excretion rate or re-assimilation rate was calculable for the 12 hr dark filtrate samples (Tables 11 and 12).

(E) Assessment of a Filtration Effect

Activity retained on the membrane filters increased as the volume of the aliquot being filtered increased (Table 13). In addition, activity of the filtrate remained constant or increased slightly as the volume of the aliquot filtered was increased. In most cases, activity of the filter and the filtrate increased with longer incubation periods.

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Time (hrs)	Filtrate Carbon mgC $m^{-3}hr^{-1}$ Light	Filtrate Carbon mgC $m^{-3}hr^{-1}$ Dark
4	0.940	0.967
8	0.849	0.705
12	0.435	1.053
24	0.631	0.792

Table 9. Excretion of labelled products (mgC $m^{-3}hr^{-1}$) by natural lake populations during a 24 hr incubation period (30/7/71), at a depth of 5 meters, Station 5.

Time (hrs)	Filtrate Carbon mgC $m^{-3}hr^{-1}$ Light	Filtrate Carbon mgC $m^{-3}hr^{-1}$ Dark
4	--- (0.940)	--- (0.967)
8	0.091	0.262
12	0.505	*
24	0.309	0.175

Table 10. Re-assimilation of labelled excreted products (mgC $m^{-3}hr^{-1}$) by natural lake populations (30/7/71) , at a depth of 5 meters, Station 5.

* no value could be calculated. Values in brackets represent original amount of excreted carbon present.

Time (hrs)	Filtrate Carbon mgC $m^{-3}hr^{-1}$ Light	Filtrate Carbon mgC $m^{-3}hr^{-1}$ Dark
2	54.226	220.314
4	14.728	77.298
6	9.716	14.874
8	4.248	5.034
10	6.842	7.249
12	5.812	*

Table 11. Excretion of labelled products (mgC $m^{-3}hr^{-1}$) by natural lake populations during a 12 hr incubation period (1/9/71), at a depth of 5 meters, Station 5.

*no value available.

Time (hrs)	Filtrate Carbon mgC $m^{-3}hr^{-1}$ Light	Filtrate Carbon mgC $m^{-3}hr^{-1}$ Dark
2	--- (54.226)	--- (220.314)
4	39.498	143.016
6	44.510	205.440
8	49.978	215.280
10	47.384	213.065
12	48.414	*

Table 12. Re-assimilation of labelled excreted products (mgC $m^{-3}hr^{-1}$) by natural lake populations (1/9/71), at a depth of 5 meters, Station 5.

*no value available

Values in brackets represent original amount of excreted carbon present.

Volume Filtered	4 H-Light F	4 H-Light T	8 H-Light F	8 H-Light T	12 H-Light F	12 H-Light T	24 H-Light F	24 H-Light T
1 ml	925	679	1,525	685	1,800	761	1,800	368
3 ml	991	450	2,767	550	2,133	750	2,175	550
5 ml	1,175	675	2,305	500	1,910	775	2,315	525
7 ml	1,129	575	2,946	700	2,132	700	2,321	550
10 ml	1,105	475	2,280	500	2,010	1,000	2,203	650
20 ml	1,201	300	2,153	975	2,388	10,500	2,371	825

F - Activity of filter (DPM) per 25 ml

T - Activity of filtrate (DPM) per 25 ml

Table 13. "Filtration Effect", the relationship between volume of the sample filtered and the amount of activity retained on the filter and the amount in the filtrate.

Discussion

A) Saturation Curve Response

The suitability of the nine organic acids which were used as substrate for the determination of individual saturation responses were basically determined according to order of descending V_{max} values. These ranged from the highest of 142 x 10^{-3} $\mu gCl^{-1}hr^{-1}$ for lactic acid to the lowest, 3 x 10^{-3} $\mu gCl^{-1}hr^{-1}$ for formic acid (Table 1). These results were found to be comparable in some respect with values reported in the literature. The results reported here were not directly comparable with those of Parsons and Strickland (1962); however the relative velocity (v) values were and indicated that for the nine organic acids, the maximum substrate concentration used (in all but one case substantially less than 250 µg C/l) produced uptake rate velocities (v) which were substantially higher than the rate values found by Parsons and Strickland (1962) for acetate and glucose.

The V_{max} values attained by Wright and Hobbie (1965, 1966) for glucose and acetate in Lake Erkin were within the range of V_{max} values determined for the nine organic acids in this research. In all cases, the V_{max} for acetic acid; the only substrate common to both sets of experiments, was within the ranges found by Wright and Hobbie (1965, 1966). Vaccaro and Jannasch (1966) working in a marine environment found that with increasing amounts of glucose (up to 60 µg C/l) that a V_{max} of 0.110 µg C $l^{-1}hr^{-1}$ was attained, this value being slightly less than the highest reported here, lactic acid. Subsequent measurements from other marine locations produced V_{max} values of 0.07 and 0.02 µgC $l^{-1}hr^{-1}$ (Vaccaro and Jannasch, 1966) which still were within the range of V_{max}

values reported here for the nine organic acids. In addition, the relative uptake velocities (v) for the nine organic acids were similar to those reported by Vaccaro and Jannasch (1966) for glucose at five different marine stations. On the other hand, the V_{max} values as presented for the nine organic acids where anywhere from one to four orders of magnitude less than those reported by Allen (1969); Munro and Brock (1968), and Hamilton and Preslan (1970).

Perhaps the most important information can be gained from the turnover times of these substrates in the natural environment (Hobbie, 1967). The turnover times, derived from the Lineweaver-Burk plots and/or mathematically for the nine organic acids have been shown to range from long periods of time for glycolic and formic acid to those which are intermediate such as succinic, acetic, lactic, pyruvic and citric acid to those of short turnover times as fumaric and malic acid (Table 1). The range of turnover times which have been presented (Table 1) for the nine organic acids were all similar in magnitude to those presented in the literature for glucose and/or acetate (Allen, 1969; Hobbie, 1967; Hobbie, and Wright, 1969; Munro and Brock, 1968; and Wright and Hobbie, 1966), and for selected organic acids, amino acids and sugars (Hamilton and Preslan, 1970). It has been suggested (Wright and Hobbie, 1966) that short turnover times are indicative of the importance of the soluble organic compound as a small but biologically active fraction of the pool of dissolved organic matter. In addition, it was suggested (Wright and Hobbie, 1966) that microorganisms appear to be adapted to the efficient usage of substrates at natural concentrations and that short turnover times are

reflective of this. Therefore, of importance in the dissolved organic pool in West Blue Lake could be malic and fumaric acid, which had the shortest turnover times (0 hrs and 75 hrs respectively), whereas glycolic acid (950 hrs) could be the least important having the longest turnover period. Although the data on maximum velocities and turnover times were measured only once during the summer for this study, it has been found that maximum velocities (V_{max}) and turnover times follow yearly regimes within a body of water and may vary by two orders of magnitude during this time (Hobbie and Wright, 1962).

The (K+S) values attained for the nine organic acids reflected the maximum natural substrate concentrations for each of the organic acids used in the saturation experiments. The (K+S) values ranged from 42.5 µgC/l for lactic acid to approximately 1.0 µgC/l for citric and malic acid. The (K+S) values reported here for the nine organic acids are of the same order of magnitude and well within the range of (K+S) values reported in the literature for glucose and/or acetate in fresh or seawater at various depths and at various times of the year (Allen, 1969; Hobbie, 1968; Parsons and Strickland, 1962; Wright and Hobbie, 1965; Wright and Hobbie, 1966; and Vaccaro and Jannasch, 1966), and also for selected organic acids, amino acids and sugars (Hamilton and Preslan, 1970). However, the literature has reported an occasional (K+S) value as high as 120 µgC/l for glucose and acetate (Allen, 1969; and Vaccaro and Jannasch, 1966), and in one case as high as 170 µgC/l for acetate (Munro and Brock, 1968). It has been suggested that low (K+S) values positively reflect the presence of a substrate and its importance to the microorganisms, and that the micro-

organisms are adapted to and have specific transport systems for the uptake of these organics at low concentrations (Hobbie, 1967). Therefore, the low turnover times and (K+S) values for malic acid, succinic acid and citric acid could reflect the relative importance of these as substrates for the microorganisms of West Blue Lake. This has not, however, been substantiated by subsequent experimentation.

It has been shown that the uptake response of algae differs markedly from that of bacteria when these responses have been analyzed by enzyme kinetics (Wright and Hobbie, 1965; Hobbie and Wright, 1965; Wright and Hobbie, 1966; Hobbie, 1967; and Hobbie and Wright, 1968). With a mixed population of algae and bacteria, two superimposed responses were shown to occur (Hobbie and Wright, 1968; and Wright and Hobbie, 1966). The initial response, at low substrate concentrations (<500 µgC/l) being attributed to active uptake by bacteria while the second response, at higher substrate concentrations, (>500 µgC/l) was due to passive uptake by algae. In the case of determining the saturation responses for the nine organic acids, it was found that after kinetic analysis (Figs. 1-9 and Figs. 10-18), the Michaelis-Menten and Lineweaver-Burk plots were similar to those described in the literature for the active uptake of glucose and acetate by bacteria (Allen, 1969; Hobbie, 1967; Hobbie and Wright, 1967; Hobbie and Wright, 1968; Wright and Hobbie, 1965; Wright and Hobbie, 1966; and Vaccaro and Jannasch, 1966). Consequently, the kinetic parameters attained from the saturation curve data (Table 1 and Appendix 3) and the methods used suggest that in actuality an active system was in operation and that all heterotrophic responses measured in West Blue Lake

were a consequence of heterotrophs other than algae. In addition, the (K+S) values, which indicate the maximum amount of naturally occurring substrate and which were shown to be less than 50 μgC/l for each of the nine organic acids tested in West Blue Lake water; were proof that heterotrophy in that lake under natural circumstances was by bacteria and other heterotrophs and not by the algae. Although heterotrophy of algae at low substrate concentrations has been shown to occur (Hellebust and Guillard, 1967; Hellebust, 1971; Hellebust and Lewin, 1972; and Sloan and Strickland, 1966) only the data of North and Stephens (1967), Stephens and North (1971) for a marine flagellate has shown the occurrence of heterotrophy at substrate levels comparable to those used to determine the saturation responses presented in this thesis. Therefore, at present it can be conditionally stated that heterotrophy by the algae of West Blue Lake at the endogenous levels of naturally occurring substrate is highly unlikely.

The experimental procedures used for the various experiments discussed in this dissertation differed significantly in two ways from those of previous investigators. It has been common practice by others in the past (Allen, 1969; Hamilton et al, 1966, 1970; Hobbie, 1967; Hobbie and Wright, 1965a, 1965b, 1968; Parsons and Strickland, 1962; Wright and Hobbie, 1965, 1966 and Vaccaro and Jannasch, 1966) to fix their controls following inoculation and to fix their samples prior to filtration either with Lugol's solution (I_2KI) or formalin, and secondly to dry and desiccate their filters following filtration in preparation for radioactivity counting by Geiger-Mueller methods. Both of these procedures are possible sources of significant error. The fixation of the controls following inoculation and the fixation of the samples prior to filtration could aid in and lead to the

rupture of algal and bacterial cell walls with the subsequent loss of labelled product. Loss due to cell rupture because of fixation would essentially be indeterminate, varying with the cell composition of the sample, length of storage period, type and strength of fixative used, metabolic condition of the cells prior to fixation, length of incubation period, the substrate used plus other environmental factors such as light and temperature. Losses, calculated by Allen (1969) and Hobbie and Wright (1965) were shown to be about 10%, however with the possibility of such unpredictability, such checks should and would have to be done with each experiment. Such past procedures introduced a needless source of error which could be negated by alternatively using cell-free blanks and filtration of samples immediately after incubation. The second source of error stems from the Geiger-Mueller technique. It has been shown by Ward and Nakanishi (1971) that drying and desiccation of filters can lead to a substantial loss of activity, possibly due to evaporation of volatile compounds, therefore this method along with the filtration procedure could lead to an underestimation of any heterotrophic response which is being monitored by the use of radioactively labelled compounds.

B) Effect of Time on the Heterotrophic Uptake of Nine Labelled Organic Acid Substrates

The data for the uptake of the nine organic acids with time indicated that in most cases there was a progressive increase in uptake velocities for each substrate with the time in the light and the dark. The only exception was formic acid which produced little change in uptake velocity with time. However, periods during which the uptake of a

substrate lagged was in evidence from the data (Table 2). The general trend shown by the uptake of these substrates with time agreed well with similar trends shown for glucose, acetate and various amino acids (Parsons and Strickland, 1962; Munro and Brock, 1968, Williams and Askew, 1968; and Williams and Gray, 1970). Williams et al (1968, 1970) found that the progress curves for low amounts of added glucose and a mixture of amino acids, increased either linearly or almost linearly with time. Munro and Brock (1968) showed essentially the same thing for acetate during an eight hour period. Progress curves of this type are suggestive of a population growing exponentially (Parsons and Strickland, 1962) and that this is evidence that the population is showing no noticeable effects to being contained in a glass vessel (Williams and Gray, 1970). However, of the substrates used, glycolic acid and succinic acid showed a lag in uptake rate between 12 and 24 hrs for both light and dark samples (Table 2). Pyruvic acid, fumaric acid and formic acid exhibited a similar lag between 6 and 12 hrs for both light and dark samples. Malic acid and lactic acid illustrated this lag in the uptake rate between the 6 and 12 hr sampling periods for only the dark samples, while citric acid and acetic acid exhibited the same phenomenon for only those samples kept in the light. This pronounced presence of a lag period for most of the substrates followed by increasing uptake rates was believed to be the result of increasing cell numbers within the sample bottles during the incubation periods. The fact that these experiments were conducted individually during the summer of 1971 and not all at one specific time was felt to be the overall general cause of the variation of where the lag period occurred during the 48 hr experiment and the magnitude to which it was expressed.

Factors making up the 'overall general cause' could be any of the following such as changes in species composition, changes in naturally occurring substrate levels and subsequent changes of the physiology of the species concerned. Similar lag periods as were noted here, have been shown by Williams and Gray (1970), although to a lesser degree, especially with water samples of low heterotrophic activity; and by Hobbie and Crawford (1969) with samples from oligotrophic waters.

In addition to monitoring changes in uptake velocities of the nine organic acids during a 48 hr period, these experiments were also designed to measure any differences between uptake velocities in the light and in the dark by the natural populations. It was previously thought that algal heterotrophy might be of significance in West Blue Lake and that this would be reflected in higher dark uptake velocities. But this was later questioned (Section A Discussion). The results for this section were highly variable when comparing the light data with the dark data. There were a moderate number of overlapping values between light and dark samples indicating similar uptake velocities. However, four of the acids tested, glycolic, pyruvic, citric and acetic acid had higher uptake velocities in the dark than in the light (Table 2). Three of the acids, malic, lactic and formic acid showed no significant difference between light uptake rates and dark uptake rates. Succinic acid and fumaric acid, had similar uptake velocities for both the light and the dark until 48 hrs when both showed higher uptake velocities in the light than the dark. Therefore, for five of the substrates tested; malic, lactic, formic, succinic and fumaric acid, uptake velocities were

similar in the light and the dark, as should be expected if bacteria were the organisms responsible for the uptake of the substrates. The three remaining substrates, glycolic, citric and acetic acid; which were taken up at a higher rate in the dark than the light was probably the result of physiological and species differences amongst the heterotrophic population during the summer and not due to algal heterotrophy. Therefore, higher uptake velocities in the dark may be due to one or several species of bacteria having a preference to dark uptake predominating while the experiment was being conducted, or possibly because of environmental factors which could be effecting the mechanisms of active uptake. The literature to date has contained no record as to there being any difference between dark uptake velocities or light uptake velocities by either natural lake populations or by bacteria cultures, therefore the reasons given are only speculative and suggest that this aspect of heterotrophy requires further experimental review.

C) Effect of Depth on the Heterotrophic Uptake of Three Labelled Organic Acid Substrates

Of the three substrates used, lactic acid and malic acid showed similar patterns of uptake with depth. These two substrates exhibited a surface maximum followed by a decrease of the uptake rate down to approximately 20 to 25 meters, and then a sharp increase in the rate to 30 meters. On the other hand, malic acid produced a similar rate of uptake from 0 meters down to 20 meters followed by a moderate decrease of the uptake rate down to 30 meters. However, the overall pattern for the three

acids did indicate a decrease in uptake velocity with depth (Figs. 19,20 and 21). Conceivably, the similar pattern exhibited for all three substrates was in fact due to one of two reasons and in all liklihood a combination of both. Firstly, thermal stratification is thought to have played a direct role in controlling bacterial biomass in the water column thus affecting the pattern of uptake throughout the water column. In all cases, there were substantial similarities between the temperature profile and the velocity of uptake with depth (Figs. 19,20 and 21). That is,with decreasing temperatures with depth there was a similar decline in the uptake velocity. This has been substantiated in the literature by the findings of Allen (1969) who detected a correlation between bacterial biomass and therefore maximum velocity values, and temperature. He found that in Lake Lotsjon, a shallow lake which becomes homothermous in early spring that as the water column warmed the V_{max} values for acetate and glucose increased, reaching their highest rate when the lake registered its highest water temperature, and subsequently turnover times decreased, being the lowest when the water temperature was the highest.

Secondly, we suggest that uptake velocity with depth is associated with phytoplankton standing crop. This was found to be true by Hobbie (1967) and was suggested by Vaccaro et al (1968). Hobbie (1967) found that the highest V_{max} values for glucose and acetate in Lake Erkin occurred closely after the phytoplankton peaks, and that decomposition of the dying or dead algae, released sufficient substrate to increase bacterial biomass and thus V_{max} values. Vaccaro et al (1968) found there to be a decrease in glucose concentration and therefore in glucose uptake with depth in the eastern Atlantic Ocean and that this decrease in glucose

concentration may have reflected decreasing photosynthetic production with a resulting decrease in the amount of organic material excreted. Similar patterns for oceanic waters have been noted by Andrews and Williams (1971); Williams (1970); and Vaccaro and Jannasch (1966). Therefore, in West Blue Lake, it was felt that the decrease of uptake rate with depth for both lactic acid and succinic acid was due to declining temperatures and photosynthetic productivity with depth (Figs. 19 and 20). The shallow surface zone of highest uptake velocity for succinic acid (Fig. 19) seemed to be the result of high productivity and possibly photooxidation rather than temperature, as this region was homothermous down to approximately 7 meters. The surface maximum for lactic acid agreed well with temperature, as the depth of this zone of maximum uptake velocity extended to the bottom of the epilimnion and secondly with the zone of maximum primary productivity. The peak at 0 meters, due to high surface productivity and again possibly photooxidation and the peak at 7 meters due to high phytoplankton numbers which would have been concentrated on top of the thermocline, which began at this depth (Fig. 20). The remainder of both curves, down to 25 meters, declined with depth as would be the case with decreasing temperatures. Both substrates exhibited a significant increase in uptake rate from 25 meters down towards the sediments. Both increases were felt to be due to the increasing concentration of soluble organic compounds as a result of increasing bacterial mineralization of detrital particles which would concentrate in this zone. A similar increase in uptake velocity and in this case V_{max} values for acetate were noted by Hobbie (1967) in Lake Erkin. However, Allen (1969)

explained that the high V_{max} values which he found above the sediments were due to increasing temperatures and subsequently increased bacterial biomass and activity. In West Blue Lake the hypolimnion never warmed above 4.5°C therefore temperature was discredited as having caused this sharp increase of uptake rate. The uptake data of malic acid (Fig.12) was completely dissimilar from that of the other two substrates. The surface maximum, a zone 17 meters in depth, could not be rationalized as being completely influenced by temperature and/or productivity, although the surface uptake velocities may have been effected in some part by both of these factors. The sharp decline in uptake velocity below 17 meters followed the theoretical decline in activity with temperature. The extent of the maximum zone of uptake for malic acid, down to 17 meters; (Fig. 21) is believed to suggest a large bacterial population capable of assimilating this substrate and that in nature malic acid is evenly dispersed throughout this zone. The low turnover times and (K+S) value for malic acid (Table 1) as was determined by the saturation curve experiments indicated that this substrate never accumulates during the summer and that the bacteria utilizing this substrate were highly efficient in doing so. It was felt that this was probably the most significant substrate of those tested for heterotrophy in West Blue Lake. Also the data would appear to indicate that the bacteria capable of utilizing malic acid were associated with detrital particles which were completely oxidized before reaching the hypolimnion. On the other hand, the bacteria which were capable of assimilating lactic acid and succinic acid were associated with detrital particles which underwent slow oxidation and subsequently were carried into the hypolimnion. Wright and Hobbie (1966) showed that bacteria utilizing acetate were associated with detrital particles whereas those which utilized glucos

were not. Hobbie (1967) found higher V_{max} values with acetate than glucose in deeper water, as would be the case if the acetate utilizing bacteria were associated with the sinking detrital particles. From these differences in response, it appeared that the bacteria which utilized malic acid were different from those which utilized lactic and succinic acid. When comparing the velocity values of these experiments with those collected from the saturation response experiments for malic acid, the velocities recorded for the malate uptake with depth experiment were two orders of magnitude higher. Lactic acid and succinic acid had similar velocities for both experiments (Table 3 and Appendix 2). The constantly higher velocity values for malic acid throughout the water column ruled out the possibility of sample contamination, for if contamination had occurred, it is doubtful that it would effect all samples in an equal manner. Although not confirmed, the differing velocities of malic acid between the two experiments, may reflect population or physiological differences between the bacteria of Station 5, where the saturation responses were measured, and the bacteria of Station 2 where the uptake with depth responses were measured.

The measured heterotrophic productivity of the three substrates used, only reflect relative heterotrophic productivity for one day and could not be extrapolated to a yearly estimate. Allen (1969) and Hobbie (1967) have reported that enzymatic parameters of heterotrophic uptake kinetics can vary greatly during the year. Both authors have reported that for glucose and acetate, highest uptake velocities were reported during the summer months when phytoplankton productivity and water temperature were

the highest. Therefore, the values which we have reported here (Table 4) measured for mid to late summer might be close to the maximum rate of uptake for these substrates. Andrews and Williams (1971) crudely estimated the yearly flux of glucose and amino acid oxidation by respiration measurements of English Channel waters, and noted that the oxidation of glucose increased throughout the spring, occasionally reaching percentages as high as 250% per day. Andrews and Williams (1971) reported the annual oxidation of glucose as being 2.6 gC/m^2 and of amino acids as being 12 gC/m^2. If one accepts the growth yields as calculated by Williams (1970) for glucose (67%) and for amino acids (78%), as being correct then the oxidation values converted into gross heterotrophic productivity for these two substrates would be significantly higher. Therefore, it was felt that the values reported for lactic acid, succinic acid and malic acid (0.0244, 0.0071 and 2.37 gCm^2hr^{-1}, respectively) might be higher than infact indicated. It would appear from this one set of data that heterotrophic productivity as was measured for the three organic acids can exceed primary productivity per square meter on a day to day basis during the summer in West Blue Lake.

It is believed that if these uptake with depth experiments were modified so as to include measurements of saturation responses at various depths in

the water column for various substrates, the information which would be obtained by kinetic analysis would allow for a greater understanding of the heterotrophic responses as they occurred from the surface of the water column down to the sediments. It seems pointless to measure V_{max} values, (K+S) values and turnover times for only one or two depths when bacterial hetertrophy is known to occur throughout the complete water column. Only with saturation responses and uptake velocities being measured at various depths throughout the water column, on a regular basis could one make an estimate of yearly heterotrophic productivity per substrate. In addition, such a study would produce information on changes of heterotrophic responses with depth during the year for a particular substrate. Such information, collected in this manner would be more compatible for comparison with estimates of primary productivity, and would allow for an estimation of the relative importance of each of these trophic levels.

D) Uptake and Excretion of ^{14}C by Natural Populations

These experiments were designed to measure the excretion rates of labelled soluble organic compounds by natural populations over a 12 to 24 hr period, with added $NaH^{14}CO_3$, and incubated "in situ"; and subsequently to determine re-assimilation rates of these compounds from the immediate medium. No such experiment with similar objectives has been mentioned in the literature to date, but the phenomenon of algal excretion, usually expressed as a percentage of the CO_2 fixed photosynthetically for both cultures and natural populations is well documented (Antia et al, 1963; Fogg and Belcher, 1961; Fogg and Nalewajko, 1964; Fogg and Watt, 1965; Hellebust,

1965; Horne, Fogg and Eagle, 1969; Guillard and Wangersky, 1958; Marker, 1965; Nalewajko, 1966; Tolbert and Zill, 1956; and Anderson and Zeutchel, 1970). Accordingly, excretion expressed as a percentage of CO_2 fixation has been reported anywhere from 2% to 35%, and in some cases as high as 90% (Fogg and Nelewajko, 1964). Although the data discussed here has not been expressed in percentages of fixation, it was evident that excretion of labelled products could reach a significant level (e.g. 220-314 mgC $m^{-3}hr^{-1}$ - Table 8). In all cases, the initial excretory rates (Tables 5,6,7 and 8) were higher in the dark than in the light. A similar occurrence was noted by Horne, Fogg and Eagle (1969). Subsequent excretion rates decreased but were higher in the light than in the dark. The decrease in excretion rates, both in the light and the dark, following the initial high rates were taken to represent re-assimilation of the excretory products (Tables 5a, 6a, 7a and 8a), as all samples would initially contain approximately the same amounts of excretory products per unit volume. Excretion and subsequent re-assimilation of the labelled products with time was believed to indicate an associated response between the algae and the bacteria of the sample. The algae were presumed to be responsible for excretion of the products, while the bacteria and maybe to some degree the algae were responsible for the re-assimilation of these labelled products. Anderson and Zeutchel (1970) felt that the excretion values which they measured were possibly low because of re-assimilation by bacteria and algae. Saunders and Storch (1971) suggested that in fact a coupled oscillatory response between algae and bacteria exists and is controlled by the production and utilization of soluble organic compounds. Tolbert and Zill (1957) and Miller,

Mayer and Tanner (1963) have both shown with algal cultures that glycolic acid, thought to be a major algal excretory product could be re-assimilated by algae under various conditions. Tolbert and Zill (1957) and Fogg and Nalewajko (1964) have suggested that there was an equilibrium between bicarbonate ions and glycolic acid both inside and outside the cell, and that when there was a CO_2 deficit within the cell, external CO_2 diffused in and glycolate was excreted. However, with what has been determined about algal and bacterial heterotrophy since this time, the occurrence of such an operation is doubtful, and it would appear that at present the coupled oscillatory response mechanism as suggested by Saunders and Storch (1971) would be the most acceptable hypothesis regarding excretion and re-assimilation.

Fogg, Nalewajko and Watt (1965) have shown with unialgal cultures that more soluble organic matter was found in the filtrates with longer incubation times, however the results of the four experiments reported here have indicated that the opposite can occur. In most cases, the rate of excretion decreased as the incubation period increased (Tables 5,6,7 and 8).

The data indicate that a type of equilibrium response, and a coupled oscillatory response were in operation to control the excretion and re-assimilation response. Firstly, the initial excretion velocities were highest, decreasing thereafter, indicating that an excretion equilibrium within the new environment of the bottle, had been established. Secondly, the gradual decrease of excretion rates indicated that a coupled response

had been established between the bacteria and the algae of the sample and that the bacteria were heterotrophically utilizing the excreted soluble organic compounds. This equilibrium response may in fact have been the direct effect of having upset an established equilibrium by having enclosed the samples in a glass bottle, and as such probably bears no similarity with the hypothesized equilibrium of Tolbert and Zill (1957) and Fogg and Nalewajko (1964).

The experiment as conducted has one serious pitfall which could and probably did effect the results. It has been shown by ZoBell (1946) that when sea water samples were placed in glass bottles there shortly follows a burst of microbial multiplication, which has been termed the "wall effect". In order to rule out interference due to the "wall effect" experiments designed to measure heterotrophy have been suggested not to exceed 12 hrs duration, and ideally should be of 4 hrs duration (Parsons and Strickland, 1962, and Williams and Askew, 1968). Therefore, instead of measuring an occillating exchange between the algae and the bacteria, an experiment with extended incubation periods would measure only the decline of algal excretory products from the medium concomitant with increasing bacterial numbers. This would explain the decreasing excretion rates of the four experiments (Tables 5,6,7 and 8). Inconsistancies in this trend, which occurred at approximately 12 hrs for experiments 2,3 and 4 (Tables 6,7 and 8 respectively) conceivably represented changes in the bacterial population.

E) Assessment of the Filtration Effect

The filtration effect ("Arthur-Rigler effect") was tested for and no such response with volume change was measured (Arthur and Rigler, 1967).

Conceivably, this was understood to indicate that there was no cellular rupture of the microplankton as a result of filtration. Such results were believed to be due to two possibilities. Firstly, the presence of phytoplankton which were resistant to cellular rupture upon vacuum filtration and secondly to the use of a vacuum pressure which was gentle enough not to cause cellular rupture. However, it must be emphasized that such measurements were determined for only one $NaH^{14}CO_3$ excretion and re-assimilation experiment, and that the other experiments were not checked for this occurrence. Such a filtration effect possibly could have had some bearing on these other experiments consequently producing underestimations where filter activity measurements were involved or over-estimations in the case of filtrate activity measurements.

The results which have been discussed here for the various experiments conducted at West Blue Lake, Manitoba, have not been corrected for $^{14}CO_2$ losses due to respiration by the heterotrophic organisms. Hamilton and Austin (1967) found that bacteria respiration losses of $^{14}CO_2$ could amount to over 50% of the labelled carbon assimilated. Hobbie and Crawford (1969) reported that natural heterotrophic populations could respire between 8 and 80% of the originally assimilated labelled substrate. Therefore, the various heterotrophic parameters reported here might be underestimated by as much as 80%. Respiration losses have been found to vary with the type of substrate, location of the label and the time of the year (Hamilton and Austin, 1967; and Hobbie and Crawford, 1969). However, it is felt that the data reported here is comparable with that reported in the literature, because the data reported there-within contained no corrections for respiration losses.

In light of the failure to monitor respiration losses of $^{14}CO_2$, conclusions regarding the availability, modes, and rates of utilization of the organic acids by the heterotrophic populations of West Blue Lake might be over speculative in nature.

CONCLUSIONS AND SUMMARY

From the preceding experiments demonstrating some of the heterotrophic processes of natural populations to the presence of various labelled organic acids and NaH $^{14}CO_3$, the following conclusions can be drawn.

(a) Saturation Curve Responses

1) Uptake rates for nine test organic acids at low substrate concentrations expressed as V_{max}, T, and K+S values; in most cases were comparable to other values reported in the literature for glucose and/or acetate.

2) Of importance as substrates supporting heterotrophic activity in West Blue Lake were malic acid, fumaric acid, citric acid and succinic acid.

3) Because of the nature of the various heterotrophic responses to the organic acids used as substrates, it was felt that all heterotrophic responses measured were those exhibited by bacteria. This seemed to be substantiated by the fact that K+S values indicated that none of the substrates used were present in amounts in excess of 50 µg/l in West Blue Lake.

(b) Effect of Time on the Heterotrophic Uptake of Nine Labelled Organic Acid Substrates

1) In most cases there was an increase in assimilation rates of the test substrates with time in the light and the dark.

2) Lag periods which were apparent during the 48 hr incubation periods for the test substrates were believed to be indicative of physiological changes of the test organisms due to confinement within the limits of a sample bottle.

3) Velocity of uptake of the test substrates differed only slightly between samples incubated in the light and those incubated in the dark, therefore, ideally light or darkness should not affect the rate at which an organic substrate is assimilated.

(c) Effect of Depth on the Heterotrophic Uptake of Three Labelled Organic Acid Substrates

1) In West Blue Lake, the decrease of uptake velocities with depth for both lactic and succinic acid was believed to be due to declining rates of photosynthetic productivity and temperature with depth, both factors directly controlling the size of the endogenous bacterial population.

2) Photo-oxidation of the algae at the immediate surface of the water column was believed to be the indirect reason for the high surface rates of uptake for lactic and succinic acid.

3) The increased rates of uptake within the hypolimnion were believed to be due to an increase in bacterial population size concommitent with mineralization activity.

4) The decrease in uptake rate with depth for malic acid followed the theoretical decline in activity with temperature.

5) Malic acid, because of its low K+S and T values in addition to the extensive zone in which a high rate of uptake was evident indicated that this organic acid would be of prime importance as a substrate for heterotrophic organisms within West Blue Lake.

6) The hypolimnetic peak in uptake rate for lactic and succinic acids suggested that the bacteria which assimilated these substrates were associated with detrital particles which passed into the hypolimnion where rates of mineralization were high making available sufficient amounts of lactic and succinic acids. Whereas, the bacteria capable of assimilating malic acid were not associated with detrital particles which passed into the hypolimnion, therefore accounting for the rapidly declining rates within this zone.

7) From the data presented, it appears that heterotrophic production as was estimated for lactic, malic, and succinic acid possibly could exceed primary productivity per square meter on a day to day basis during the summer in West Blue Lake.

(d) The Uptake and Excretion of ^{14}C by Natural Populations

1) Initial excretion of labelled products by planktonic heterotrophs were higher in the dark than in the light, with subsequent excretion rates being higher in the light than in the dark.

2) Re-assimilation of labelled excretory products indicated a probable coupled oscillatory response between algal excretion and bacterial uptake of excreted products within the sample.

3) It was felt that experiments of this type should be of the shortest time interval, ideally 4 hrs or less in order to rule out any possible aberration of results caused by a "wall effect".

APPENDIX I

Procedure for Standardization of Isotopes

1. From mean count of six 5 λaliquots of isotope, activity was expressed as DPM/ml isotope solution.

2. Specific activity was expressed as μCi/mg acid; then as DPM/mg acid and finally as DPM/ μg.

3. DPM/ml/DPM/μg = μg acid/ml isotope solution.

4. This value was finally expressed as μg Acid/m^3 and as μg Acid C/m^3.

Velocity ($\mu gC\ l^{-1} hr^{-1}$) x 10^{-3}

Substrate Volume of Inoculum (ml)	Glycolic Acid	Malic Acid	Succinic Acid	Pyruvic Acid	Citric Acid	Acetic Acid	Lactic Acid	Formic Acid	Fumaric Acid
0.01	0.4	11.9	3.4	3.0	2.0	0.1	1.3	0.1	20.8
0.02	0.9	21.2	4.8	6.4	2.9	1.6	2.4	0.1	36.7
0.05	2.4	20.2	10.2	10.8	4.2	1.7	6.3	0.4	37.9
0.07	2.6	34.9	5.6	18.3	5.2	1.8	8.5	0.6	32.4
0.1	3.3	33.0	16.6	32.2	7.8	3.6	18.2	1.2	49.4
0.2	5.3	38.8	24.4	44.0	7.1	10.2	24.4	1.9	53.6
0.5	8.2	44.8	26.8	66.3	9.9	12.0	43.7	2.8	58.9
0.7	9.8	48.8	27.0	60.1	13.6	15.6	55.5	3.0	47.3
1.0	7.6	36.9	20.3	89.2	11.2	17.0	57.5	2.8	56.2
1.2	10.3	60.0	25.6	294.4	10.7	20.2	80.9	3.0	44.7
1.5	10.4	45.9	29.7	98.2	7.0	22.4	86.0	8.0	73.2
1.7	9.8	37.7	61.1	65.1	15.0	21.5	86.7	2.6	51.3
2.0	8.0	39.3	27.9	390.0	8.5	11.6	103.6	2.5	54.9

Appendix 2. Velocities of uptake of nine organic acids with increasing amounts of available substrate.

Substrate / Volume of Inoculum (ml)	Glycolic Acid	Malic Acid	Succinic Acid	Pyruvic Acid	Citric Acid	Acetic Acid	Lactic Acid	Formic Acid	Fumaric Acid
				$\frac{C\mu t}{c}$					
0.01	1,559	39	249	363	647	3099	319	1129	72
0.02	1,370	44	349	333	912	229	340	1812	81
0.05	1,255	115	413	495	1,584	517	316	932	196
0.07	1,621	93	1055	410	1,779	681	330	1009	322
0.1	1,847	141	510	332	1,688	488	219	666	302
0.2	2,304	240	693	486	3,742	344	328	866	556
0.5	3,691	519	1574	808	6,650	734	457	1453	1265
0.7	4,328	666	2187	1247	6,808	791	504	1876	2206
1.0	8,016	1260	4155	1200	11,845	1039	694	2870	2653
1.2	7,091	929	3956	436	14,793	1048	592	3259	3997
1.5	8,739	1517	4270	1635	28,340	1179	696	4011	3054
1.7	10,564	2092	2348	2794	15,021	1393	782	5343	4941
2.0	15,056	2364	6055	549	31,031	3036	771	6481	5433

Appendix 3. Velocities of uptake of nine organic acids expressed as $\frac{C\mu t}{c}$ for Lineweaver-Burk conversions.

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