INHIBITION OF *BACILLUS SUBTILIS* RESPIRATION ON GLUCOSE, FORMATE AND SUCCINATE BY CERTAIN ANESTHETIC AGENTS

by

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INHIBITION OF *BACILLUS SUBTILIS* RESPIRATION ON GLUCOSE, FORMATE AND SUCCINATE BY CERTAIN ANESTHETIC AGENTS

HISTORY AND GENERAL ASPECTS OF THE PROBLEM

The importance of the use of narcotic agents*, including anesthetics, at the present time is well recognized. The literature concerning the choice of a particular agent for use in a given situation is quite voluminous and a certain amount of proficiency in the use of narcotics has been achieved. More recently considerable work has been devoted toward an elucidation of the mode of action of narcotic agents and though some advances have been made the over-all picture is not yet clear. In the last analysis we still do not know how many narcotics bring about their physiological action.

Efforts to determine the causative mechanism of narcosis have led to the investigation of many narcotic agents and their effect on many types of living organisms as well as on isolated tissues and tissue extracts. The range of living forms, which have been studied

*

The present day terminology which is encountered in many reviews dealing with the physiological action of drugs is somewhat confusing. For purposes of clarity the author will follow the terminology of V. E. Henderson (12) who reserves the term narcotic to designate chemical compounds which depress the activity of the central nervous system when present in certain concentrations. When their concentration falls below a certain point the cells involved are restored to their normal activity. Henderson considers general anesthetics and hypnotics as being included under the more general term of narcotic and discusses the physiological theory of such compounds under the heading, "Theories of Narcosis".

while under the influence of narcotics, extends from the larger animals, including man, down to the single-celled organisms such as yeasts and bacteria (12). In the earlier investigations the larger part of the work was carried out with the higher animals as the objects of study. Recent advances in the knowledge of cellular metabolism and enzyme chemistry, and the realization of the similarity between the basic aspects of cell physiology in higher and lower forms of life, have formed a new background for the study of narcotic action. Thus we find that the use of brain tissue (3,7,11,12,15,16,23,24,26) and microorganisms (7,14,19,20) are prominent at the present time.

Although the earlier theories of narcosis have proved inadequate in many respects, it is true that a brief consideration of the older work is necessary in order to complete the over-all picture.

The earliest attempt to provide a theory of narcotic action was presented by Bilers and Marless (12). These workers suggested that anesthetic agents dissolve certain fatty constituents out of the brain cells and that these brain constituents are later deposited in the liver. This theory is historically important because it served as a forerunner of the more modern lipoid theory which will be discussed subsequently.

Another early theory was concerned with the possibility of coagulation of the nerve cell "substance" when the cell in question was under the influence of a narcotic. This hypothesis was temporarily disregarded because the experimental coagulation was irreversible and was obtained with isolated protein systems in the presence of narcotics

in higher concentrations than the concentrations required to produce narcosis in living animals. More recent work indicates that certain intracellular substances may undergo a coagulation which is reversible (2,13,14).

Kochman (12) believed that narcotics produce a reversible dehydration of the colloids within the nerve cells. It was assumed that the dehydration upset the normal permeability of the cell membranes which in turn led to a deranged metabolism. However it was soon pointed out that many other conditions which produced dehydration did not produce symptoms indicative of narcotic action.

The work of Traube (12,18) although it does not necessarily present additional information as to the specific intracellular mechanism of narcotic action, should not be omitted. Traube pointed out the relationship between capillary activity and narcosis. In a homologous series of chemical compounds it appears that those members of the series which have the greatest ability to depress the surface tension of aqueous solutions also possess the greatest narcotic activity.

Another phenomenon which apparently has a bearing on narcotic action but which does not explain, in a specific manner, the mode of action has been presented in the Meyer-Overton theory (12,18). This theory was advanced by both Meyer and Overton while working independently. These workers believe that narcotics exert their chief action upon the cells of the central nervous system by virtue of the fact that they are taken up by the cell lipoids such as lecithin and cholesterin. According to Meyer and Overton, the characteristic properties of anesthetics increase with an increase in lipoid solubility and water

insolubility. At the present time it is stressed that the Meyer-Overton phenomenon shows how the brain cell may be reached but does not explain the specific action on the cells involved.

More recently there has been a tendency to consider the specific cellular influence of narcotics in the light of what effect they may have upon the oxidative metabolic processes of the nerve cells (7,11,12,15,17,23,24,26). The asphyxial theory of Verworn (12,18) served as a predecessor to the more modern view. Verworn theorized that a cell under the influence of a narcotic loses its ability to take up and store oxygen. The appearance of metabolic products which were usually correlated with a process of incomplete combustion within the cell, namely, acetone and lactic acid, supported this view. Verworn further suggested that the atomic groups within the cell, which are concerned with transfering oxygen to the cell, may enter into a loose chemical union with the anesthetic agent and thereby hinder the normal oxidative processes. It was also shown that such action may take place in any cells of the body, but that the cells of the cerebrum are especially sensitive to a lack of oxygen and are depressed with a much lower concentration of the narcotic than is necessary to depress the nerves and muscles.

Although Verworn's theory of narcotic action is not as specific as the modern view, it is to be remembered that Verworn presented his asphyxial theory at a time when the mechanism of cellular oxidations were less fully known than they are today. It appears to the author and others (12) that indeed, the concept of oxygen

deprivation was a long step leading to the current postulations concerning the mode of action of narcotics.

One objection to Verworn's theory, which is summarized in the review of Henderson (12), was that narcosis could be induced in cells carrying on an anaerobic type of metabolism. For example, Warburg and Wiesel (12), in 1912 showed that yeast fermentation was hindered by the presence of narcotics and Veszi (12) in 1918 brought about anesthetization of obligately anaerobic bacteria. However, the knowledge that many of the oxidative processes of the living cell do not actually involve molecular oxygen (9,10) makes the belief that narcotics generally interfere with cell respiratory processes more tenable.

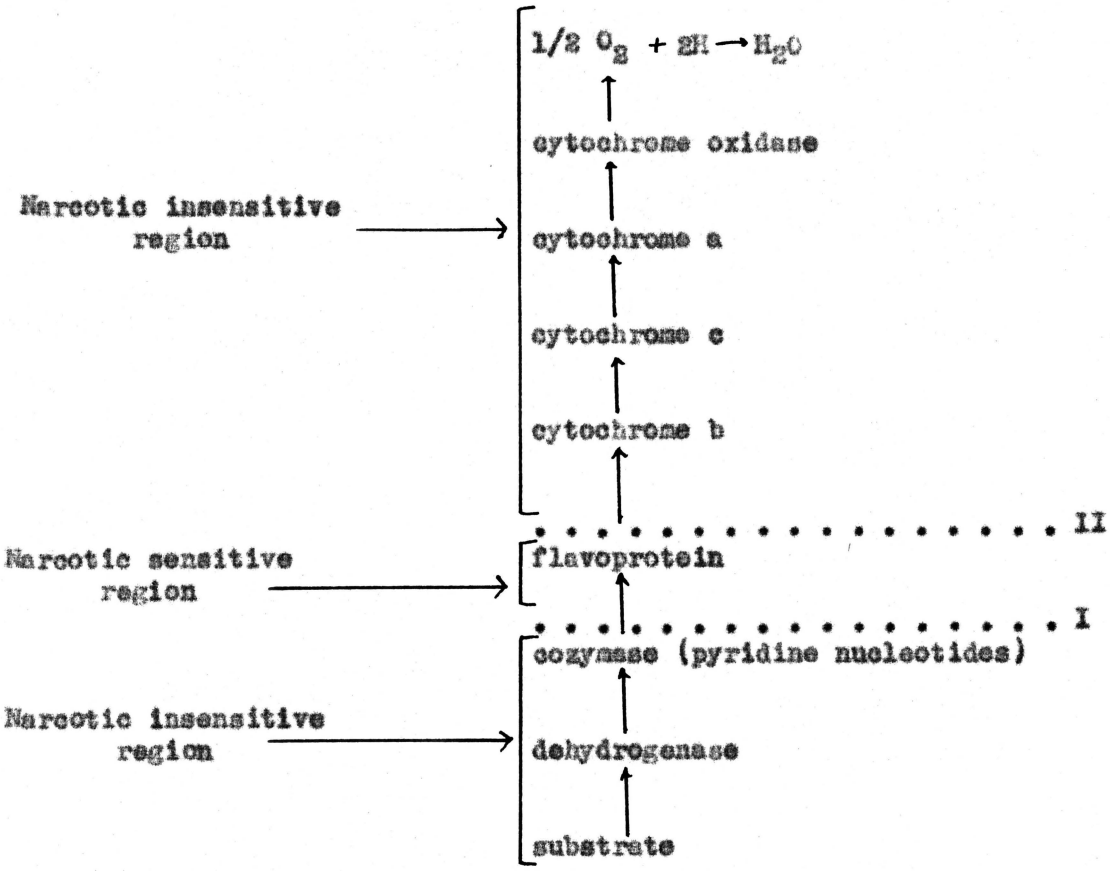
Additional evidence that the respiratory activity of the central nervous system is reduced during narcosis was presented by McClure et al (12). These workers demonstrated that anesthesia in animals, brought about by barbiturates and other narcotics produced anoxia (oxygen deficiency). Also, according to Shaw et al (23), ether anesthesia is associated with a decrease in the difference between the oxygen contents of arterial and venous bloods.

In 1938 Jowett (15) demonstrated, by the use of the brain slice technic and manometric methods (5,29), that quite definite inhibitions in the respiration of brain cortex tissue take place in the presence of nartotics at concentrations which produce narcosis in animals (about 0.001 molar). The substrate or material oxidized by the cells in these studies was either glucose or some compound believed to be important in the oxidative breakdown of glucose. Such a choice of substrates

fits the fairly well established fact that glucose is the main substrate of the brain in the living animal (23). It is true that narcotics have the ability to inhibit oxidation in tissues other than brain, but the rather striking action of low concentrations of narcotics seems to be limited to the inhibition of enzyme systems which play an important role in carbohydrate metabolism (23).

Proceeding on the evidence that many narcotics exert their physiological action by interfering with some phase of glucose metabolism in the central nervous system, Michaelis and Quastel (17), in 1941, conducted a series of investigations by which they were able to locate, within limits, the portion of the glucose oxidation chain which is acted upon by some narcotics. Using rat or guinea pig brain cortex slices as the oxidizing tissue and chloretone (trichlortertiary butyl alcohol) as the narcotic agent, these workers found that the oxidation of glucose, lactate and pyruvate was inhibited while the oxidation of succinate was not. They have postulated that there are two possible positions where narcotics may bring about their inhibiting effect. The location of these two positions is best seen with the aid of Hall's scheme (16) of the oxidation-reduction systems involved in biological oxidations as utilized by Orieg (11). This scheme shows diagrammatically how the oxidation of the substrate is though to take place under aerobic conditions. Initially the substrate is oxidized by the removal of hydrogen and the hydrogen thus removed ultimately unites with molecular oxygen to form water. The compounds acting within the extremities of the chain are capable of undergoing reduction and mediate the oxidation of the

substrate and the final reduction of molecular oxygen. As the reduced forms of the intermediates are in turn oxidized they are continually being regenerated for further use.



Scheme of Biological Oxidation

According to Quastel and his associates, narcotics exert their action at either point I or point II. An effect at point I would block the transfer of hydrogen from the hyridine nucleotide to flavoprotein while an effect at point II would inhibit the transfer from flavoprotein to cytochrome b or some unknown intermediate.

Grieg, working with nembutal and chloroform as inhibitors, has extended this basic idea. She has shown that there is no accumulation of reduced cozymase during the metabolism of carbohydrate by brain tis-

sue and has reasoned from this that there is no interference at point I. She also has shown that there is no decrease in the rate of hydrogen transfer from cozymase to flavoprotein in the presence of narcotics and that narcotics do not inhibit the oxidation of succinate. In summary, Grieg suggests that the narcotic inhibitors selectively hinder the transfer of hydrogen by cytochrome b or by some other intermediate which is similar to cytochrome b.

Philpot (21) has shown that local anesthetics such as cocaine and procaine lower the oxidation rate of liver tissues respiring in the presence of adrenaline and from this postulates that some portion of the cytochrome c system is affected.

The enzyme system involved in the oxidation of succinate, namely: succinic dehydrogenase or succinoxidase, has received considerable attention from investigators (11,17) who are interested in narcotic action. As stated previously, this enzyme system is thought to be insensitive to the action of narcotics (11,17). The enzyme dehydrogenates succinate to fumarate provided a hydrogen acceptor is present (6,9,16,22) and if the hydrogen acceptor is oxygen Straub (22) believes the succinoxidase system to be composed of the following:

Cytochrome oxidase
↑
Cytochrome a
↑
Cytochrome c
↑
Unknown factor
↑
Succinic dehydrogenase

The terminology encountered in a discussion of the above system appears to be irregular as the term <u>succinic dehydrogenase</u> may

refer to either a portion of the system or the system in its entirety. It is to be noted that Straub's conception of the succinoxidase system contains no cytochrome b but rather an unknown factor which transfers hydrogen to cytochrome c. Axelrod, Potter, and Elvehjem (29) believe that one or more components of the succinoxidase system are flavoproteins.

An enzyme system which may be similar to the succinoxidase system is the formic dehydrogenase system. Most of the information concerning formic dehydrogenase has come from the work of Gale (22,28). The enzyme is generally reported as being found in Escherichia coli and possesses the ability to dehydrogenate formate as follows:

Formate ——— carbon dioxide + 2 hydrogen

Under aerobic conditions the hydrogen is taken up by the cytochrome - cytochrome oxidase system. The exact components of the cytochrome system involved in the oxidation of formate and the effect of narcotics on the system have not been reported.

The major portion of the studies to date which have dealt with the effects of narcotics on bacteria have been concerned with the luminescent bacteria. Several investigators (13,14,19,20) have employed the enzyme system responsible for bacterial luminescense (luciferase) to study narcotics as inhibitors of cellular activity. The enzymes taking part in bacterial luminescense are quite sensitive to the presence of narcotics and have thus provided a basis for research as to the specific manner in which narcotics may inactivate enzymes. Achromobacter fischeri (Bacterium phosphorescens indigenus) and Photobacterium phosphoreum (Bacterium phosphoreum) have been studied in this connection.

It is to be noted that the physiologist, who is today interested in gathering information concerning the specific mode of action of narcotics often directs his attention, in one manner or another, toward the enzymatic systems generally believed to be active in the biological oxidation of glucose. More specifically the general plan of attack often involves some technic by means of which a given segment of the over-all oxidative scheme may be isolated and studied. Once the particular segment is isolated the problem may then be one of comparing the activity of the segment under normal environmental conditions with the activity of the segment operating under the influence of the narcotic in question.

PURPOSE OF THE INVESTIGATION

One approach to the problem of the mechanism of anesthetic action is through a study of cell respiration and fermentation (7). The bacterial cell is relatively easy to manipulate in the laboratory and possesses a form of metabolism which is strikingly similar to the type of metabolism found in the cells of higher animals.

The present investigation was carried out to determine what concentrations of certain anesthetic agents are required to bring about a noticeable inhibition of the respiration of Bacillus subtilis. If the organism was allowed to respire on glucose, formate and succinate, such a variety of substrates would permit the study of the action of several enzyme systems under the influence of the anesthetic agents.

The results could then be compared with the work of other investigators (11,24) in which the respiration of certain mammalian brain tissues was measured.

THE THEORY AND USE OF THE WARBURG RESPIROMETER

Manometric methods are extremely useful for quantitative analytical work in which it is desirable to estimate the exchange of gases involved in chemical and biological reactions. Several modifications of manometric instruments are available for determinations of this type (5,29). The modification known as the Warburg respirometer (see Figure 1) is one of the most extensively used.

The theoretical aspects of the Warburg apparatus have been thoroughly covered in the literature (5,29). Briefly this instrument is a constant volume type of respirometer consisting essentially of a graduated U-tube manometer and a reaction flask. The left-hand portion of Figure 1 shows a front view of the Warburg respirometer while the right-hand portion depicts a side view of the same apparatus. One end of the manometer is open to the atmosphere while the other end is attached, through a ground glass joint, to the reaction flask. It is also provided with a rubber reservoir and screw clamp arrangement by which the level of the manometric fluid may be adjusted. When the respirometer is in use it is attached to a shaking apparatus and water bath in such a manner that the reaction flask is completely immersed. The manometer is not immersed in the water bath but is on the outside of the water bath for good observation of the manometric fluid. The water bath provides a medium of constant temperature while the shaking apparatus promotes a rapid exchange between the liquid and gas phase within the reaction flask.

The operation of the Warburg respirometer is as follows:

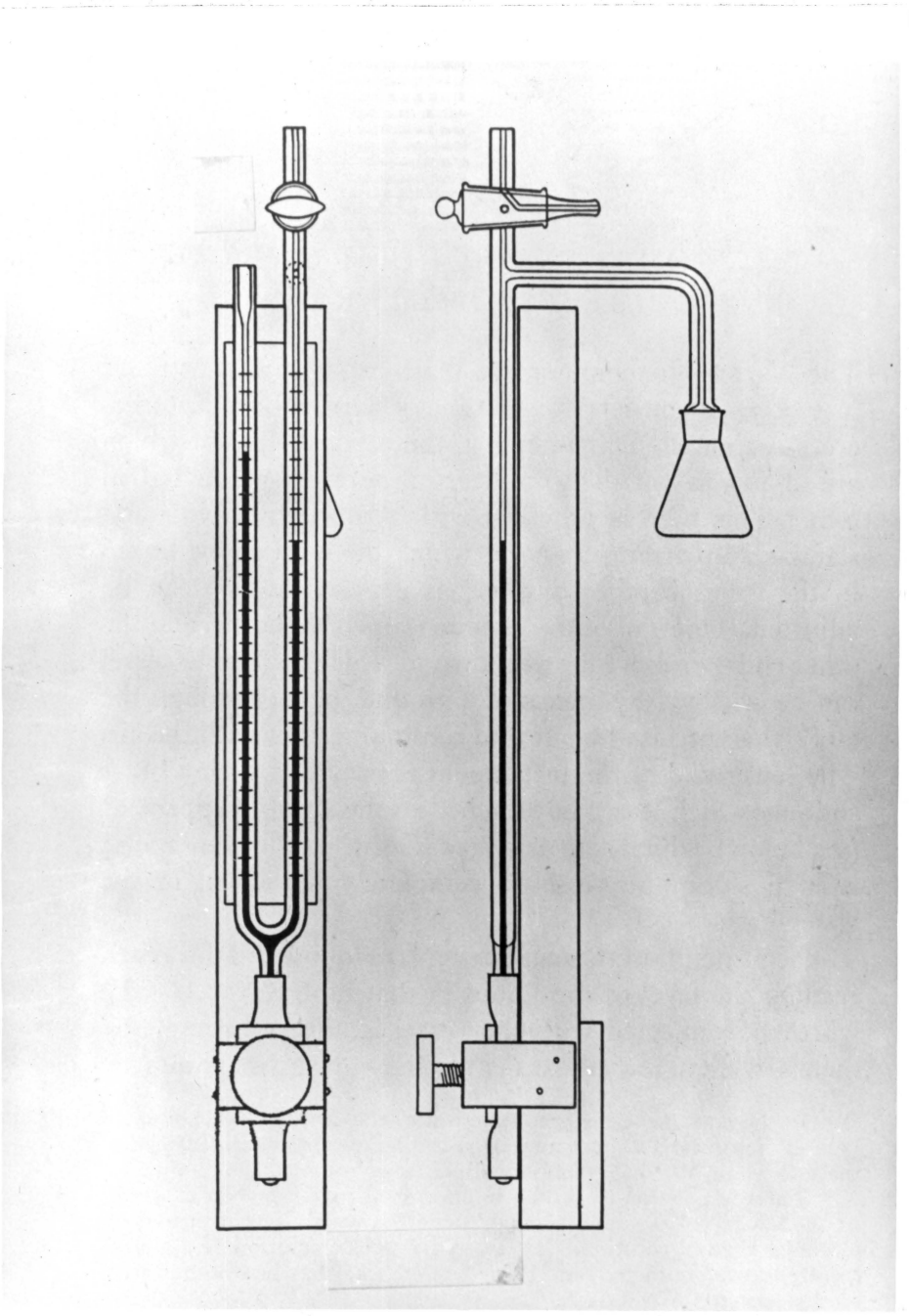


Figure 1. The Warburg Respirometer

The reaction flask contains a known amount of liquid in which a reaction takes place. The reaction in question produces either an evolution or absorption of a gas. The magnitude of this amount of gas is determined by reading the level of the manometric fluid before and after the reaction takes place.

So that each reading may be made at constant volume, the level of the liquid in that side of the manometer which is connected with the reaction vessel is always brought to the same given point (usually the half-way mark) by means of the screw adjustment. Only the level in the open side of the manometer is read. The difference between the before and after readings is due to the change in pressure within the reaction flask.

The equation used for the calculation of the volume of gas liberated or taken up is

$$x = hk$$

where **x** is the amount of gas evolved in cubic millimeters (c.mm.) at normal pressure and temperature (760 mm. and 0°C.). If the gas is absorbed x will be negative.

h is the observed change in the reading of the manometer in millimeters.

k is the constant of the apparatus. The constant of the apparatus, generally known as the flask constant, is necessary to convert the actual change in manometer reading into volume of gas. The value of k is dependent upon the characteristics of the particular flask and the conditions of the experiment.

A detailed derivation of the foregoing equation may be found in the literature (5,29).

The calibration of the apparatus, which gives rise to the value of the apparatus constant k, can be deduced by three methods (29).

1. By calculation from the following formula:

$$k = \frac{V_g \frac{273}{T} + V_f \, a}{P_0}$$

where V_g is the volume of the gas space in the reaction flask. This volume includes that portion of the manometer located between the reaction flask itself and the manometric fluid.

T is the absolute temperature of the water bath.

V_f is the volume of the liquid in the reaction flask.

a is the solubility of the gas being measured in the reaction liquid. It is the cubic millimeters of gas at normal pressure and temperature dissolved in the reaction liquid when the liquid is in equilibrium with a partial pressure of the gas equal to P_0.

P_0 is the normal pressure expressed in terms of manometric fluid. If D is the density of the manometric fluid,

$$P_0 = 760 \times \frac{13.60}{D}$$

2. By the Munzer and Newman method (29) in which a measured amount of gas is added to or withdrawn from the flask by means of a graduated pipette and the resulting reading of the manometer observed.

3. By liberating or absorbing a known amount of gas in the vessel by means of a known chemical reaction.

In these studies the apparatus constants were determined by the method of calculation.

THE DETERMINATION OF OXYGEN CONSUMPTION All oxygen consumption values were determined by the direct method (5,29). In the direct method 20% KOH is placed in the center well of the reaction flasks. The alkali absorbs the carbon dioxide produced by the respiring cells and any displacement of the manometric fluid is then due to oxygen consumption. This is based on the assumption that oxygen and carbon dioxide are the only two gases involved in the respiratory activity of the cells whose metabolism is to be studied. The reading of the manometer gives a direct measure of the oxygen consumed. Only one flask is necessary for determining the oxygen uptake by the direct method.

THE DETERMINATION OF CARBON DIOXIDE LIBERATION The direct method of carbon dioxide measurement (5,29) was adhered to throughout this work. This method requires the use of two manometers plus the accompanying reaction flasks, with the same amount of respiring material in each flask. In one flask the oxygen uptake is determined directly by the method discussed above. In the second flask the alkali is left out and the manometer reading is influenced by both the oxygen consumption and the carbon dioxide liberation. For purposes of explanation, let the volume of oxygen consumed per unit time be equal to X_{O_2} and the volume of carbon dioxide liberated for the same period equal X_{CO_2}. The liberation of X_{CO_2} gives an observed change in the manometer reading

h_{CO_2} $\frac{X_{CO_2}}{k_{O_2}}$. The absorption of X_{O_2} will change the manometer reading by an amount $h_{O_2} = \frac{X_{O_2}}{k_{O_2}}$. h_{O_2} and X_{O_2} are negative quantities. The resultant change in the reading of the second manometer, which contains no alkali, is the algebraic sum of the two component readings given above.

$$h = \frac{x_{O_2}}{k_{O_2}} + \frac{x_{CO_2}}{k_{CO_2}}$$

thus

$$x_{CO_2} = \left(h - \frac{x_{O_2}}{k_{O_2}} \right) \times k_{CO_2}$$

Since x_{O_2} is known from calculations involving the reading of the manometer with alkali present, k_{O_2} and k_{CO_2} are known from calibrations and h is observed, it is possible to calculate x_{CO_2}.

THE USE OF THE THERMOBAROMETER Due to the fact that one end of the Warburg manometer is open to the atmosphere, the level of the manometric fluid is greatly influenced by small changes in barometric pressure or the temperature of the water bath, during the course of an experiment. An allowance is made for these changes by use of a thermobarometer. The thermobarometer is a Warburg respirometer containing only a small amount of water in the reaction flask. This manometer is placed in the water bath along with the experimental respirometer. The thermobarometer is read whenever the other respirometers are read and its reading is subtracted from the readings of the experimental respirometers. Such a procedure corrects for errors due to changes in external conditions. Any changes will affect the readings of all manometers in the same way (29).

Calibration of the Warburg Respirometers

In order to determine the flask constant (k) from the expression $k = \frac{V_g \frac{273}{T} + V_f a}{P_0}$ it is necessary to know the values for V_g, T, a, V_f and P_0. These values remain constant for a given set of conditions.

The values for V_g for each of the individual respirometers was determined by calibration with mercury according to the procedure outlined by R. H. Burris (29). V_g is the sum of the reaction flask volume (x), the volume of the ungraduated portion of the manometer (y), and the volume of the graduated portion of the right limb of the manometer between the 300 mm. and 150 mm. marks. The volumes represented by x, y, and Z were obtained by filling the various parts of the apparatus with mercury at known temperature and weighing. Since the density of mercury at various temperatures was known the volumes x, y, and z were calculated from the equation, volume equals mass/density.

The determination of the reaction flask volumes. The weight of the mercury required to fill the reaction flask was obtained by weighing the flask in question before and after filling with mercury. The values used to calculate the reaction flask volumes are found in Table I.

Table I

Values for calculating reaction flask volumes
(temperature 24.8° C., density of mercury)
$Hg = 13.53$

Reaction Flask Number	Weight Flask Empty	Weight Flask Filled	Weight of Mercury	Flask Volume
1	26.04 gm.	256.74 gm.	230.70 gm.	17050mm^3
2	25.82	269.51	243.69	18020
3	25.59	263.57	237.98	17590
4	25.91	261.68	235.77	17430
5	28.02	257.79	229.77	16480
6	31.37	239.27	207.90	15370
7	26.68	254.73	228.05	16860

The determination of the volumes of the graduated portions of the manometers.

In order to determine the volumes of the graduated portions of the manometers it was first necessary to obtain the internal cross sectional areas of the manometer tubes. The cross sectional areas were calculated from length and weight measurements of mercury columns contained within the manometer tubes. For a cylinder, area equals volume/length. The mercury columns were measured in several different positions on the manometer scale in order to show up gross variations in the bore of the manometer if they existed. Variations were negligible. The values for the length of the columns were measured by means of the graduated scales on the manometers and the weights of the columns were ascertained by weighing upon removal from the manometers. The values used in the calculation of the manometer cross sections are indicated in Table II which lists the average lengths of the mercury columns.

Table II

Values for calculation manometer tube cross sectional areas
(temperature 25° C., density of mercury 13.53)

Manometer Number	Average Length Mercury Column	Weight of Mercury	Volume of Mercury	Cross Sectional Area
1	151.6mm	3.4814 gm.	257mm^3	1.70mm^2
2	203.1	5.4358	401	1.98
3	131.6	3.0502	227	1.73
4	103.9	2.1369	158	1.52
5	92.1	2.4949	184	2.00
6	96.0	2.7605	204	2.13
7	92.2	2.8885	210	2.28

Once the cross sectional areas of the manometer tubes were known the volumes of the graduated portions of the manometers were readily found by computing the product of the length and cross sectional area. As stated previously all volumes were contained between the 300mm and 150mm marks. Calculation values and results are found in Table III.

Table III

Values for calculation of volumes of graduated portion of manometers

Manometer Numbers	Length of Desired Portion	Cross Sectional Area	Volume of Graduated (150 - 300) Portion
1	150mm	1.70mm^2	255.0mm^3
2	150	1.98	297.0
3	150	1.73	259.5
4	150	1.52	228.0
5	150	2.00	300.0
6	150	2.13	319.5
7	150	2.38	342.0

The determination of the volumes of the ungraduated portion of the manometers.

The determination of the internal volumes of the ungraduated protions of the right-hand limbs of the manometers was carried out by an indirect method. Length and weight measurements were taken on mercury columns which extended from some point on the graduated scale to the ground glass joint connecting the manometer with the reaction flask. Since the cross sectional areas of the manometer tubes were known from

previous calculations and the length of the segment of the mercury column lying in the graduated section of the manometer was measurable, it was possible to determine the volume of the ungraduated portion by difference. The data necessary for the calculation of these volumes is contained in Table IV.

Table IV

Values for calculation of volumes of ungraduated portion of manometers
(temperature 25°C. density of Hg 13.53)

Manometer Number	Length of Mercury Column	Weight of Mercury	Volume of Mercury	Volume of Graduated Portion	Volume of Ungraduated Portion
1	joint-287.0mm mark	6.5366gm	468.3mm^3	22.1mm^3	446.2mm^3
2	joint-233.6	10.1353	748.3	131.5	616.8
3	joint-192.1	10.1555	750.4	125.7	624.7
4	joint-197.0	8.4578	625.9	157.6	468.3
5	joint-218.3	9.6455	712.9	163.4	549.5
6	joint-294.6	6.5073	481.0	11.5	369.5
7	joint-214.0	11.2222	829.4	196.1	633.3

The determination of the total respirometer volumes. The total respirometer volumes were calculated by taking the sum of the volume of the graduated portion of the manometer, the volume of the ungraduated portion of the manometer and the volume of the reaction flask. These values are summarized in Table V.

Table V

Values for calculation of total respirometer volumes

Manometer Number	Volume of Graduated Portion	Volume of Ungraduated Portion	Volume of Reaction Flask	Total Respirometer Volume
1	255mm^3	446mm^3	17050mm^3	17751mm^3
2	297	617	18020	18934
3	260	625	17590	18475
4	228	468	17430	18125
5	300	550	16980	17830
6	320	370	15370	16060
7	342	635	16860	17835

The determination of the flask constants. The actual calculation of the flask constants were performed according to the suggestions of Umbreit (19). It has been stated previously that the flask constant (k) is equal to $\frac{V_g \frac{273}{T} + V_f a}{P_0}$ and it is possible to calculate k for any set of experimental conditions from the expression given above. However, it is convenient to alter the constant (k) from one experimental condition to another without recalculating the complete expression. Umbreit has presented a method for calculating different factors for different

volumes of fluid in the flasks. It has been shown (29) that the relationship between the presence of one cc. more fluid in the reaction flask and the flask constant is quite simple. For each cc. of additional fluid in the flask, the flask constant will change a definite amount. In this paper the value of this change is designated U_{1000} and is equal to $\frac{- 1000 \frac{273}{T} + 1000\,a}{P_0}$. It is to be noted that U_{1000} does not vary with the characteristics of each reaction flask and hence is the same for all flasks. Umbreit's values for $U^{O_2}_{1000}$ for determinations involving oxygen consumption at 30°C. show $U^{O_2}_{1000}$ to be -0.087.

Since values for $U^{CO_2}_{1000}$ when carbon dioxide is liberated at 30°C. were not available it was necessary to make complete calculations. It was first necessary to calculate values for P_0 and a. In order to arrive at P_0 it was necessary to determine the density of the manometric fluid. A 5 cc. sample was weighed in triplicate.

Sample 1.	5.1629 gm
Sample 2.	5.1578 gm
Sample 3.	5.1595 gm
Average sample weight	5.160 gm
Average weight/ cc.	1.032 gm

Since P_0 is normal pressure expressed in terms of manometric fluid

$$P_0 \text{ equals } 760 \times \frac{13.60}{1.032}$$

$$P_0 \text{ equals } 10015$$

Because these experiments were carried out in buffered solutions at a pH of 6.8 it was also necessary to correct for carbon dioxide

retention (29). A useful method of correcting for carbon dioxide retention has been suggested by Johnson (29). This method consists primarily of substituting the effective value of a (known as a') whenever a is ordinarily used. Johnson further states that $\frac{a'}{a}$ equals antilog [(pH - 6.343)]+1 where the pH is that in the reaction flask during the experiment.

For experiments run at a pH of 6.8 and a temperature of 30°C.

$$\frac{a'}{a} = [\text{antilog } (pH - 6.343)] + 1$$

$$= [\text{antilog } (6800 - 6.343] + 1$$

$$= \text{antilog } 6.457 + 1$$

$$= 2.864 + 1$$

$$= 3.864$$

When a is 0.66 (28)

$$a' = 3.86 \times 0.66$$

$$a' = 2.550$$

Therefore at pH 6.8 and temperature 30°C.

$$U_{1000}^{CO_2} = \frac{-1000 \frac{293}{303} + 1000 (2.550)}{10015}$$

$$U_{1000}^{CO_2} = \frac{-900.990 + 2550}{10015}$$

$$U_{1000}^{CO_2} = \frac{1649.010}{10015}$$

$$U_{1000}^{CO_2} = 0.165$$

When using the factor U_{1000} it is necessary to use a flask constant (k) based on 0.0cc. of fluid being present in the flask. Therefore since

$$k = \frac{V_g \frac{273}{T} + a \quad V_f}{P_0}$$

at 0.0 cc. of fluid

$$k_{0-cc.} = \frac{V_g \frac{273}{T}}{P_0}$$

and $k_{0\text{-cc.}}$ is the same regardless of the gas being measured. At 30°C. and with P_0 to 10015 as calculated previously.

$$k_{0\text{-cc.}} \text{ equals } \frac{V_g \times \frac{273}{303}}{10015}$$

$$k_{0\text{-cc.}} \text{ equals } V_g \times \frac{0.900990}{10015}$$

$$k_{0\text{-cc.}} \text{ equals } V_g \times 0.00008996$$

$$k_{0\text{-cc.}} \text{ equals } V_g \times 0.0000900$$

The flask constants for the various flasks under conditions of 30°C. and 0.0 cc. of fluid in the flasks are given in Table VI.

Table VI

Values for calculation of $k_{0\text{-cc.}}$

Flask Number	V_g	x	0.0000900	=	$k_{0\text{-cc.}}$
1	17751 mm³	x	0.0000900	=	1.598
2	18934	x	0.0000900	=	1.704
3	18475	x	0.0000900	=	1.663
4	18108	x	0.0000900	=	1.630
5	17830	x	0.0000900	=	1.605
6	16060	x	0.0000900	=	1.445
7	17835	x	0.0000900	=	1.605

The experiments involving oxygen consumption were carried out at 30°C. with a total of 3.2 cc. of fluid in the reaction flask, i.e. 3.0 cc. of reaction solution plus 0.2 cc. of 20% KOH for carbon dioxide absorption. Since the fluid volume was 3.2 cc. it was necessary to multiply U_{1000} by 3.2. Therefore for oxygen measurements:

$$U_{1000} \times 3.2 \text{ equals } -0.087 \times 3.2$$

$$U_{1000} \times 3.2 \text{ equals } -0.278$$

Furthermore:

$$k_{O_2} \text{ equals } k_{0\text{-cc.}} + U^{O_2}_{1000}$$

$$k_{O_2} \text{ equals } k_{0\text{-cc.}} - 0.278$$

The final k_{O_2} for the various flasks are listed in Table VII.

Table VII

Values for calculation of k_{O_2}

Flask Number	$k_{0\text{-cc.}}$	- 0.278	=	k_{O_2}
1	1.598	- 0.278	=	1.320
2	1.704	- 0.278	=	1.426
3	1.663	- 0.278	=	1.385
4	1.630	- 0.278	=	1.352
5	1.605	- 0.278	=	1.327
6	1.445	- 0.278	=	1.167
7	1.605	- 0.278	=	1.327

The experiments in which carbon dioxide evolution was measured were performed at 30°C. with a total fluid volume of 3.0 cc. in the reaction flasks. Hence:

$$k_{CO_2} \text{ equals } k_{O\text{-cc.}} + U^{CO_2}_{3000}$$

and:

$$U^{CO_2}_{3000} \text{ equals } U^{CO_2}_{1000} \times 3.0$$

$$U^{CO_2}_{3000} \text{ equals } 0.165 \times 3.0$$

$$U^{CO_2}_{3000} \text{ equals } 0.495$$

The final k_{CO_2} for the various flasks are listed in Table VIII.

Table VIII

Values for calculation of k_{CO_2}

Flask Number	$k_{O\text{-cc.}}$	+	0.495	=	k_{CO_2}
1	1.598	+	0.495	=	2.093
2	1.704	+	0.495	=	2.199
3	1.663	+	0.495	=	2.158
4	1.630	+	0.495	=	2.125
5	1.605	+	0.495	=	2.100
6	1.445	+	0.495	=	1.940
7	1.605	+	0.495	=	2.100

Table IX contains typical values of oxygen consumption as determined by the various respirometers and is presented to show that

the variation in oxygen volumes obtained was not large. A similar comparison of carbon dioxide liberation values is presented in Table X.

Table IX

Typical variation between Warburg respirometers. Respiration of Bacillus subtilis; oxygen consumption on 0.01M glucose.

	Respirometer Number					
	7	6	4	3	2	1
Time in Minutes	Gas Volumes in Cubic Millimeters					
A (replicate)						
0 - 15	30	31	30	32	31	29
15 - 30	26	28	28	28	30	30
30 - 45	28	33	34	32	28	29
45 - 60	27	30	28	30	28	30
60 - 75	29	30	28	30	30	33
75 - 90	30	28	30	31	29	32
Totals	170	180	178	183	176	183
B (replicate)						
0 - 15	24	29	33	30	33	34
15 - 30	30	31	36	38	30	33
30 - 45	33	34	30	32	30	35
45 - 60	30	36	33	39	33	34
60 - 75	36	40	36	35	32	37
75 - 90	33	40	39	34	33	36
Totals	186	210	207	208	201	209

Table X

Typical variation between Warburg respirometers. Respiration of *Bacillus subtilis*: carbon dioxide liberation on 0.01M glucose.

Time in Minutes	Respirometer Number 6	3	1
	Gas Volumes in Cubic Millimeters		
A (replicate)			
0 - 15	44	46	44
15 - 30	38	36	40
30 - 45	36	38	40
45 - 60	34	34	32
60 - 75	28	35	32
75 - 90	29	30	34
Totals	209	219	222
B (replicate)			
0 - 15	42	48	48
15 - 30	36	42	39
30 - 45	42	36	39
45 - 60	36	30	36
60 - 75	36	36	33
75 - 90	30	33	33
Totals	222	225	228

EXPERIMENTAL

ORGANISM The organism used throughout this investigation was Bacillus subtilis Koch-Novy (Texas), V.P.I., No. 3. This organism was selected for study because its size makes it relatively easy to separate from a suspending liquid by centrifugation and it possesses a highly aerobic type of metabolism and is therefore suitable for experiments which involve oxygen consumption. This strain does not readily form a pellicle in nutrient broth and is thus suitable for the preparation of uniform suspensions.

MEDIA Two types of media were used, a semi-solid medium and a liquid medium. The semi-solid medium was employed for carrying the organism in stock and consisted of tryptone-glucose-extract agar (Difco). The liquid medium was used to supply the cells used in the experimental runs. The liquid medium was a broth having the following composition: 0.5% Bacto peptone (Difco), 0.1% Bacto beef extract (Difco), 1.0% powdered glucose (Baker's C.P.). The broth was prepared by dissolving the components in distilled water and adjusting the pH to a value of 6.8 to 7.0. The medium was then portioned into 400 cc. batches, placed in cotton-plugged liter Erlenmeyer flasks and sterilized. Sterilization of both types of media was carried out at 15 pounds pressure for 15 minutes.

STANDARD BUFFER SOLUTION The standard buffer solution was a mixture of KH_2PO_4 and NaOH prepared according to the buffer tables given by Clark and Lub (8). The final pH value of the buffer was 6.8. This solution was used as the suspending liquid for cell suspensions and employed as solvent in the preparation of the substrate and inhibitor solutions.

CELL SUSPENSIONS Experimental runs were carried out with non-proliferating or "resting cell" suspensions (30) of Bacillus subtilis. A fresh suspension was prepared for each run so that the cells under invistigation would have approximately the same rate of respiration.

The cells were harvested from a 15-20 hour broth culture by centrifugation. The centrifugations were carried out in 50 cc. conical-bottomed tubes using an International clinical centrifuge. The centrifuge speed was approximately 2000 revolutions per minute and the centrifugation time for a 200 cc. volume was 10 minutes. A total of 800 cc. of broth was required to supply the cells for a single experimental run. After draining off the supernatant liquid from the sedimented cells, a 10 cc. portion of standard buffer was employed to wash down the sides of the centrifuge tubes, to resuspend and to wash the cells. This procedure was repeated and subsequently the cells were redistributed in the buffer and the resulting suspension adjusted to the desired concentration.

This adjustment was facilitated by the use of a Klett-Summerson photoelectric colorimeter. The colorimeter was calibrated so that light absorption readings could be interpreted in terms of milligrams of dry weight of bacterial protoplasm. It was thus possible to prepare a cell suspension, measure its light-absorbing ability, and determine its concentration in terms of dry weight of bacteria by referring to a standard turbidity vs dry weight curve. Furthermore it was possible to adjust the concentration of the suspension to a desired value by means of the equation:

$$\frac{\text{concentration 1}}{\text{concentration 2}} \quad \frac{\text{volume 2}}{\text{volume 1}}$$

The standard suspension concentrations used in this work were 1.5 milligrams of dry weight per cubic centimeter when the substrate was glucose or formate and 3.0 milligrams per cubic centimeter when the substrate was succinate. Suspensions possessing the above concentrations exhibited a respiration rate which was easily measured in the Warburg apparatus.

DRY WEIGHTS In order to prepare the standard turbidity vs dry weight curve it was necessary to ascertain the dry weights of several cell suspensions of known turbidity. Dry weight values were determined by evaporating to dryness duplicate 10 cc. portions of distilled water suspensions and weighing. The weighing vessels (30 cc. beakers) and contents were allowed to air dry in an oven at 80°C. for a period of 16 to 20 hours. They were then placed in a calcium chloride dessicator and allowed to come to constant weight.

MANOMETRIC MEASUREMENTS Oxygen consumption and carbon dioxide liberation measurements were performed with a Warburg respirometer. The Warburg flasks were shaken at a rate of 110 full one inch strokes per minute and the temperature of the water bath was 30°C. Determinations were carried out in the presence of air and the flasks were shaken for a period of 15 minutes before readings were initiated so as to obtain equilibration between the temperature of the reaction flasks and the water bath. In all cases the fluid volume in the main compartment of the reaction flask was 3 cc. In the case of determinations involving glucose or formate volume was composed of 1 cc. of cellsuspension, 1 cc. of substrate solution and 1 cc of inhibitor solution. In the case of determinations involving succinate the cell suspension was prepared with

succinate solution instead of plain buffer and the cells and substrate were added to the reaction flask in the same 2 cc. volume along with 1 cc. of inhibitor solution. Oxygen determinations were carried out with 0.2 cc. of a 20% KOH solution present in the alkali cup of the reaction flask.

When glucose or formate were present as substrates manometric readings were made at 15 minute intervals for a period of an hour and when succinate was the substrate readings were made at 30 minute intervals for a period of an hour and one half. In all cases when the final reading was completed the reaction flasks were removed from the manometers and bromthymolblue indicator was added to the reaction liquid so as to check for any large change in the pH (about 0.5 pH unit) of the liquid during the course of the gaseous exchange.

After the pH was determined the flasks were cleaned. Cleaning of the flasks was accomplished by removing the stopcock grease from the ground glass joints with Energine cleaning fluid, rinsing thoroughly with tap water and immersing the flasks in a hot aqueous solution of Calgon according to the recommendations of Umbreit (29). The use of sulfuric acid-dichromate cleaning mixture may have a deleterious effect on metabolic studies (25). Later the flasks were rinsed with tap water and distilled water and allowed to dry. Drying was facilitated by a final rinse with methyl alcohol.

SUBSTRATE SOLUTIONS The three substrates investigated were glucose (Baker's C.P.), sodium formate (Baker's C.P.) and succinic acid (Mallinckrodt's Pure). Glucose and formate solutions were prepared by weighing out the desired amount, dissolving in standard buffer solution and diluting to the desired concentration. Succinate solutions were

prepared by weighing out the desired amount of succinic acid plus sufficient NaOH (Baker's C.P.) to neutralize the acid and diluting to the desired concentration with standard buffer solution.

Working concentrations of the three substrates were ascertained by allowing a given cell suspension to respire in the presence of different concentrations of substrate and determining the concentration of substrate which gave the maximum value of oxygen consumption per unit time.

INHIBITOR SOLUTIONS The inhibitor compounds investigated were:

1. Chloral hydrate -- $Cl_3CCH(OH)_2$ (Baker's U.S.P.)
2. Chloretone -- $Cl_3CC(CH_3)_2OH$ (Park, Davis & Co.)
3. Chloroform -- $CHCl_3$ (Will Corp., Pure)
4. Ether -- $(C_2H_5)_2O$ (Baker's C.P.)
5. Potassium cyanide - KCN (Baker's C.P.)
6. Urethane -- $NH_2CO_2C_2H_5$ (Merck)

The solutions of chloretone, chloroform and ether were made up from saturated buffer solutions and the concentrations were calculated on the basis if solubilities in water at 25°C. (1,27). The presence of the low concentrations of KH_2PO_4 and NaOH in the solutions was assumed to have a negligible effect on the solubilities listed for aqueous solutions.

The solutions of chloral hydrate, potassium cyanide and urethane were prepared by weighing according to the usual procedure. The potassium cyanide solutions were freshly prepared prior to usage as there was visible evidence of decomposition after storage for a week at 3°C.

FLASK CONSTANTS The calculated values for the flask constants (pages 27 and 28) were checked in order to ascertain whether or not there was any great variation in gas measurements between flasks. This was

accomplished by allowing equal aliquots from the same cell suspension to respire in the presence of 0.01M glucose and comparing the gaseous exhange as determined by the various flasks. Flask No. 5 was used as the thermobarometer. Checks for oxygen consumption were made on flasks Nos. 7, 6, 4, 3, 2, and 1 and checks for carbon dioxide liberation were made on flasks Nos. 6, 3, and 1.

EFFECT OF INHIBITORS ON RESPIRATION Experiments were run in order to determine what concentration of each inhibitor was required to decrease the gaseous exchange of non-proliferating cells of Bacillus subtilis respiring in the presence of each substrate, glucose, formate and succinate. The respiration of 1.5 milligrams (dry weight) of cell material was employed when the substrate was glucose or formate and 3.0 milligrams was used when the substrate was succinate. Manometer readings were taken at 15 minute intervals over a 60 minute experimental period.

Table XI

Typical data sheet showing calculation of O_2 and CO_2 volumes

		Flask with KOH			Flask without KOH				
Time	Th'meter Reading	h Reading	Correct Reading	O_2 Vol.	h Reading	Correct Reading	x_{O_2}/k_{O_2}	Difference $h-x_{O_2}/k_{O_2}$	CO_2 Vol. $(h-x_{O_2}/k_{O_2})k_{CO_2}$
(1)	(2)	(3)	(4)	(5)	(6)	(7)	(8)	(9)	(10)
Minutes	Used for correction of reading	Actual respirometer reading in mm.	mm. observed uptake corrected for thermobarometer change	Corrected h times k_{O_2} for flask	Actual respirometer reading in mm.	mm. observed uptake corrected for thermobarometer change	Column 5 divided by k_{O_2} of this flask	Subtract column 8 from column 6	Multiply column 9 by k_{CO_2} for this flask
0	149.2	146.1			148.6				
	3.0	-19.9			-1.6				
15	152.2	126.2	- 22.9	-31.8	147.0	-4.6	-22.3	17.7	38.9
	2.0	-22.0			-4.2				
30	154.2	104.2	-24.0	-33.2	144.8	-6.2	-23.3	17.1	37.6
	3.3	-20.2			-1.8				
45	157.5	84.0	-23.5	-32.6	143.0	-5.1	-22.8	17.7	38.9
	2.8	-23.8			-5.3				
60	160.3	60.2	-26.6	-36.8	137.7	-8.1	-25.8	17.7	38.9
Totals				134.4					154.3

Date: 3/25/48
Flasks Nos. 7 and 6
Run No. 149
Flask Contents: Suspension B_{32} - - 1 cc.
0.03M glucose - - 1 cc.
Buffer - - - - - - - 1 cc.

Conditions: Temp. 30°C.
Strokes 110/min.
In air

TURBIDITY AND DRY WEIGHT. The results of the turbidity and dry weight measurement of *B. subtilis* are found in Table XII. Only those suspensions which were undiluted (1:0) were aliquoted for dry weight measurements. The dry weights for diluted suspensions were obtained by calculation from the weight of the undiluted suspensions. A turbidity versus dry weight plot is shown in Figure 2. The segment of the curve falling between the 30.0% and 10.0% absorption ordinates was the portion generally used in determining concentrations of unknown suspensions.

Table XII

Turbidity and Dry Weight Values for Distilled Water Suspensions of *Bacillus subtilis*

Original Suspension	Dilution	Turbidity in Percent Absorption	Dry Weight in Milligrams/Milliliter
1	Undiluted	70.5	2.01
1	1:10	14.9	0.20
2	Undiluted	30.8	0.47
2	1:10	4.4	0.05
2	1:2	18.3	0.24
2	1:5	8.2	0.09
3	Undiluted	65.6	1.36
3	1:2	44.1	0.68
3	1:5	22.1	0.27
3	1:10	12.1	0.14
4	Undiluted	65.8	1.53
4	1:2	44.6	0.77
4	1:4	38.3	0.61
4	1:5	22.7	0.31
4	1:10	12.6	0.15

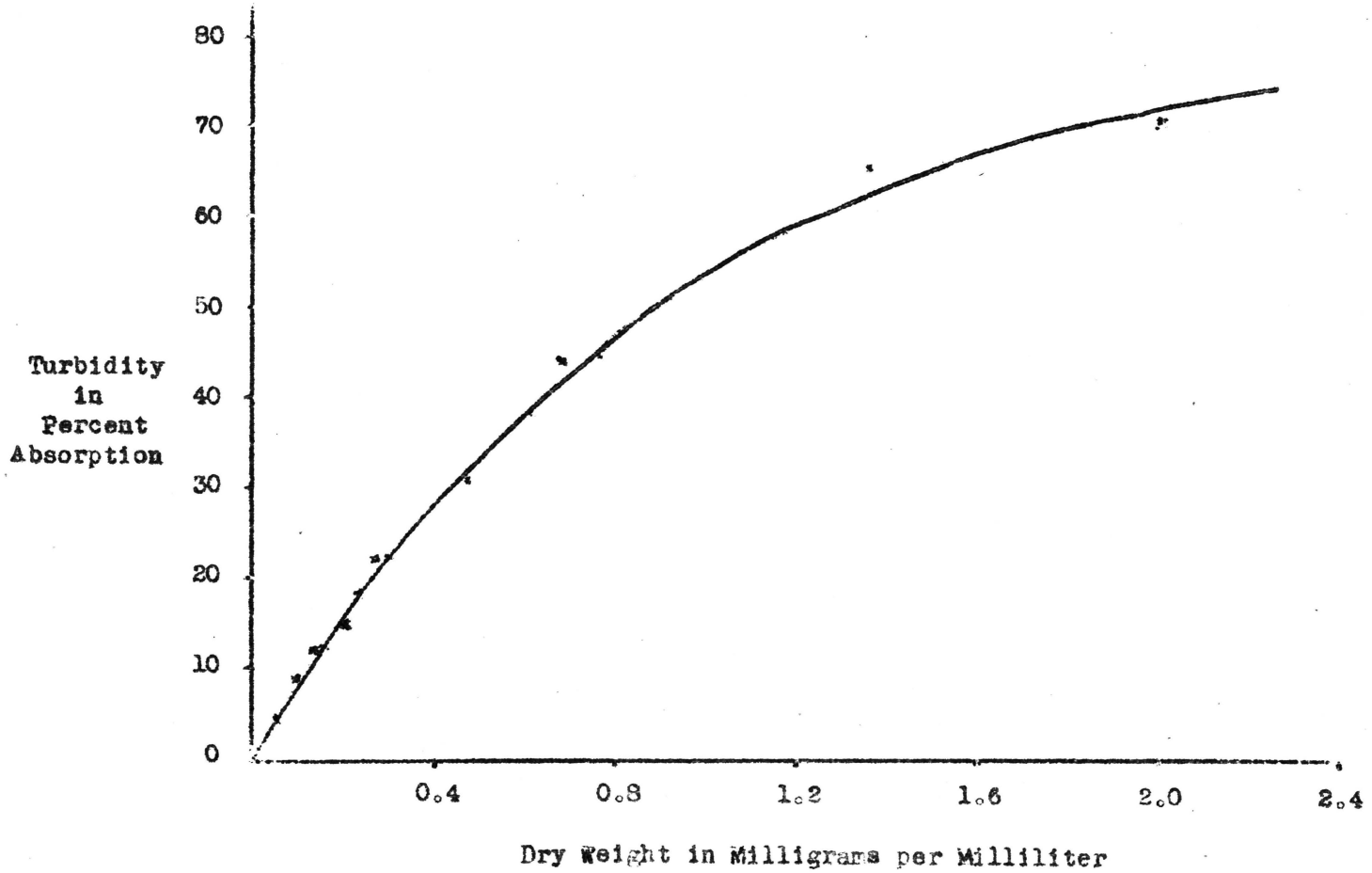


Figure 2. Turbidity - Dry Weight Curve.

SUBSTRATE CONCENTRATIONS Oxygen consumption values for *B. subtilis* respiring in the presence of various concentrations of glucose are listed in Table XIII. Similar tables are presented for formate (Table XIV) and for succinate (Table XV).

INHIBITORS Values for the oxygen consumption and carbon dioxide liberation of *B. subtilis* respiring on the various substrates and in the presence of various concentrations of inhibitors are shown in Tables XVI to XXX inclusive. Each individual table presents the gaseous exchange values for non-proliferating cells of *B. subtilis* metabolizing on one substrate in the presence of one inhibitory agent. The data for chloretone is a special case as the changes in the levels of the manometric fluid were not consistant with changes observed with the other inhibitors. The data for chloretone is contained in Tables XXXI and XXXII and is discussed on pages 118-119.

The data mentioned above are presented in graphical form in Figures 3 to 52. The graphical representations are of two types. Figures 3 to 27 are bar diagrams in which the total cubic millimeters of gas measured are plotted against concentrations of the inhibitors. Figures 28 to 52 are bar diagrams in which the values for the gas taken up or liberated are expressed in terms of per cent of gas consumed or liberated compared with the control flask (no inhibitor).

Table XIII

Respiration of Bacillus subtilis:
1.5 mg. cells on GLUCOSE

Time in Minutes	Moles of Glucose						
	0.001	0.005	0.01	0.05	0.1	0.5	1.0
A (replicate)	Oxygen Volumes in Cubic Millimeters						
0 -15	18	20	16	21	22	18	11
15 - 30	26	26	31	22	20	17	10
30 - 45	26	27	26	23	25	19	9
45 - 60	26	26	28	21	23	17	10
Totals	96	99	101	87	90	71	40
B (replicate)							
0 - 15	20	24	25	20	25	19	10
15 - 30	25	22	22	20	24	19	10
30 - 45	25	26	23	21	24	17	10
45 - 60	24	23	22	24	21	18	8
Totals	94	95	92	85	92	73	38

Table XIV

Respiration of Bacillus subtilis:
1.5 mg. cells on FORMATE

Time in Minutes	Moles of Glucose						
	0.01	0.05	0.1	0.5	1.0	2.0	4.0
	Oxygen Volumes in Cubic Millimeters						
A (replicate)							
0 - 15	24	30	30	26	24	16	5
15 - 30	22	26	24	28	30	20	18
30 - 45	14	20	24	28	22	20	10
45 - 60	16	22	20	24	26	18	6
Totals	76	98	98	106	102	74	39
B (replicate)							
0 - 15	24	24	25	28	28	22	10
15 - 30	20	26	31	30	32	24	15
30 - 45	15	26	31	28	26	23	11
45 - 60	14	28	30	28	26	19	7
Totals	73	104	117	114	112	88	43

Table XV

Respiration of *Bacillus subtilis*:
3.0 mg. cells on SUCCINATE

Time in Minutes	Moles of Succinate			
	0.001	0.01	0.1	0.2
	Oxygen Volumes in Cubic Millimeters			
A (replicate)				
0 - 30	4	13	17	31
30 - 60	6	11	17	32
60 - 90	6	11	20	40
Totals	16	35	54	103
B (replicate)				
0 - 30	3	12	16	29
30 - 60	7	12	15	28
60 - 90	2	14	18	37
Totals	12	38	49	94

Table XVI

Respiration of Bacillus subtilis:
1.5 mg. cells on GLUCOSE in presence of CYANIDE

	Moles of Potassium Cyanide									
	0M		0.0001M		0.001M		0.005M		0.01M	
Time in Minutes	Gas Volumes in Cubic Millimeters									
	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
A (replicate)										
0 - 15	32	39	28	35	31	-	-	-	7	-
15 - 30	34	38	31	40	28	-	-	-	8	-
30 - 45	33	39	32	40	27	-	-	-	8	-
45 - 60	37	39	35	36	34	-	-	-	8	-
Totals	136	155	126	151	120	-	-	-	31	-
B (replicate)										
0 - 15	27	34	-	-	21	-	14	-	8	10
15 - 30	28	30	-	-	26	-	19	-	10	14
30 - 45	32	37	-	-	31	-	20	-	7	11
45 - 60	29	34	-	-	30	-	17	-	5	6
Totals	116	135	-	-	108	-	70	-	30	41
C (replicate)										
0 - 15	19	24	-	-	10	-	15	21	5	-
15 - 30	17	20	-	-	14	-	13	18	5	-
30 - 45	19	22	-	-	18	-	12	19	8	-
45 - 60	21	20	-	-	16	-	16	19	6	-
Totals	76	86	-	-	58	-	56	77	24	-

- not determined

Table XVII

Respiration of Bacillus subtilis:
1.5 mg. cells on FORMATE in presence of CYANIDE

	Moles of Potassium Cyanide									
	0		0.00001		0.00005		0.0001		0.001	
Time in Minutes	Gas Volumes in Cubic Millimeters									
	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
A (replicate)										
0 - 15	24	78	22	-	18	30	3	-	-	-
15 - 30	20	46	20	-	14	28	3	-	-	-
30 - 45	24	42	18	-	12	26	1	-	-	-
45 - 60	18	36	16	-	6	14	1	-	-	-
Totals	86	202	76	-	50	98	10	-	-	-
B (replicate)										
0 - 15	22	39	17	-	19	-	7	-	1	5
15 - 30	20	39	16	-	15	-	2	-	0	0
30 - 45	19	41	14	-	15	-	0	-	1	1
45 - 60	18	40	13	-	4	-	1	-	1	3
Totals	79	159	60	-	53	-	10	-	3	9
C (replicate)										
0 - 15	24	51	22	-	32	-	4	10	-	-
15 - 30	18	39	18	-	22	-	0	7	-	-
30 - 45	20	41	16	-	18	-	0	3	-	-
45 - 60	12	29	14	-	13	-	1	3	-	-
Totals	74	160	70	-	75	-	5	23	-	-

- not determined

Table XVIII

Respiration of Bacillus subtilis:
3.0 mg. cells on SUCCINATE in presence of CYANIDE

	Moles of Potassium Cyanide				
	0M	0.0001M	0.001M	0.005M	0.01M
Time in Minutes	Gas Volumes in Cubic Millimeters O_2	O_2	O_2	O_2	O_2
A (replicate)					
0 - 30	31	26	-	-	-
30 - 60	32	26	-	-	-
60 - 90	40	26	-	-	-
Totals	103	78	-	-	-
B (replicate)					
0 - 30	11	-	14	-	5
30 - 60	11	-	17	-	0
60 - 90	13	-	12	-	0
Totals	35	-	43	-	5
C (replicate)					
0 - 30	30	-	32	25	16
30 - 60	25	-	33	18	4
60 - 90	31	-	28	16	0
Totals	86	-	93	59	20

- not determined

Table XIX

Respiration of Bacillus subtilis:
1.5 mg. cells on GLUCOSE in presence of URETHANE

	Moles of Urethane									
	0M		0.001M		0.01M		0.5M		1.0M	
Time in Minutes	Gas Volumes in Cubic Millimeters									
	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
A (replicate)										
0 - 15	28	35	28	-	30	-	-	-	1	3
15 - 30	26	36	30	-	32	-	-	-	0	2
30 - 45	32	40	30	-	27	-	-	-	0	0
45 - 60	24	29	27	-	22	-	-	-	0	0
Totals	110	140	115	-	111	-	-	-	1	5
B (replicate)										
0 - 15	30	36	-	-	30	-	22	-	0	0
15 - 30	26	33	-	-	30	-	18	-	0	0
30 - 45	35	41	-	-	30	-	19	-	0	0
45 - 60	31	33	-	-	25	-	16	-	0	0
Totals	122	143	-	-	115	-	75	-	0	0
C (replicate)										
0 - 15	23	30	-	-	-	-	18	23	2	8
15 - 30	18	26	-	-	-	-	15	19	2	9
30 - 45	26	35	-	-	-	-	21	29	0	2
45 - 60	21	31	-	-	-	-	16	19	1	0
Totals	89	122	-	-	-	-	70	90	5	19

- not determined

Table XX

Respiration of *Bacillus subtilis*:
1.5 mg. cells on FORMATE in presence of URETHANE

	Moles of Urethane									
	0M		0.01M		0.1M		0.5M		1.0M	
Time in Minutes	Gas Volumes in Cubic Millimeters									
	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
A (replicate)										
0 - 15	30	92	28	-	30	-	-	-	2	-
15 - 30	26	74	24	-	26	-	-	-	0	-
30 - 45	24	58	24	-	26	-	-	-	1	-
45 - 60	24	62	18	-	20	-	-	-	1	-
Totals	104	286	94	-	102	-	-	-	4	-
B (replicate)										
0 - 15	26	90	-	-	30	-	14	-	0	2
15 - 30	28	78	-	-	26	-	10	-	0	0
30 - 45	26	72	-	-	18	-	6	-	0	0
45 - 60	24	62	-	-	18	-	2	-	0	0
Totals	104	302	-	-	92	-	32	-	0	2
C (replicate)										
0 - 15	34	74	-	-	-	-	22	27	1	5
15 - 30	37	80	-	-	-	-	25	38	0	0
30 - 45	31	58	-	-	-	-	19	24	3	0
45 - 60	28	61	-	-	-	-	18	21	0	1
Totals	130	273	-	-	-	-	84	110	4	6

- not determined

Table XXI

Respiration of Bacillus subtilis:
3.0 mg. cells on SUCCINATE in presence of URETHANE

	Moles of Urethane				
	0M	0.01M	0.1M	0.5M	1.0M
Time in Minutes	Gas Volumes in Cubic Millimeters				
	O_2	O_2	O_2	O_2	O_2
A (replicate)					
0 - 30	31	-	-	13	-
30 - 60	32	-	-	8	-
60 - 90	40	-	-	5	-
Totals	103	-	-	26	-
B (Replicate)					
0 - 30	11	-	29	9	6
30 - 60	11	-	27	3	1
60 - 90	13	-	28	1	0
Totals	35	-	84	13	7
C (replicate)					
0 - 30	22	-	25	10	10
30 - 60	23	-	30	7	2
60 - 90	27	-	33	4	2
Totals	72	-	88	21	14

- not determined

Table XXII

Respiration of Bacillus subtilis:
1.5 mg. cells on GLUCOSE in presence of CHLORAL HYDRATE

	Moles of Chloral Hydrate									
	0M		0.001M		0.01M		0.05M		0.1M	
Time in Minutes	Gas Volumes in Cubic Millimeters									
	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
A (replicate)										
0 - 15	30	47	-	-	29	-	26	-	0	0
15 - 30	52	46	-	-	36	-	23	-	0	0
30 - 45	33	49	-	-	30	-	19	-	0	0
45 - 60	34	40	-	-	32	-	19	-	0	0
Totals	129	182	-	-	126	-	87	-	0	0
B (replicate)										
0 - 15	29	42	-	-	36	-	29	-	1	3
15 - 30	31	40	-	-	30	-	27	-	2	1
30 - 45	33	48	-	-	33	-	23	-	0	0
45 - 60	29	39	-	-	31	-	22	-	0	0
Totals	122	169	-	-	130	-	101	-	3	4
C (replicate)										
0 - 15	17	26	-	-	-	-	11	25	0	0
15 - 30	18	20	-	-	-	-	11	23	0	0
30 - 45	21	28	-	-	-	-	9	19	0	0
45 - 60	20	20	-	-	-	-	8	20	0	0
Totals	76	94	-	-	-	-	39	87	0	0

- not determined

Table XXIII

Respiration of Bacillus subtilis:
1.5 mg. cells on FORMATE in presence of CHLORAL HYDRATE

	Moles of Chloral Hydrate									
	0		0.001		0.01		0.05		0.1	
Time in Minutes	Gas Volumes in Cubic Millimeters									
	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
A (replicate)										
0 - 15	28	-	-	-	29	-	25	-	17	-
15 - 30	27	-	-	-	29	-	21	-	15	-
30 - 45	24	-	-	-	32	-	20	-	13	-
45 - 60	21	-	-	-	22	-	20	-	7	-
Totals	100	-	-	-	112	-	84	-	52	-
B (replicate)										
0 - 15	32	68	36	-	30	-	26	-	16	-
15 - 30	32	54	30	-	30	-	27	-	10	-
30 - 45	32	56	30	-	30	-	22	-	6	-
45 - 60	24	48	24	-	22	-	16	-	2	-
Totals	120	226	120	-	112	-	93	-	34	-
C (replicate)										
0 - 15	30	60	-	-	-	-	24	51	9	31
15 - 30	30	63	-	-	-	-	18	39	9	20
30 - 45	30	54	-	-	-	-	21	40	6	13
45 - 60	30	54	-	-	-	-	18	37	3	9
Totals	120	231	-	-	-	-	81	167	27	73

- not determined

Table XXIV

Respiration of Bacillus subtilis:
3.9 mg. cells on SUCCINATE in presence of CHLORAL HYDRATE

	Moles of Chloral Hydrate				
	0	0.001	0.01	0.05	0.1
Time in Minutes	Gas Volumes in Cubic Millimeters				
	O_2	O_2	O_2	O_2	O_2
A (replicate)					
0 - 30	31	-	-	-	7
30 - 60	32	-	-	-	0
60 - 90	40	-	-	-	0
Totals	103	-	-	-	7
B (replicate)					
0 - 30	21	-	15	12	3
30 - 60	20	-	12	10	0
60 - 90	18	-	14	13	1
Totals	59	-	41	35	4
C (replicate)					
0 - 30	28	-	29	22	5
30 - 60	26	-	32	17	6
60 - 90	30	-	32	14	1
Totals	84	-	93	53	12

- not determined

Table XXV

Respiration of *Bacillus subtilis*;
1.5 mg. cells on GLUCOSE in presence of CHLOROFORM

	Moles of Chloroform									
	0		0.002		0.01		0.02		0.04	
Time in Minutes	Gas Volumes in Cubic Millimeters									
	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
A (replicate)										
0 - 15	26	35	27	-	29	-	23	25	-	-
15 - 30	33	40	30	-	25	-	27	27	-	-
30 - 45	28	36	24	-	29	-	22	22	-	-
45 - 60	28	29	27	-	26	-	20	20	-	-
Totals	115	140	108	-	109	-	92	94	-	-
B (replicate)										
0 - 15	30	37	-	-	-	-	24	29	11	15
15 - 30	29	35	-	-	-	-	21	26	7	9
30 - 45	33	38	-	-	-	-	20	28	3	5
45 - 60	27	31	-	-	-	-	19	22	0	2
Totals	119	141	-	-	-	-	84	105	31	31
C (replicate)										
0 - 15	19	24	-	-	-	-	20	25	1	5
15 - 30	17	20	-	-	-	-	18	23	0	5
30 - 45	19	26	-	-	-	-	14	26	0	0
45 - 60	21	24	-	-	-	-	15	21	0	0
Totals	76	94	-	-	-	-	67	95	1	10

- not determined

Table XXVI

Respiration of Bacillus subtilis:
1.5 mg. cells on FORMATE in presence of CHLOROFORM

	Moles of Chloroform									
	0		0.002		0.01		0.02		0.04	
Time in Minutes	Gas Volumes in Cubic Millimeters									
	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
A (replicate)										
0 - 15	21	54	22	-	21	-	19	33	-	-
15 - 30	22	41	22	-	21	-	18	33	-	-
30 - 45	19	33	20	-	20	-	17	31	-	-
45 - 60	17	23	19	-	18	-	16	25	-	-
Totals	79	153	83	-	80	-	70	122	-	-
B (replicate)										
0 - 15	39	79	-	-	36	-	37	-	29	47
15 - 30	38	73	-	-	30	-	40	-	19	31
30 - 45	32	67	-	-	30	-	29	-	18	24
45 - 60	23	59	-	-	26	-	25	-	12	16
Totals	132	274	-	-	122	-	131	-	78	118
C (replicate)										
0 - 15	26	54	-	-	-	-	26	50	18	40
15 - 30	22	46	-	-	-	-	22	43	12	31
30 - 45	26	48	-	-	-	-	24	51	16	20
45 - 60	20	40	-	-	-	-	18	33	10	17
Totals	94	188	-	-	-	-	90	177	56	108

- not determined

Table XXVII

Respiration of Bacillus subtilis:
3.0 mg. cells on SUCCINATE in presence of CHLOROFORM

	Moles of Chloroform				
	0	0.002	0.01	0.02	0.04
Time in Minutes	Gas Volumes in Cubic Millimeters				
	O_2	O_2	O_2	O_2	O_2
A (replicate)					
0 - 30	31	-	-	22	-
30 - 60	32	-	-	17	-
60 - 90	40	-	-	17	-
Totals	103	-	-	56	-
B (replicate)					
0 - 30	21	-	24	-	5
30 - 60	20	-	19	-	4
60 - 90	18	-	15	-	0
Totals	59	-	58	-	9
C (replicate)					
0 - 30	32	30	30	25	6
30 - 60	34	32	27	21	9
60 - 90	37	28	33	27	3
Totals	103	90	90	73	18

- not determined

Table XXVIII

Respiration of Bacillus subtilis:
1.5 mg. cells on GLUCOSE in presence of ETHER

	Moles of Ether									
	0		0.0026		0.026		0.13		0.26	
Time in Minutes	Gas Volumes in Cubic Millimeters									
	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
A (replicate)										
0 - 15	29	35	31	-	30	-	-	-	18	36
15 - 30	31	39	28	-	32	-	-	-	2	8
30 - 45	34	39	27	-	35	-	-	-	1	3
45 - 60	29	31	30	-	33	-	-	-	0	0
Totals	123	144	116	-	130	-	-	-	21	47
B (replicate)										
0 - 15	34	40	-	-	31	-	28	-	20	29
15 - 30	29	33	-	-	32	-	23	-	18	31
30 - 45	36	41	-	-	32	-	27	-	6	15
45 - 60	28	31	-	-	29	-	21	-	5	5
Totals	127	145	-	-	124	-	99	-	47	60
C (replicate)										
0 - 15	20	25	-	-	-	-	21	30	16	25
15 - 30	18	24	-	-	-	-	14	23	5	13
30 - 45	25	28	-	-	-	-	20	27	0	4
45 - 60	21	24	-	-	-	-	12	19	0	4
Totals	84	101	-	-	-	-	67	99	21	46

- not determined

Table XXIX

Respiration of Bacillus subtilis:
1.5 mg. cells on FORMATE in presence of ETHER

	Moles of Ether									
	0		0.0025		0.026		0.13		0.26	
Time in Minutes	Gas Volumes in Cubic Millimeters									
	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2	O_2	CO_2
A (replicate)										
0 - 15	21	37	22	-	22	-	-	-	9	20
15 - 30	18	39	18	-	17	-	-	-	6	18
30 - 45	17	35	18	-	15	-	-	-	3	5
45 - 60	16	28	16	-	15	-	-	-	0	2
Totals	72	139	74	-	69	-	-	-	18	45
B (replicate)										
0 - 15	20	48	-	-	20	45	14	-	2	12
15 - 30	18	64	-	-	18	43	14	-	0	0
30 - 45	24	38	-	-	24	46	16	-	1	2
45 - 60	16	30	-	-	16	39	12	-	0	7
Totals	78	180	-	-	78	173	56	-	3	21
C (replicate)										
0 - 15	38	71	-	-	-	-	30	64	3	8
15 - 30	34	75	-	-	-	-	29	63	2	3
30 - 45	28	75	-	-	-	-	23	43	0	3
45 - 60	31	60	-	-	-	-	22	47	0	0
Totals	131	266	-	-	-	-	104	217	5	14

- not determined

Table XXX

Respiration of Bacillus subtilis:
3.0 mg. cells on SUCCINATE in presence of ETHER

	Moles of Ether				
	0	0.0026	0.026	0.13	0.26
Time in Minutes	Gas Volumes in Cubic Millimeters				
	O_2	O_2	O_2	O_2	O_2
A (replicate)					
0 - 30	31	-	-	-	14
30 - 60	32	-	-	-	1
60 - 90	40	-	-	-	0
Totals	103	-	-	-	15
B (replicate)					
0 - 30	21	-	29	-	8
30 - 60	20	-	26	-	8
60 - 90	18	-	25	-	5
Totals	59	-	80	-	21
C (replicate)					
0 - 30	30	-	31	18	20
30 - 60	29	-	36	15	5
60 - 90	34	-	28	16	2
Totals	93	-	95	49	27

- not determined

Table XXXI

Typical changes in manometric fluid levels obtained with Bacillus subtilis respiring in the presence of formate and 0.015M chloretone.

Time in Minutes	Changes in manometric fluid level in millimeters Flask with KOH	Flask without KOH
0 - 15	14.6	10.2
15 - 30	15.9	10.0
30 - 45	15.2	6.2
45 - 60	15.5	4.7

Table XXXII

Typical changes in manometric fluid levels obtained with Bacillus subtilis respiring in the presence of 0.015M chloretone

Time in Minutes	Flask with KOH	Flask without KOH
0 - 15	18.8	0.5
15 - 30	20.3	1.6
30 - 45	18.1	0.6
45 - 60	16.8	0.4

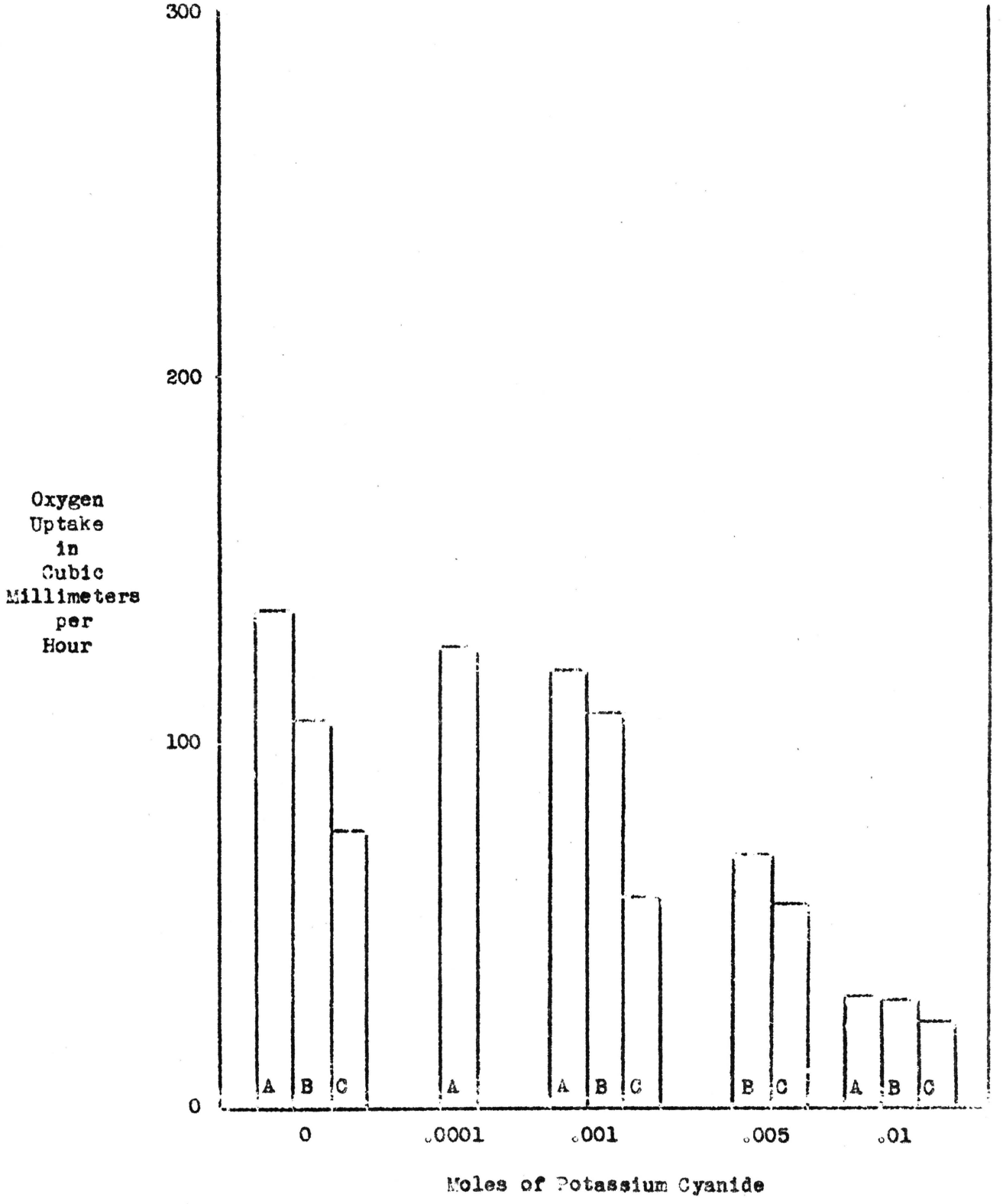


Figure 3. Oxygen consumption by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of CYANIDE.

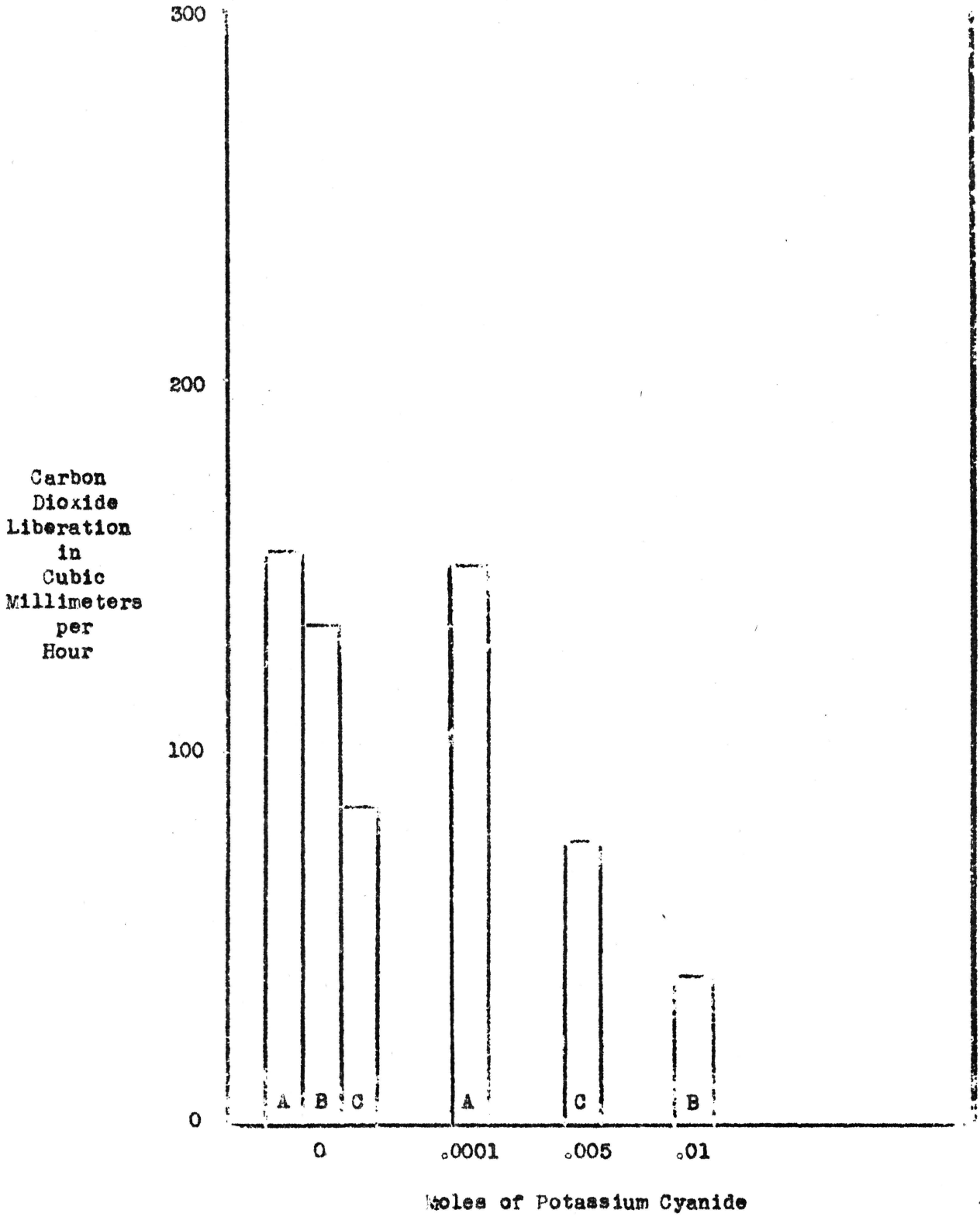


Figure 4. Carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of CYANIDE.

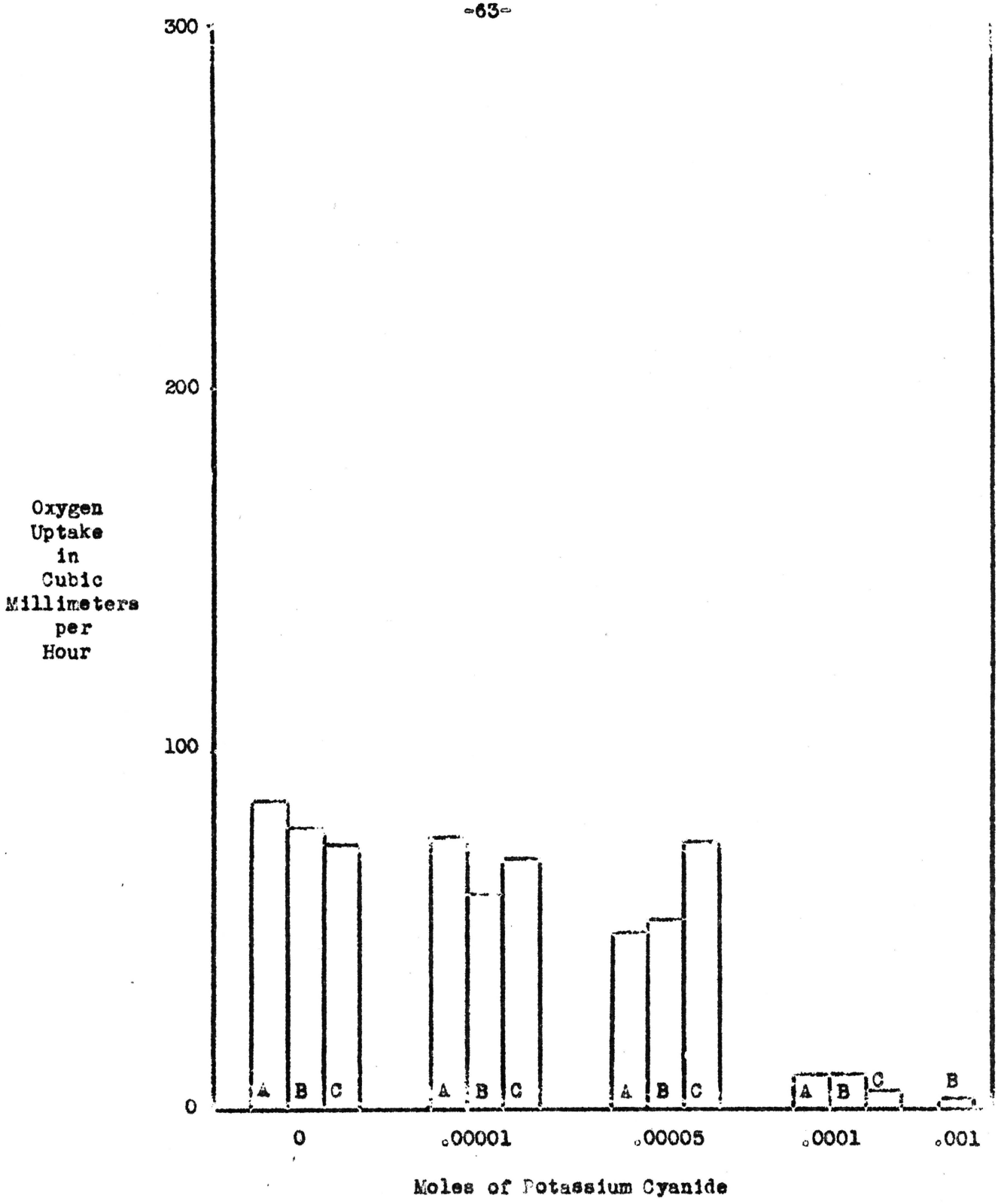


Figure 5. Oxygen consumption by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of CYANIDE.

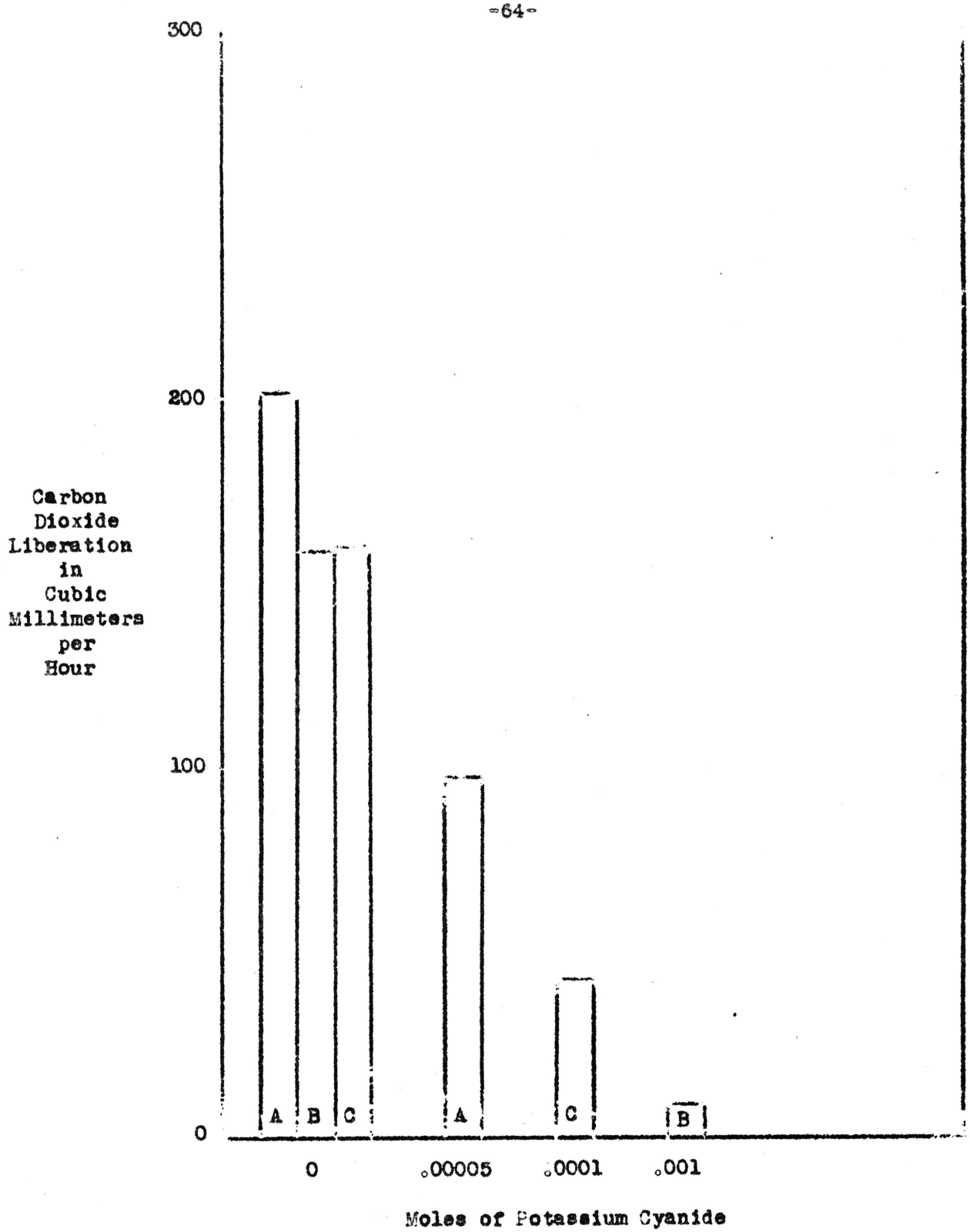


Figure 6. Carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of CYANIDE.

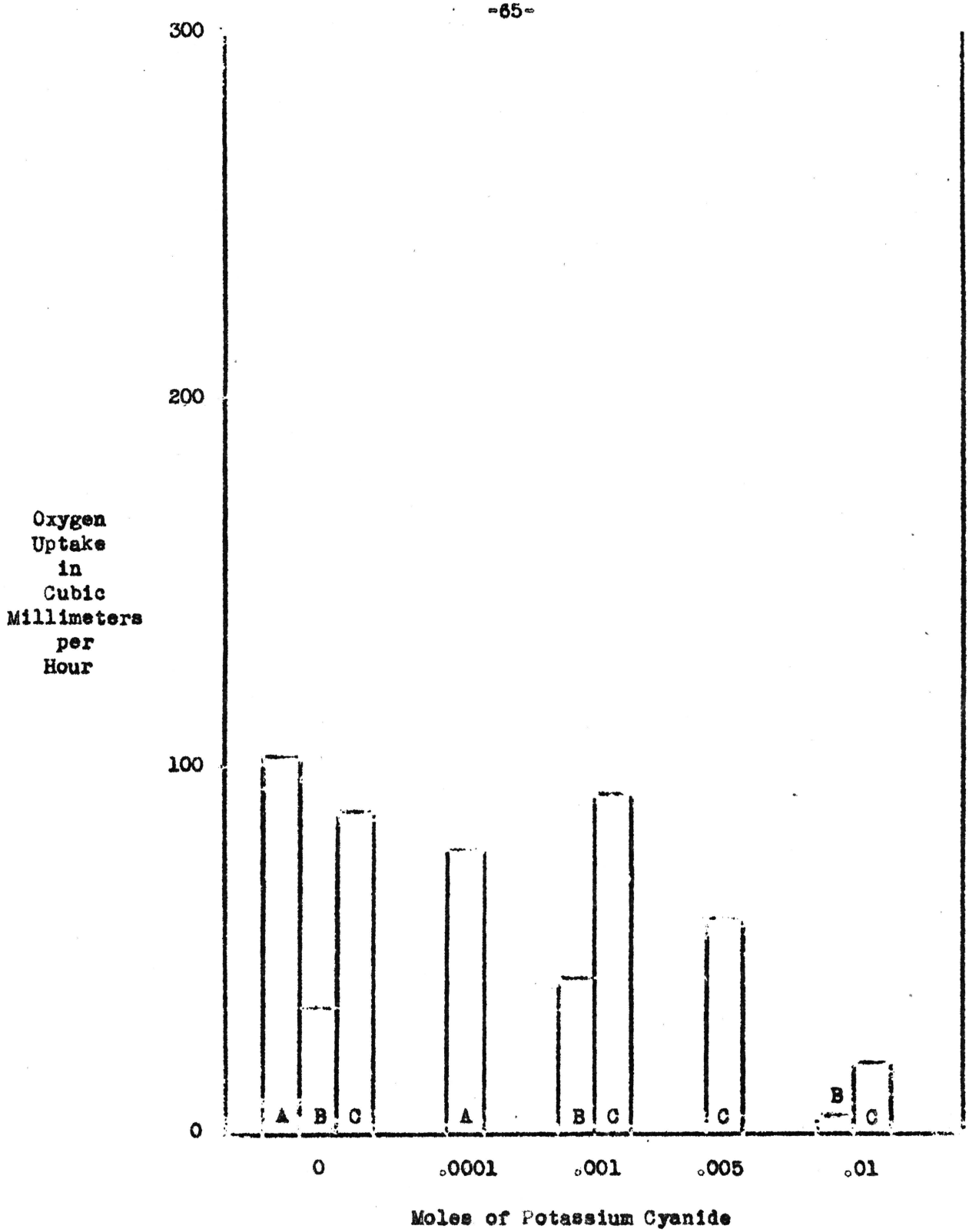


Figure 7. Oxygen consumption by Bacillus subtilis: 3.0 mg. cells on SUCCINATE in presence of CYANIDE.

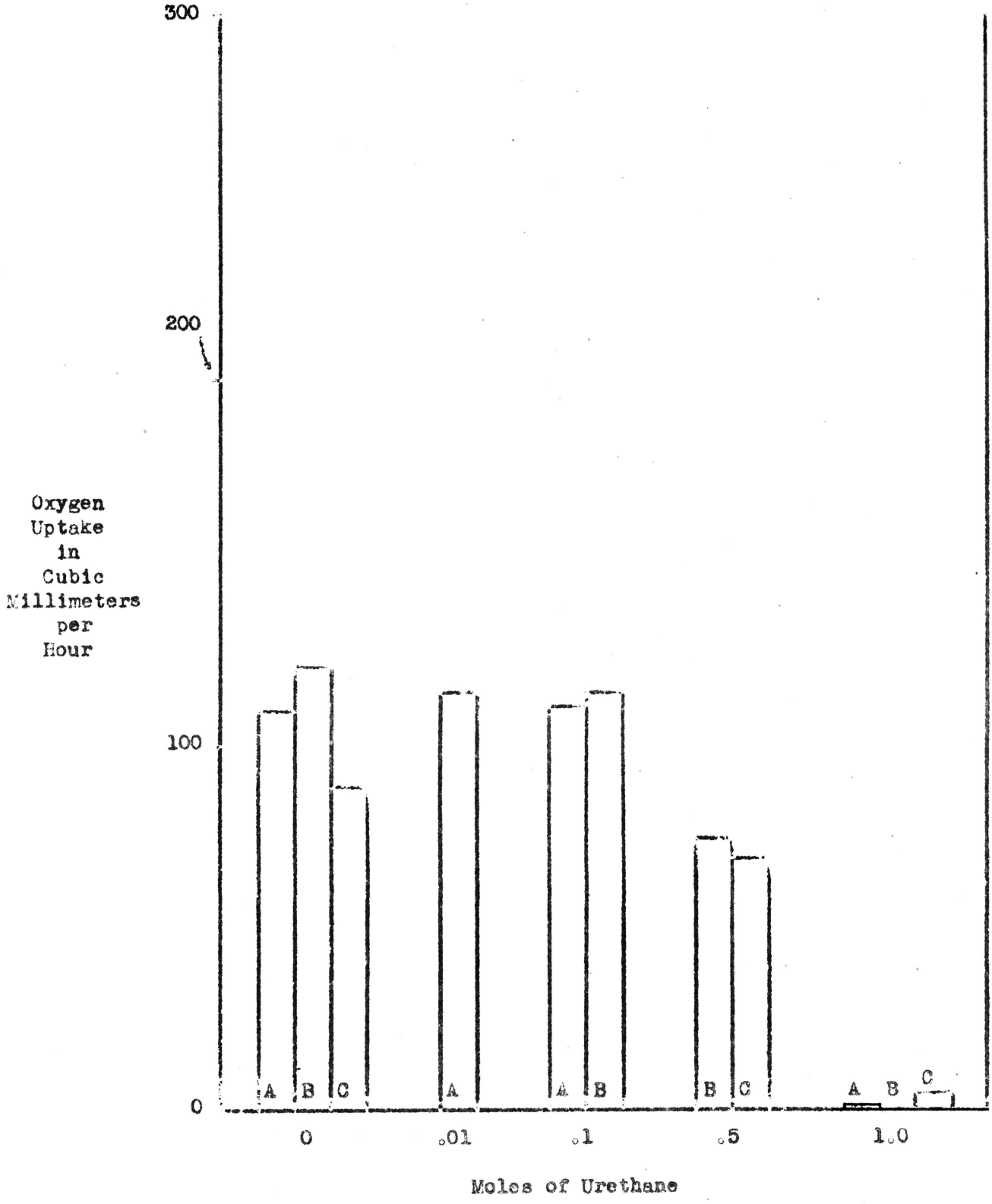


Figure 8. Oxygen consumption by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of URETHANE.

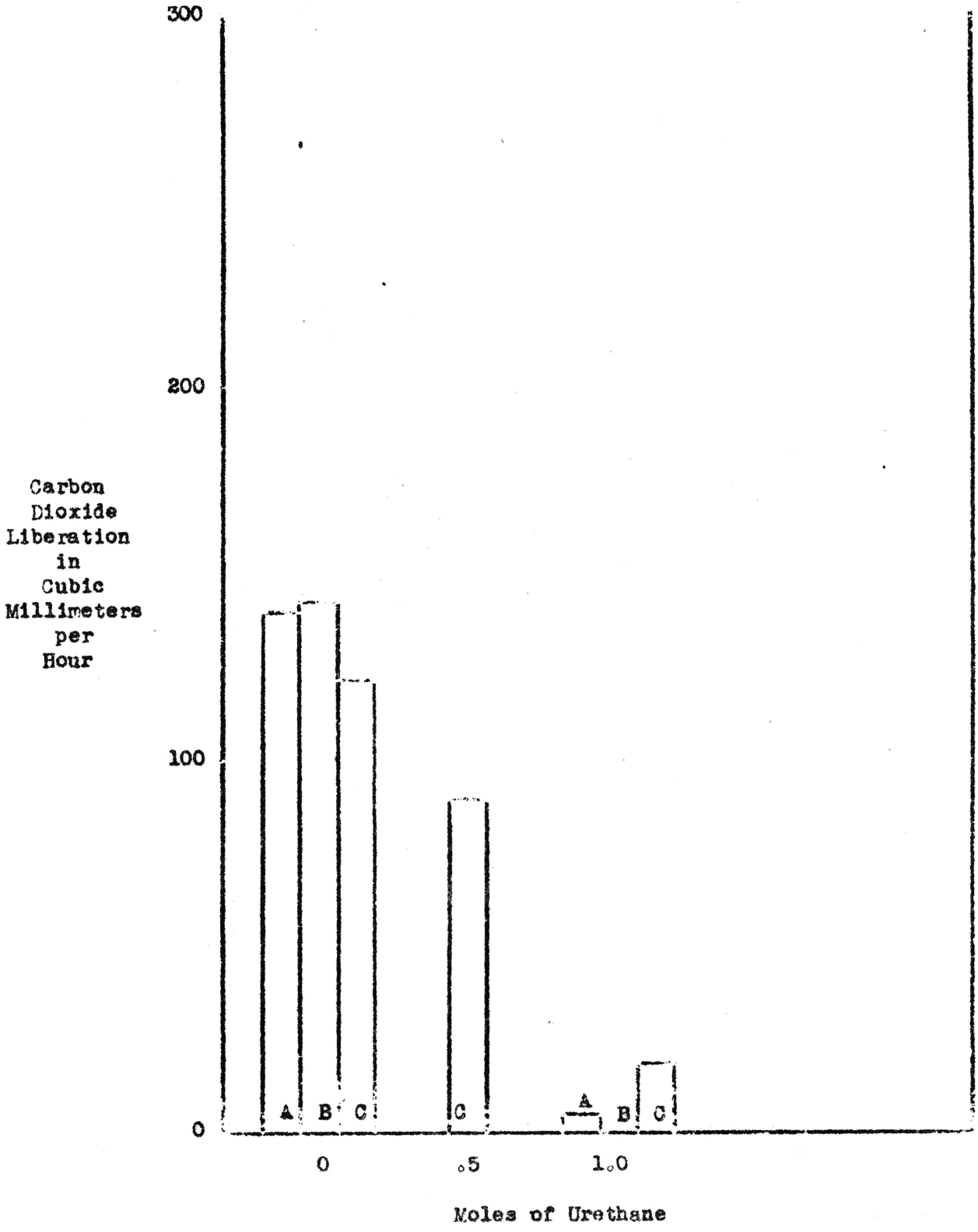


Figure 9. Carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of URETHANE.

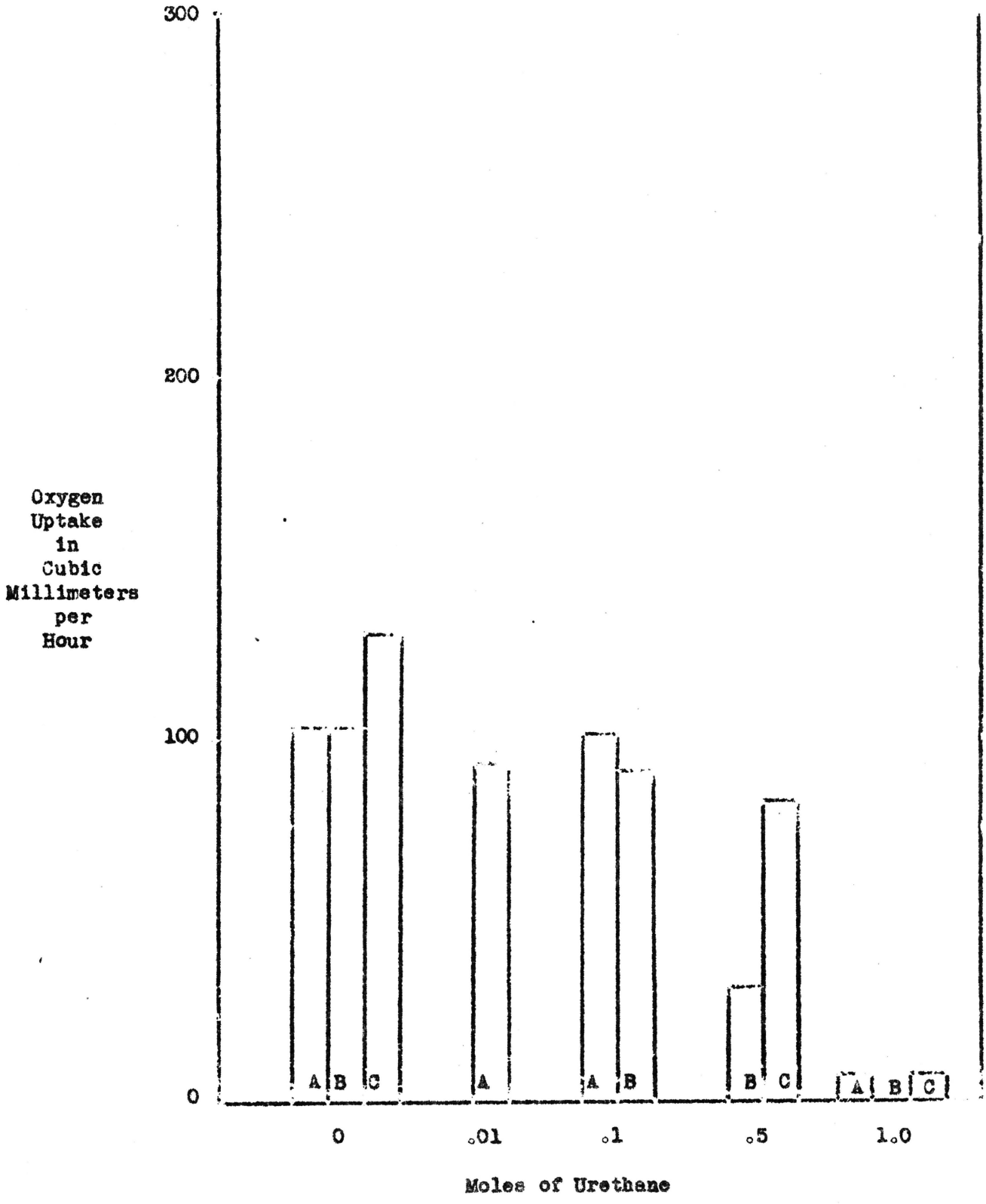


Figure 10. Oxygen consumption by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of Urethane.

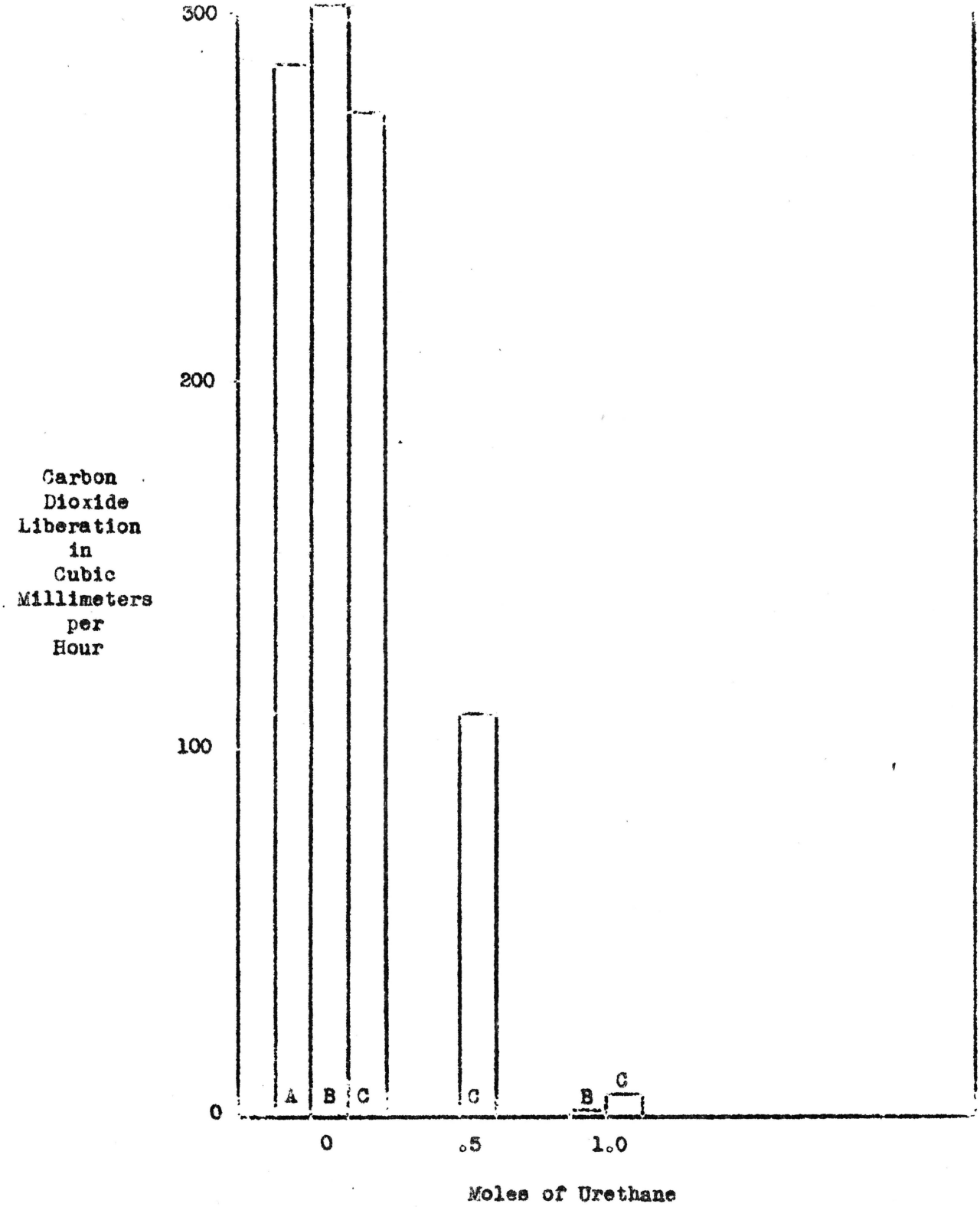


Figure 11. Carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of URETHANE.

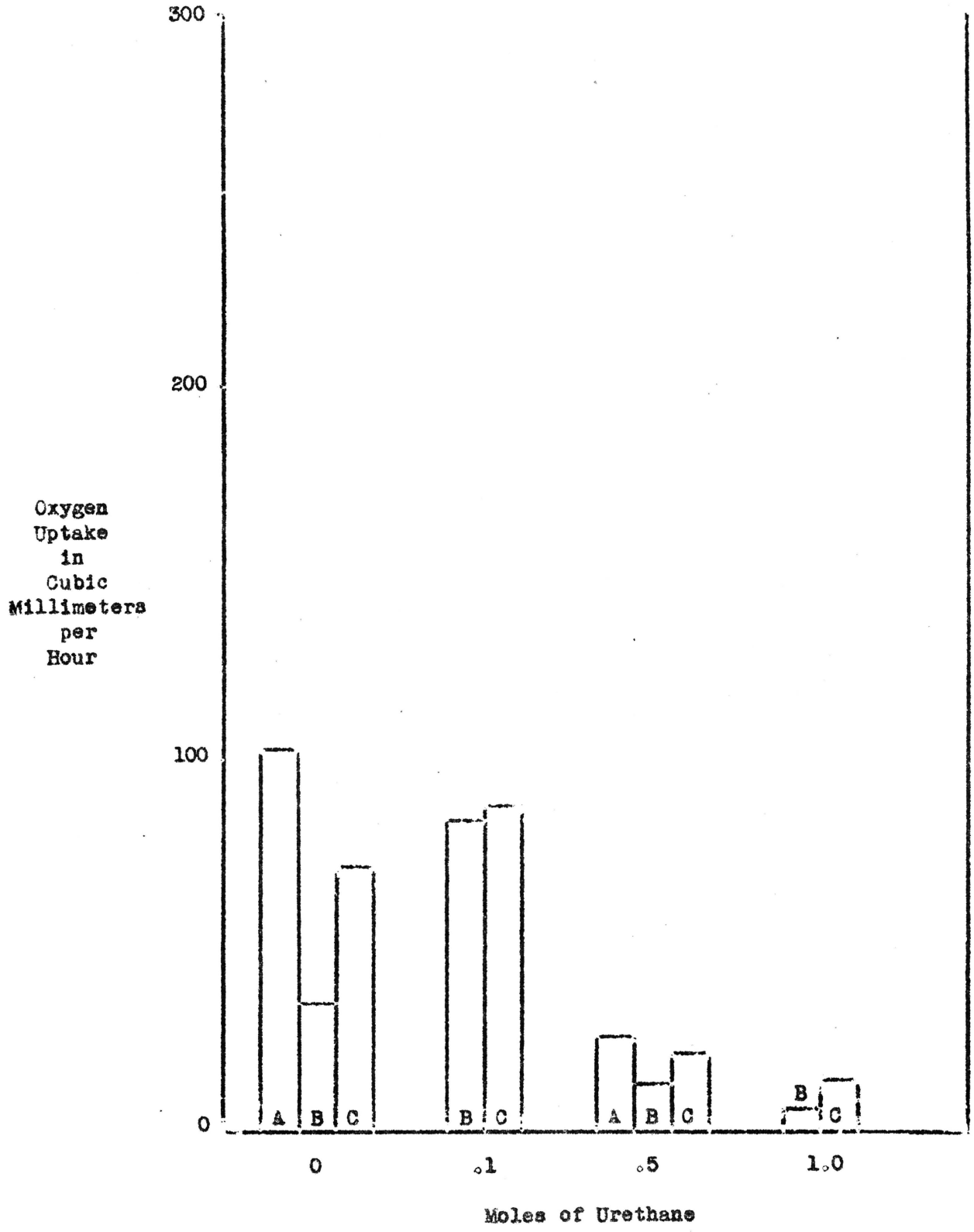


Figure 12. Oxygen consumption by Bacillus subtilis: 3.0 mg. cells on SUCCINATE in presence of URETHANE.

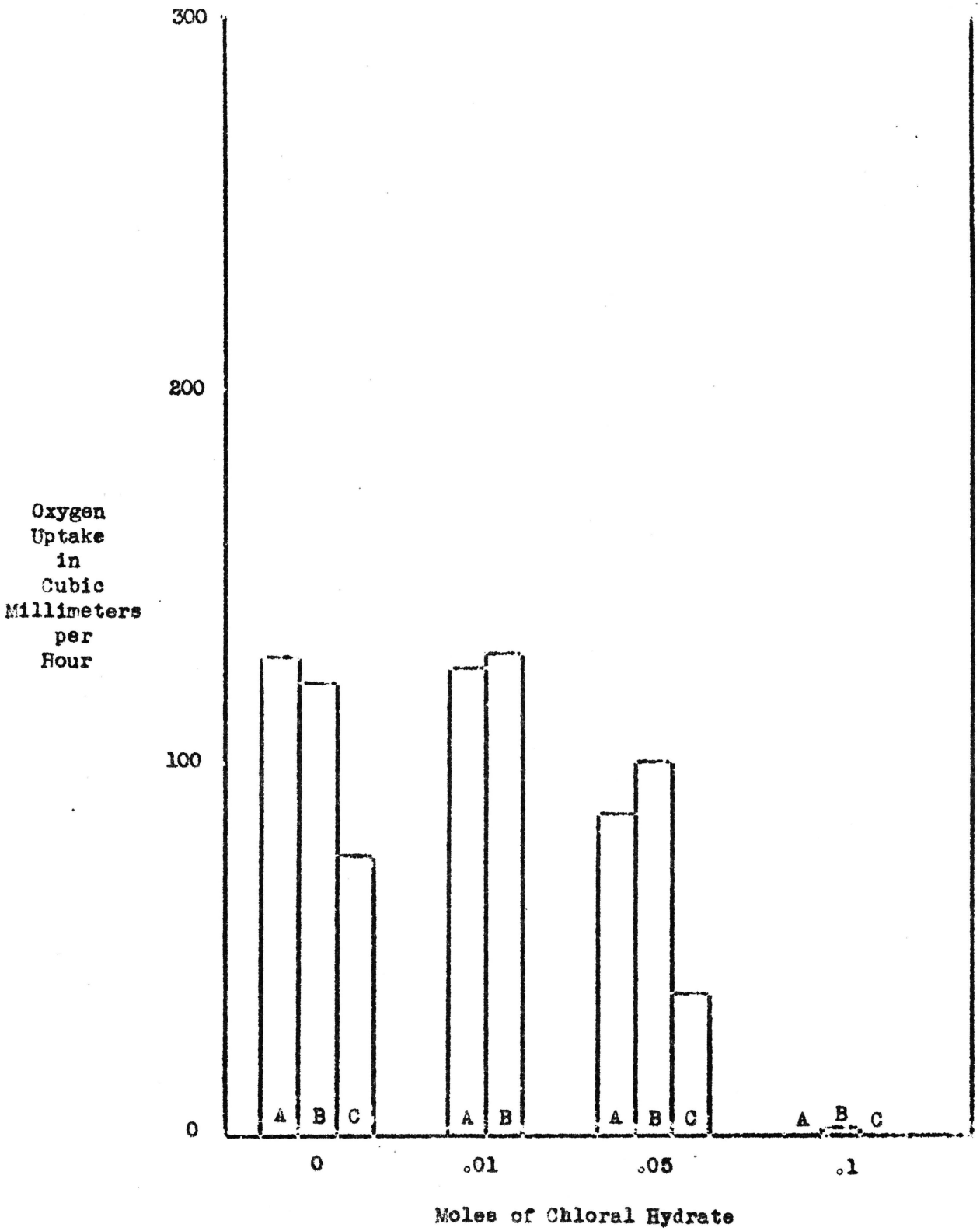


Figure 13. Oxygen consumption by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of CHLORAL HYDRATE.

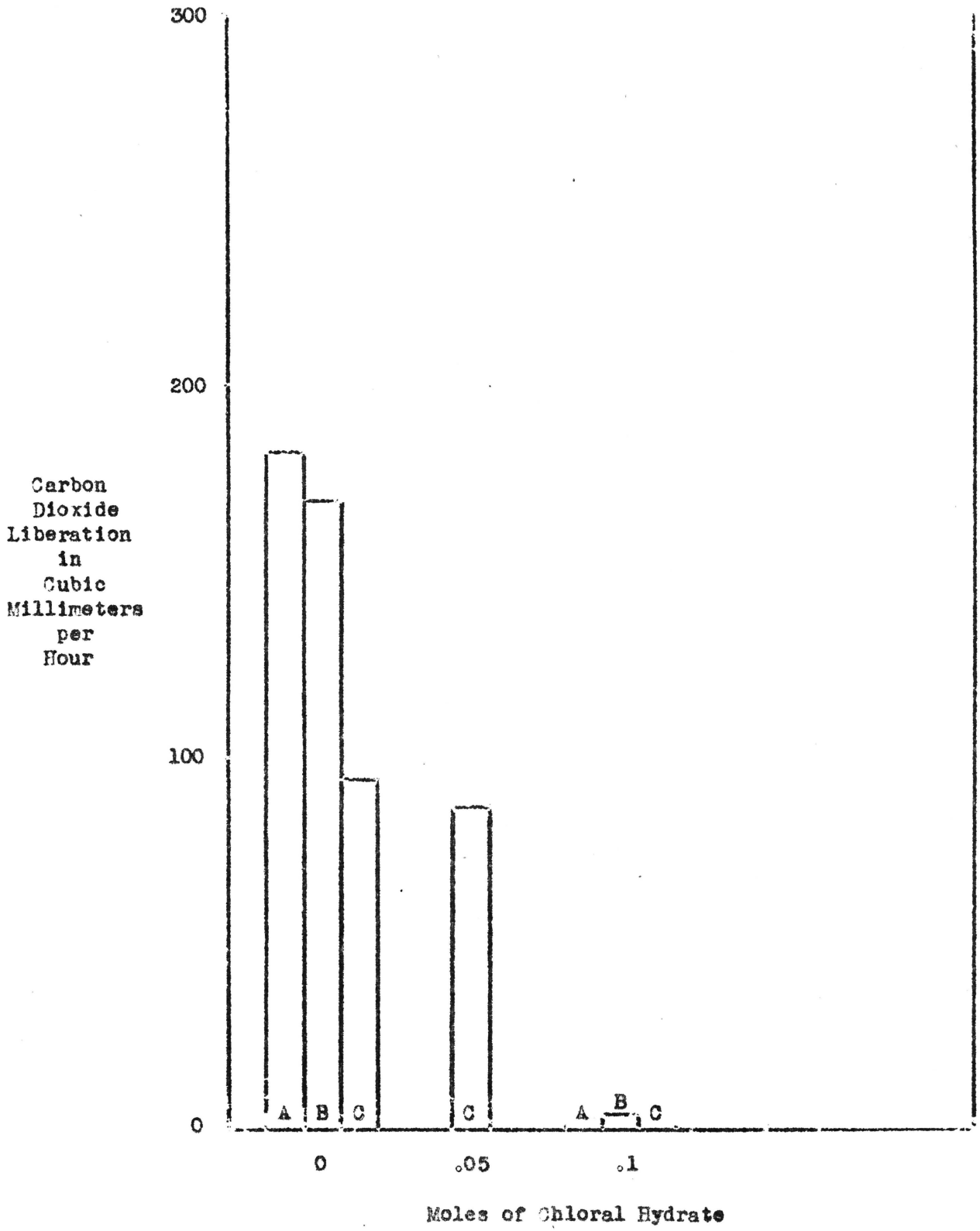


Figure 14. Carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of CHLORAL HYDRATE.

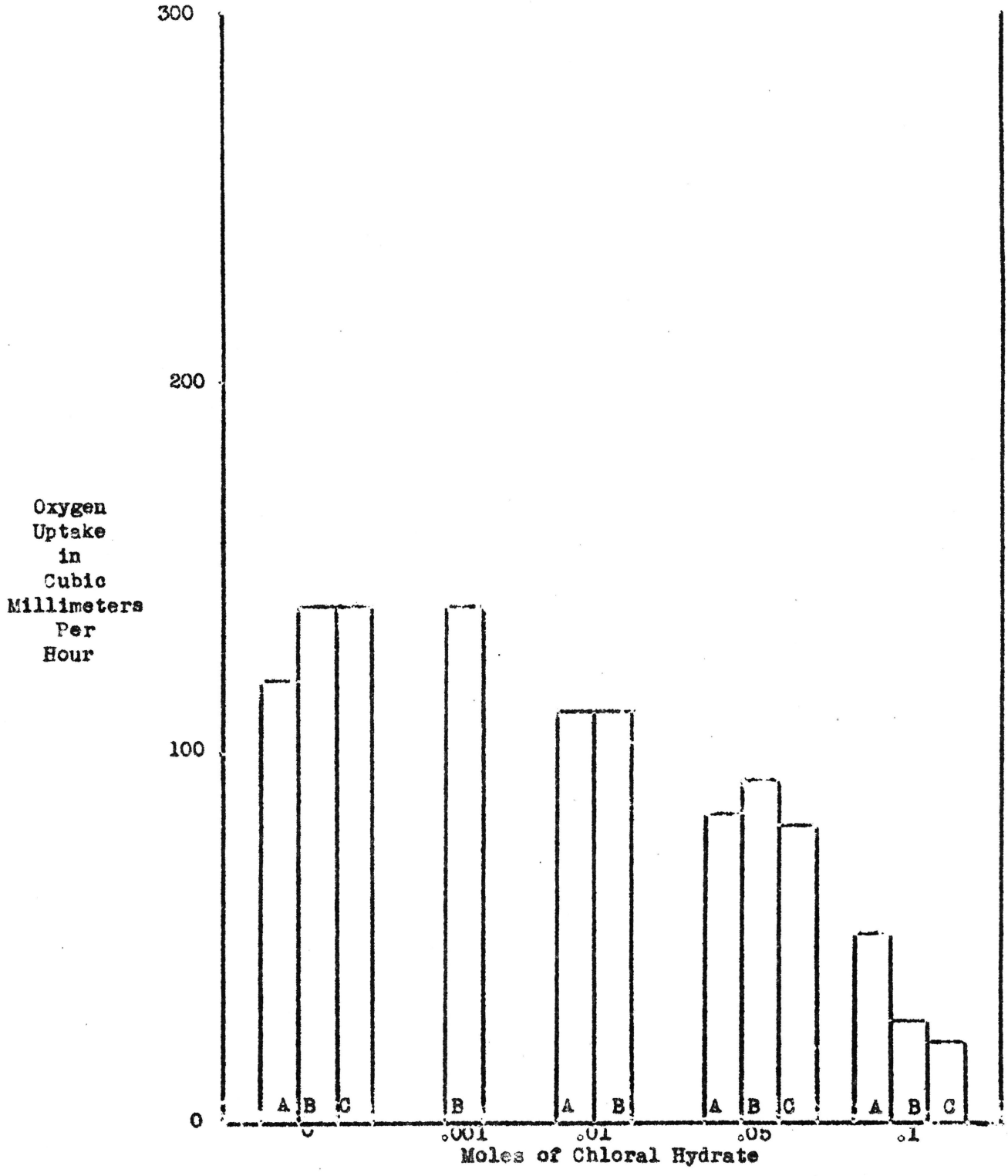


Figure 15. Oxygen consumption by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of CHLORAL HYDRATE.

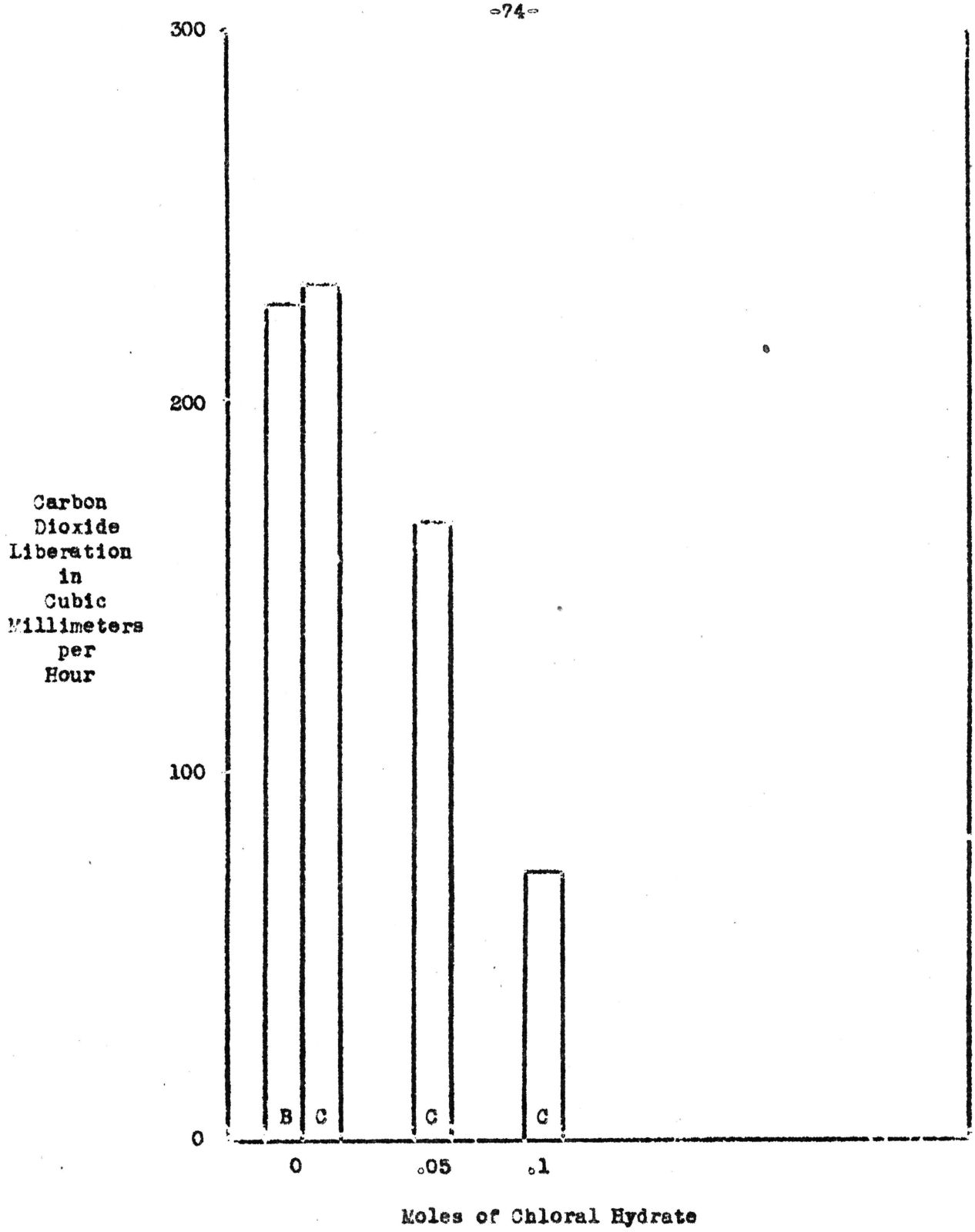


Figure 16. Carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of CHLORAL HYDRATE.

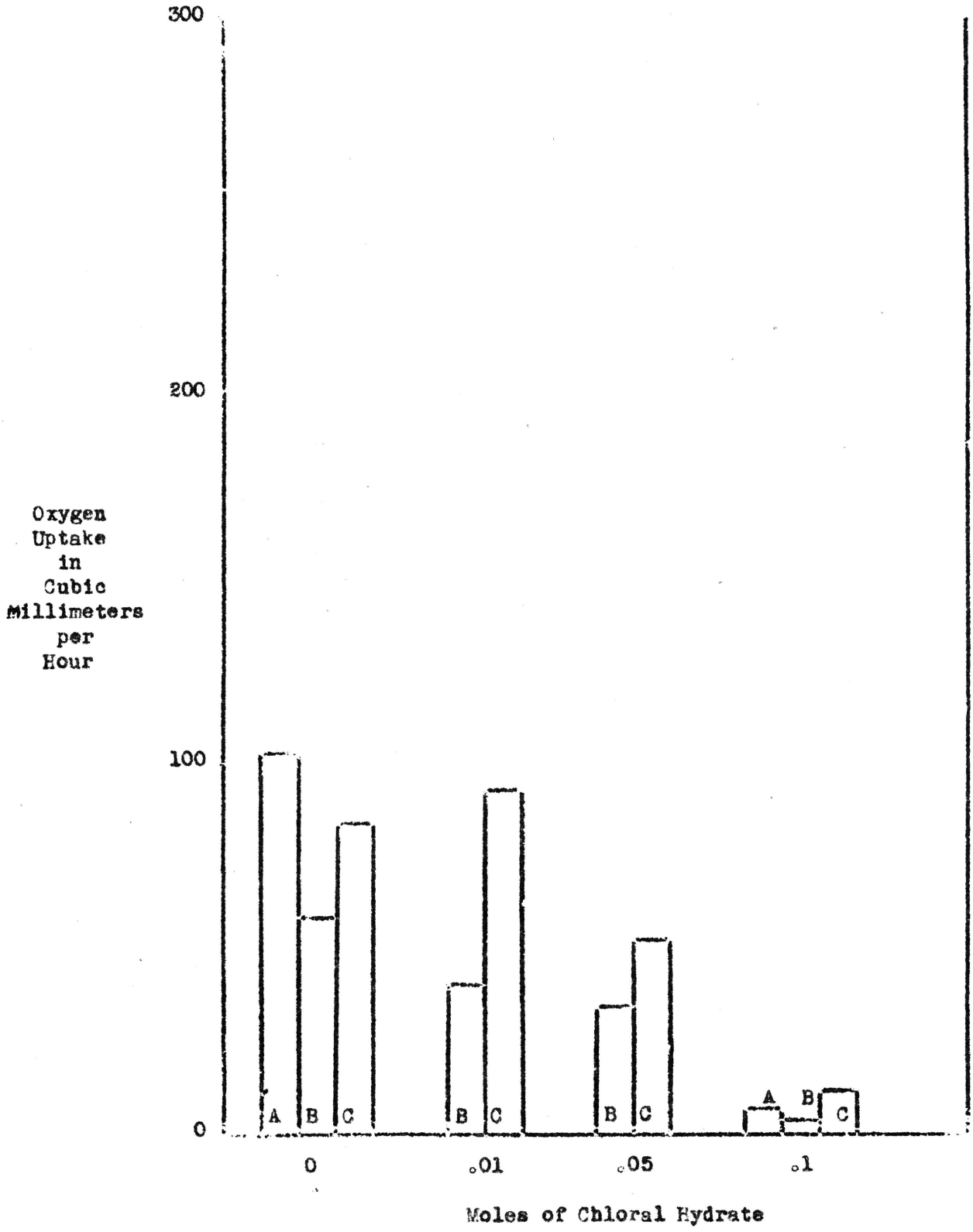


Figure 17. Oxygen concumption by Bacillus subtilis: 3.0 mg. cells on SUCCINATE in presence of CHLORAL HyDRATE.

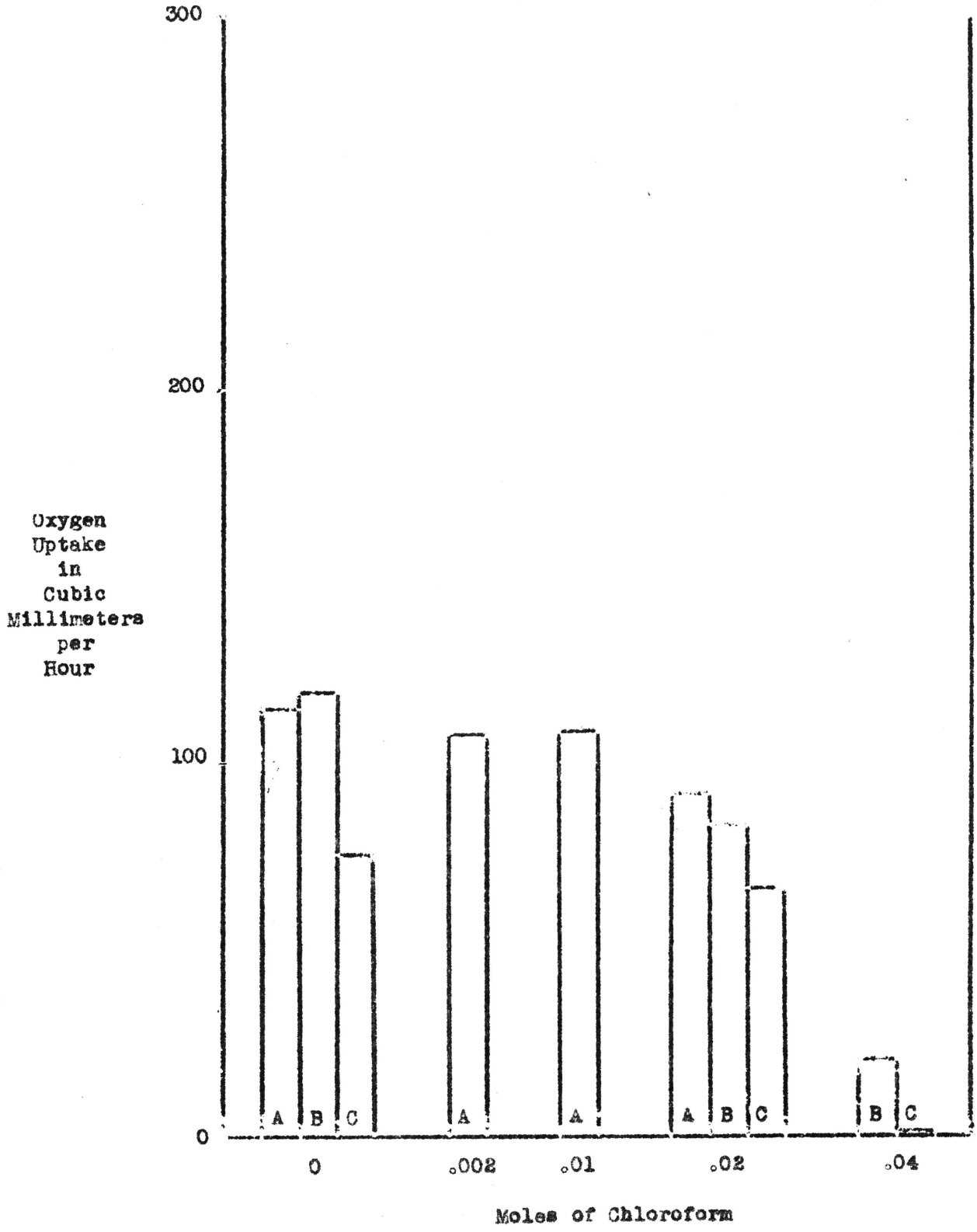


Figure 18. Oxygen consumption by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of CHLOROFORM.

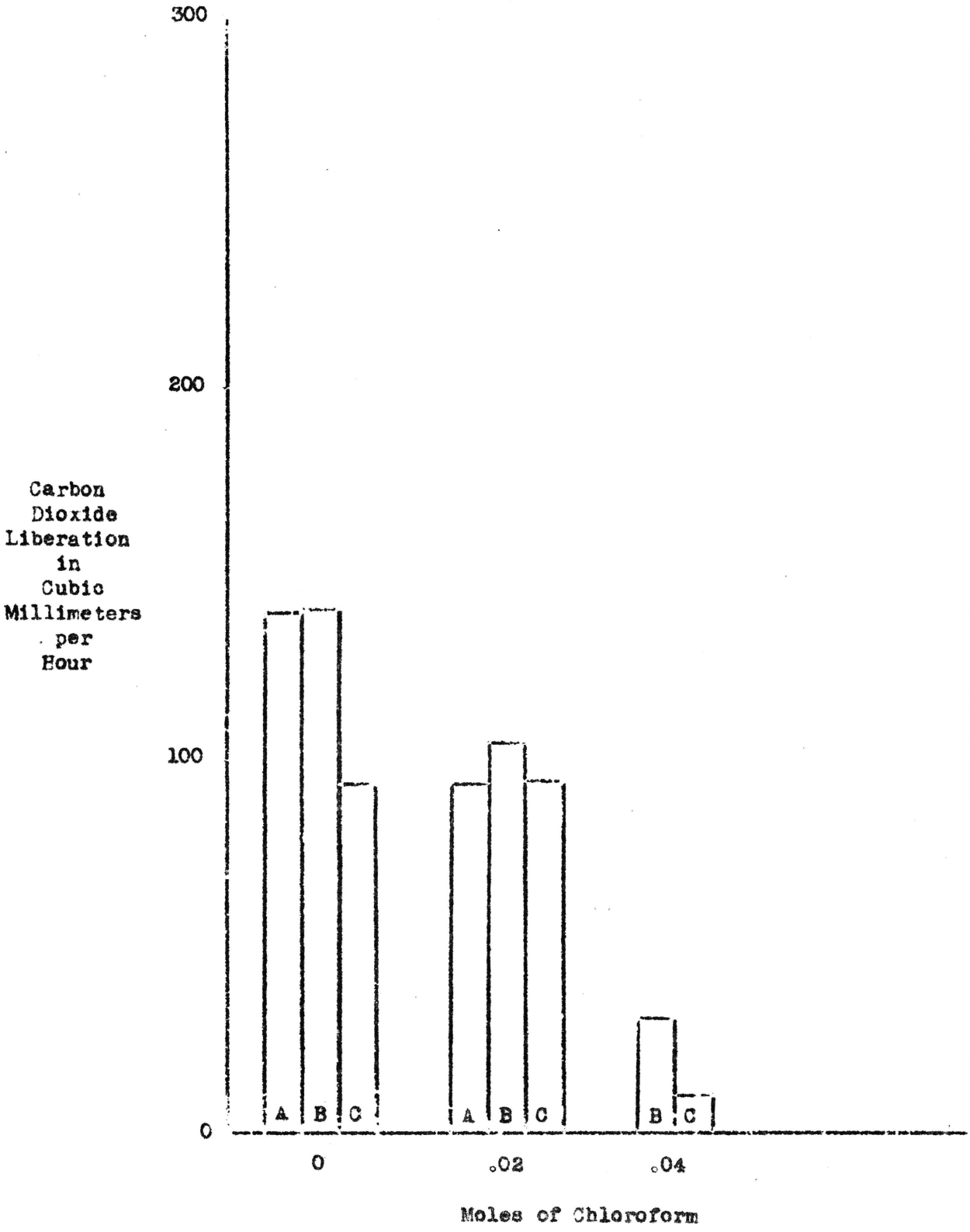


Figure 19. Carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of CHLOROFORM.

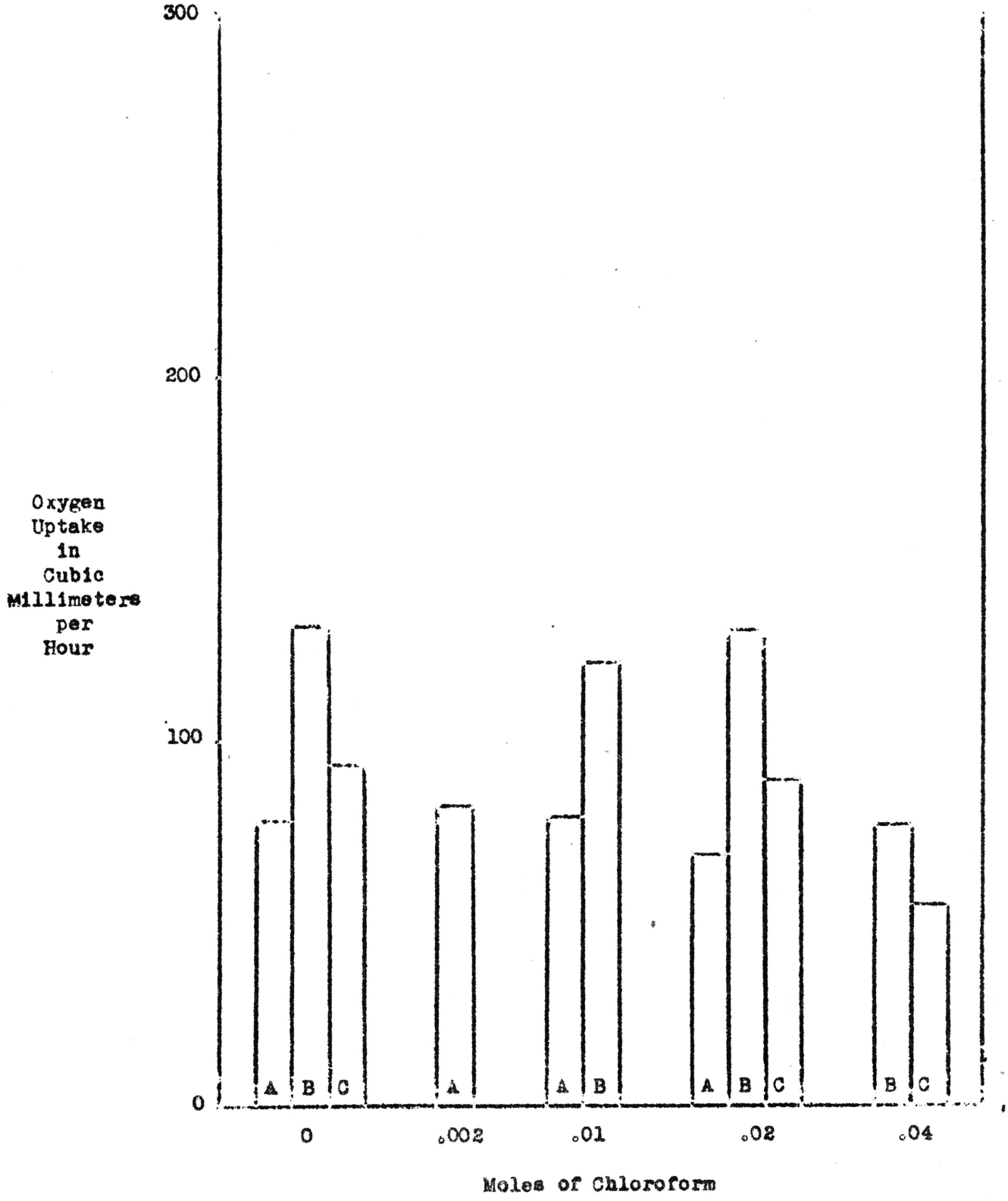


Figure 20. Oxygen consumption by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of CHLOROFORM.

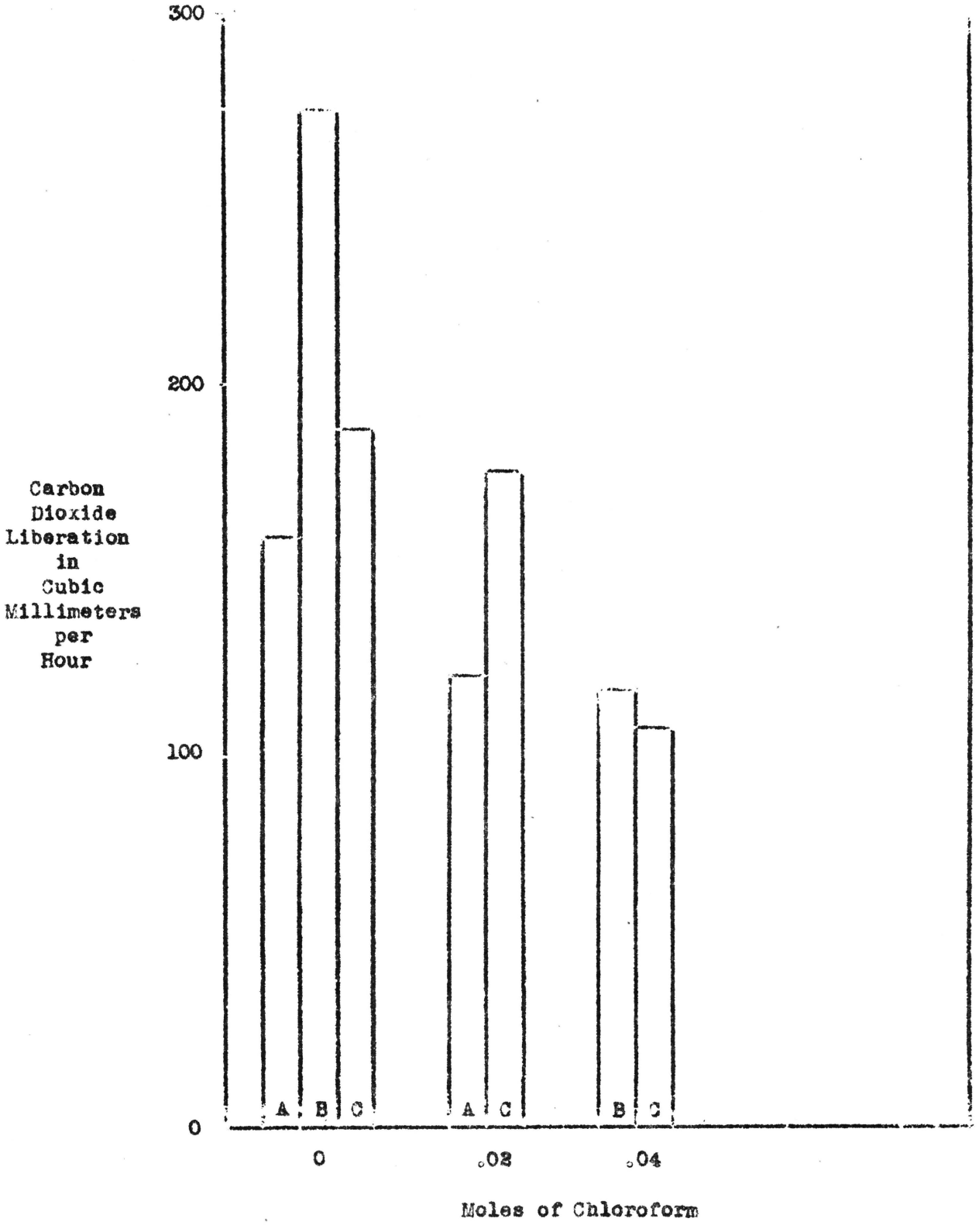


Figure 21. Carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of CHLOROFORM.

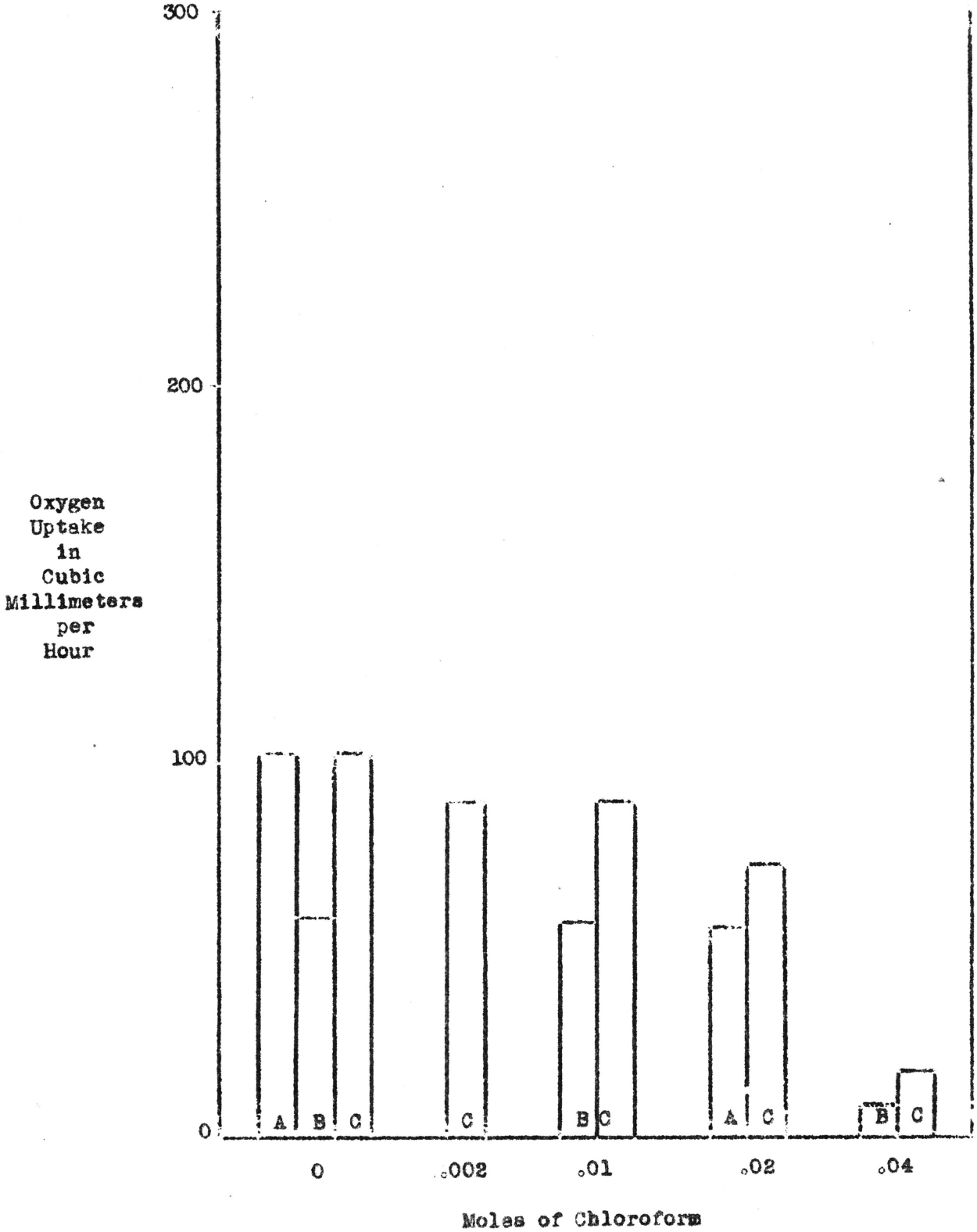


Figure 22. Oxygen consumption by Bacillus subtilis: 3.0 mg. cells on SUCCINATE in presence of CHLOROFORM.

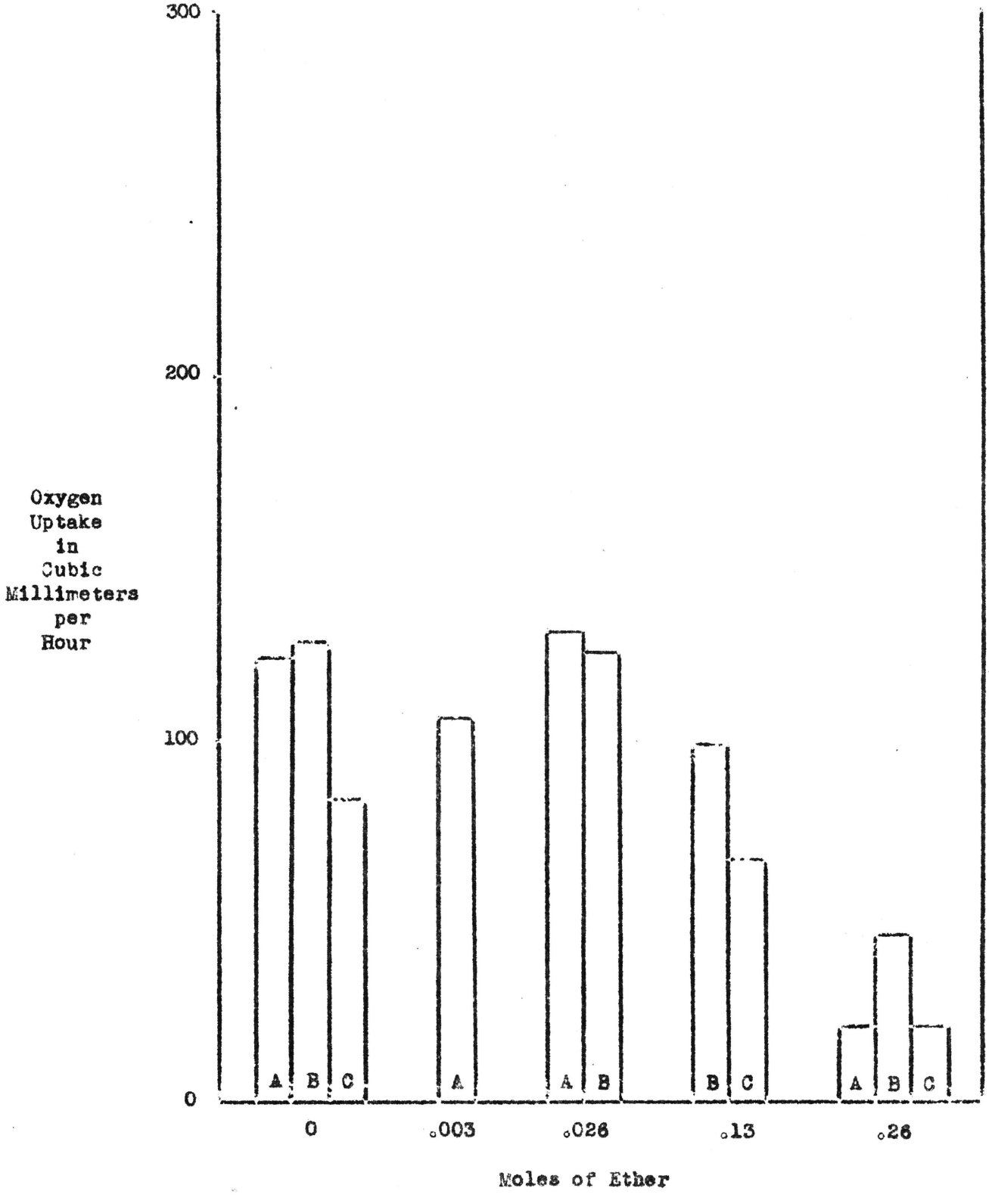


Figure 23. Oxygen consumption by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of ETHER

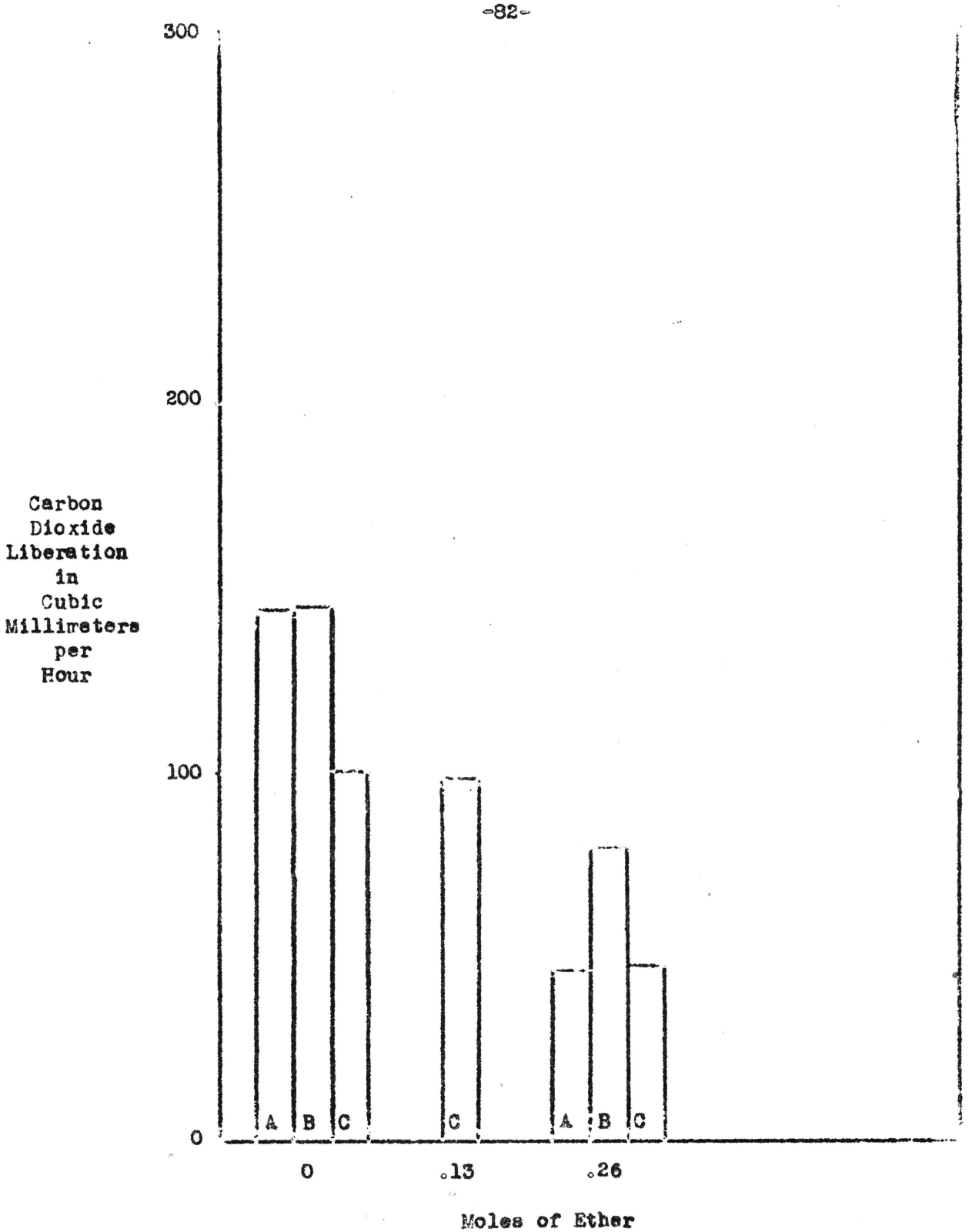


Figure 24. Carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of ETHER.

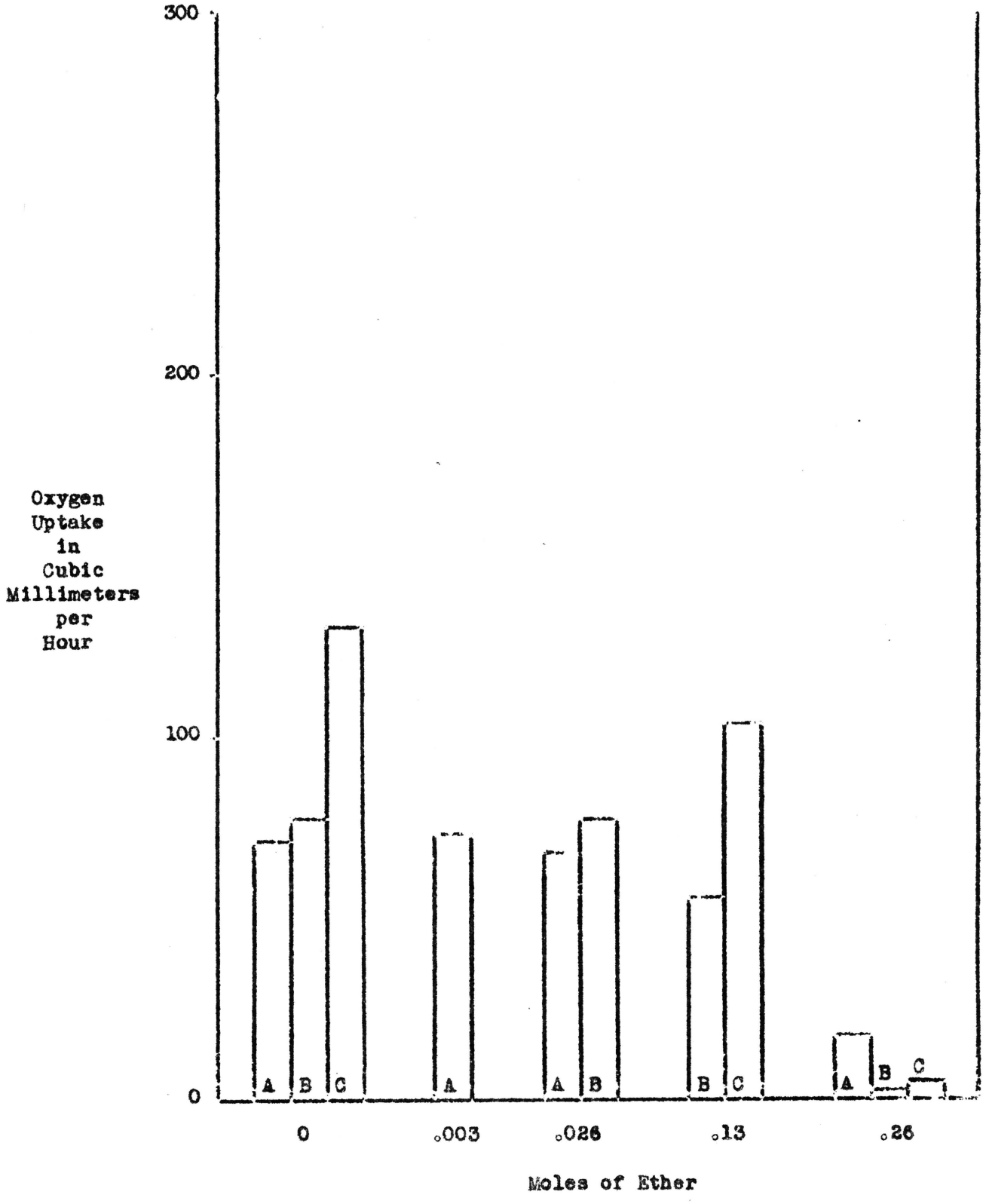


Figure 25. Oxygen consumption by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of ETHER.

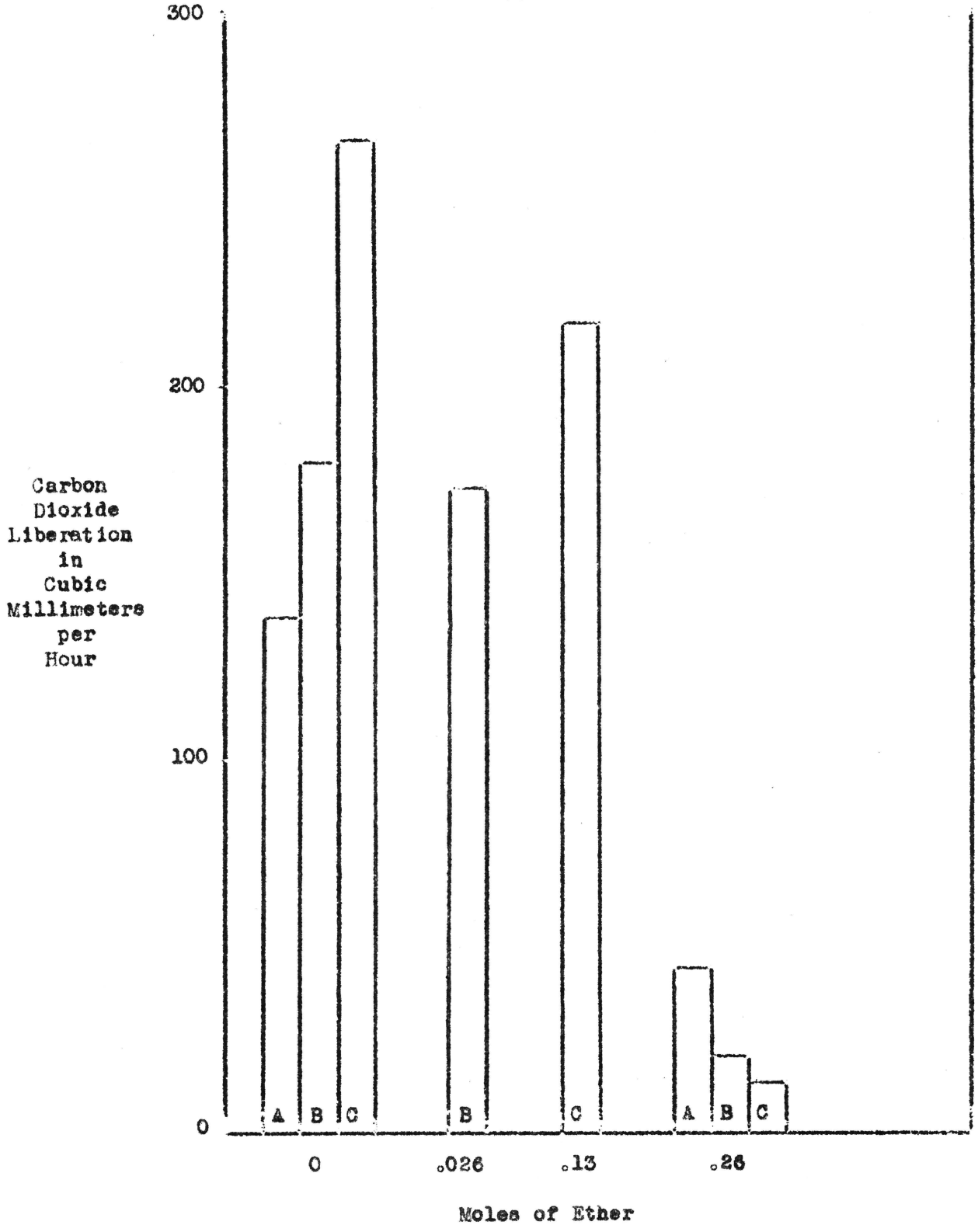


Figure 26. Carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of ETHER.

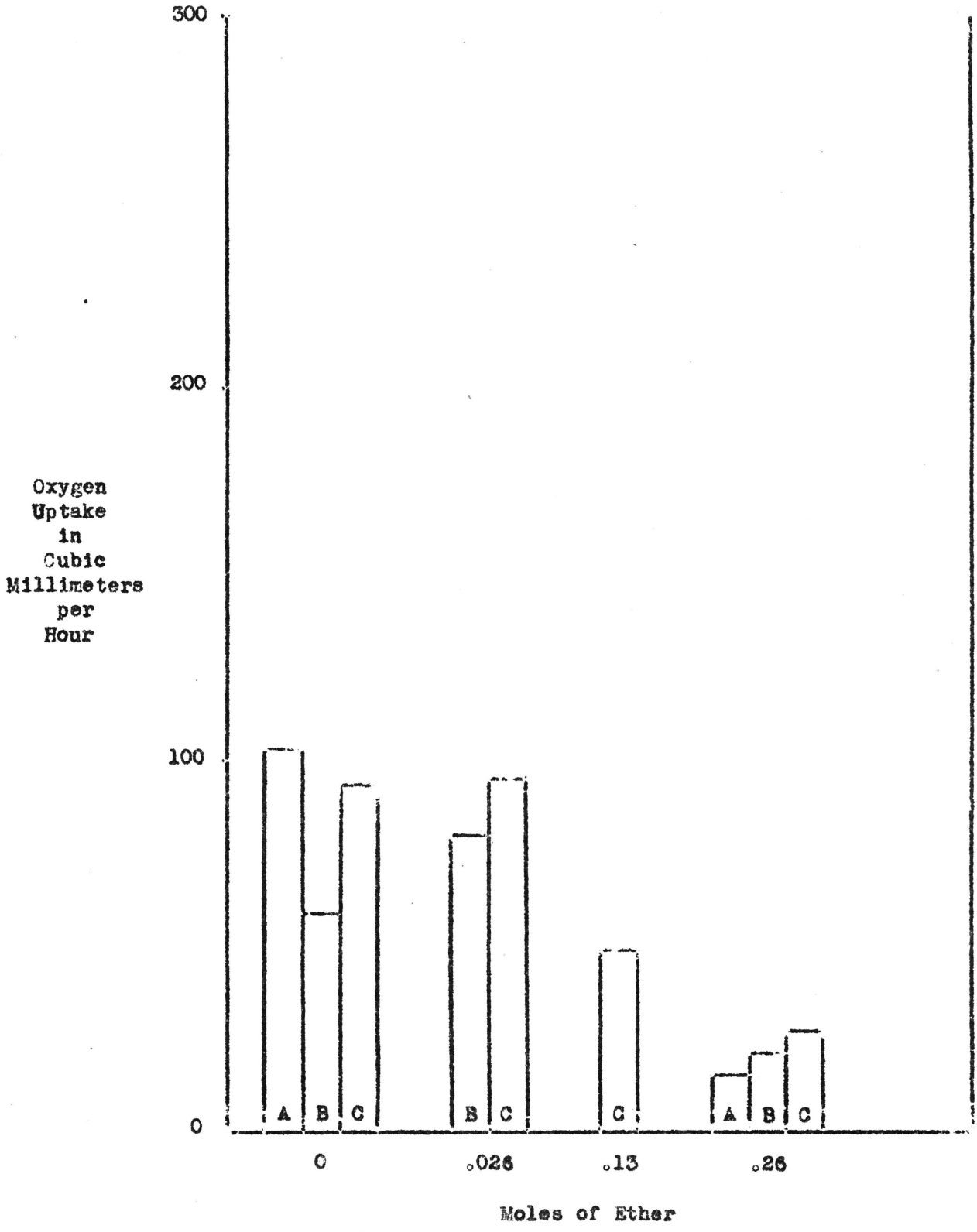


Figure 27. Oxygen consumption by Bacillus subtilis: 3.0 mg. cells on SUCCINATE in presence of ETHER.

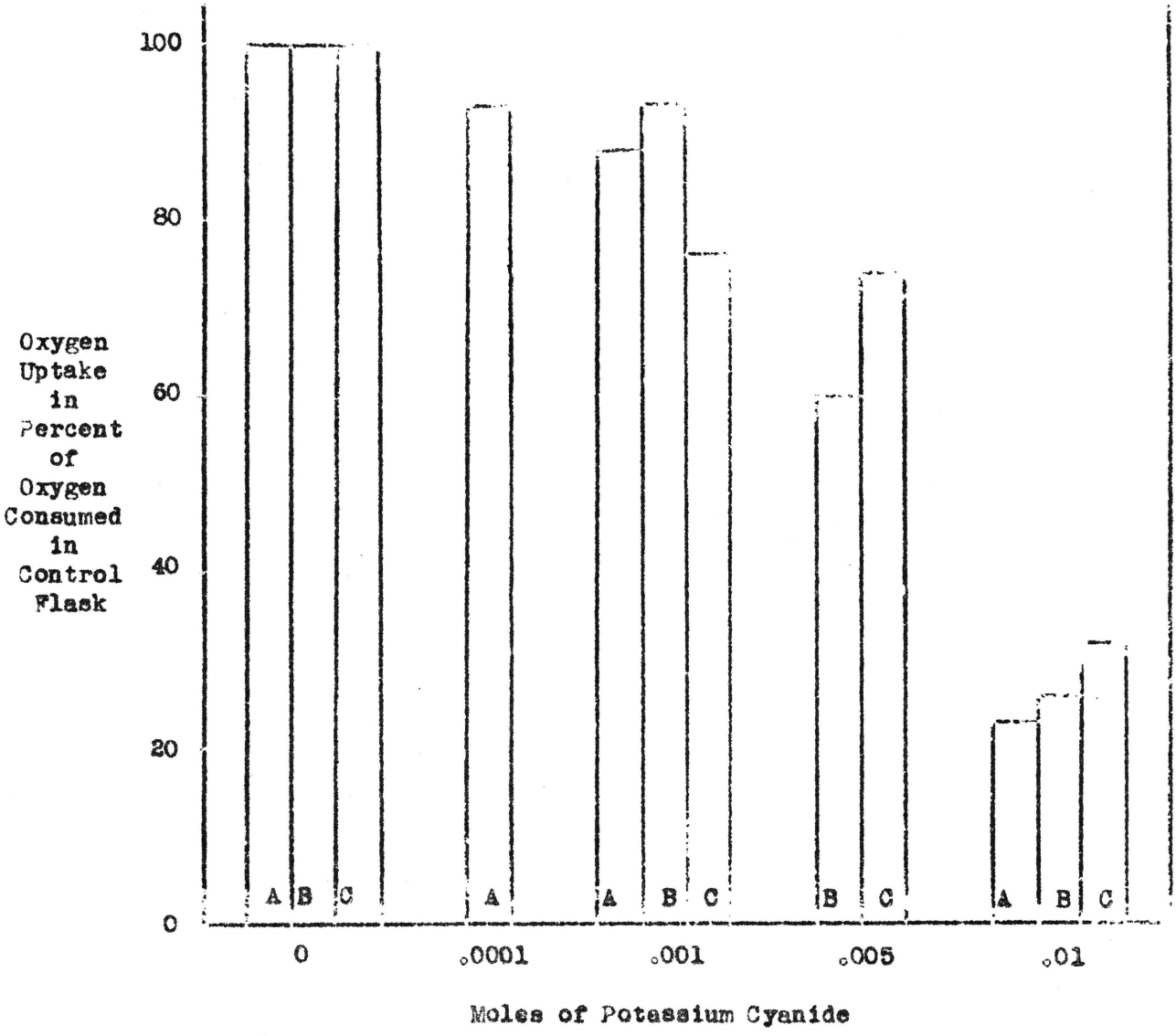


Figure 28. Percent oxygen consumption by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of CYANIDE.

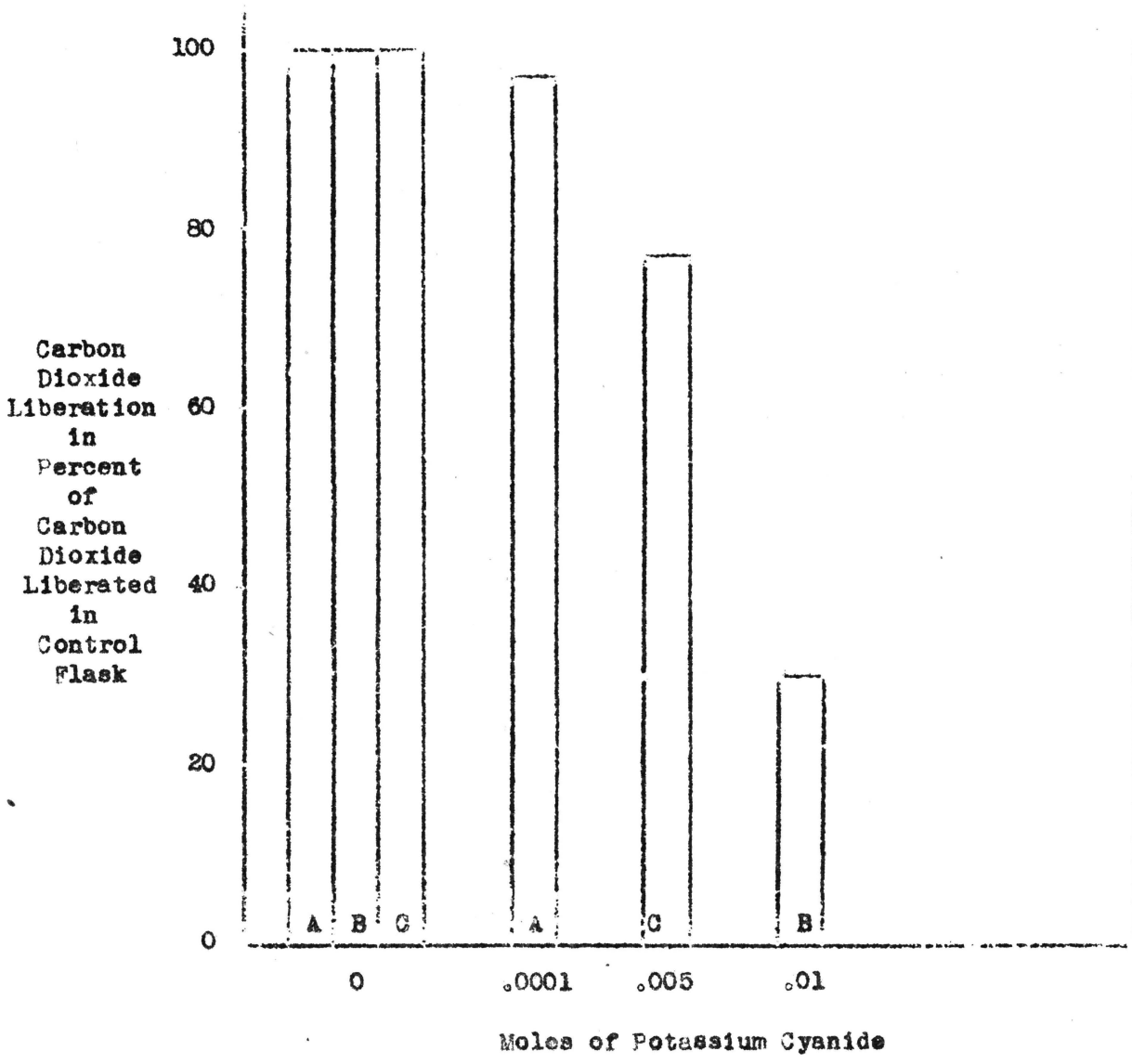


Figure 29. Percent carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of CYANIDE.

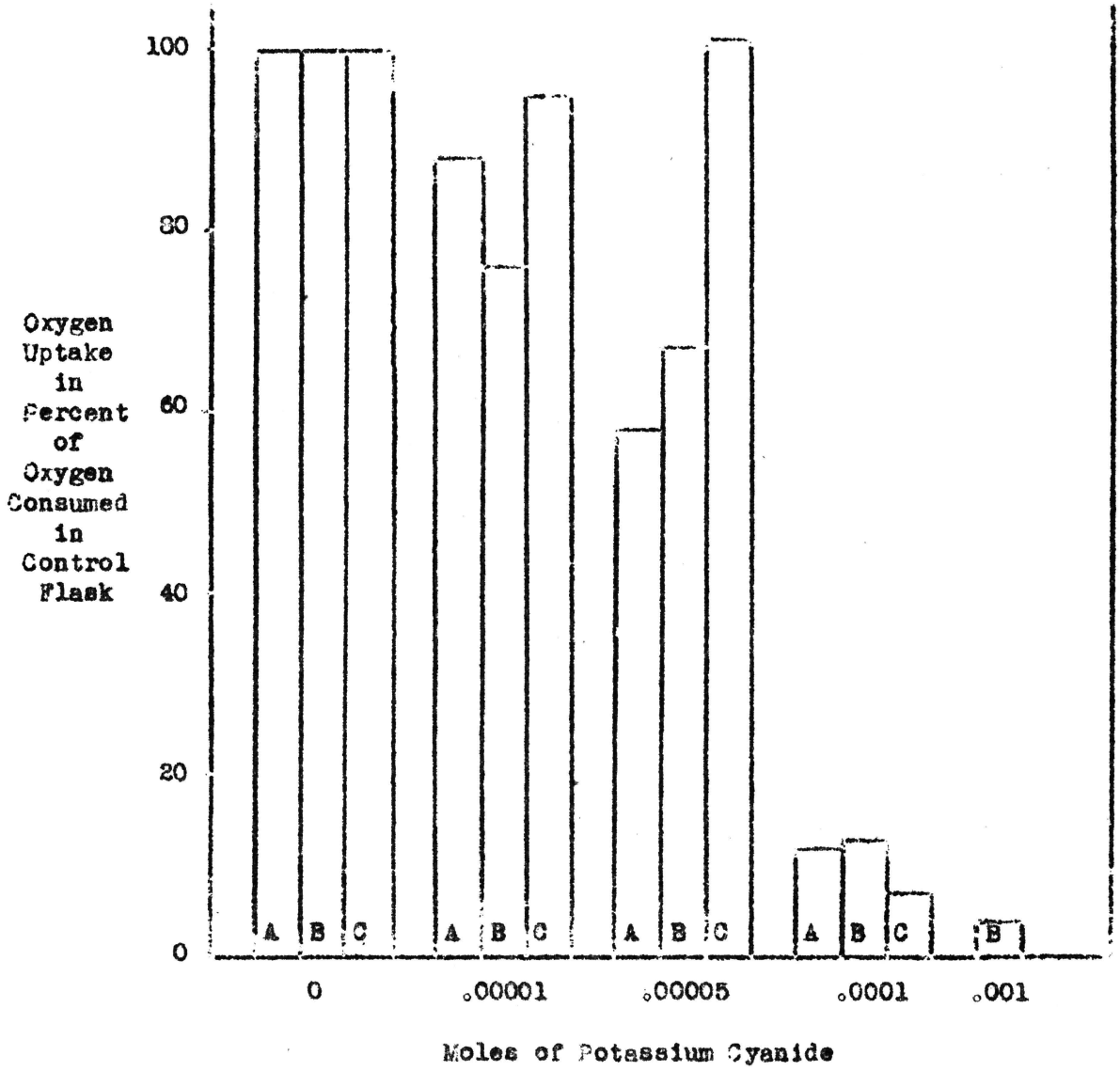


Figure 30. Percent oxygen consumption by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of CYANIDE.

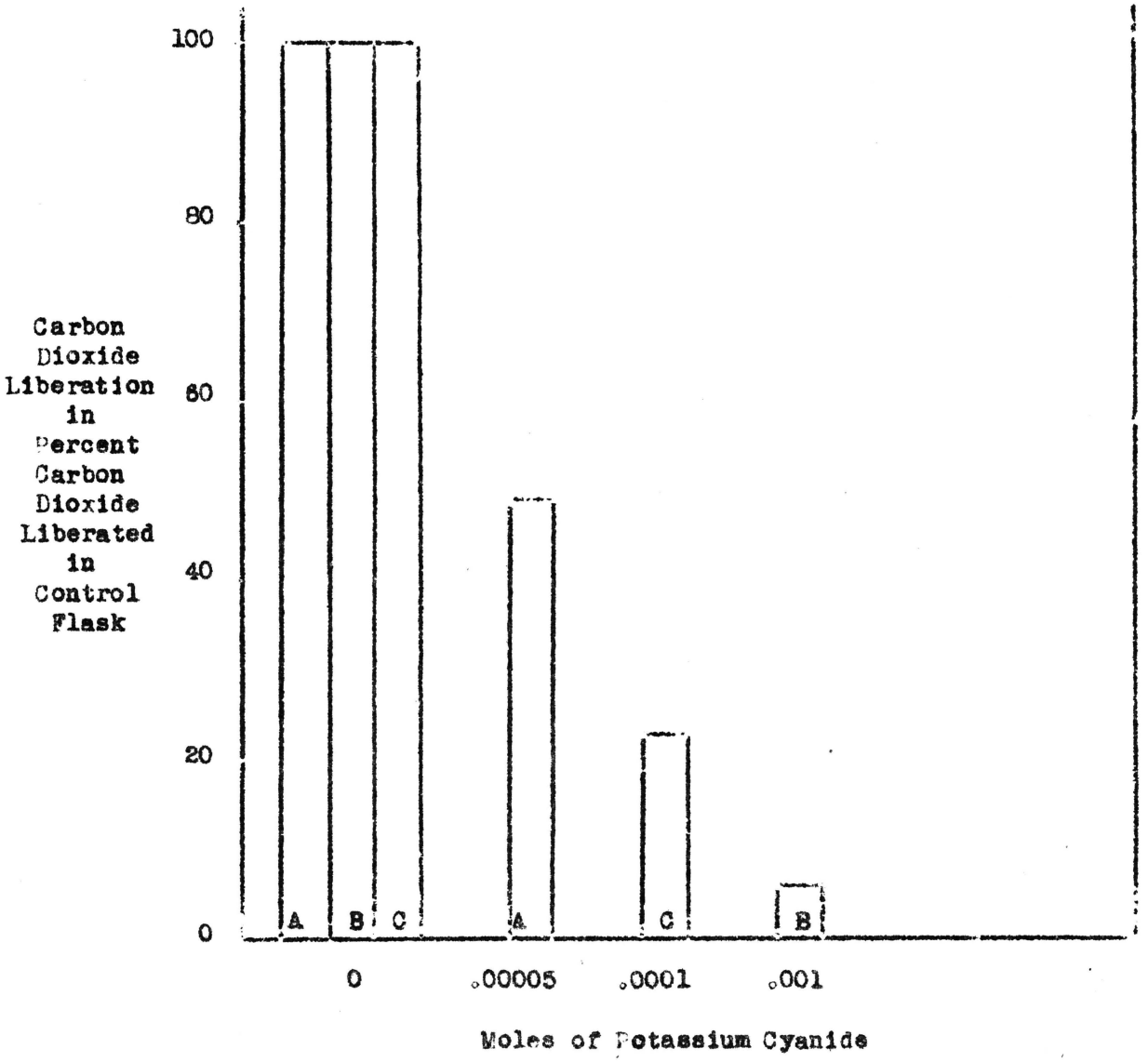


Figure 31. Percent carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of CYANIDE.

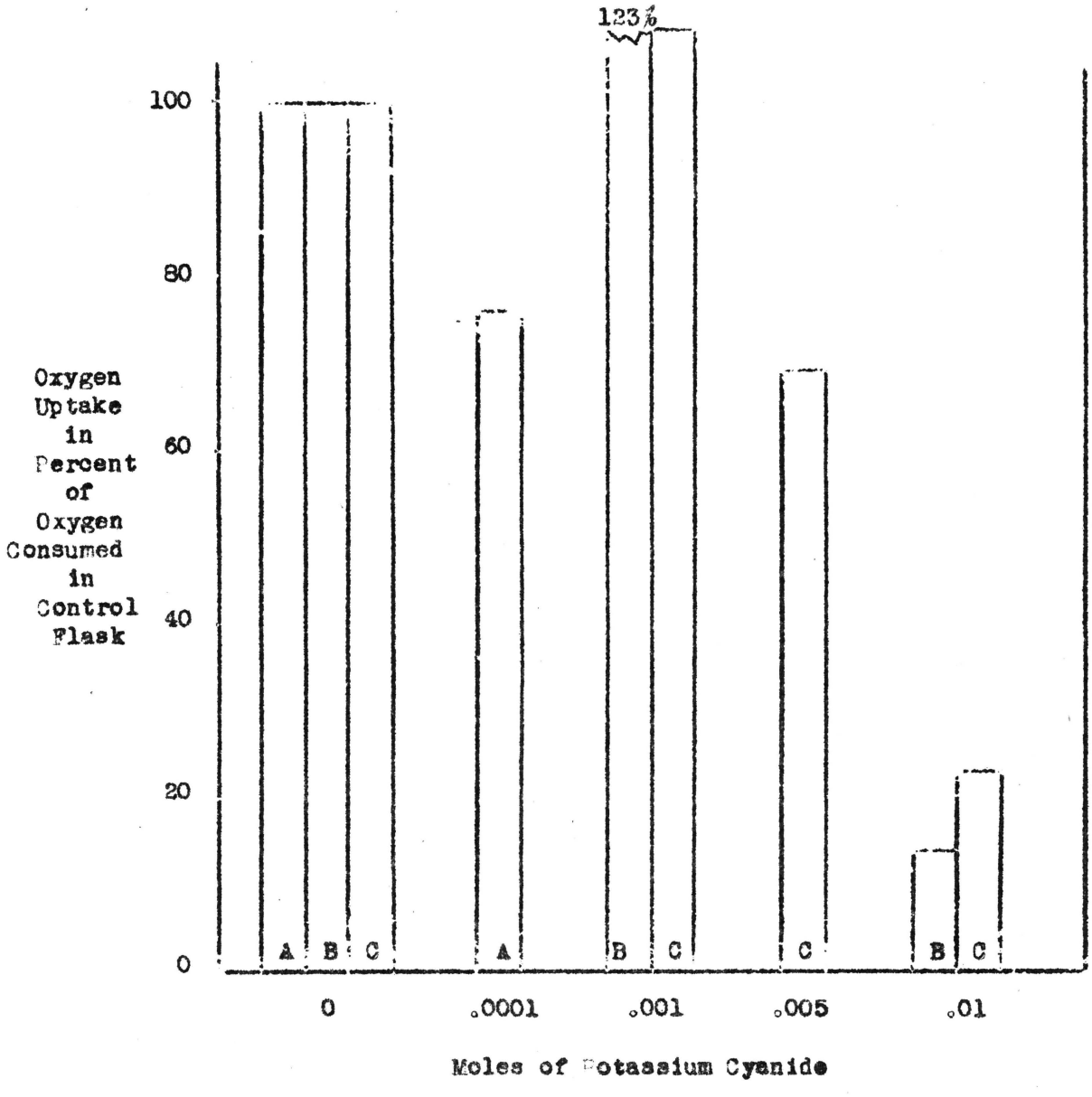


Figure 32. Percent oxygen consumption by Bacillus subtilis: 3.0 mg. cells on SUCCINATE in presence of CYANIDE.

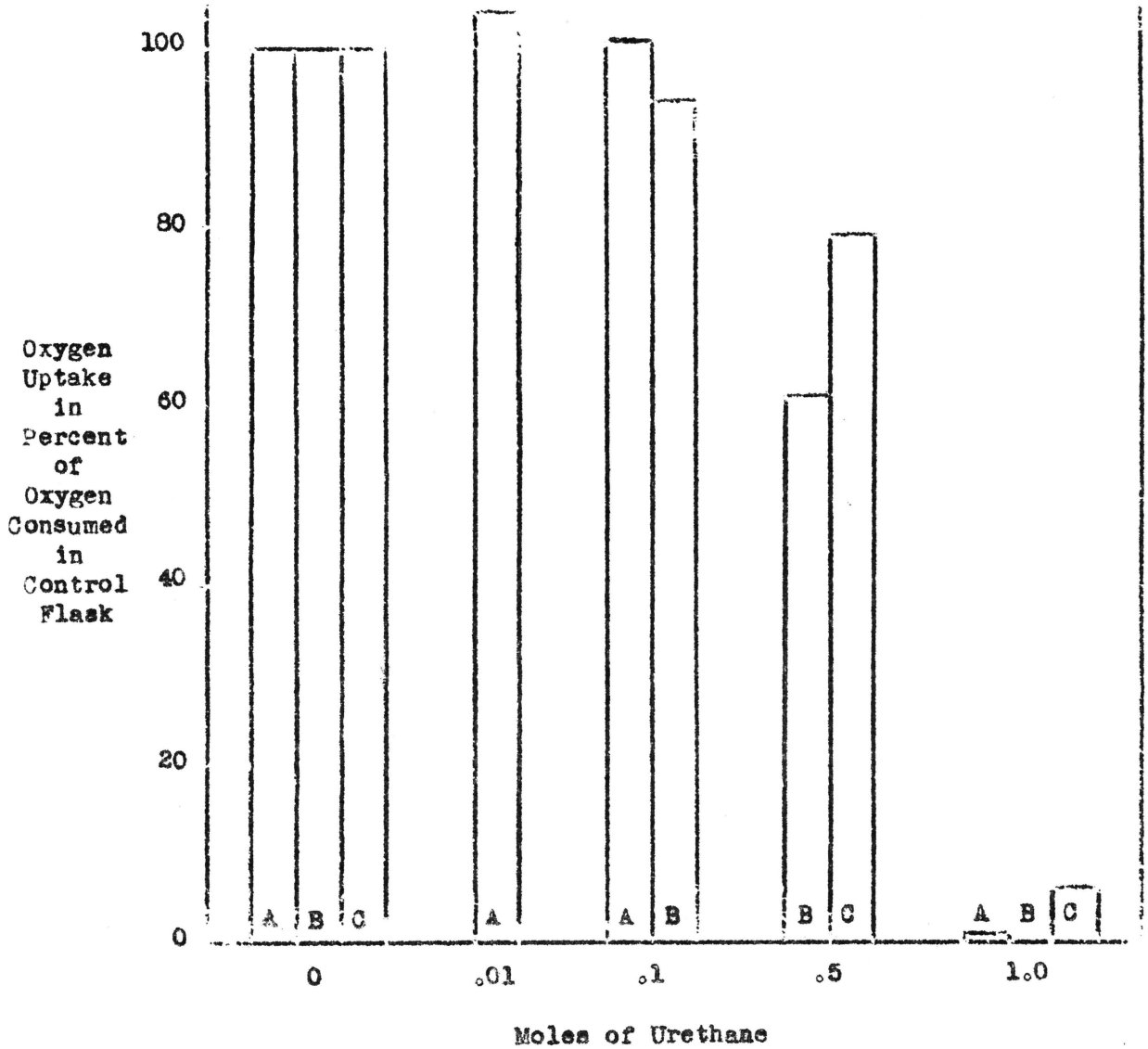


Figure 33. Percent oxygen consumption by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of URETHANE.

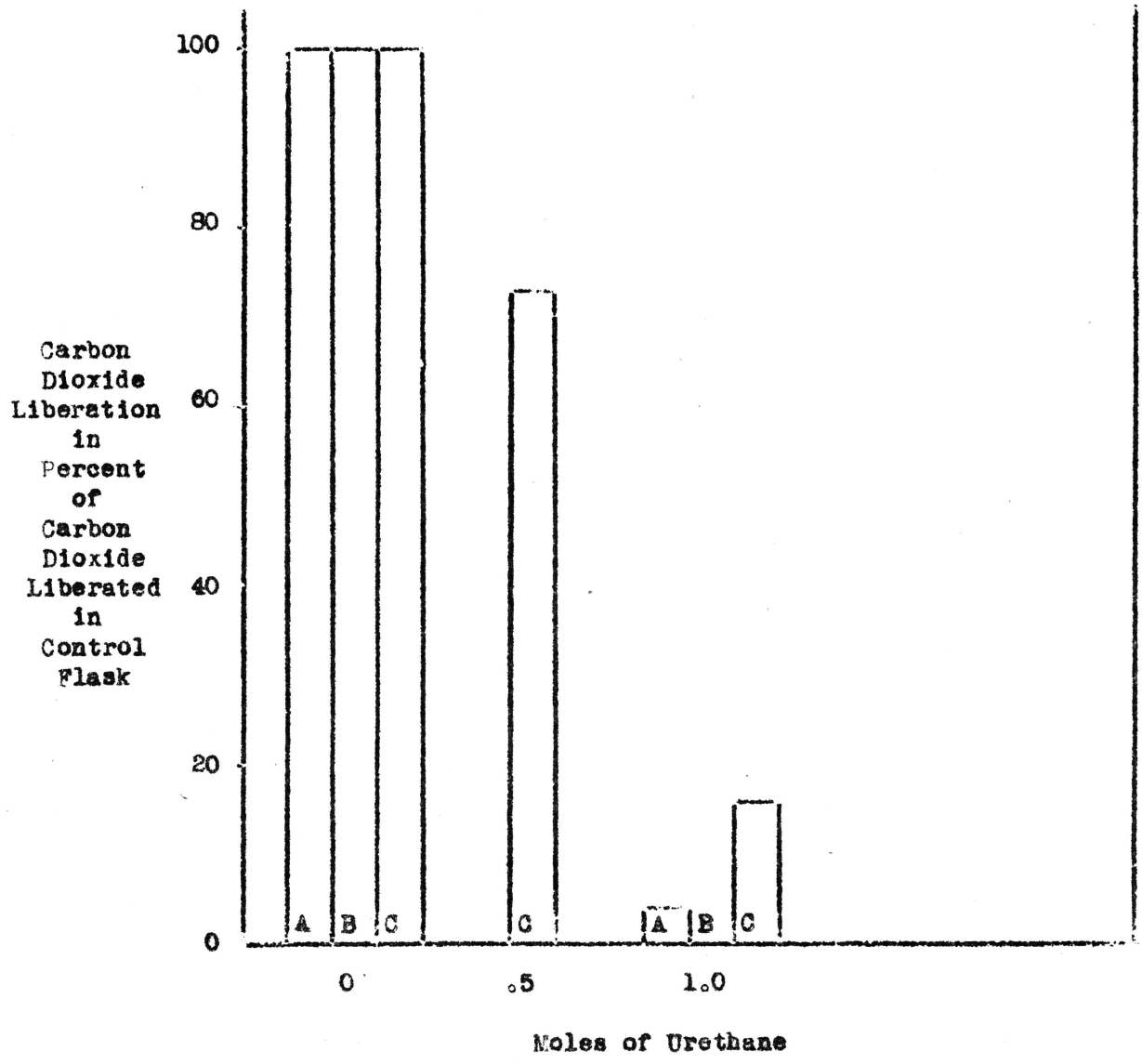


Figure 34. Percent carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of URETHANE.

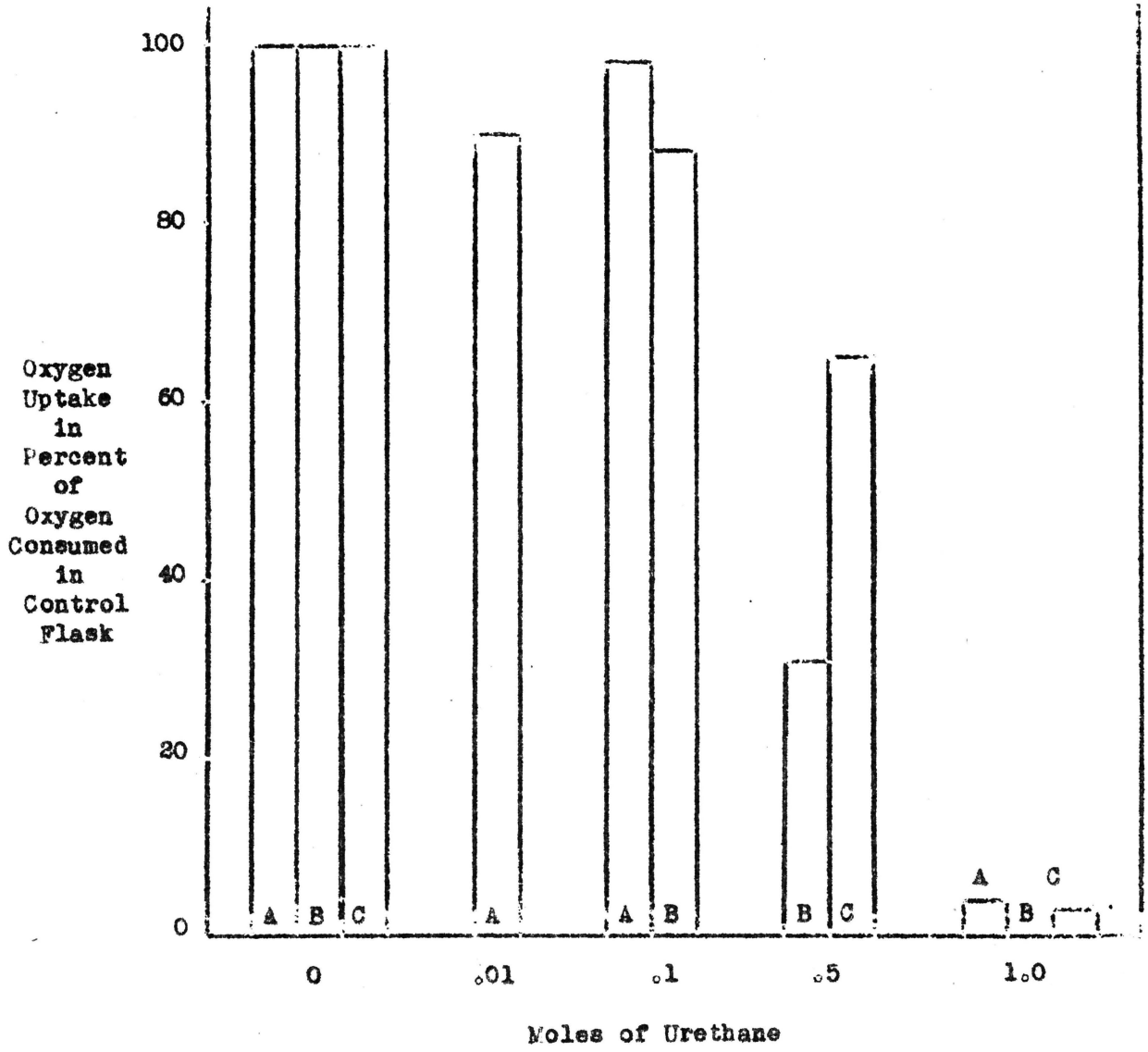


Figure 35. Percent oxygen consumption by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of URETHANE.

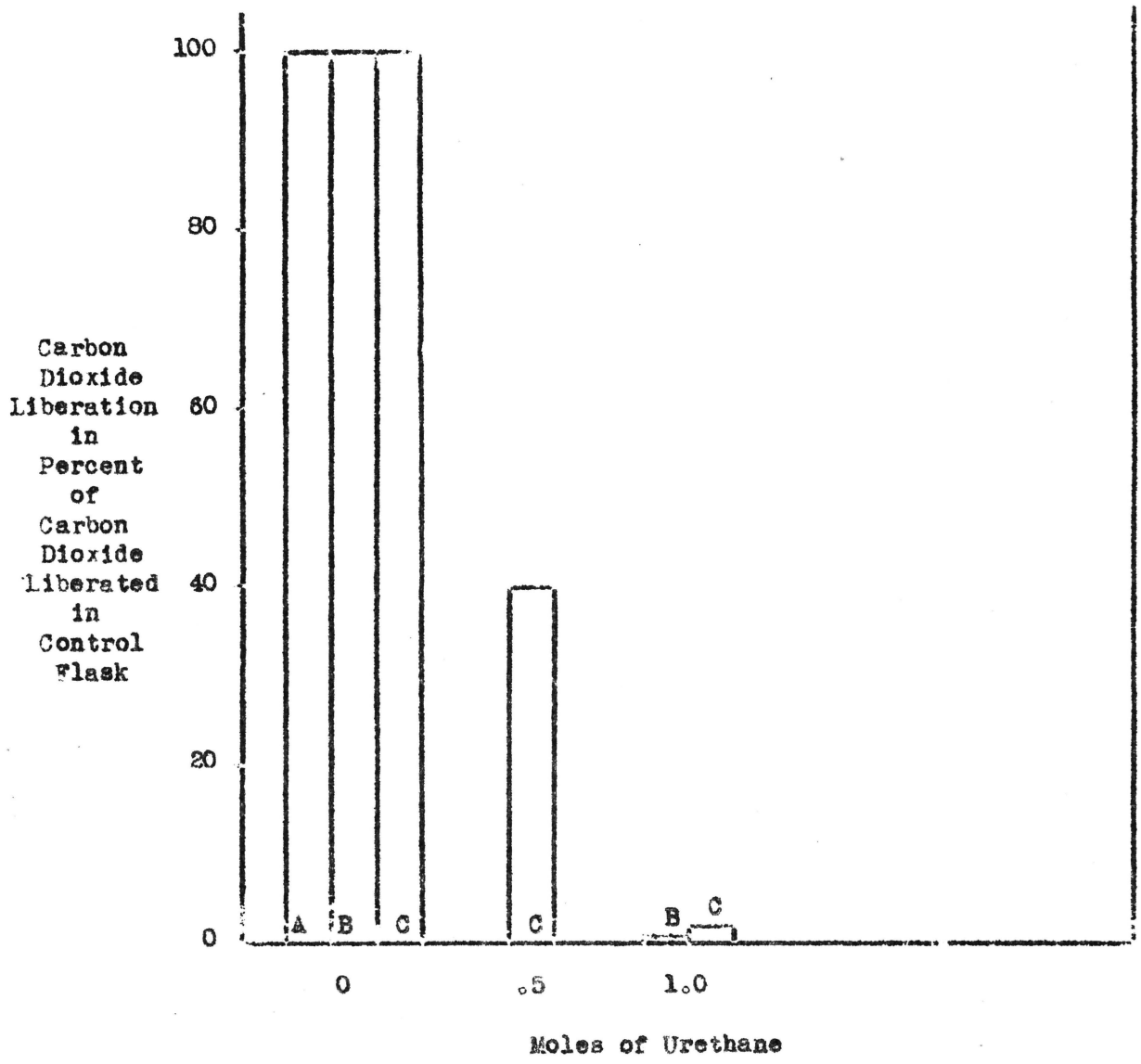


Figure 36. Percent carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of URETHANE.

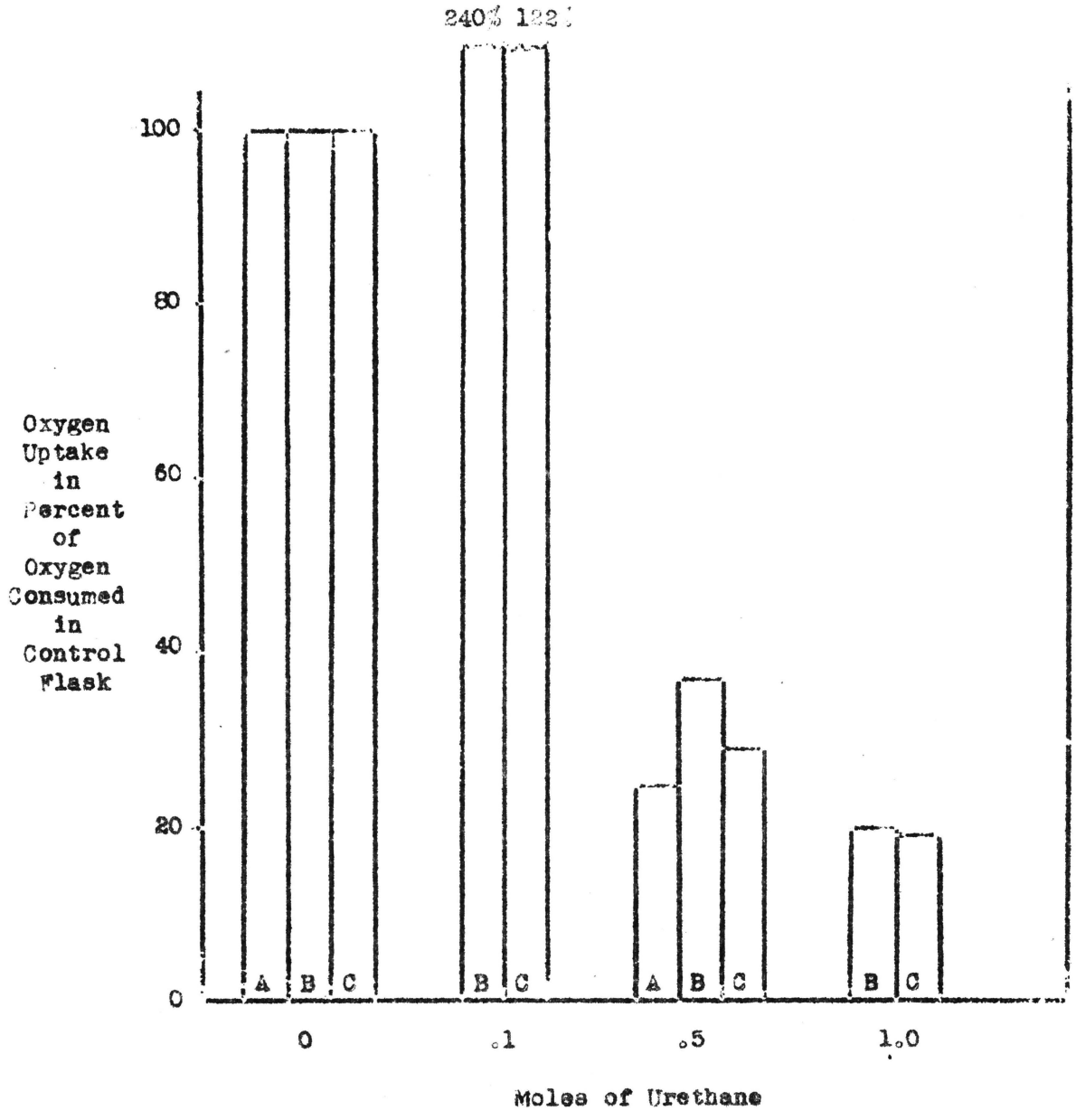


Figure 37. Percent oxygen consumption by Bacillus subtilis: 3.0 mg. cells on SUCCINATE in presence of URETHANE.

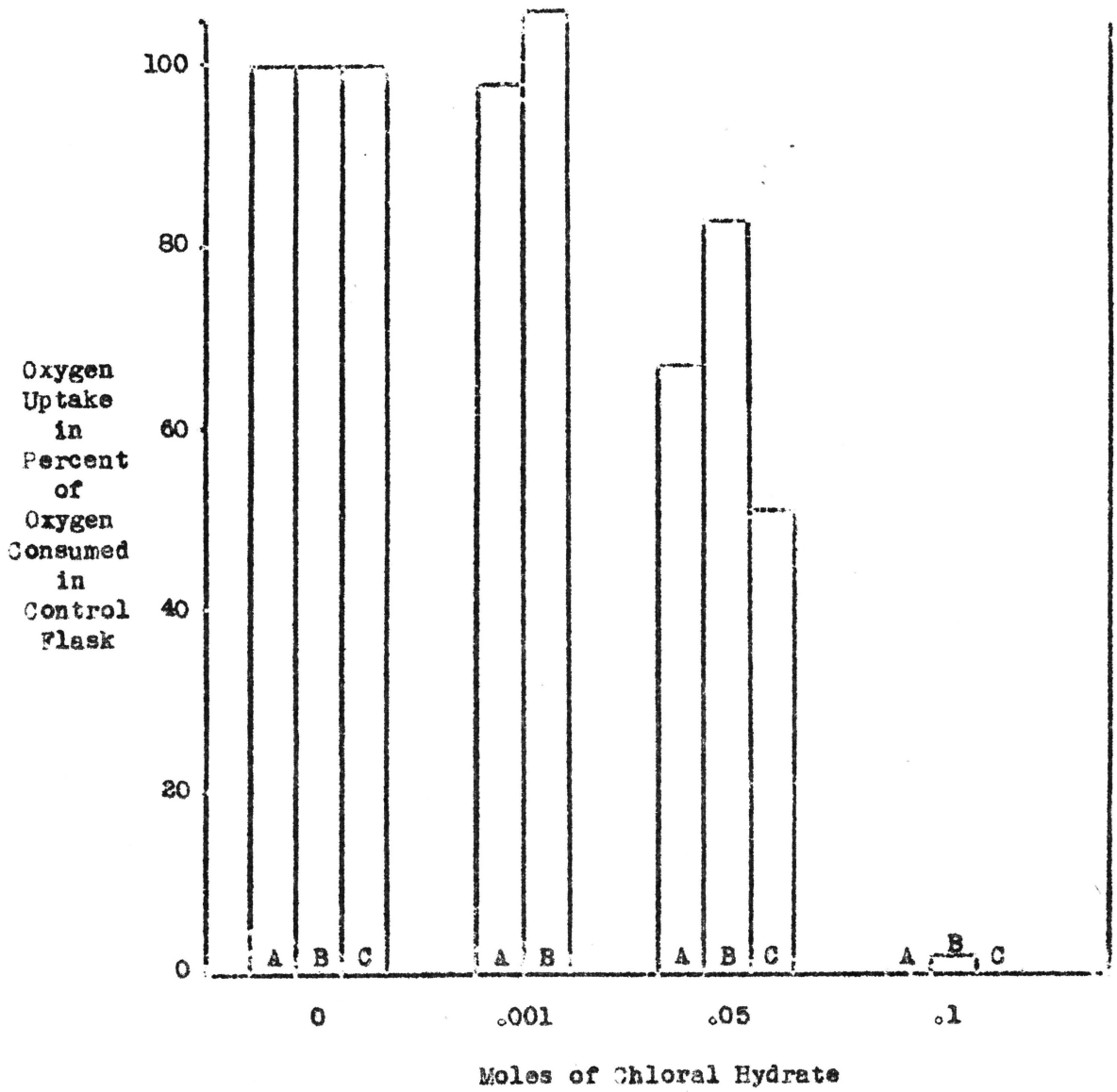


Figure 38. Percent oxygen consumed by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of CHLORAL HYDRATE.

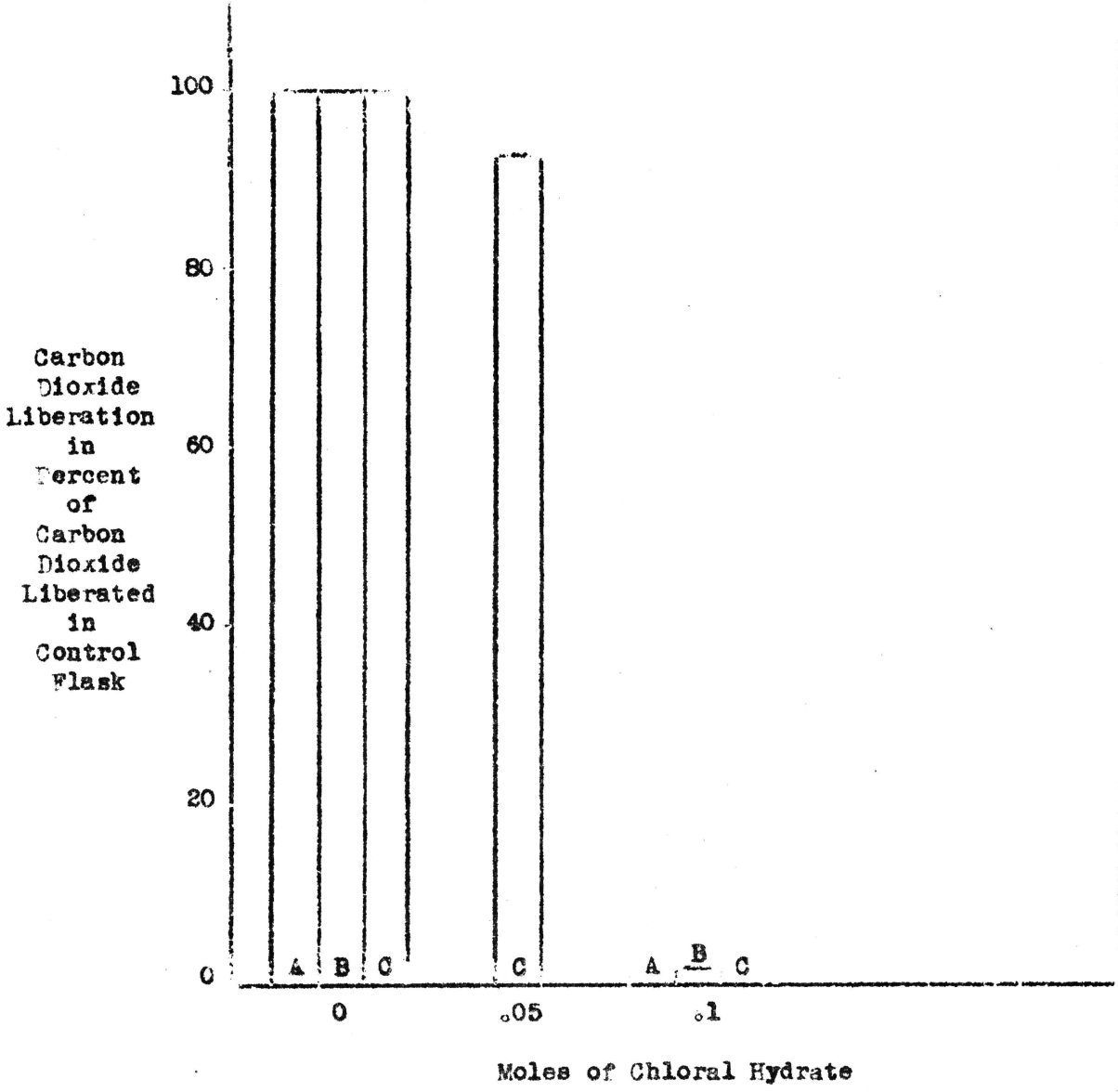


Figure 39. Percent carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of CHLORAL HyDRATE.

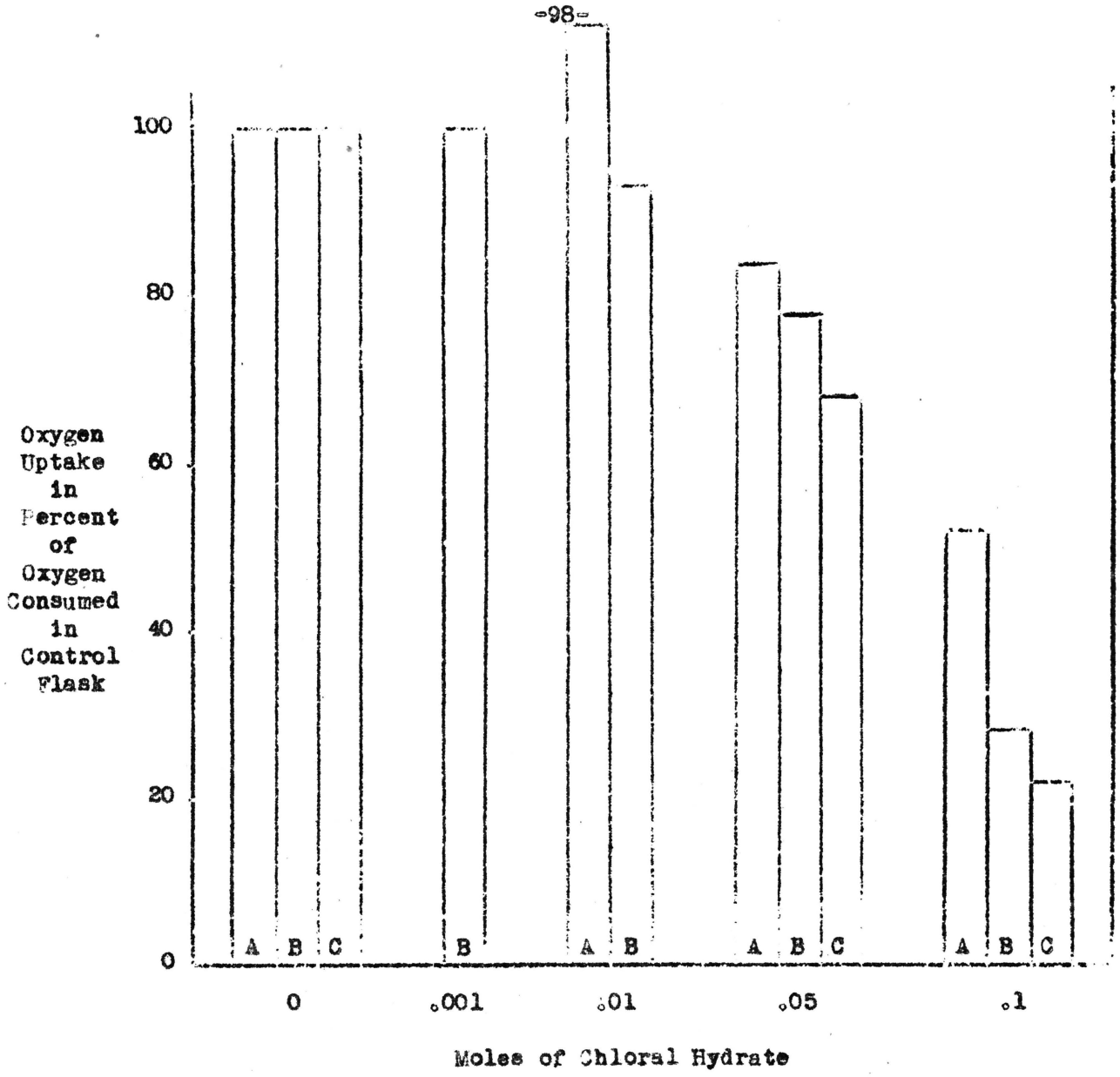


Figure 40. Percent oxygen consumption by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of CHLORAL HYDRATE.

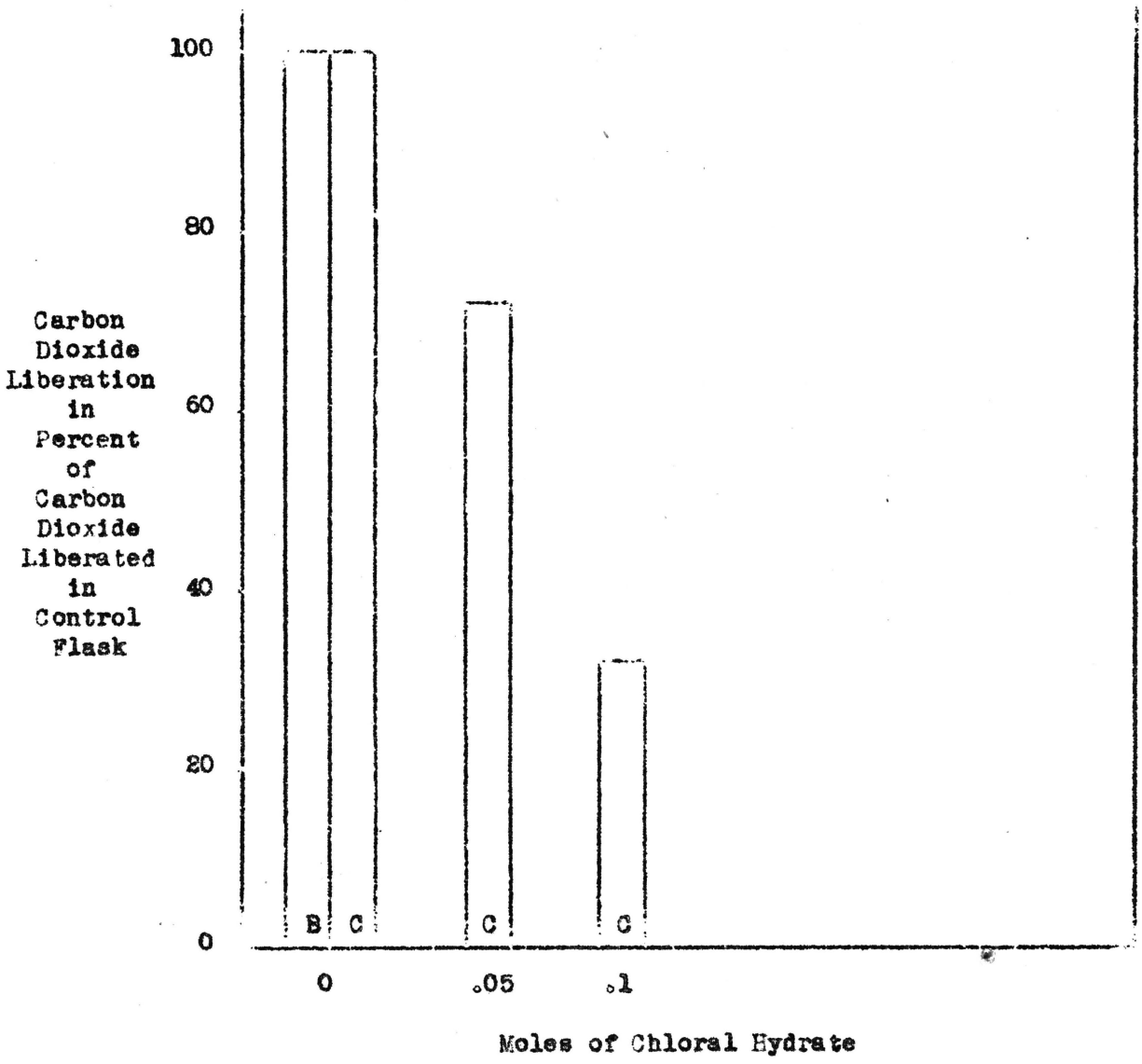


Figure 41. Percent carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of CHLORAL HYDRATE.

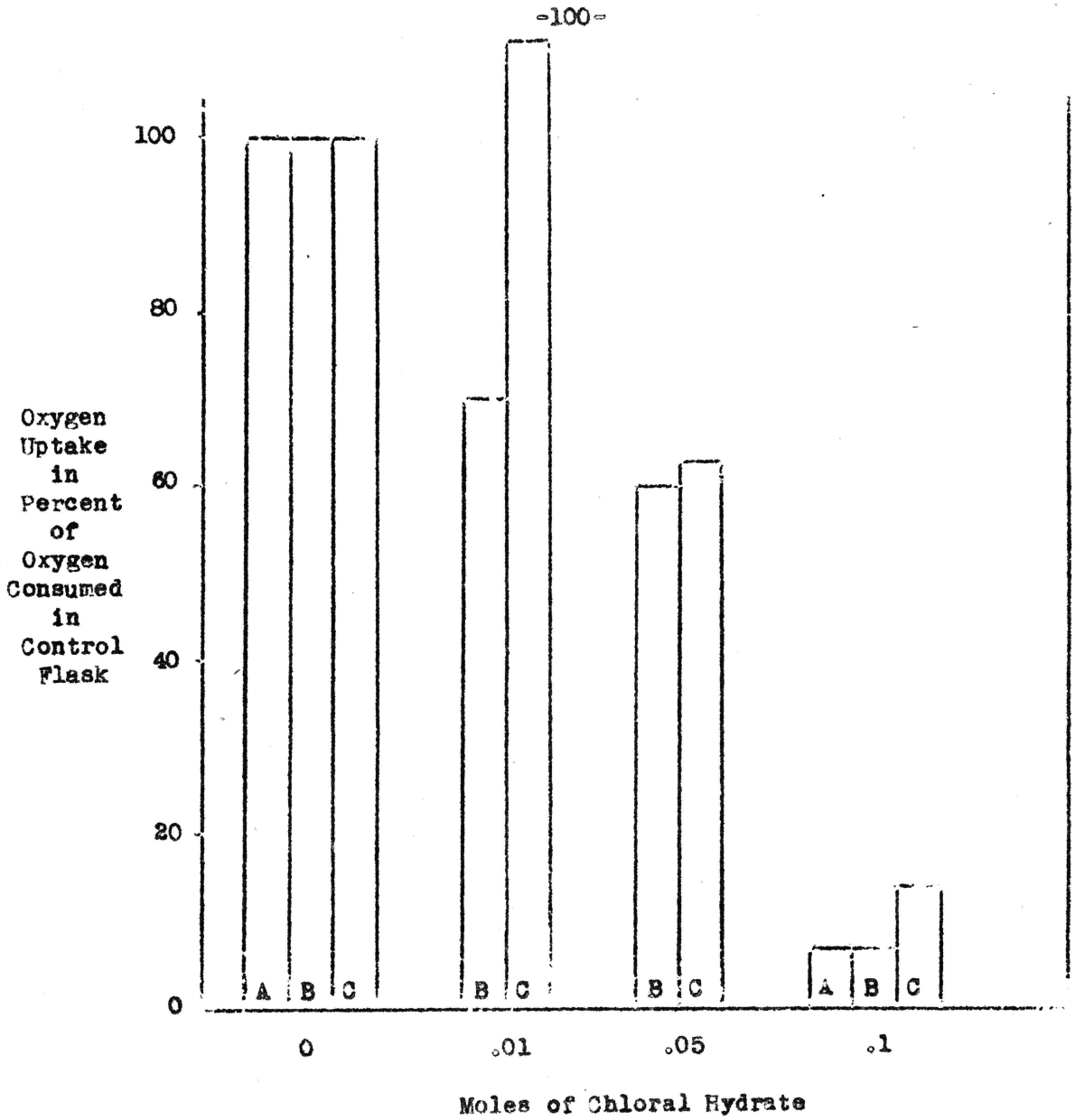


Figure 42. Percent oxygen consumption by Bacillus subtilis: 3.0 mg. cells on SUCCINATE in presence of CHLORAL HYDRATE.

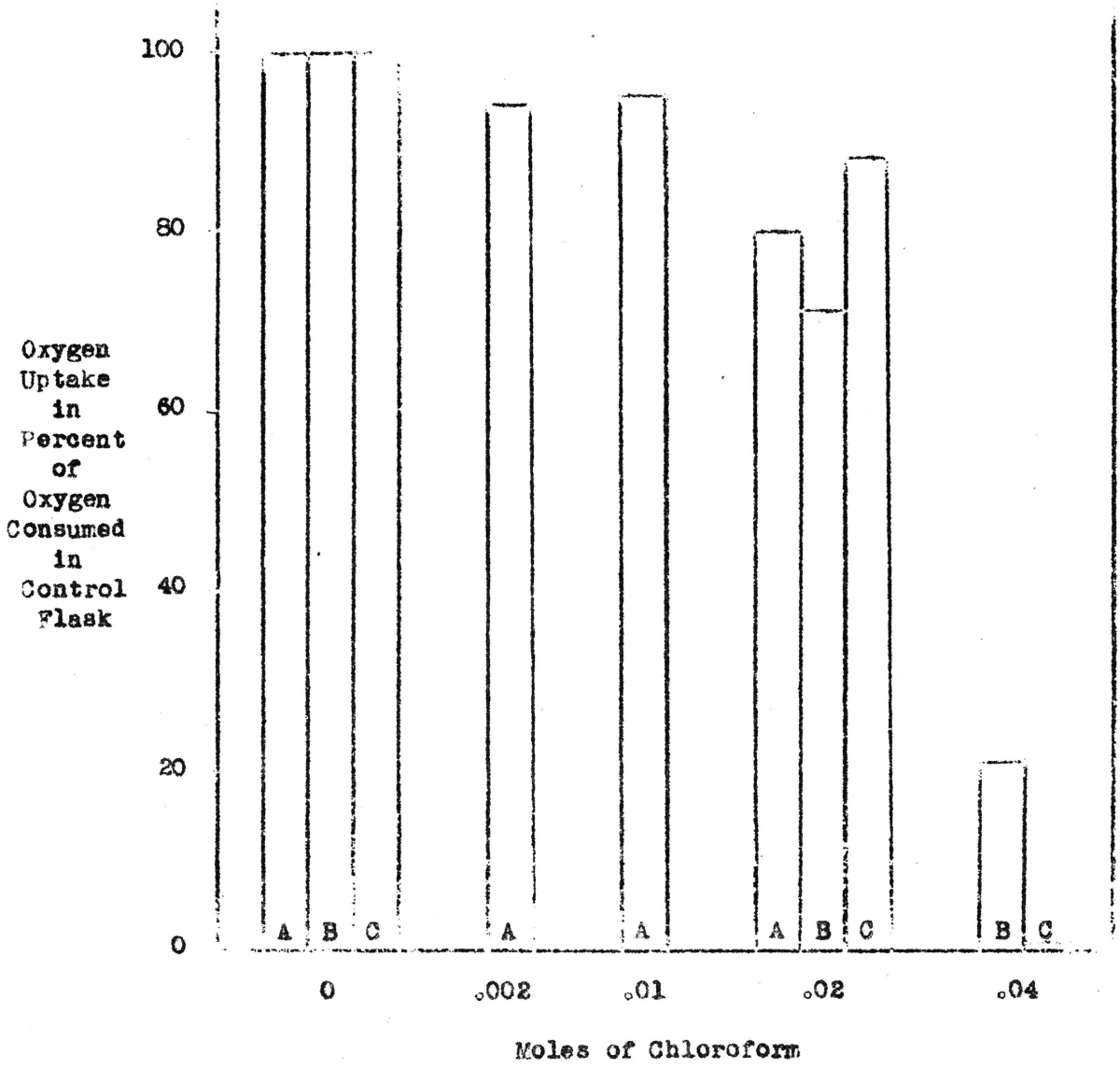


Figure 43. Percent oxygen uptake by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of CHLOROFORM.

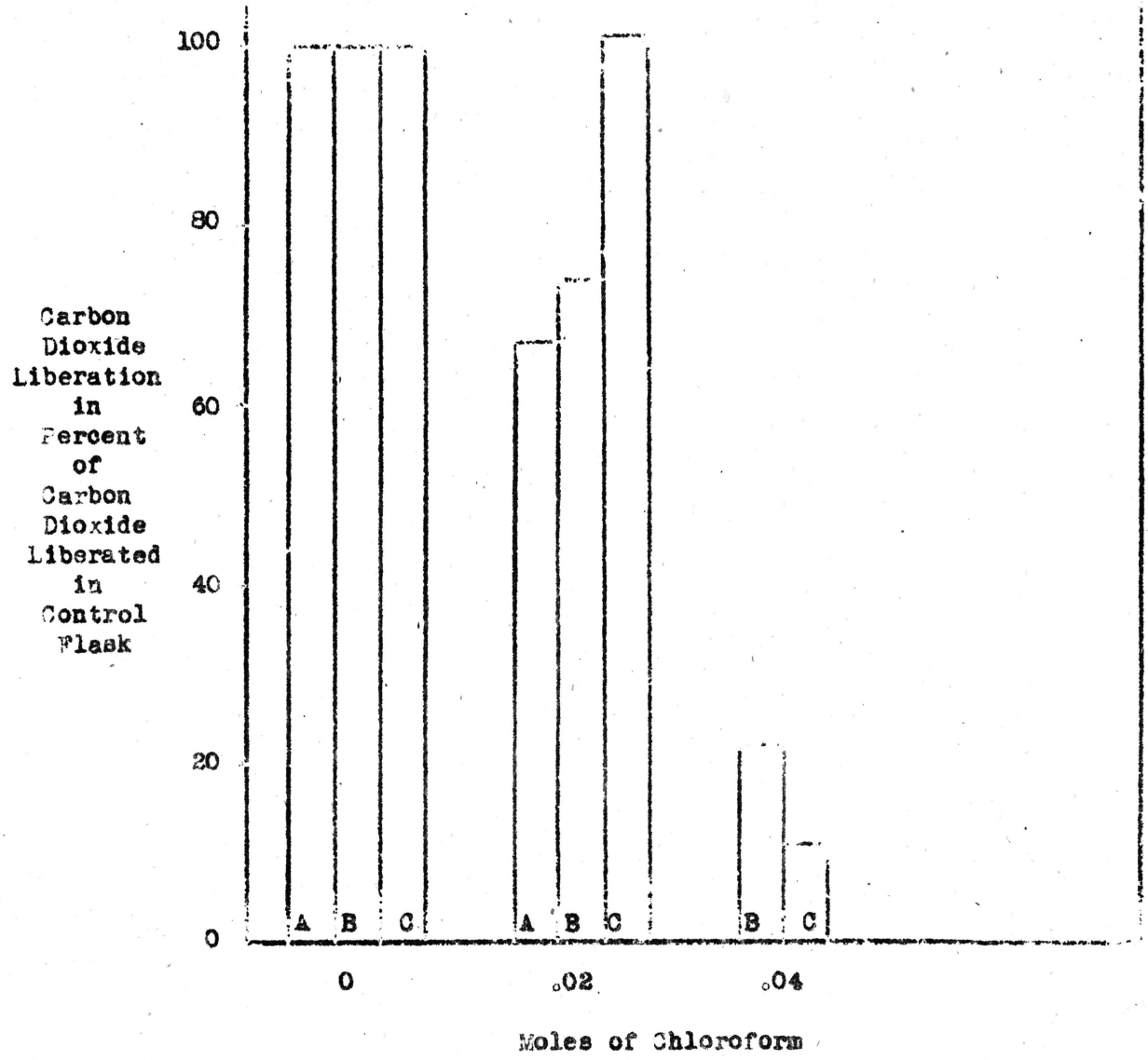


Figure 44. Percent carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of CHLOROFORM.

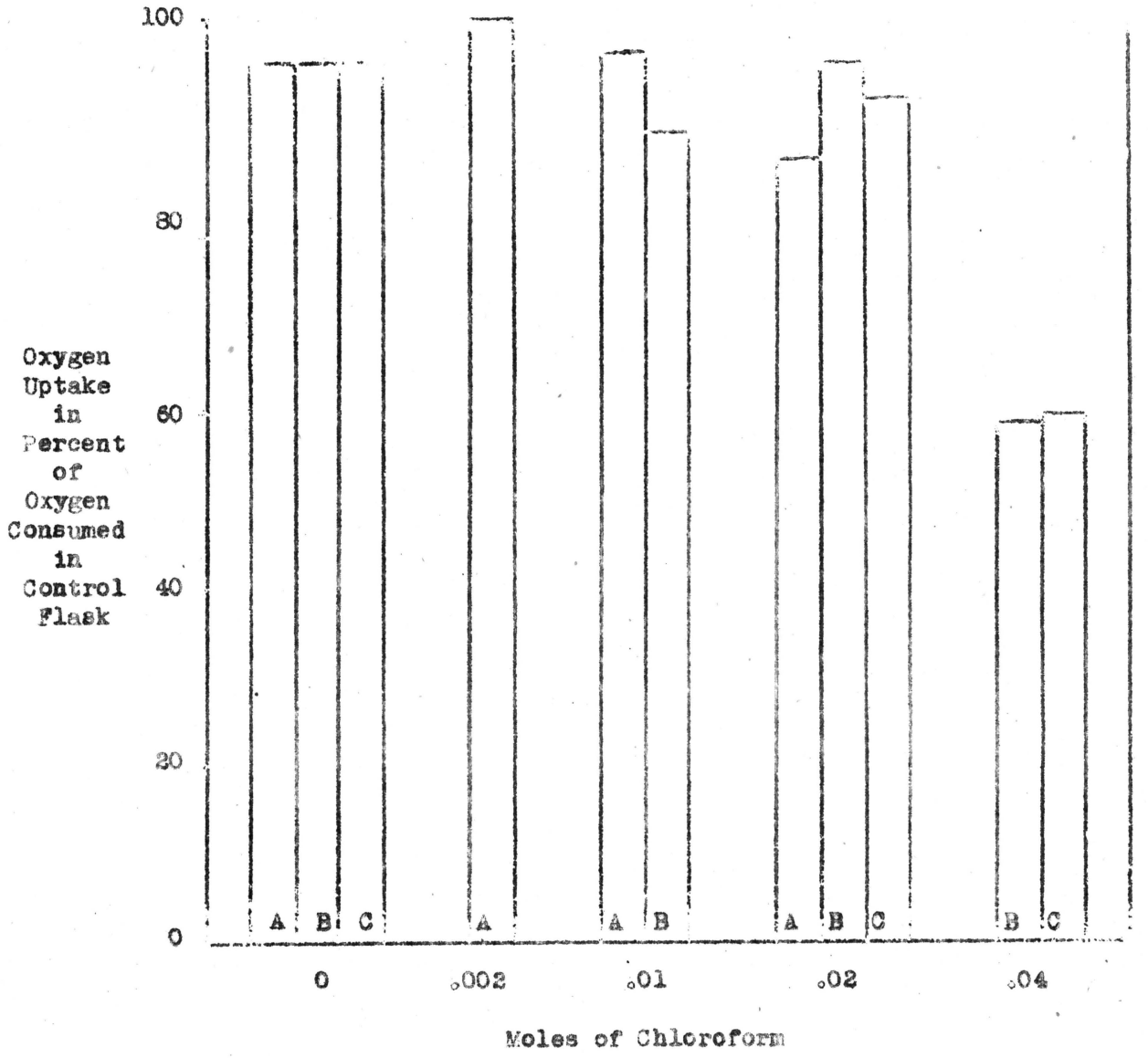


Figure 45. Percent oxygen consumption by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of CHLOROFORM.

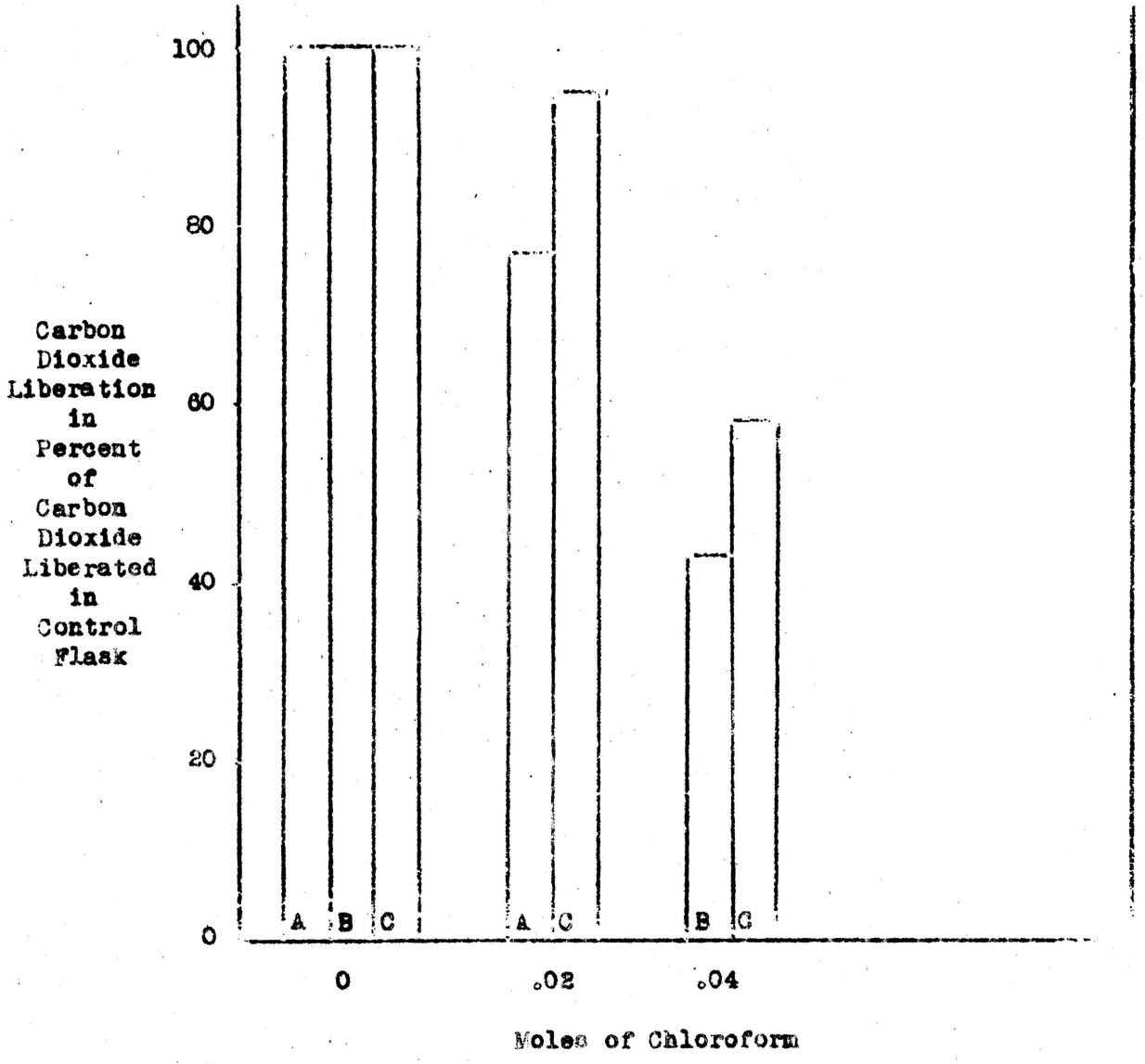


Figure 46. Percent carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of CHLOROFORM.

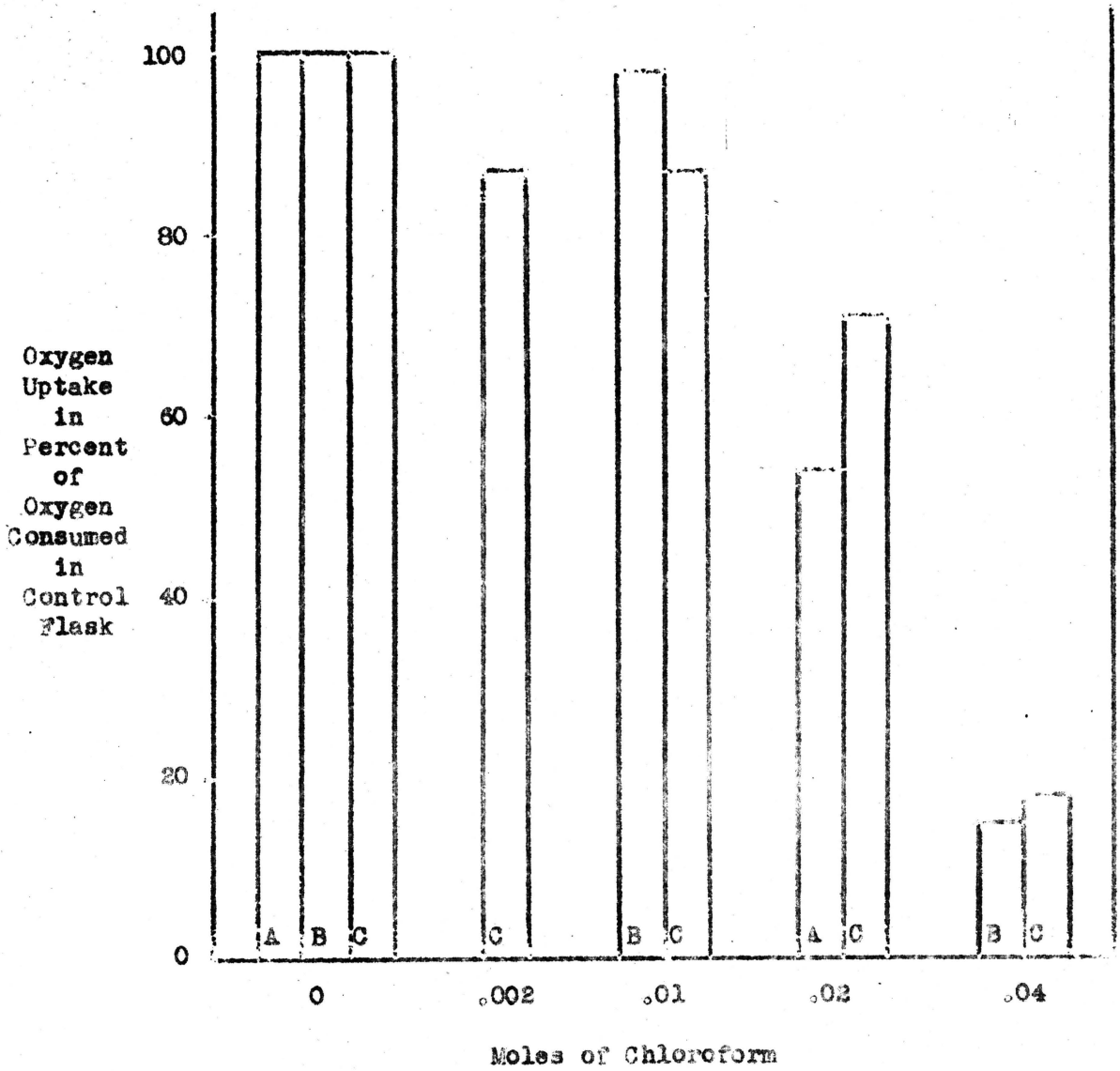


Figure 47. Percent oxygen consumption by Bacillus subtilis 3.0 mg. cells on SUCCINATE in presence of CHLOROFORM.

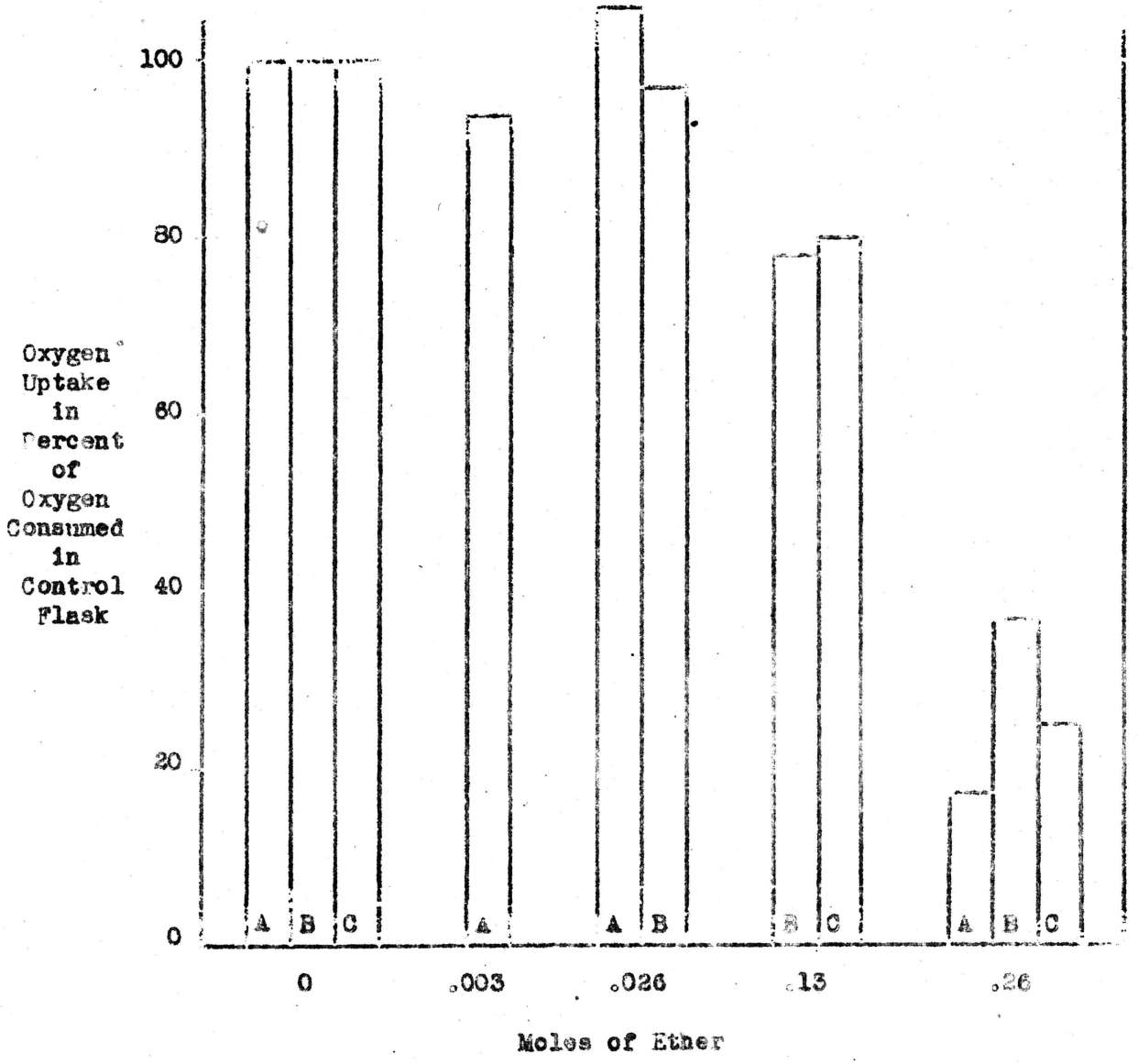


Figure 48. Percent oxygen consumption by Bacillus subtilis: 1.5 mg cells on Glucose in presence of Ether.

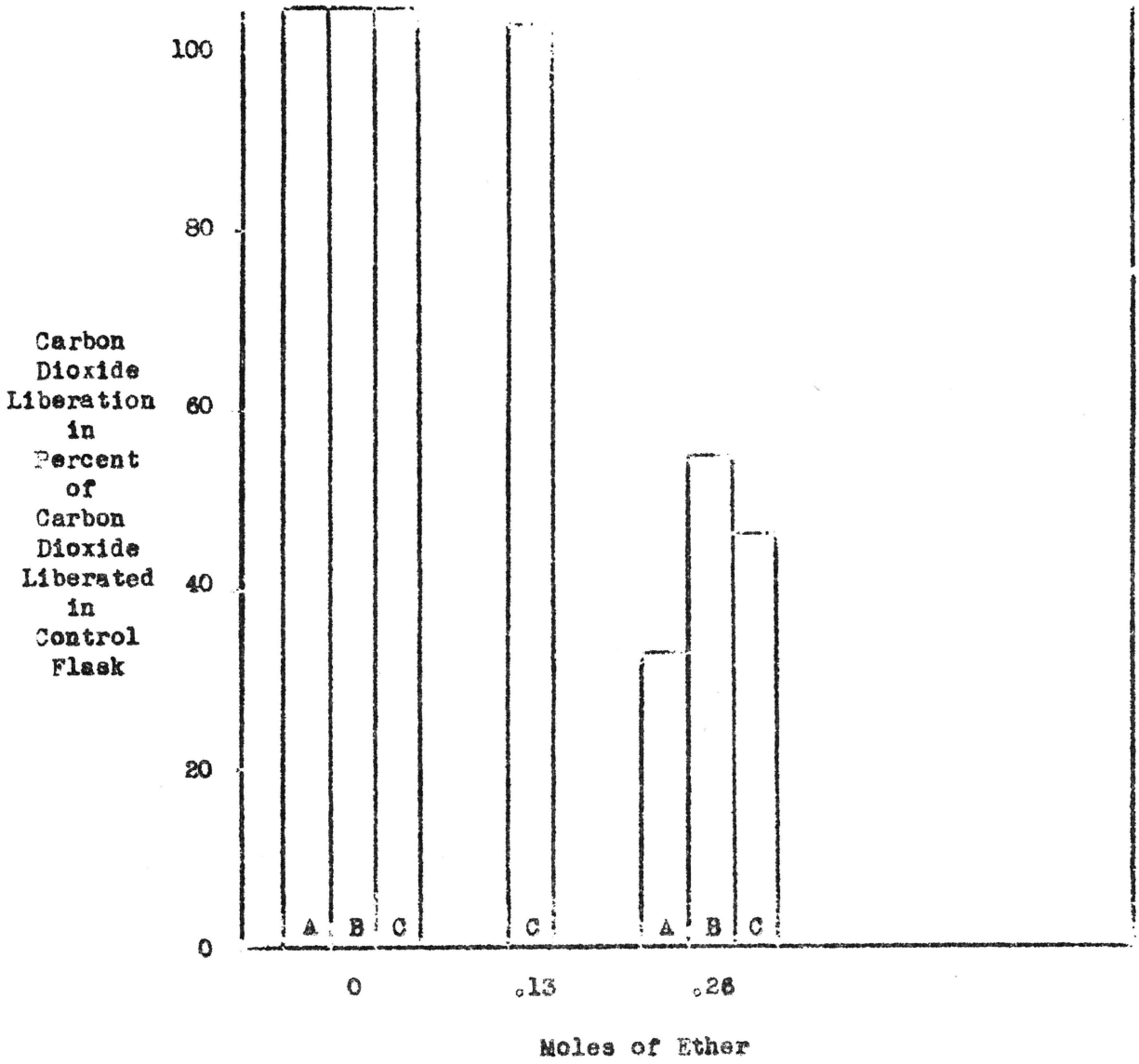


Figure 49. Percent carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on GLUCOSE in presence of ETHER.

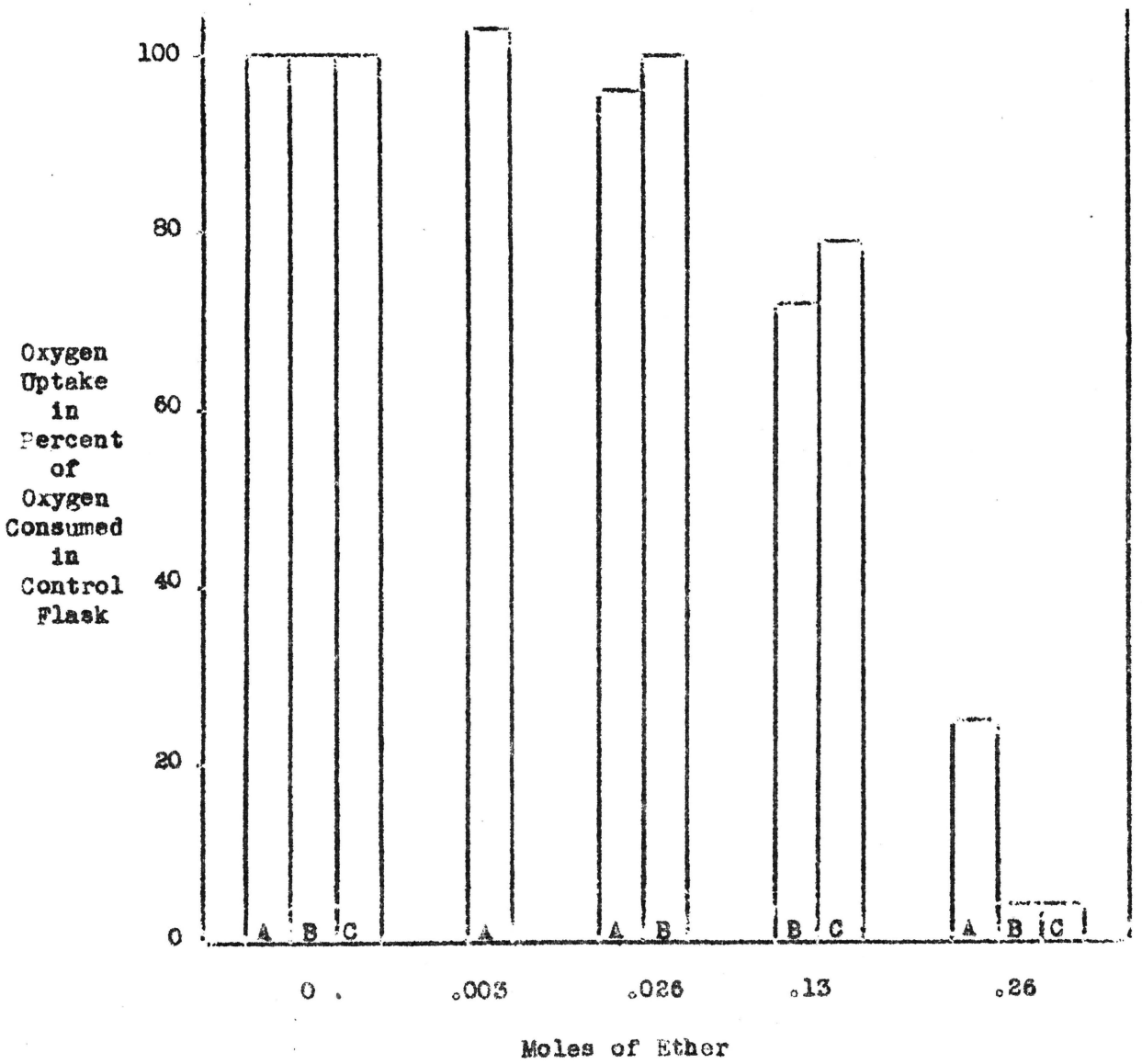


Figure 50. Percent oxygen consumption by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of ETHER.

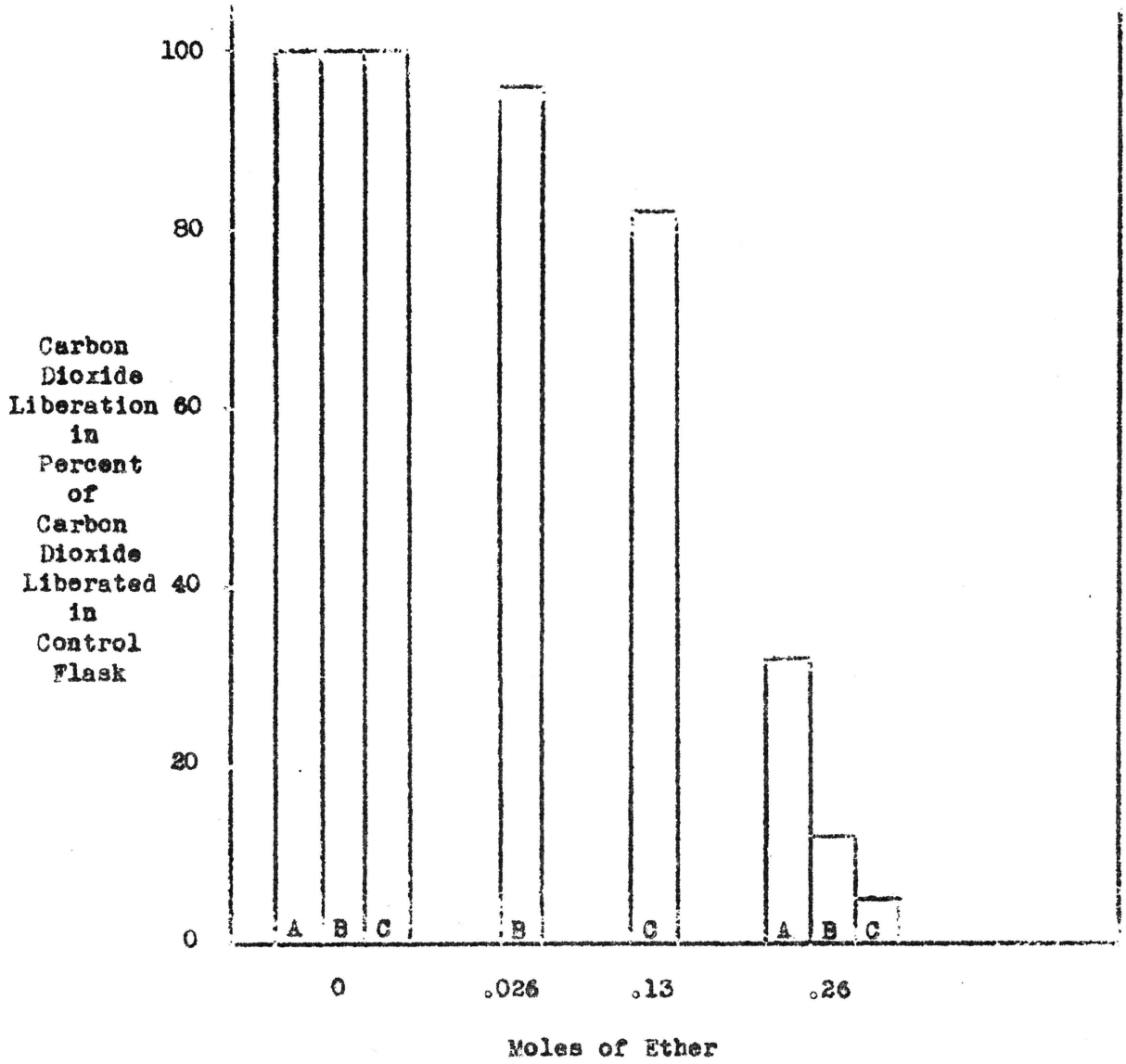


Figure 51. Percent carbon dioxide liberation by Bacillus subtilis: 1.5 mg. cells on FORMATE in presence of ETHER.

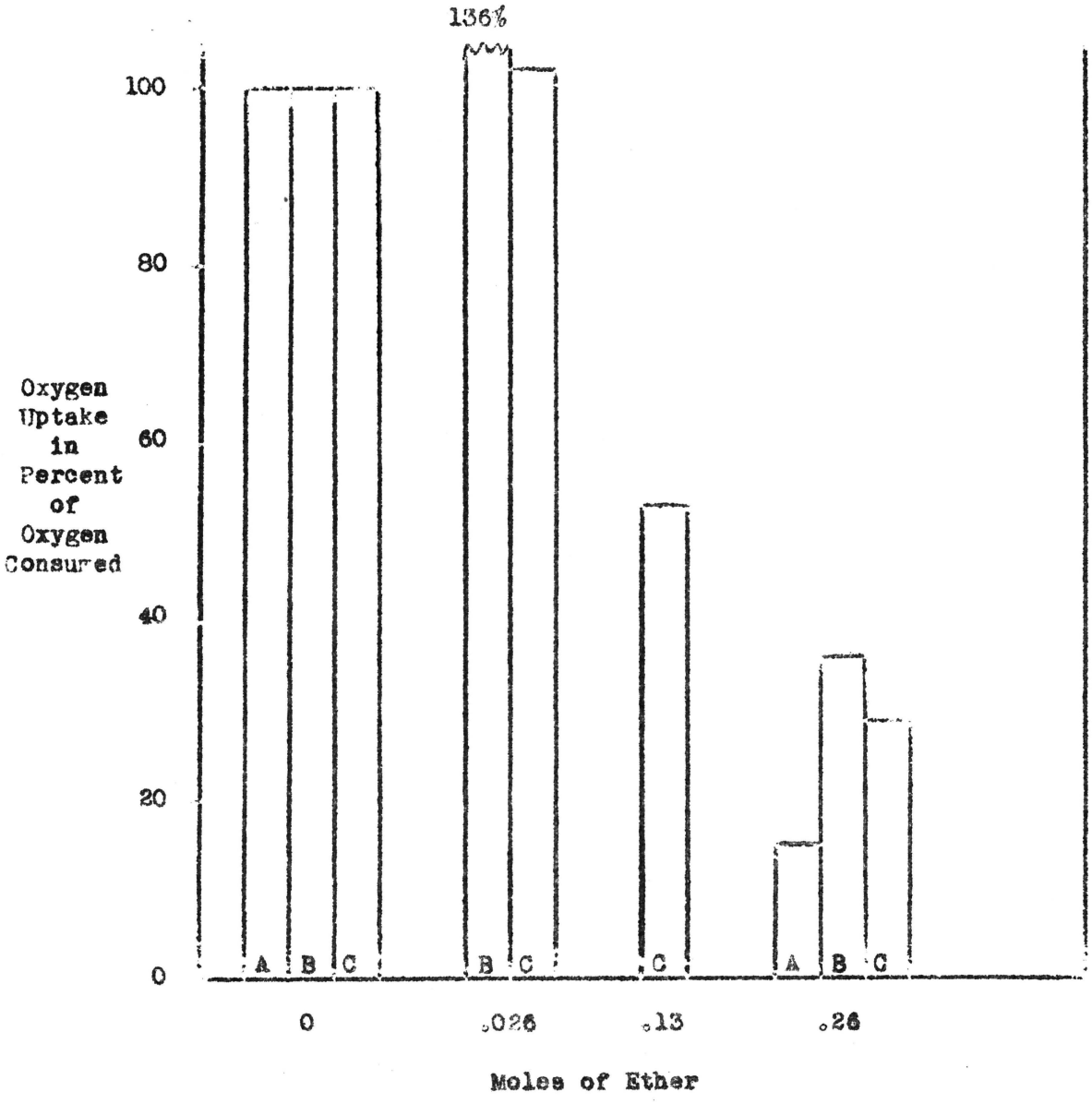


Figure 52. Percent oxygen consumption by Bacillus subtilis: 3.0 mg. cells on SUCCINATE in presence of ETHER.

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DISCUSSION

Some of the effects of three anesthetic agents and two known inhibitory agents of cellular activity on the respiration of a typical aerobic, spore-forming bacterium have been investigated by means of the Warburg respirometer. In order to do this it was first necessary to calibrate the Warburg apparatus and become familiar with the technic involved in the operation of this instrument. The more important points will be discussed with a view toward making comparisons between activity of bacteria respiring in the presence of anesthetic and inhibitory agents, and the activity of certain mammalian brain tissues respiring under the influence of similar agents. Such a comparison will lead to the formulation of an opinion regarding the suitability of the use of bacteria as tools for the study of the site of action of anesthetic agents.

For purposes of discussion and comparison, gaseous exchange values which are 50% or lower than the values for the control (no inhibitor) are arbitrarily taken as denoting significant inhibition of the respiration of B. subtilis.

RESPIRATION ON GLUCOSE. The respiratory rate of non-proliferating B. subtilis was approximately constant over a rather wide range of glucose concentrations (see Table XIII). Oxygen uptake values for glucose concentrations between 0.1M and 0.0001M were the same within experimental error but above a concentration of 0.1M there was a decrease in the amount of oxygen utilized. Values for concentrations of glucose below 0.001M were not determined. On the basis of these

findings, 0.01M glucose was used in all experiments where glucose was involved as the substrate.

In the presence of glucose alone, the volume of carbon dioxide liberated by B. subtilis was only slightly more than the volumes of oxygen consumed (see Table XVI). This indicates that the respiration of B. subtilis on glucose is nearly but not completely aerobic. This finding is in agreement with description of B. subtilis found in Bergey's Manual of Determinative Bacteriology (4).

RESPIRATION ON FORMATE. The respiration of B. subtilis on sodium formate was approximately constant between formate concentrations of 1.0M to 0.05M (Table XIV). Above a concentration of 1.0M and below a concentration of 0.05M there was a decrease in the oxygen uptake. On the basis of data shown in Table XIV 0.1M formate was employed in all experiments in which formate was the substrate. The rate of oxygen uptake by non-proliferating cells of B. subtilis in the presence of 0.1M formate was roughly the same as the rate of oxygen consumption in the presence of 0.01M glucose. This finding is evident from a comparison of Table XIII and Table XIV.

Gaseous exchange values for B. subtilis respiring in the presence of 0.1M formate show that the ratio of carbon dioxide released to oxygen consumed approaches 2 (CO_2/O_2 2). This finding is in accord with the theoretical equations for the oxidation of formate to carbon dioxide (22,28).

$$HCOOH + \begin{matrix}\text{Oxidized}\\ \text{Hydrogen}\\ \text{Acceptor}\end{matrix} \longrightarrow CO_2 + \begin{matrix}\text{Reduced}\\ \text{Hydrogen}\\ \text{Acceptor}\end{matrix}$$

and when the hydrogen acceptor is molecular oxygen reacting through the cytochrome system:

$$2\ HCOOH + O_2 \xrightarrow[\text{system}]{\text{cytochrome}} 2\ CO_2 + 2\ H_2O$$

RESPIRATION ON SUCCINATE. In general the rate of respiration of B. subtilis on succinate was considerably lower than on glucose or formate. This relatively poor ability of succinate to donate hydrogen can be observed in Table XV. It is to be remembered that 3.0 milligrams dry weight of cells were used whenever succinate was the substrate under investigation. The relationship between succinate concentrations and the amount of oxygen consumed can also be seen in Table XV. Of the concentrations studied, the highest rate of respiration was secured with a 0.2M succinate concentration. Concentrations above this were not determined since working with concentrations above 0.2M would entail handling of near saturated solutions.

The enzyme succinic dehydrogenase aids in converting succinate into fumarate according to the equation (21,27):

$$\underset{\text{succinate}}{HOOC\text{-}CH_2\text{-}CH_2\text{-}COOH} \xrightarrow[\text{dehydrogenase}]{\text{succinic}} \underset{\text{fumarate}}{HOOC\text{-}CH{=}CH\text{-}COOH} + 2H$$

In the presence of molecular oxygen the enzyme functions by way of the cytochrome system (22,28), thus:

$$2H + 2 \text{ Oxidized Cytochrome} \longrightarrow 2 \text{ Reduced Cytochrome}$$

and:

$$2 \text{ Reduced Cytochrome} + \tfrac{1}{2}O_2 \longrightarrow 2 \text{ Oxidized Cytochrome} + H_2O$$

Since there is no cleavage of a carbon-to-carbon bond by the aerobic succinic dehydrogenase system, there is no carbon dioxide liberated in this oxidative step. No attempts were made to determine carbon dioxide production when succinate was employed as substrate.

POTASSIUM CYANIDE. The effect of potassium cyanide on the respiration of B. subtilis in the presence of various substrates was studied in this investigation so that a consideration of the results could be made on a

basis of comparison with a much studied inhibitor of cellular activity. Cyanide is known to inhibit the metabolic activity of aerobic forms of life (6,22,28) and is thought to act specifically on the portion of the cytochrome system known as cytochrome oxidase.

Cyanide possessed the ability to lower the respiration rate of B. subtilis in the presence of glucose, formate and succinate (Figures 28,29,30,31 and 32). There was some variation as to the concentration of cyanide required for 50% inhibition depending on the substrate. When glucose or succinate was the substrate 0.01M cyanide brought about a significant inhibition of both the oxygen uptake and the carbon dioxide liberation (Figures 28,29 and 32). With formate as substrate less cyanide appeared to be required for inhibition as a 0.0001 concentration was effective. (Figures 30 and 31).

Since cytochrome oxidase plays a role in the aerobic oxidation of glucose, formate and succinate by bacteria and as this enzyme is inactivated by cyanide, the inhibition of B. subtilis respiration by cyanide is quite logical. Quastel and Woolridge showed that the oxidation of glucose, formate and succinate by Escherichia coli were retarded by 0.01% (0.1M) cyanide (22).

URETHANE. Urethane, like cyanide, has been studied quite extensively with regard to its effect on respiratory enzyme systems (6). Urethane is believed to interfere with enzymatic processes responsible for activating the hydrogen of various substrates so that the hydrogen atoms may be transfered to a suitable hydrogen acceptor (6).

Figures 33 and 34 show that urethane present to the extent of 1.0M hinders the oxidation of glucose by B. subtilis. The oxidation of formate is also significantly inhibited by 1.0M urethane and possibly

by 0.5M urethane (Figures 35 and 36). The oxygen uptake exhibited by B. subtilis respiring on succinate is quite appreciably lowered by the presence of 0.5M urethane (Figures 37).

The inhibitions obtained with urethane in the present investigation appear to be in line with previous reports (6) concerning the effect of urethane on enzymatic activity.

CHLORAL HYDRATE. The action of chloral hydrate on the respiratory activity of B. subtilis is shown in Figures 38,39,40,41 and 42. It is evident from these bar diagrams that 0.1M chloral hydrate has a decided inhibitory effect on the gaseous exchange of B. subtilis when the substrate is glucose, formate or succinate.

The inhibition of succinate oxidation by 0.1M chloral hydrate is of special interest. Quastel and Wheatley (24) have demonstrated that 0.12% (0.007M) chloral hydrate has a selective action on the oxidations by mammalian brain tissue depending upon the substrate oxidized. These workers have presented evidence that chloral hydrate inhibits the oxidation of glucose by brain tissue but not the oxidation of succinate. It is on the basis of specific effects such as the one mentioned above that Quastel first postulated that there are two possible sites of action for narcotics.

In view of the fact that the exact components of the enzyme system involved in the oxidation of succinate have not been fully established (22,28), it appears possible that the succinic dehydrogenase system of B. subtilis differs from the succinic dehydrogenase system of rat and guinea pig brain tissue or that brain tissue can use an alternate mechanism. Such a difference might account for the discrepancy

in the effect of chloral hydrate on the two systems.

It is also to be noted that when glucose is the substrate, 0.1M chloral hydrate is required to depress significantly the respiration of B. subtilis but that only 0.007M chloral hydrate is necessary for inhibition when brain tissue is involved. It may be that chloral hydrate possesses a different mode of action when bringing about its effects on bacteria that it has when it acts against the normal functioning of brain tissue.

CHLOROFORM. The effect of various concentrations of chloroform on the respiration of B. subtilis are presented in Figures 43,44,45,46 and 47. Chloroform significantly lowered the rate of glucose and succinate oxidation by B. subtilis when the chloroform was present in 0.04M quantities. The amount of respiration with formate as substrate and 0.04M chloroform as inhibitor was approximately 55% of the respiration of the control (no chloroform).

The action of chloroform, like that of chloral hydrate, deserves special mention. Grieg (11) found that 0.24M chloroform did not decrease the oxygen consumption exhibited by homogenized brain tissue in the presence of succinate. Here again the effect of a narcotic (chloroform), with succinate as substrate, appears to depend upon the type of cell bringing about the oxidation of succinate. The possible explanations for the phenomenon as presented on page 116 would also apply to the discrepancy in the action of chloroform.

ETHER. Ether also exhibited an ability to retard significantly B. subtilis respiration in the presence of glucose, formate and succinate

(Figures 48,49,50,51 and 52). Ether in a concentration of 0.26M lowered the respiratory activity in the cases of the three substrates investigated.

CHLORETONE. Experiments employing chloretone gave results quite different from those encountered in the cases of the other inhibitory agents.

The respirometers were set up in the usual manner. Under normal conditions when oxygen is consumed by the cells within the reaction flask the level of the manometric fluid in the right-hand limb of the manometer will rise. However, it was observed that when 0.015M chloretone was present, the level of the manometric fluid in the right-hand limb moved oppositely to the direction expected. Typical changes in manometric fluid levels illustrating this abnormal shift of manometric fluid are listed in Table XXXI. If the over-all change in the gas pressure had been due to an absorption process (as with oxygen consumption) the values listed for the flask with KOH would have been negative rather than positive.

In an effort to investigate further this abnormal effect of chloretone, experiments were designed with only organisms and chloretone present in the reaction flasks. Typical changes in manometric fluid levels for these experimental conditions are given in Table XXXII. These changes in levels of manometric fluid inidcate that some type of gaseous exchange takes place when chloretone and B. subtilis are in contact. If the organism is able to oxidize chloretone aerobically one would expect that oxygen would be consumed and in this event the

changes listed for the flask with KOH should again be negative. As the changes are positive it is improbably that B. subtilis oxidizes chloretone by an aerobic mechanism.

The positive changes cannot be due to carbon dioxide liberations as any carbon dioxide given off should be absorbed by the KOH in the alkali cup and give rise to no change at all in the level of the manometric fluid. Furthermore, if carbon dioxide liberation had taken place, greater changes would be expected in the cases of the manometric fluid of the flask containing no KOH in the alkali cup.

If gases other than carbon dioxide, such as hydrogen or chlorine, were liberated, they too would be expected to produce large changes in the level of the manometric fluid of the flask with no KOH present in the alkali well.

At present it appears that some type of gaseous exchange takes place between chloretone and the cells of B. subtilis but the exact nature of this reaction has not been explained.

CONCLUSIONS

1. The respiration rates of non-proliferating Bacillus subtilis Koch-Novy on glucose, formate and succinate are of such magnitude that they may be studied conveniently with the Warburg respirometer.

2. The optimum glucose concentration for oxygen consumption by non-proliferating Bacillus subtilis Koch-Novy is approximately 0.01M. The optimum concentration for formate or succinate is 0.1M.

3. The rate of oxygen consumption by non-proliferating Bacillus subtilis Koch-Novy is of the same order of magnitude whether the substrate is glucose of formate. When the substrate is succinate the rate of oxygen consumption is roughly one-half of the rate on glucose of formate.

4. The enzyme system utilized by Bacillus subtilis Koch-Novy in the oxidation of formate is sensitive to cyanide and urethane. This sensitivity indicates that the oxidation of formate by aerobic, spore-forming bacteria takes place in a manner similar to aerobic oxidation of formate by Escherichia coli.

5. The oxidation of glucose, formate and succinate by non-proliferating Bacillus subtilis Koch-Novy is inhibited by the presence of anesthetic agents such as chloral hydrate, chloroform, and ether. The concentrations of these agents required for significant inhibition is about 0.3 to 0.05 molar. It appears that the concentrations of anesthetic agents required to inhibit oxidations by aerobic bacteria are somewhat higher that the concentrations (about 0.001M) of narcotics required to produce narcosis in animals. This fact should be

considered in investigations designed to study the action of narcotics or anesthetics through effects on aerobic bacterial respiration.

6. The enzyme system involved in the oxidation of succinate by Bacillus subtilis Koch-Novy differs from the succinic dehydrogenase system found in mammalian brain tissue. A comparison of the results of the present investigation with the findings of Quastel et al (23,24) indicates that the enzymatic oxidation of succinate by aerobic spore-forming bacteria is inhibited by anesthetic agents whereas the analogous oxidative system in the mammalian brain is not.

7. A buffered, non-proliferating cell suspension of Bacillus subtilis Koch-Novy in contact with a dilute solution of chloretone gives rise to a gaseous exchange. The exact nature of this reaction is not understood.

SUMMARY

The non-proliferating or "resting" cell technic and the Warburg respirometer have benn employed to study the respiration of Bacillus subtilis Koch-Novy with glucose, formate and succinate as substrates.

The enzymatic oxidation of these substrates in the presence of cyanide, urethane, chloral hydrate, chloroform and ether have been investigated and the effects of these agents over a range of concentrations have been noted. Graphs have been prepared for comparison of the oxygen consumption and carbon dioxide liberation values for Bacillus subtilis Koch-Novy in the presence and absence of the various inhibitory agents.

Differences between the oxidation of succinate by bacteria and by mammalian brain tissue have been pointed out. Information concerning the oxidations of brain tissue was obtained from the previous work of Quastel et al and Grieg (11,23,24).

Gaseous exchange which suggests some sort of metabolic action by Bacillus subtilis Koch-Novy on chloretone has been noted.

The author has expressed an opinion regarding the advisibility of employing members of the genus Bacillus for studies concerning the mode of action of anesthetic agents.

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