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SODIUM INFLUENCED RESPIRATION OF NORMAL AND ULTRAVIOLET IRRADIATED *ESCHERICHIA COLI*

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Sodium Influenced Respiration of Normal and Ultraviolet Irradiated *Escherichia coli*

INTRODUCTION

Cytological studies have indicated that external Na plays an important role in the nuclear organization of ultraviolet irradiated bacteria. Whitfield (1955) has shown an aggregation of chromatin following UV irradiation of *Shigella dysenteriae* cultured on basic LB media plus 170 meq. Na agar. With Na deficient LB agar fragmentation was observed instead of aggregation. Clumping followed by dispersion of nuclear bodies occurred in UV irradiated *Escherichia coli* cultured in nutrient broth containing 40 meq Na/l. (Cortelyou *et al*. 1955). Transient aggregation of chromatinic bodies followed by fragmentation in UV irradiated *E. coli* was also reported by Payne, *et al*. (1955).

With non-irradiated *Shigella dysenteriae* Whitfield (1955) demonstrated similar chromatin aggregation when cells were cultured on LB media + 1700 meq Na agar. Non-irradiated cells on LB + 170 meq. Na showed no clumping. Payne *et al*. demonstrated chromatin aggregation of non-irradiated *E. coli* cultured on nutrient agar + 680 meq. Na/l.

MacLeod and Snell (1948) and Clark and MacLeod (1954) have reported Na to be inhibitory in glycolysis and K can be a stimulus to overcome Na inhibition. This work, however, was done with Gram positive organisms. Rahn (1936) summarizing the work of many investigators and his own, stated that Gram negative bacteria can grow without potassium. The dependence of Gram positive organisms on K has been

demonstrated by many investigators reported in the review by Haynes, et al. (1954).

In accord with the evidence of differential stimulation and inhibition of K and Na on Gram positive and Gram negative organisms, Starck and Curtis (1936) reported that addition of 0.5% sodium formate to brilliant green bile tended to increase the rate of growth of Escherichia-Aerobacter organisms although it did not affect the Gram positive organisms responsible for false tests in this medium.

Stapleton (1952) and Kaplan, et al.(1953) have suggested that adaptive enzyme systems are injured by UV irradiation to a much greater degree than are the constitutive enzyme systems. Heimets, et al. (1954), consider that a large number of structural elements and metabolites are altered by irradiation. These investigators studied the effects of added metabolites of the tricarboxylic acid cycle and coenzymes on ultraviolet irradiated E. coli. Results, based on viable cell counts, although not decisive, indicate that several links in the cyclic processes are injured by irradiation.

Serazdarian, et al.(1954) found that diphosphopyridine nucleotide was inactivated by UV irradiation which caused the formation of four products: ADP, adenylic acid, adenine and nicotinamide.

The work presented here was carried out to determine the possibility of a protective effect of increasing Na in the media of irradiated E. coli and whether this is an ionic effect or a true Na effect. Because of the inactivation of pure coenzyme I by ultraviolet irradiation it seemed desirable to also study the effect of adding coenzyme I to irradiated E. coli and also of adding glyceraldehyde phosphate dehydrogenase and phosphoglyceric acid to the media. Correlations between these additions and the sodium variations were sought.

MATERIALS AND METHODS

Stock cultures of Escherichia coli strain AR were used for all experiments reported. Daily transfers were maintained in M-9 media (Anderson, 1946) and cells at 2 hrs. incubation were used for all studies. Population densities of 2 hr. cultures ranged from 3-7 X 10^7 cells/ml as were determined by the plate method.

To study the effect of Na and K ions on both normal and ultraviolet irradiated cells of E. coli, the composition of M-9 was modified in the following manner: based on preliminary respiratory measurements as a function of cell density in a 2 hr. culture the basal medium was established at 7 parts of solution I (inorganic salts) to 3 parts of solution II (4% glucose). This medium was then varied in Na level by omitting this ion, wherein buffer and osmotic balance were controlled by the addition of K_2HPO_4 or by adding NaCl and Na_2HPO_4 to yield final Na concentrations equal to 0, 93, 124, 139 and 186 meq./liter.

Since the ratio of Na:K is affected by variations in Na, studies were also made on variations in K concentration. Data on metabolic rate would then indicate specific ion effects or would represent a simple monovalent cation effect. Normal M-9 media contains 22 meq.K/liter and 93 meq. Na/l. Maintaining the Na level at 93 meq/l the K level was varied to yield final concentrations of 53 meq/l (total ionic concentration equal to that of medium containing 124 meq. Na/l), and 115 meq. K/l. (total ionic concentration equal to that of medium containing 186 meq. Na/l.). The relationship between cation concentrations and ratios used in this work is given in Table I.

Table I

Sodium and Potassium Concentrations

M-9 Media				M-9 Media		
Na/K	K Variations meq./l.		Na + K Ionic Conc.	Na Variations meq/l.		Na/K
	Na	K		Na	K	
4.2	93	22*	115	0	115	
			115	93	22*	4.2
1.79	93	53	146	124	22	5.69
			161	139	22	6.31
0.81	93	115	208	186	22	8.45
				LB Media		
			31.5	20.5	11	1.86
			173.5	162.5	11	14.77
			201.5	190.5	11**	17.32
			243.5	232.5	11	21.14

* Normal M-9 Media

** Normal LB Media

In addition to the use of M-9 media, a buffered tryptone, yeast extract, glucose medium was used (LB of Whitfield, 1955). This medium contains ca. 11 meq. K and 20.5 meq. Na/l. Sodium, as NaCl, was added in the following concentrations: 0, 142, 170 and 212 meq./l.

In all experiments reported here, cells of a 20 hour complete M-9 culture were washed and aliquots resuspended in media of various Na and K concentrations. These were subsequently incubated at 37°C for two hours.

To study the role of ions on metabolism of both normal and ultraviolet irradiated cells, 2 hr. cultures were divided into aliquots and one of each pair was treated. The ultraviolet source was a 4-watt, G.E. G4T4/1 germicidal lamp. All cultures were treated with 420 mw/ft^2

for 15 seconds yielding a total energy of 6.7×10^4 ergs/cm^2. Gentle agitation was maintained throughout the radiation period. M-9 media is transparent to ultraviolet radiations and thus irradiation was carried out in this medium with variations in ionic composition. When LB media was used, the cultures were washed in Na-free M-9 and irradiated in this medium. Subsequently, they were resuspended in LB media of the previous ionic composition.

Following irradiation, oxygen uptake was measured in Warburg reaction vessels at 37°C, using the Warburg direct method, absorbing CO_2 in 0.4 ml 10% KOH. In the main room of each vessel was placed 1.8 ml of the cell suspension and 1.0 ml of media of the corresponding ionic composition. In the studies with LB media 1.0 ml of the cell suspension and 1.8 ml of media were used.

Results indicating increased oxygen uptake at particular salt concentrations suggested ultraviolet inactivation of at least some of the intermediates in glycolysis. Manometric determinations were subsequently made using complete M-9 as the basal medium wherein cosenzyme I (250 and 500 micrograms/ml.), glyceraldehyde phosphate dehydrogenase (2000 and 4000 units/ml.), and phosphoglyceric acid (1 and 2 mM) (Nutritional Biochemicals Corp.) were used singly and in combination.

RESULTS

1. Varying Concentrations of Sodium.

Based on the composition of M-9 medium, 93 meq. Na/l. has been accepted as the normal concentration of this ion for routine successful cultivation of bacteria. Oxygen consumption per 10 min. interval was measured through a period of 4 hr. when young cells (2 hr. incubation) were cultivated in solutions of greater and lesser Na concentrations. The data are presented in μl O_2/10 min. (figure 1 A) and total O_2 uptake through a 4 hr. period (figure 1 B). It can be seen from figure 1 A that a considerable difference in metabolic rate exists between normal cultures when media of various Na concentrations are employed. The data show that a Na concentration of 124 meq./l. supports the greatest O_2 uptake while variations from this cause a proportionate reduction in aerobic metabolism until a concentration of 186 meq./l. is reached. In this last case inhibition is disproportionate and effectively complete. By 3 1/2 hr. bacteria grown in 186 meq Na/l. utilized 6 μl. O_2/10 min. This was not followed by any significant rise in the following 2 hours. On the other hand, while bacteria grown in Na-free M-9 medium showed a substantially lower O_2 uptake (35 μl/10 min.) than those treated with 93, 124, and 139 meq Na/l., at 3 1/2 hr., these cells reached 91 μl O_2/10 min. by 4 hours and 10 min. Thus, the Na-deficiency does not abolish aerobic metabolic pathways but does produce a marked lag in the rate of O_2 consumption.

The data presented in figures 1 A and B for cells of E. coli cultivated on media of various Na concentrations, after sub-lethal ultraviolet irradiation, present essentially the same general trend of Na effects.

whether the data for normal and irradiated cells are considered comparatively at 3½ hr. based on O_2 uptake/10 min. or at 4 hr. based on total uptake the same relative difference exists. This can be seen in th the following tabular data.

Table 2

Percent of Control O_2 Uptake by Ultraviolet Irradiated Cells in H-9 Medium.

Na Conc. (meq/l.)	O_2/10 min. 3½ hrs.	Total O_2 4 hrs.
0	19	36
93	41	53
124	81	103
139	43	44
186*	100*	112*

* Na concentration where aerobic metabolism of normal cells is almost completely abolished.

These data indicate that ultraviolet treated cells cultivated in medium containing 93 meq Na/l. show a marked depression in O_2 uptake which is however, significantly less than that observed in Na-deficient medium. Furthermore, the magnitude of the depression is approximately the same as that of cells cultivated in 139 meq Na/l. Of considerable interest is the fact that irradiated cells cultivated in media containing 124 meq Na/l. show no marked difference in rate or magnitude of O_2 consumption when compared with identical untreated cultures.

The activity of irradiated cells cultivated in media containing 186 meq Na/l., when expressed as per cent of non-irradiated controls in the same medium, cannot be considered in the same manner as the preceding data. Recognizing almost complete inhibition of untreated cells cultivated in this medium, a difference of 2-7 μl. O_2 cannot be considered significant.

These data show that with an increase in the Na concentration, and with an increase in molar ratios of Na to K there is a progressive increase in aerobic metabolism to a critical level at 124 meq/l. in synthetic M-9 medium. Subsequent increases in this ion cause a proportionate reduction in O_2 uptake to the limiting concentration of 185 meq./l. Sodium concentrations influence metabolic rate of ultraviolet irradiated cells in a manner comparable to normal cells with the difference in O_2 consumption between these two types being abolished when the medium contains 124 meq./l.

Results obtained with the use of LB media to which Na was added are somewhat similar to those obtained when cultivation was in M-9. This medium contains ca. 20.5 meq. Na/l. and ca. 11 meq. K/l. The data of figure 2A show the rate and magnitude of O_2 consumption of 2 hr. cells of E. coli when 142, 170 and 212 meq. Na/l. are added to basic LB media. Identical studies were made with normal and ultraviolet treated cells. The data show that when no Na is added to basic LB media there is an initial lag in O_2 uptake which is followed, however, by a sudden rise. This is comparable to the lag observed in Na-free M-9 media but is not so marked as in that medium. When Na is added in the three concentrations used here, the character of the metabolic response as expressed by O_2 uptake is almost identical. It is probable that the general augmenting effect of the tryptone, yeast extract medium when compared with M-9 renders the cells less sensitive to changes in Na concentration and that a protein buffering action would account for a tolerance to the higher Na levels before definite inhibition is evident.

In a manner similar to Na induced effects when M-9 was used, irradiated cells show a response to variations in Na concentration. The

data presented in figures 2 A and B demonstrate that the addition of Na, as NaCl, to a level of 170 meq/l. increases post-irradiation O_2 consumption while increases beyond this do not stimulate aerobic metabolism to the same degree. The relative effect of Na concentration on O_2 consumption of ultraviolet irradiated cells in LB medium can be seen in Table 3.

Table 3

Per cent of Control O_2 Uptake by Ultraviolet Irradiated Cells in LB Medium in 2 Hours.

Na added (meq/l.)	% Total O_2
0	36.4
142	44.1
170	48.7
212	33.0

The data show that with increases in Na concentration there is a reduction in the depression of O_2 consumption due to ultraviolet irradiation. The maximum effect is at 170 meq/l. and increases in Na level above this result in a depression in O_2 uptake greater than that with the basic LB medium (ca. 20.5 meq Na/l.) alone.

Thus, in LB media there is also an optimum Na concentration with respect to O_2 consumption for both normal and ultraviolet irradiated cells. However, as might be expected, with a medium in which bacteria are less sensitive to changes in Na concentration the optimum level for irradiated cells is higher (170 meq Na/l.) and the metabolic response to it is less than in M-9 media. If external Na preserves or stimulates at least in part, the aerobic metabolism of U-V irradiated bacteria it would appear to do so in a protein-free basal medium but not to any marked degree in a highly favorable medium containing organic sources

of nitrogen and carbon as well as numerous other poorly defined components.

It can be noted that in these studies the Na/K ratio ranged from 4.2 to 8.45 with Na variations in M-9 with the maximal O_2 uptake, in both normal and irradiated cells, where the ratio was 5.69. With Na variations in LB media the Na/K ratio varied from 1.86 to 21.14 with an optimum O_2 uptake at 17.32. Such variance in monovalent cation ratios at which O_2 consumption is maximum indicates more strongly that these effects are the results of absolute ion concentration rather than the effect of ion ratios.

2. Varying Concentrations of Potassium.

With the use of M-9 medium, K variations were studied using the basal concentration of 22 meq/l. and 53 and 115 meq/l. (Na/K ratios from 4.2 to 0.81). The data are shown in figure 3 and demonstrate, both in normal and ultraviolet irradiated cells, that increases in this ion to 53 meq/l. or above elevate the O_2 consumption to but one given level. Normal cells exposed to 53 and 115 meq K/l. have an O_2 uptake of 37 and 36% more than that consumed by cells cultivated in 22 meq K/l. This is based on total O_2 taken up through 3 hr. In a similar manner ultraviolet treated cells take up, in the same period of time, 40, 35 and 39% of the O_2 taken up by comparable controls treated with 22, 53 and 115 meq K/l. respectively. In addition ultraviolet treated cells take up 22 and 36% more O_2 than irradiated cells cultivated in 22 meq K/l. when cultivated in media containing 53 and 115 meq K/l. respectively.

It is apparent that while K has an effect on aerobic metabolism, it does not modify this after ultraviolet irradiation. The data indicate

that variations in O_2 consumption with a rise in K level before and after irradiation are of the same order of magnitude.

3. Coenzyme I, Glyceraldehyde Phosphate Dehydrogenase, Phosphoglyceric Acid

These elements of the glycolytic cycle when added to irradiated cultures of E. coli have been shown to have no augmenting effect on rate or magnitude of O_2 consumption. Figure 4 shows the respiratory activity of normal and ultraviolet irradiated cells during 4 hours after the addition of phosphoglyceric acid in concentrations of 1 and 2 mM. With the addition of glyceraldehyde phosphate dehydrogenase in a concentration of 2000 and 4000 units, there is no significant difference in metabolic rate whether cells are normal or irradiated. However, normal cells in the presence of the enzyme continued to consume O_2 at a steady rate of increase after the O_2 consumption of normal cells in complete M-9 had started to decline due to the limiting conditions of the reaction vessels. The addition of coenzyme I with glyceraldehyde phosphate dehydrogenase yielded similar results while the addition of Co I alone had no effect at all.

The data of figure 4 show a depression in respiratory activity when phosphoglyceric acid is added to both normal and irradiated cells. This depression in proportionate to the concentration used in both cases. Depression of O_2 uptake in the presence of phosphoglyceric acid cannot be ascribed to a change in pH since measurements made at the start of the experiment and again 4 hr later show no change in this metabolic variable.

Discussion

The data obtained from these studies show that the ionic composition of the medium in which E. coli are cultivated has a marked effect upon the rate and magnitude of oxygen consumption. While increases in both Na and K concentrations cause an increase in the respiratory activity of normal cells of E. coli, the effects are not proportionate nor do the ions substitute for each other. The data indicate that these effects are more likely due to the kind of ion and its absolute concentration rather than to ion ratios. A 25 per cent increase from the basal Na level in synthetic medium (93 to 124 meq/l.) results in a 40 per cent increase in oxygen consumption while K increases of 2.5 to 5 fold (22 to 53 and 115 meq/l.) elevate oxygen consumption to the same degree. On the other hand, a two fold increase in Na (93 to 186 meq/l.) is essentially inhibitory. When ultraviolet irradiated cells are studied in the same media, responses to variations in the external concentration of Na and K are similar to those of normal cells but of a different order of magnitude. With both normal and irradiated cells in synthetic M-9 medium, a concentration of 124 meq Na/l. is the point of maximal aerobic metabolism. However, at this concentration the increase in oxygen consumption of control cultures is 160% of that in Na-free medium while with irradiated cultures it represents an increase of 646% of that in Na-free medium. Cultivation in more complex LB medium with varying concentrations of this same ion does not lead to the same type of response in normal cells but ultraviolet treated cells do show an increase in O_2 consumption to an optimum at 170 meq Na/l. with a decrease at higher concentrations. In both media however, aerobic metabolism is greater when Na is added than in its absence. One notable exception to this is

the addition of 186 meq Na/l. to M-9 medium which is effectively inhibitory to both normal and irradiated cells. Contrariwise, the addition of 212 meq Na/l. to LB media does not depress oxygen consumption of normal cells to or below that of the basal concentration of 20.5 meq/l.

Increases in metabolism of bacterial cells as a function of external ionic concentration have been reported by other investigators. In a study on the mechanism of halophilism, Robinson et al. (1952) demonstrated an increase in intracellular nitritase activity as the external NaCl concentration was raised from 0.55 to 2.2 per cent with decreases in activity from 4.4 to 17.6 per cent. An external concentration of 3.28% NaCl was considered normal. Analysis of intact cells and cell-free homogenates demonstrated that intracellular enzyme activity is maximal at 0.9 per cent NaCl and that a concentration difference across the membrane was maintained by an expenditure of energy. The metabolic inhibitors used by these investigators suggested that coenzymes I or II are involved in maintaining the concentration gradient. The metabolic response of normal cells of *E. coli* to external Na concentrations simulates that of intracellular nitritase under parallel conditions of increasing concentrations of this salt. When treated with ultraviolet radiations, cells of *E. coli* show a significantly heightened response to these same conditions. Changes in membrane permeability as a result of radiation injury would permit an excess of Na to enter the cell. This result would require an increase in the expenditure of energy to maintain a viable concentration gradient across the cell membrane. Changes in permeability as a result of ultraviolet have been reported by Heinmets et al. (1954). In addition, Billen et al. (1953) have shown post-irradiation leakage of ATP and other substances from cells of

E. coli B/r. That Na enters normal as well as irradiated cells has been shown by numerous workers. With normal cells of marine bacteria, Johnson and Gray (1949) demonstrated increases in chromatin aggregation with an elevation of salt concentration or subsequent to urethane treatment. Aggregation and dispersion are reversibly induced by salt variations within viable limits. Whitfield (1952) and Whitfield and Murray (1955) have demonstrated that the same nuclear changes occur with ultraviolet irradiation in moderate (0.17 M) Na concentrations as are seen to occur in normal cells at high Na levels (1.7 M NaCl). The effect was always less marked when the basal medium was LB as compared with a salt solution alone. This observation, among others, indicates a greater entrance of Na into irradiated cells which are affected by radiation induced permeability changes than into normal cells. Relating aerobic metabolic increases as a function of external Na concentrations to the role of active Na extrusion, it would appear that cells of E. coli demonstrate a regulatory mechanism maintaining intracellular ionic balance. Whitfield (1952) demonstrated that cells of Shigella dysenteriae cultivated on 0.34 M NaCl-LB agar at 37°C showed no nuclear aggregation while cultivation on the same medium at 4°C resulted in nuclear "contraction." This would indicate an active Na extruding mechanism. From the data obtained in this study the mechanism of active extrusion is brought to its maximum when the external concentration reaches 124 meq Na/l. whether cells are normal or have been altered by ultraviolet irradiation. Subsequent increases beyond this level may exceed the metabolic capacity of the extruding mechanism with a resultant disturbance in ionic gradients and osmoregulation or may lead to direct toxic effects. Increases in intracellular Na may then lead to general or particular enzymatic

inhibition. While enzyme or endproduct analyses have not been made in this study, Na ion influences have been shown in the energy cycle of glycolysis. Boyer et al. (1942, 1943) have demonstrated that the enzymatic transfer of phosphate from phosphopyruvate to the adenylic system requires K and that Na ions antagonize this function, depressing enzymatic activity in the presence of limiting K. If such an interference in the energy system prevailed in circumstances of overwhelming Na concentrations, the energy of Na extrusion would ultimately be effectively blocked. Whether or not ion influenced enzymatic inhibition explains the marked reduction in aerobic metabolism when concentrations exceed 124 meq/l. it is known that with external ionic imbalances cells reach a limiting capacity in regulation. This is verified in the data presented here where untreated cells demonstrated the same metabolic limitations.

It is of interest that the responses of both normal and ultraviolet treated cells to increases in Na when added to LE medium do not approach those obtained with synthetic M-9 medium. The results presented here show no gradation of respiratory activity when Na is added to normal cells above that of 142 meq/l. On the other hand irradiated cells demonstrate the highest activity at 170 meq Na/l.

It would appear first that Na ions are not as available in a protein medium as in a salt-glucose solution and as a consequence do not proportionately stress the metabolic exclusion mechanism. Considering the anionic nature of the proteins at the pH of the medium employed, it is likely that Na is not in the same available concentration. Secondly, manometric data on cells in this medium indicate an increase in the permeability of ultraviolet irradiated cells such that the greatest

aerobic respiration occurs at a level of 170 meq/l. However, total available concentration of Na and active metabolic control of intracellular ionic concentration do not entirely explain the results reported here. It is apparent that the marked metabolic response of irradiated cells, when cultivated in M-9 medium with 124 meq Na/l. and considered above to be a result of increased Na influx, should find its verification in a continued rise in O_2 consumption by normal, less permeable cells at a higher external Na concentration. In addition a similar response should be apparent in control cells in LB media where Na is considered to be less available. As a consequence of this disparity another mechanism must be sought.

Cationic action on nuclear components of normal and irradiated cells have been reported by many investigators. Subsequent to ultraviolet irradiation it has been shown that nuclear fragmentation results (Whitfield 1952, 1955; Johnson and Gray, 1949; Cortelyou et al. 1955; Whitfield and Murray, 1955; Payne et al. 1956, etc.). Cytological changes in the physical distribution of nuclear components have been shown to result from the addition of Na salts. Payne et al. (1956), among others, have shown that exposures of E. coli to hypertonic solutions of Na and K salts results in reversible aggregation of nuclear chromatin. They have offered the interpretation that subsequent to cell injury there occurs an alteration in the homeostatic regulating mechanism. As a consequence of this failure the internal ionic milieu is increased, resulting in chromatin reaggregation.

Ultraviolet irradiation has been shown to decrease the viscosity of Na-desoxyribonucleate (Greenstein and Jenrette, 1941). Miyaki and Price (1950) demonstrated a protective action of Na at concentrations greater than 0.5M against decreases in viscosity of DNA when treated with

heat. This effect was reversible. In this respect in vitro studies on Na and DNA are similar to the reversible effect shown for this ion on the aggregation of the chromatinic bodies of Shigella dysenteriae described by Whitfield (1955). Similar detailed studies have been made by Shack et al. (1953). The data presented by these workers suggests Na binding between anionic phosphate residues and as a consequence, polymerisation. The effect is greater with bivalent cations at lower concentrations than with monvalent cations. Reversal of in situ ultraviolet induced viscosity changes (fragmentation to aggregation) in nuclear DNA, with increases in external Na concentrations would protect against irreversible ultraviolet damage. Electron microscope studies of ultraviolet treated cells, cultivated in LB media with 170 meq Na/l. added, show a more normal cytology than is found in cells cultivated in basic LB with no Na added. Variations in nuclei range from chromatin granules interpreted to represent chromatin aggregation, to apparently normal cells. Irradiated cells cultivated in basic LB medium present predominantly spread nuclear elements rendering the cell uniformly dense. This cytological observation suggests a protective action of increases in environmental Na as indicated by manometric data. It may also contribute to an understanding of the disproportionate increases in oxygen consumption of irradiated cells which is not adequately explained on the basis of an active Na extruding mechanism.

That Na is generally stimulatory to glycolysis in E. coli does not seem likely in view of the evidence of many workers. On the contrary, it has been demonstrated by many that inhibition of glycolysis is proportionate to Na increases and that this ion effect is antagonized by K (Stumpf, 1954; MacLeod and Snell, 1946; Clark and MacLeod, 1954;

Tsuyuki and MacLeod, 1951). These workers have shown that in the presence of sufficient K, inhibition by Na disappears and that as the concentration of the inhibiting ion is elevated, higher concentrations of K are required to antagonize it. Inhibition by Na was not seen in the study reported here until the concentration exceeded 124 meq/l. in M-9 medium. This could be interpreted as a point of critical Na/K (eg. 5.63) ratio where increases in K would again antagonize inhibitory Na. However, if the reason for decreases in aerobic respiration rests alone in the Na/K ratio above 124 meq Na/l. there can be no apparent answer for stimulation of untreated cells in LB medium when the ratio is 14.7 or of irradiated cells when they are cultivated in the same medium with a Na/K ratio of 14.7 and 17.3. The workers cited have shown stimulation of glycolysis with increases in K. The data reported here demonstrate a general stimulation of both normal and irradiated cells when K was increased from the basal concentration of 22 to 53 and 115 meq/l. With these concentrations, though the Na/K ratio was 1.75 and 0.8, there was no significant difference in aerobic respiration at these two concentrations.

Potassium stimulation shown here is in agreement with the observations of Friedman and Fox (1954) who have concluded that there is a minimal K requirement for maximal growth and cell yield of E. coli. The stimulation of O_2 consumption by increases in K in this work may be an expression of optimal growth above that in minimal synthetic M-9 medium.

With use of certain elements of the glycolytic cycle no increase in aerobic respiration of normal and irradiated cells of E. coli resulted. Serazdarian et al. (1954) have shown ultraviolet inactivation of

coenzyme I. However, the addition of this cofactor in no way altered the O_2 consumption of either type of cell. This may be due either to a lack of penetrability of the molecule or though it may have permeated, no enhancing effect would result if either its respective dehydrogenase activity was not injured through radiation or was completely inactivated through radiation. From the rates of O_2 consumption of Na-stimulated irradiated cells it is apparent that glycolysis can proceed after ultraviolet irradiation. This has been observed by other workers who have studied metabolism through both O_2 consumption and CO_2 release. The reduction in O_2 consumption by both normal and ultraviolet irradiated cells upon the addition of phosphoglyceric acid may give evidence of the continuance of glycolysis after UV. The oxidized intermediate would participate in the cycle resulting in a reduction in the amount of endogenous carbohydrate oxidized. The real participation of added phosphoglyceric acid can only be determined by quantitative determinations of pyruvate in an aerobic system.

SUMMARY

1. Manometric studies were made of cultures of Escherichia coli in media of various Na and K concentrations after irradiation with ultraviolet at a dosage of 420 mw/ft^2/sec. for 15 seconds.

2. There is a specific protective level of Na, 124 meq.Na/l. in M-9 media, at which the oxygen consumption of E. coli is unaltered by UV irradiation.

3. 186 meq. Na/l. in M-9 media inhibits O_2 consumption of both normal and irradiated cultures of E. coli.

4. Potassium increases in the cultivation medium stimulate aerobic respiration of normal and irradiated cells, the increase being proportional in both cases.

5. Additions of coenzyme I and glyceraldehyde phosphate dehydrogenase have no marked effect on the O_2 consumption of normal or irradiated cells. Phosphoglyceric acid in concentrations of 1 and 2 mM depresses O_2 uptake in both types of cells.

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Figure 1 A The effect of sodium on the rate and magnitude of O_2 consumed per 10 min. interval when normal and ultraviolet treated E. coli are cultivated in different concentrations of this ion in M-9 media.

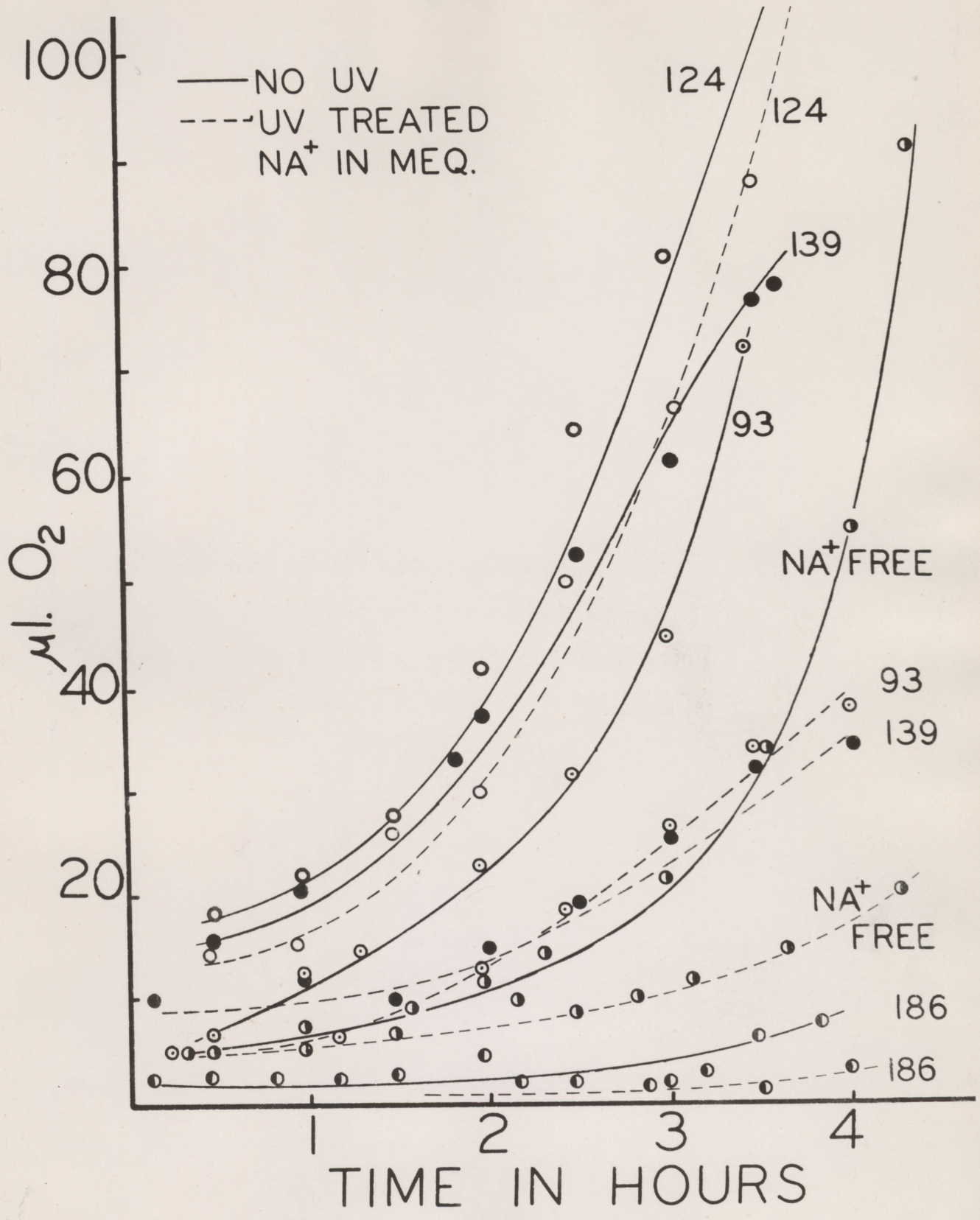
100
80
60
40
20
µl. O2
NO UV
UV TREATED
NA+ IN MEQ.
124
124
139
93
NA+ FREE
93
139
NA+ FREE
186
186
1
2
3
4
TIME IN HOURS

Figure 1 A

Figure 1 B Total oxygen taken up during 4 hours when normal and ultraviolet treated cells of E. coli are cultivated in M-9 media modified by variations in sodium concentration.

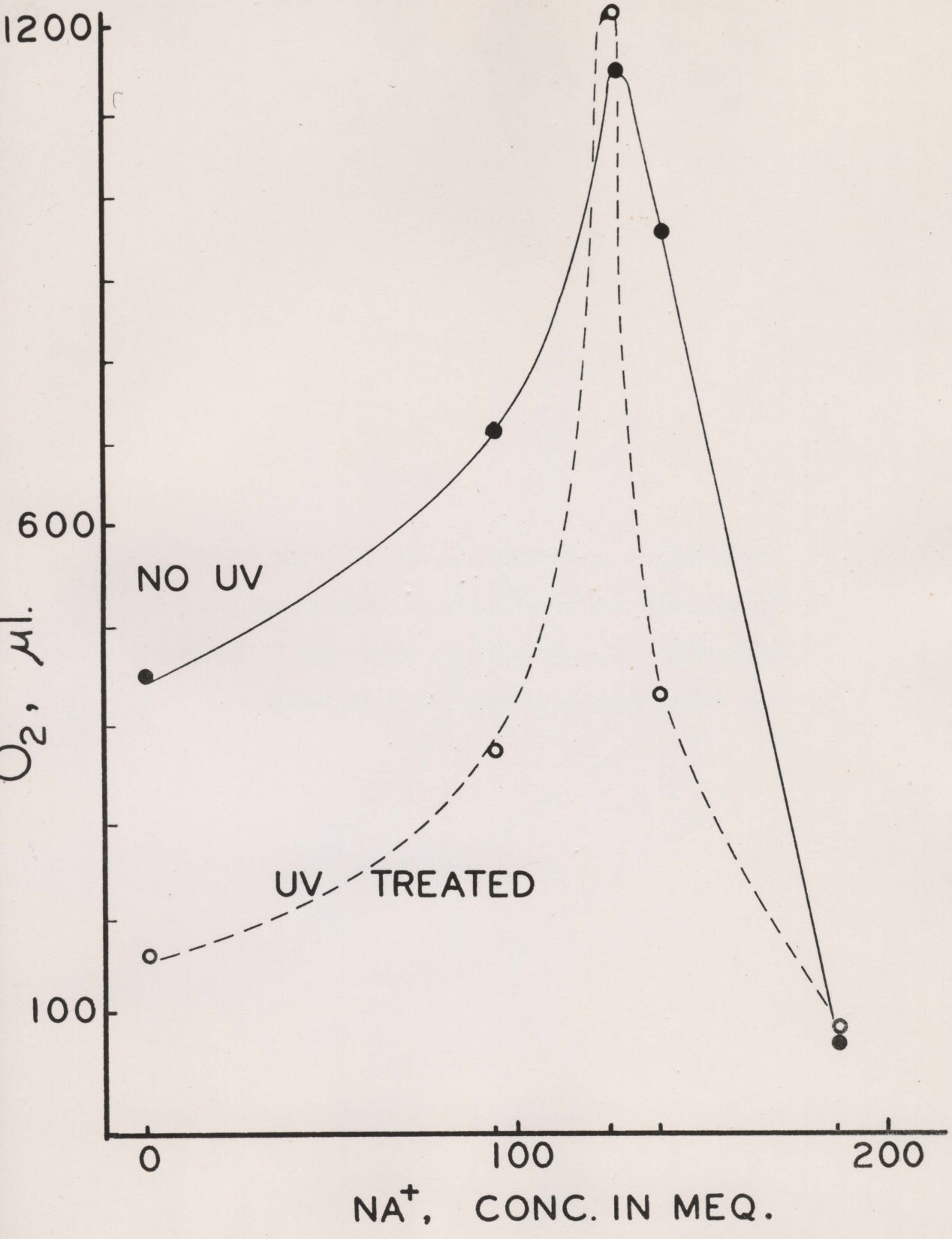

Figure 1 B

Figure 2 A The effect of sodium on the rate and magnitude of O_2 consumed per 10 min. interval when normal and ultraviolet treated cells are cultivated in different concentrations of this ion in LB media.

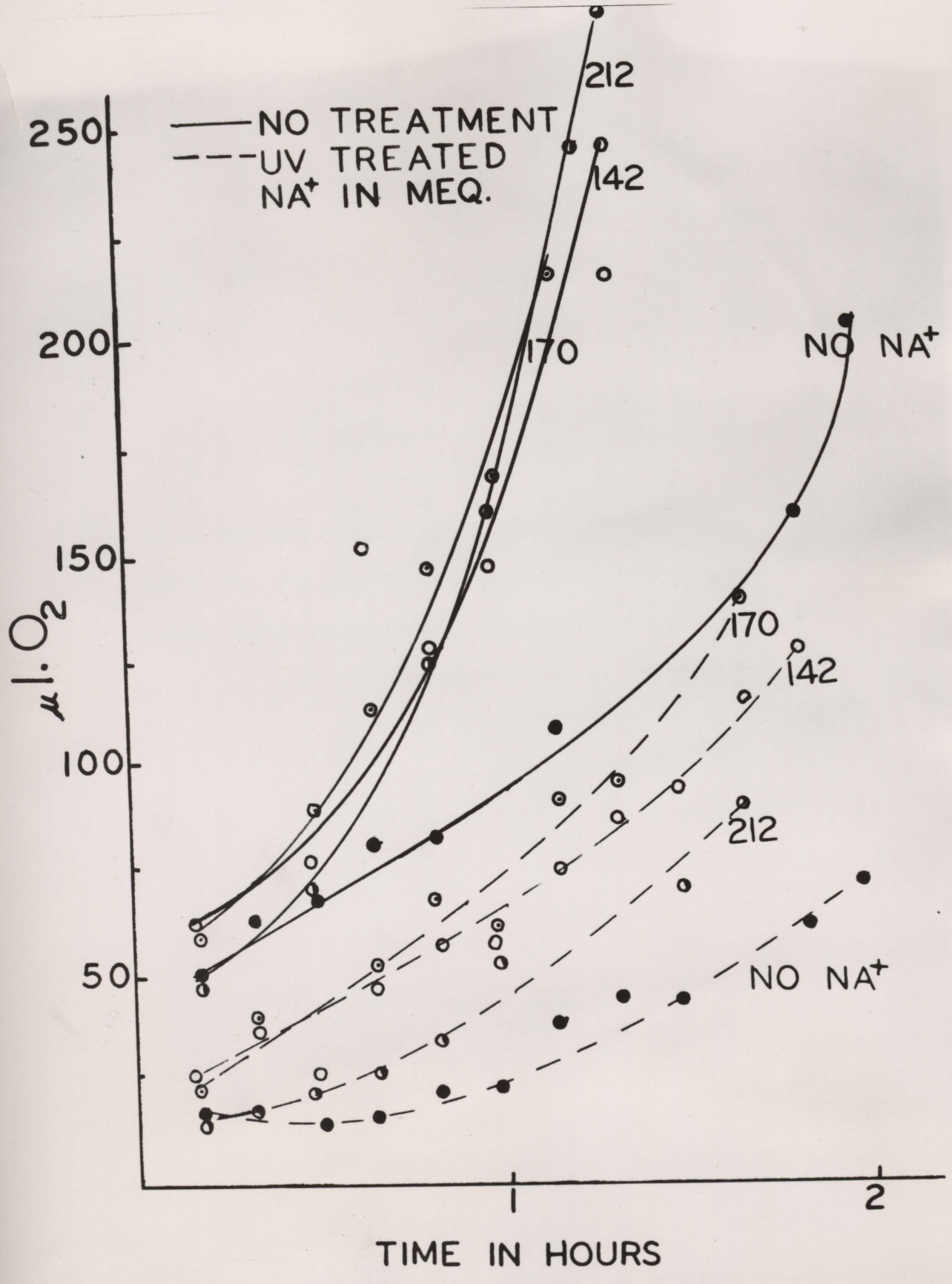
NO TREATMENT
UV TREATED
NA+ IN MEQ.
212
142
170
NO NA+
250
200
150
100
50
μl. O2
170
142
212
NO NA+
1
2
TIME IN HOURS

Figure 2 A

Figure 2 B Total oxygen taken up during 2 hours when normal and ultraviolet treated cells of E. coli are cultivated in LB media modified by variations in sodium concentrations.

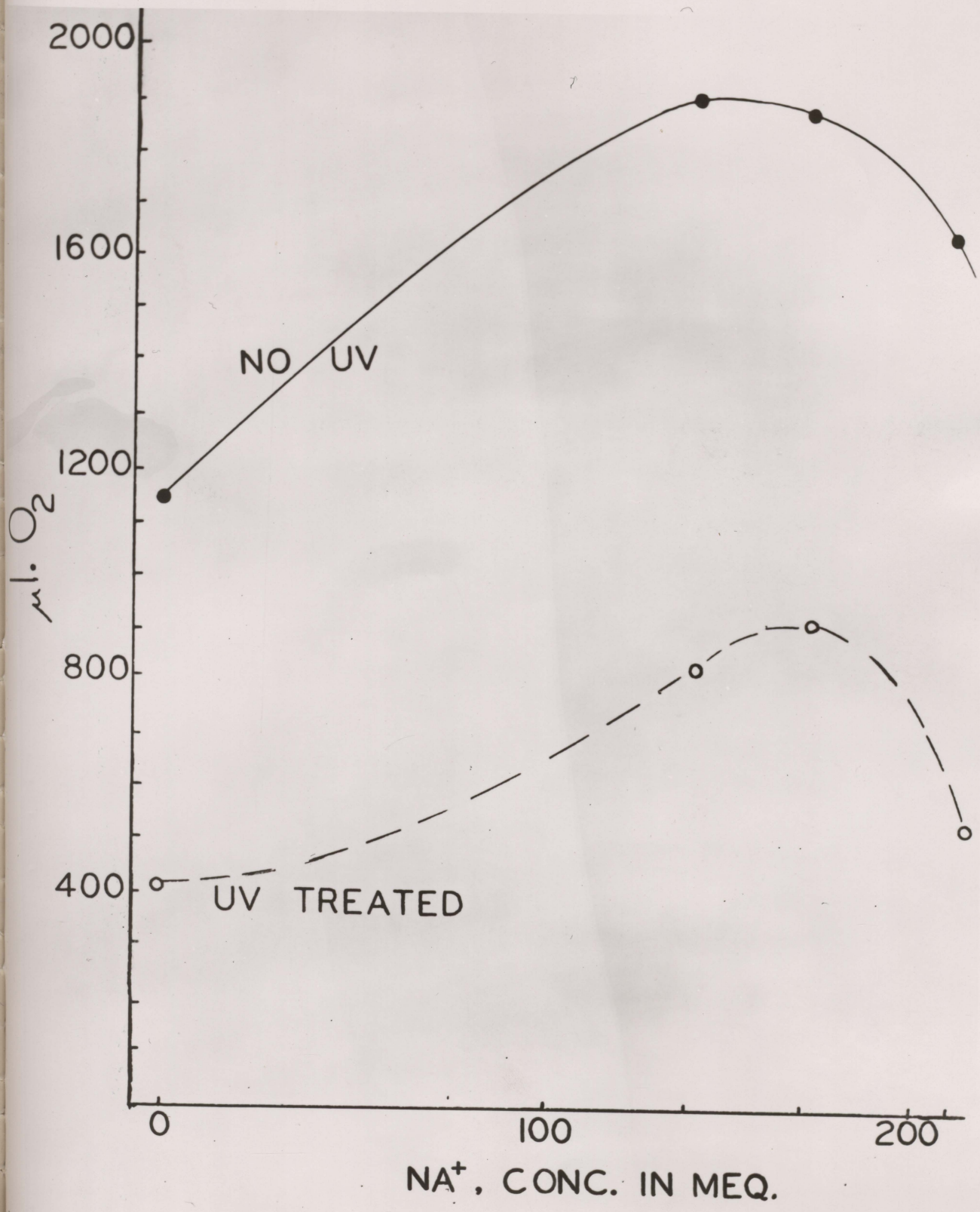
2000
1600
1200
800
400
μl. O2
NO UV
UV TREATED
0
100
200
NA+, CONC. IN MEQ.

Figure 2 B

Figure 3 The effect of potassium on the rate and magnitude of O_2 consumed per 15 min. interval when normal and ultraviolet treated E. coli are cultivated in different concentrations of this ion in M-9 media.

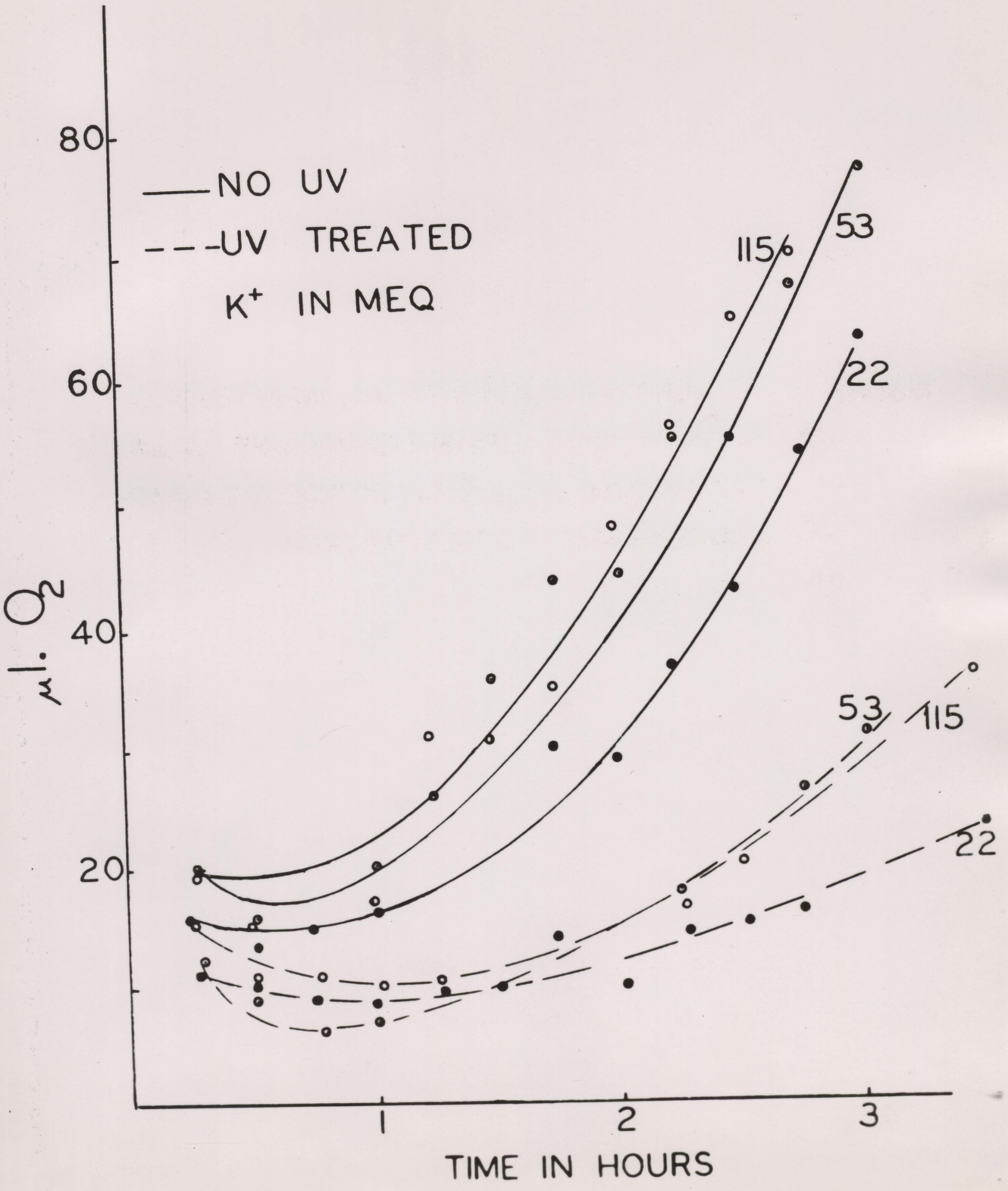
80
60
40
20
μl. O2
NO UV
UV TREATED
K+ IN MEQ
115
53
22
53
115
22
1
2
3
TIME IN HOURS

Figure 3

Figure 4 The effect of phosphoglyceric acid, in concentrations of 1mM (1) and 2 mM (2), on oxygen consumption per 30-min. interval of normal and ultraviolet treated cells of *E. coli*.

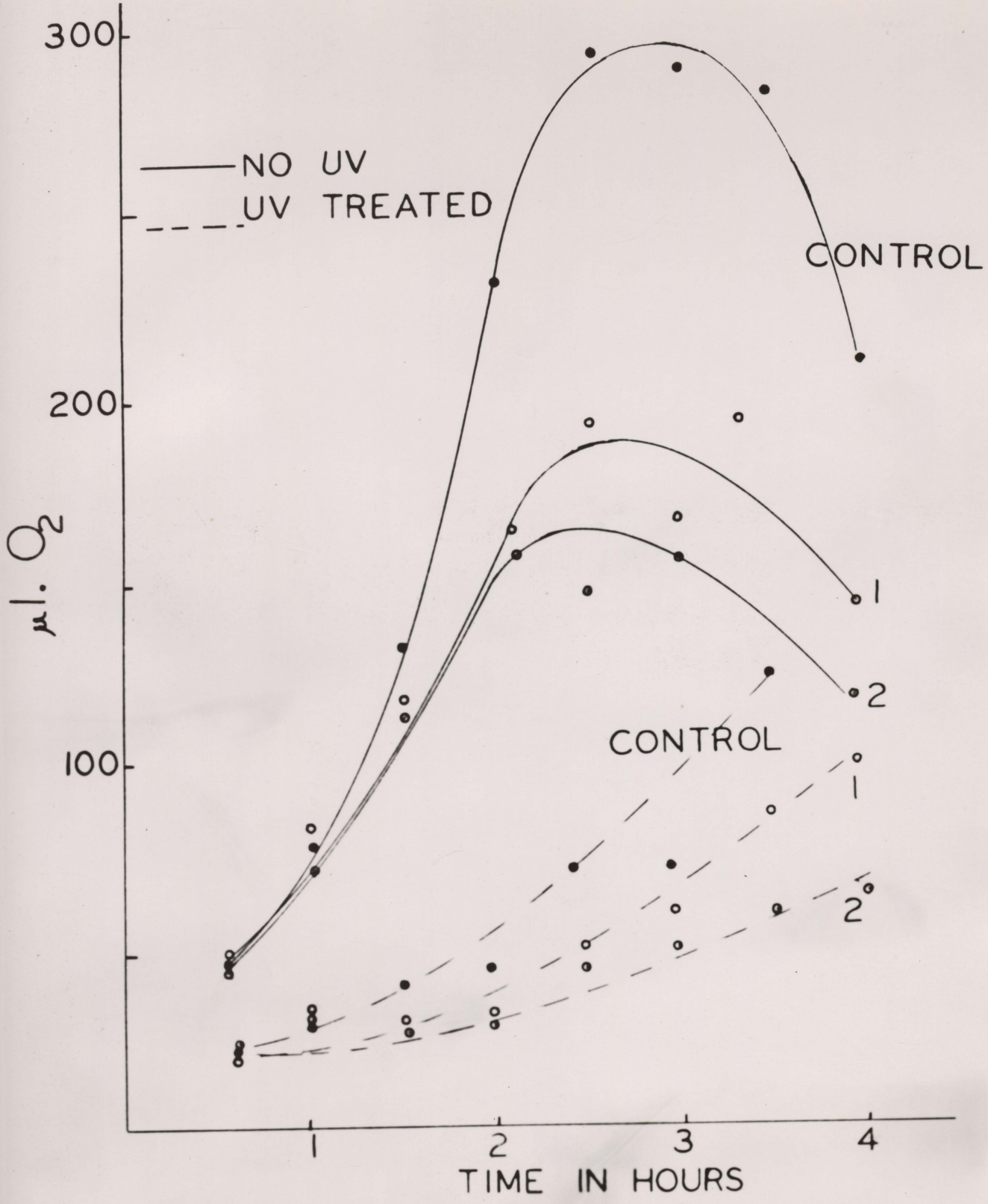
300
200
100
μl. O2
NO UV
UV TREATED
CONTROL
1
2
CONTROL
1
2
1
2
3
4
TIME IN HOURS

Figure 4

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