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Martin S. Wilder
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MARCH 1969

DEPARTMENT OF THE ARMY
Fort Detrick
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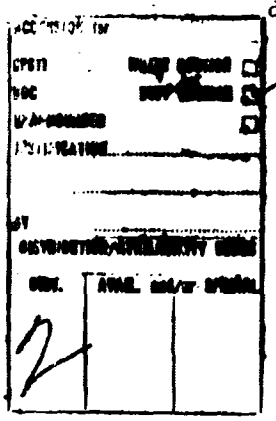

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OXIDATIVE ASSIMILATION OF GLUCOSE BY *PASTEURELLA PESTIS*

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MEDICAL SCIENCES LABORATORIES

Project 1B562602A059

March 1969

ABSTRACT

A lethal effect of glucose on aerated virulent (but not avirulent) cultures of Pasteurella pestis at 37 C observed by Wessman et al. in 1958 prompted further studies into the patterns of oxidative metabolism of the virulent Alexander strain at 37 and 26 C. Bacterial respiration was markedly stimulated by glucose; this may be associated under certain conditions with the previously demonstrated toxic effect of glucose. The inability of the pathogen to assimilate substantial quantities of cellular material, as well as its fermentative activity under aerobic conditions may also be involved. At 26 C in the presence of added NH_3, P. pestis assimilates as much glucose as at 37 C, but releases considerably less lactic acid into the supernatant fluids. The data are also consistent with the idea that at least a portion of the cellular material synthesized during oxidative assimilation may be due to the reincorporation of endogenously produced ammonia.

OXIDATIVE ASSIMILATION OF GLUCOSE BY PASTEURELLA PESTIS*

A lethal effect of glucose on aerated virulent cultures of Pasteurella pestis at 37 C was observed by Wessman, Miller, and Surgalla.** VW-positive strains respond to glucose addition to synthetic medium by dying, but VW-negative strains multiply. Although several possible mechanisms were suggested, no further work on the problem was pursued. This unique relationship of nutrition to virulence prompted further studies into the patterns of oxidative metabolism of the virulent Alexander strain of P. pestis at 37 and 26 C.

The organisms were grown at 26 C on a shaker operating at approximately 95 strokes per minute. The cells were washed in buffer and resuspended to the original culture concentration in either synthetic medium or buffer in the presence or absence of 1% glucose. Table 1 shows the oxygen uptake by washed-cell suspensions of P. pestis at 37 C. Glucose markedly stimulates respiration of the virulent strain at 37 C in synthetic medium. The stimulation is more than additive, in that respiration is greater than the combined activity with glucose alone and with medium alone.

TABLE 1. OXYGEN UPTAKE BY WASHED-CELL SUSPENSIONS OF PASTEURELLA PESTIS[a] AT 37 C

Time, minutes	Oxygen Uptake, µl			
	Endogenous Buffer	Buffer + Glucose	Synthetic Medium - Glucose	Synthetic Medium + Glucose
30	23.70	104.78	60.20	257.30
60	38.34	216.32	120.40	561.08
90	58.48	317.72	108.84	934.58
120	66.36	385.32	209.84	1223.42
150	82.16	446.16	247.68	1532.52
180	93.22	501.93	287.24	1811.06
210	104.28	547.56	319.92	2070.02
240	121.66	610.09	364.64	2244.32

a. Cells were resuspended to the original culture concentration in either synthetic medium or buffer in the presence or absence of 1% glucose. Total volume of reactants, 3 ml.

* This report should not be used as a literature citation in material to be published in the open literature. Readers interested in referencing the information contained herein should contact the senior author to ascertain when and where it may appear in citable form.

** Wessman, G.E.; Miller, D.J.; Surgalla, M.J. 1958. Toxic effects of glucose on virulent Pasteurella pestis in chemically defined media. J. Bacteriol. 76:368-375.

Oxygen uptake by washed-cell suspensions at 26 C is shown in Table 2. The rate of oxygen uptake at the lower temperature in the presence of glucose appears to be approximately one half that observed at 37 C. However, the rate of oxygen uptake of the cells suspended in synthetic medium is essentially identical at the two temperatures.

TABLE 2. OXYGEN UPTAKE BY WASHED-CELL SUSPENSIONS OF *PASTEURELLA PESTIS*[a] AT 26 C

Time, minutes	Oxygen Uptake, µl			
	Endogenous Buffer	Buffer + Glucose	Synthetic Medium - Glucose	Synthetic Medium + Glucose
30	10.20	35.20	53.34	128.88
60	22.10	81.24	110.11	293.56
90	27.20	130.24	161.59	490.46
120	35.70	164.44	203.06	646.19
150	45.90	202.19	247.39	848.46
180	51.00	230.19	273.13	1032.83
210	56.10	265.39	324.61	1240.47
240	62.90	303.89	366.08	1457.06

a. Cells were resuspended to the original culture concentration in either synthetic media or buffer in the presence or absence of 1% glucose. Total volume of reactants 3 ml.

Respiratory quotients for cells suspended in buffer and synthetic medium with glucose at 26 and 37 C are shown in Table 3. The unusually low values obtained at both temperatures where glucose was the substrate are indicative of metabolic complications. The shift to the higher values in cells respiring in synthetic medium may indicate either a change in the nature of the cellular materials being metabolized or the added ability to metabolize highly oxidizable materials more efficiently in the presence of the components of the medium.

At this point it was felt that additional studies on the assimilatory behavior of the organism would provide additional insight into the previous findings. After 23 hours at 26 C, the cells were harvested by centrifugation in the cold, washed with cold 0.04 M phosphate buffer (pH 7.4), and resuspended to ten times the growth concentration according to the procedure of Duncan and Campbell.* Manometric measurements were carried out with two Warburg baths set at 26 and 37 C. Each cup contained 1 ml of cell suspension

* Duncan, M.G.; Campbell, J.J.R. 1962. Oxidative assimilation of glucose by *Pseudomonas aeruginosa*. J. Bacteriol. 84:784-792.

in buffer. Uniformly labeled glucose C^{14} was diluted with nonradioactive glucose solution so that 5 μM of glucose contained 4.7×10^{-3} μc of uniformly labeled glucose.

TABLE 3. RESPIRATORY QUOTIENTS OF PASTEURELLIA PESTIS[a] AT 26 AND 37 C

Time, min.	Buffer + Glucose				Synthetic Medium plus Glucose			
	37 C		26 C		37 C		26 C	
	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2	Exp 1	Exp 2
30	0.57	0.42	0.56	0.82	0.14	0.46	0.58	0.38
60	.48	.41	.32	.63	0.70	0.52	.59	.44
90	.55	.55	.29	0.22	0.71	0.62	.62	.51
120	.50	.40	.38	-	0.78	0.64	.67	.57
150	0.66	.50	.24	-	0.91	0.66	.71	.61
180	-	.38	.43	-	1.0	1.0	.91	.74
210	-	.66	0.32	-	1.2	1.0	.80	.78
240	-	0.37	-	-	0.94	1.1	0.79	0.82

a. Cells were resuspended to the original culture concentration in either synthetic medium plus 1% glucose or buffer plus 1% glucose. Total volume of reactants, 3 ml.

Glucose uptake curves and distribution of C^{14} and oxygen for cell suspensions at 26 and 37 C are shown in Figures 1 and 2.

Although oxygen is taken up by the cells for the entire 2 hours, the glucose is almost entirely depleted after 30 minutes. That oxygen uptake does occur during the latter part of the experimental period indicates oxidation of some other compound or intermediate that had been secreted into the supernatant fluid. The radioactivity measurements indicate that the cells assimilate only about 10 to 16% of the labeled glucose. These data are in accord with Clifton's finding* of the assimilatory activities of E. coli grown on medium supplemented with 1% glucose.

Additional studies were undertaken to identify any key intermediate extracellular products that might have accumulated in the vessels during the oxidation of glucose.

Routine paper chromatography of the supernatant fluids was carried out by several solvent systems. Radioactive areas on paper chromatograms were located by preparation of radioautographs. On radiochromatographic analysis of the supernatant fluids at 26 C and 37 C, the C^{14} was found in a single spot, which was identified as lactic acid. Alpha-ketoglutarate was also identified by chemical assays and by chromatography of the 2,4-dinitrophenylhydrozones.

* Clifton, C.E. 1963. Influence of growth medium on assimilatory activities of Escherichia coli. J. Bacteriol. 85:1371-1377.

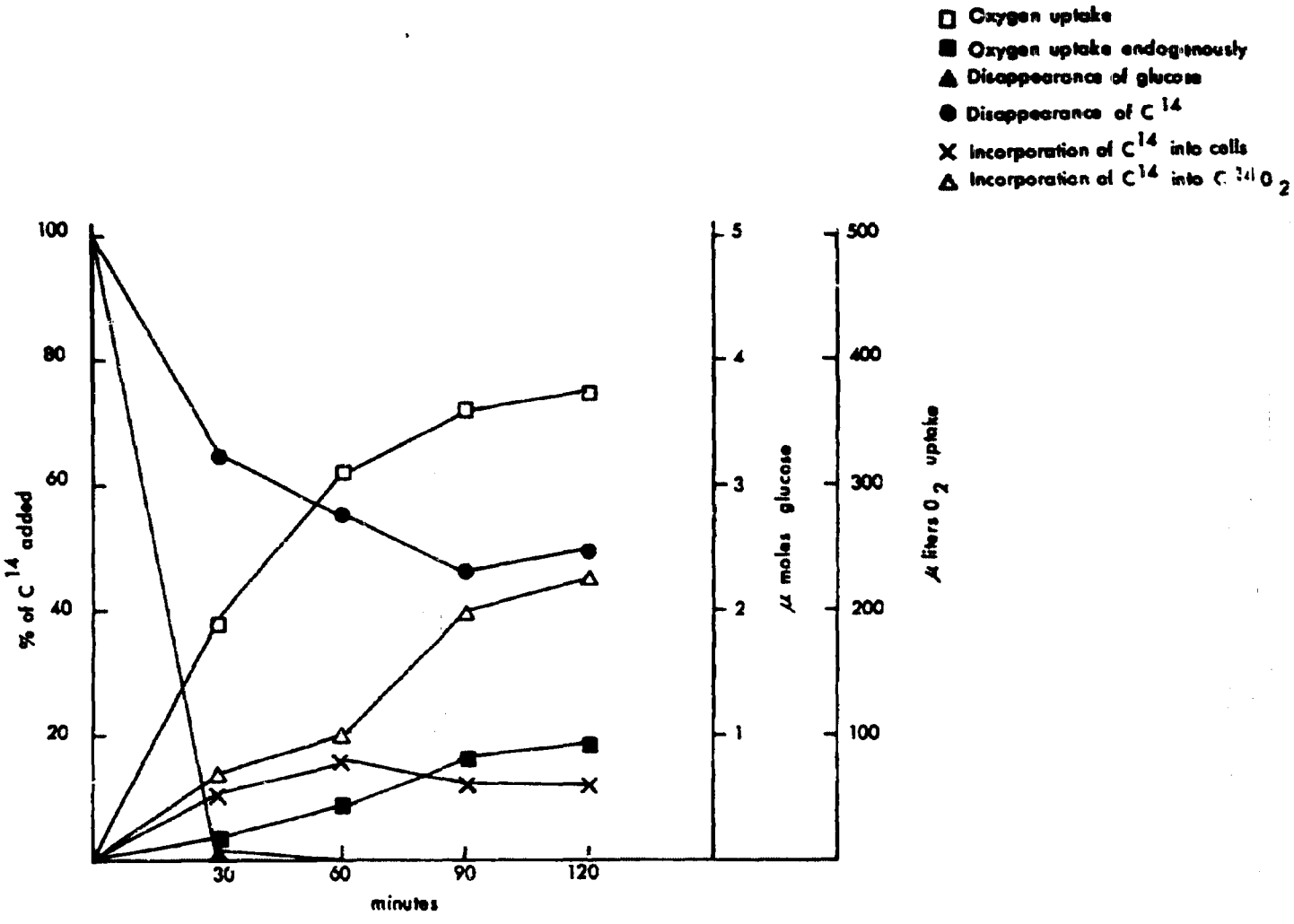

FIGURE 1. Uptake of Glucose and Oxygen and Distribution of C^{14} During Manometric Experiments with Washed-Cell Suspensions of Pasteurella pestis at 37 C.

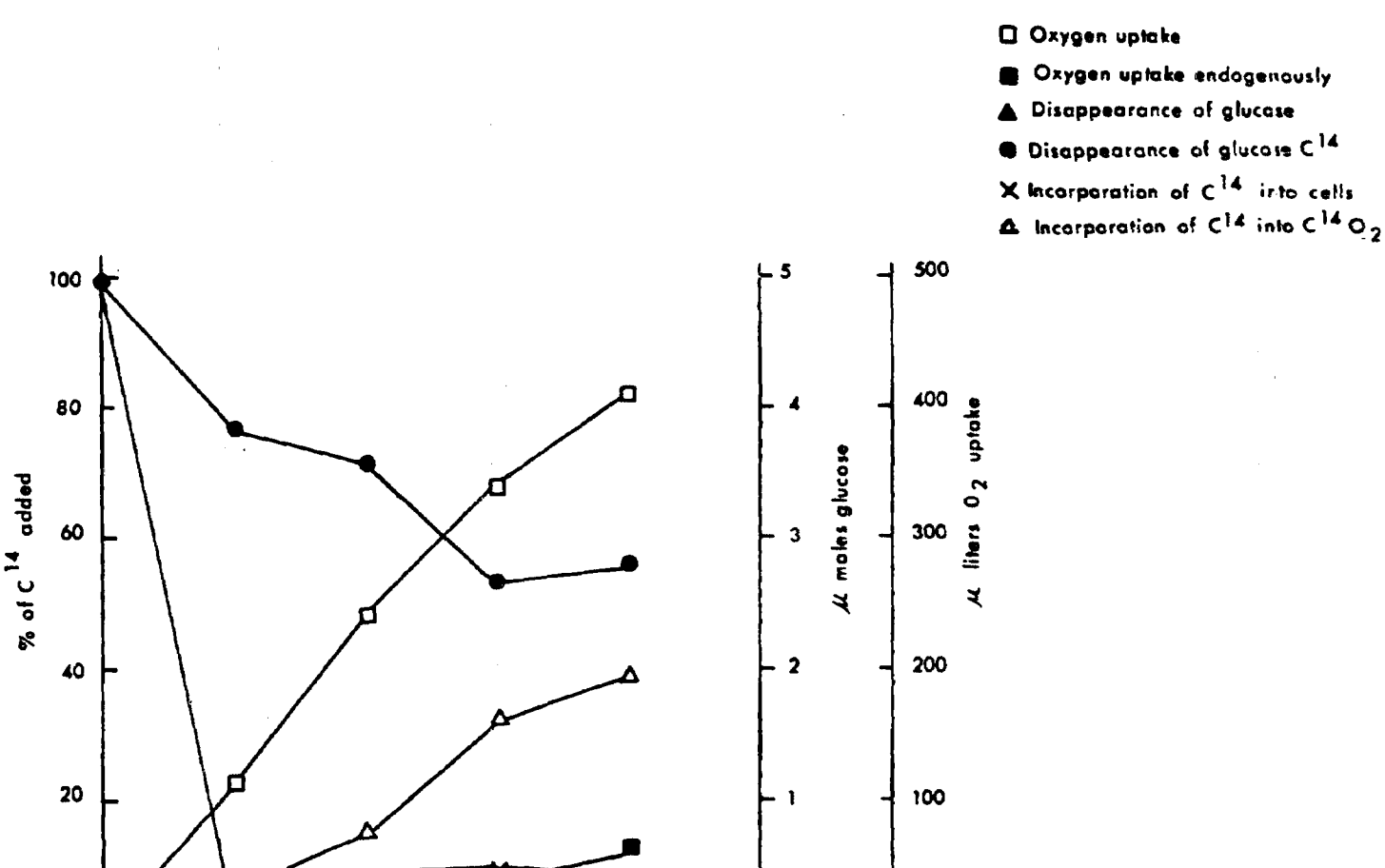

FIGURE 2. Uptake of Glucose and Oxygen and Distribution of C^{14} During Manometric Experiments with Washed-Cell Suspensions of Pasteurella pestis at 26 C.

Duncan and Campbell* reported that assimilation of glucose by Pseudomonas aeruginosa depended on the availability of endogenously produced ammonia, and since the glucose effect occurs in a nitrogen-rich medium, it seemed desirable to conduct further studies on the pattern of assimilation in the presence of added ammonia.

Figure 3 presents graphically the cellular uptake of radioactive glucose and production of lactate and α-ketoglutarate in the presence of 5 μM $(NH_4)_2SO_4$ at 37 C.

The oxidative assimilation of glucose was stimulated in the presence of ammonia. Practically no α-ketoglutarate appeared in the medium in the presence of ammonia, although a considerable amount of lactate could still be measured. Although P. pestis produces ammonia endogenously, there does not appear to be much reincorporation of α-ketoglutarate, as in the case of P. aeruginosa.

The results at 26 C are presented in Figure 4. The most obvious difference at the lower temperature is the diminution of extracellular acid production. The total amount of C^{14} assimilated in the cells is identical at both temperatures.

Table 4 shows the fate of uniformly labeled glucose added to resting cells of P. pestis in Aminco-Dubnoff shaker incubators. The products of glucose oxidation are distributed in all the fractions; however, most of the assimilated material was found in the cold trichloroacetic acid (TCA) (transient intermediates) fraction and the alcohol-soluble fraction of the cells. At 37 C, addition of ammonia stimulates assimilation of glucose most dramatically in the protein and lipid fractions; at 26 C there is a marked stimulation of assimilation into all fractions.

In summary, this work shows that the marked stimulation of bacterial respiration by glucose may be associated under certain conditions with the previously demonstrated toxic effect of glucose on VW-positive strains of P. pestis. The inability of the pathogen to assimilate substantial quantities of cellular material, together with its fermentative activity under aerobic conditions, may also be involved. At 26 C in the presence of added NH_3, P. pestis assimilates as much glucose as at 37 C, but releases considerably less lactic acid into the supernatant fluids. The data are also consistent with the idea that at least a portion of the cellular material synthesized during oxidative assimilation may be due to the reincorporation of endogenously produced ammonia.

* Duncan, M.G.; Campbell, J.J.R. 1962. Oxidative assimilation of glucose by Pseudomonas aeruginosa. J. Bacteriol. 84:784-792.

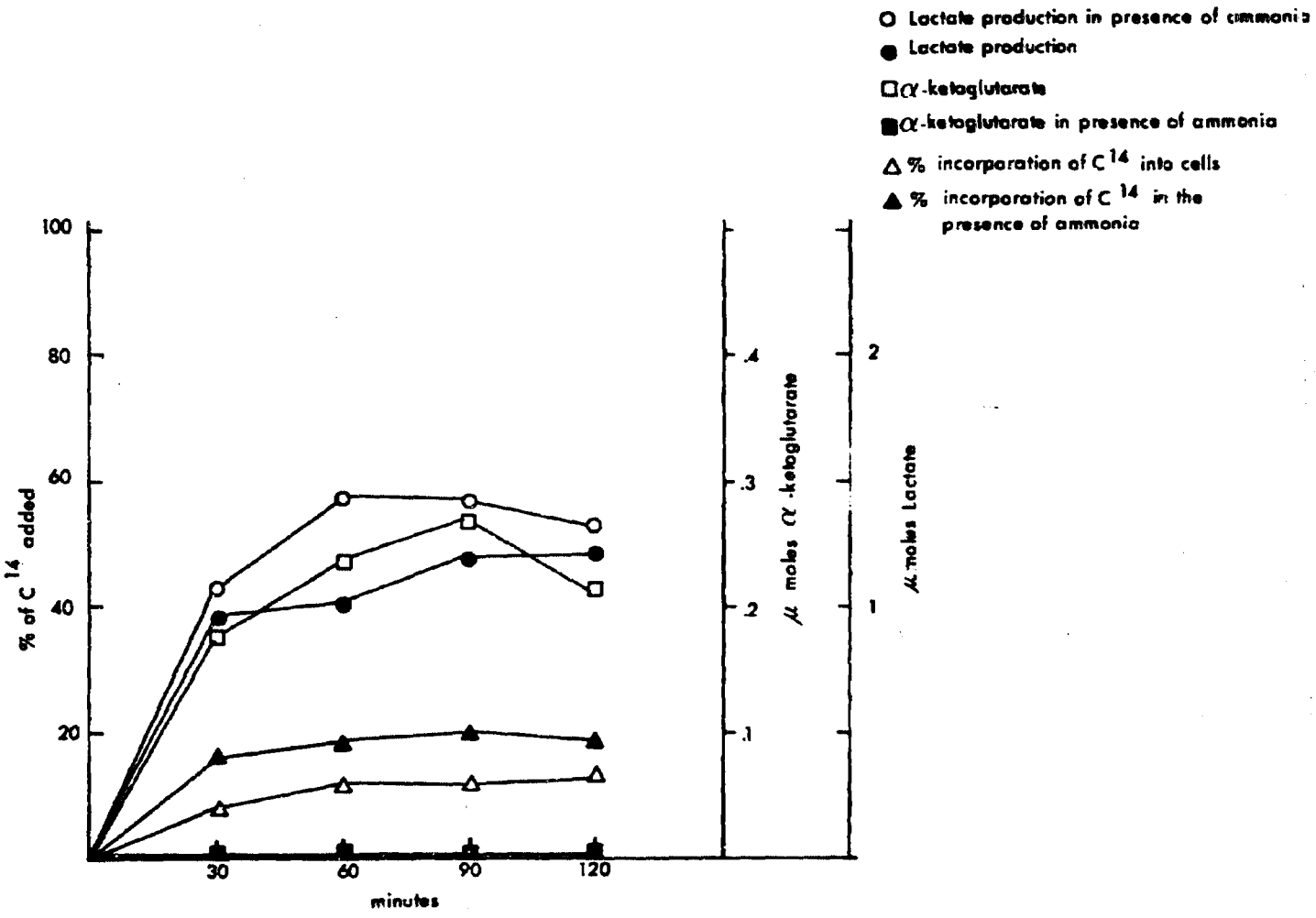

FIGURE 3. Cellular Uptake of Radioactive Glucose and Production of Lactate and α-Ketoglutarate at 37 C in the Presence and Absence of 5 μM $(NH_4)_2SO_4$.

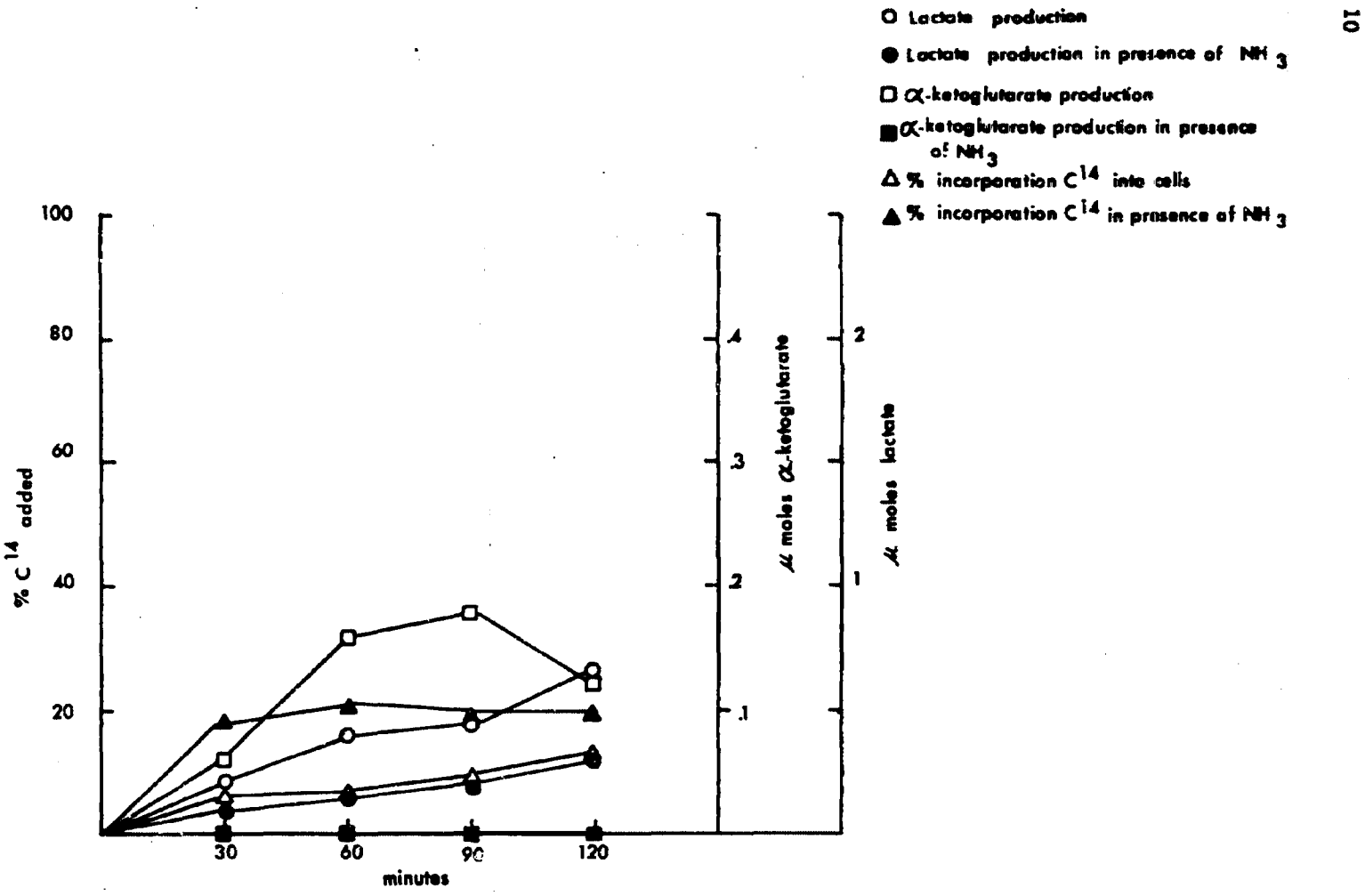

FIGURE 4. Cellular Uptake of Radioactive Glucose and Production of Lactate and α-Ketoglutarate at 26 C in the Presence and Absence of 5 μM $(NH_4)_2SO_4$.

TABLE 4. DISTRIBUTION OF C^{14} FROM 3 μc OF UNIFORMLY LABELED GLUCOSE INTO FRACTIONS OF P. PESTIS[a] AT 37 C AND 26 C IN THE PRESENCE AND ABSENCE OF 5 μM NH_3 ADDED AS $(NH_4)_2SO_4$

Condition	Time, min	Counts per Minute					
		Cold TCA-Soluble	Alcohol-Soluble	Alcohol-Ether-Soluble	Hot TCA-Soluble	Residual Protein	Unfractionated Washed Cells
37 C	30	32,760	17,054	9,388	7,020	3,398	67,168
37 C	90	38,620	27,190	13,558	14,220	5,446	105,221
37 C[b]	30	36,144	26,144	16,700	4,920	3,734	87,032
37 C[b]	90	37,700	38,800	18,460	9,600	9,764	115,230
26 C	30	14,742	6,770	3,876	2,392	1,130	33,853
26 C	90	23,932	6,210	5,850	6,512	3,098	52,506
26 C[b]	30	39,884	18,550	11,332	5,052	2,704	81,011
26 C[b]	90	50,020	37,704	21,222	11,584	5,030	128,006

a. Glucose-grown cells (10 mg dry weight) diluted in 0.04 M PO_4 buffer, pH 7.4; 4 μM of carrier glucose added.

b. + NH_3.

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13. ABSTRACT

A lethal effect of glucose on aerated virulent (but not avirulent) cultures of Pasteurella pestis at 37 C observed by Wessman et al. in 1958 prompted further studies into the patterns of oxidative metabolism of the virulent Alexander strain at 37 and 26 C. Bacterial respiration was markedly stimulated by glucose; this may be associated under certain conditions with the previously demonstrated toxic effect of glucose. The inability of the pathogen to assimilate substantial quantities of cellular material, as well as its fermentative activity under aerobic conditions may also be involved. At 26 C in the presence of added NH_3, P. pestis assimilates as much glucose as at 37 C, but releases considerably less lactic acid into the supernatant fluids. The data are also consistent with the idea that at least a portion of the cellular material synthesized during oxidative assimilation may be due to the reincorporation of endogenously produced ammonia.

14. Key Words

*Pasteurella pestis
*Oxidation
*Glucose
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