Studies on the Mechanism of Action of Phenolic Disinfectants II

Patterns of Release of Radioactivity from *Escherichia coli* Labeled by Growth on Various Compounds

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Previous studies on the leakage of radioactivity from glutamate-C^{14} labeled *Escherichia coli* cells in the presence of phenolic disinfectants were extended to involve cells labeled by growth on glucose-U-C^{14}, acetate-C^{14}, adenine-C^{14}, sulfate-S^{35}, and phosphate-P^{32}. In general, the phenolic disinfectants considered caused varying amounts of radioactivity to be released from the labeled cells. The phenol derivatives did not cause leakage of significant amounts of radioactivity from heat-treated cells, and cells exposed to *n*-butanol (which breaks the osmotic barrier) did release additional radioactivity when treated with *p*-chloro-*m*-xylenol or phenol, but the total counts released under the experimental conditions did not exceed that with further butanol treatment. Cells labeled with P^{32}, once treated with *p*-chloro-*m*-xylenol, continued to release radioactivity in saline buffer but glucose-C^{14}-labeled cells did not. The amount of radioactivity released from variously labeled cells in the presence of phenol, *p*-chloro-*m*-xylenol, or *n*-butanol was affected by temperature.

In previous studies (1), it was shown that *Escherichia coli*, when labeled by incubation with glutamate-C^{14}, would lose radioactivity when exposed to phenolic disinfectants. This loss of radioactivity was proportional to the concentration of the phenol derivative and was caused by all of the phenols tested. It was assumed that the radioactivity lost could be due to extrusion of cell contents through a damaged cell membrane. This hypothesis was further investigated by studying the release of radioactivity in the presence of phenol derivatives from *E. coli* labeled by growth on a number of radioactive compounds.

METHODS AND MATERIALS

Materials.—Only reagent grade chemicals were used and biochemicals were obtained from Nutritional Biochemicals Corp. or the California Corp. for Biochemical Research. The radioactive compounds were obtained either from the California Corp. for Biochemical Research, New England Nuclear Corp., or Abbott Laboratories. The labeled organic compounds had the following specific activities (mc./mmole): glucose-U-C^{14}, 4; sodium acetate-1,2-C^{14}, 10.5; adenine-8-C^{14}, 14.4; sodium glutamate-3,4-C^{14}, 2.3. Phosphorus^{32} was purchased as NaH_2PO_4 in solution with an activity of 10 μc./5 ml., and sodium sulfate-S^{35} was obtained with an activity of 50 μc./5 ml. The organic compounds were dissolved in distilled water to give a concentration of 2 μc./ml. A number of the phenol derivatives were a gift of the Ottawa Chemical Co., Toledo, Ohio.

Labeling of Bacteria.—The procedure for labeling *Escherichia coli* ATCC 11229 (the strain used throughout) with glutamate-C^{14} has been previously described (1). The synthetic medium (C) of Roberts, *et al.* (2), was used for growth as previously. Glucose-C^{14}, sodium sulfate-S^{35}, or sodium phosphate-P^{32} were added to the medium at the time of inoculation. In the case of P^{32}, the total phosphate content of the medium was reduced to one-fourth of the usual to permit a greater uptake of P^{32}. Approximately 1–2 μc. of glucose-C^{14} or sodium phosphate-P^{32}, or 10–20 μc. of sodium sulfate-S^{35} were added to 200 ml. of medium in a 500-ml. Erlenmeyer flask. The culture flasks were incubated on a Burrell wrist action shaker for 24 hours at 37° and harvested by centrifugation, followed by three washes with cold saline to remove any medium constituents. In the case of adenine-C^{14} and sodium acetate-C^{14}, normally two flasks (200 ml. of medium per flask) were grown for 24 hours, as above, without the addition of the labeled compounds. The cells were harvested by centrifugation and resuspended in one flask of fresh medium containing 2 μc. of radioactive acetate or adenine. The flask was shaken for an additional 3 hours at 37°, after which the cells were harvested and washed as above. During growth on labeled organic compounds, the flasks were attached to a U-tube containing soda lime to trap $C^{14}O_2$.

The washed, labeled cells were suspended in saline to give a concentration of 4×10^{10} cells/ml., as determined by turbidity readings previously calibrated with plate counts.

Measurement of Release of Radioactivity.—Basically, the previously described procedures (1) were used throughout. Phenol derivatives (except phenol) were dissolved in 25% v/v ethanol. Reaction mixtures contained, in a total volume of 3.0 ml., 2.4×10^{10} cells, pH 7.2 phosphate buffer in a final concentration of 0.02 *M*, the phenol derivative in a volume of 0.5 ml. (or made up to 0.5 ml. with 25% v/v ethanol) and enough saline

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to bring to volume. The reaction mixtures were incubated at 22 ± 2° for a total of 10 minutes and centrifuged for 20 minutes. Aliquots of the supernatant and the cells were dried on 1¼ inch, concentric ring stainless steel planchets, and radioactivity was assayed in a Nuclear-Chicago thin window gas flow counter.

In the experiments in which the amount of radioactivity released was measured at time intervals, a number of replicate reaction mixtures were prepared and removed at the indicated times for centrifugation (5 minutes at 14,000 r.p.m.).

RESULTS AND DISCUSSION

A bactericidal substance may act by a number of possible mechanisms which could be roughly categorized as either metabolic or physical-chemical (structural damage). Undoubtedly, a given substance may combine these mechanisms. In the case of phenolic disinfectants, there has been relatively little work on metabolic effects. Recently published data (1, 3–10) stress the effects of phenol derivatives on permeability mechanisms and suggest that damage to the latter are responsible ultimately for their killing effect. The results shown in Table I would tend to indicate that in the concentrations used, phenol derivatives do cause leakage of radioactivity from *Escherichia coli* labeled by growth on a number of compounds. The highest concentration used of each phenol derivative was adequate to cause at least 99% killing or more under the experimental conditions (1) except 2,4,6-trichlorophenol which caused 32% killing and 2,4-dichloro-*m*-xylenol, 97% killing.

Roberts, *et al.* (2), labeled *E. coli* by growth on a number of radioactive compounds (such as glucose, acetate, purines, sulfate-S^{35}, and phosphate-P^{32}) and fractionated the cells to determine into which type of compounds the label was incorporated. For example, inorganic sulfate was incorporated primarily into protein; phosphate into nucleic acids primarily but also phospholipids and metabolic intermediates; acetate equally into lipids and protein; and glucose, primarily into protein but also nucleic acids, lipids, and some metabolic intermediates. It was hoped that if phenol derivatives would cause release of radioactivity from cells labeled by growth on specific compounds to a much greater degree than when labeled with other compounds, some insight might be obtained as to what the substrate, in general terms, might be for phenols.

The data in Table I would seem to suggest that phenol derivatives cause release of the highest per cent of radioactivity of cells labeled by growth on adenine-C^{14} or phosphate-P^{35}, both of which are reported as being incorporated primarily into nucleic acids. Both DNA and RNA have been detected in isolated membranes (11, 12) although nucleic acids, nucleotides, and other phosphorus compounds could also originate from within the cell. Joswick (10) has reported the release of 260 mμ-absorbing substances (purines and pyrimidines) from bacteria in the presence of hexachlorophene, and the release of 260 mμ-absorbing substances is generally taken as an index of damage to the mechanisms which control permeability of the cell.

The substantial amounts of radioactivity released from cells labeled by growth on sulfate-S^{35}, which is incorporated primarily into protein, could also originate in the cell membrane which is known to contain considerable protein (13). Radioactivity from acetate-C^{14} cells, which is reported to be incorporated about equally into

TABLE I.—RELEASE OF RADIOACTIVITY FROM LABELED *Escherichia coli*[a] IN THE PRESENCE OF VARIOUS PHENOLIC DISINFECTANTS

Treatment		Net[b] Per Cent of Radioactivity Released from Cells Labeled by Growth on					
		Glucose-C^{14}	Acetate-C^{14}	Adenine-C^{14}	$Na_2S^{35}O_4$	$NaH_2P^{32}O_4$	Glutamate-C^{14}
p-Chloro-*m*-xylenol	167 mcg./ml.	1.3	1.4	2.6	3.8	3.6	...
	250 mcg./ml.	2.7	3.1	5.5	5.8	3.9	...
	333 mcg./ml.	3.0	3.7	8.7	6.2	9.1	39.2
Phenol	3.3 mg./ml.	...	0.8	0.5	...	3.8	...
	8.3 mg./ml.	...	3.5	3.1	4.0	10.1	...
	12.5 mg./ml.	3.7	6.1	6.3	5.0	12.7	54.3
p-Chloro-*m*-cresol	333 mcg./ml.	1.4	1.0	2.4	0.5	8.2	24.3
	666 mcg./ml.	3.2	2.8	7.4	4.5	11.1	37.6
p-Chloro-*o*-cresol	333 mcg./ml.	1.2	0.8	2.2	0.3	6.9	11.8
	666 mcg./ml.	2.6	4	5.1	4.4	12.3	26.2
2,4-Dichloro-*m*-xylenol	41.7 mcg./ml.	0.3	...	...	0.2	...	15.8
	83 mcg./ml.	1.5	...	...	3.1	...	...
2,4-Dichlorophenol	333 mcg./ml.	2.0	0.8	...	0.4	...	8.9
	666 mcg./ml.	3.3	1.1	...	4.1	...	29.0
2,4,6-Trichlorophenol	167 mcg./ml.	0.3	...	...	0	...	...
	333 mcg./ml.	0.6	...	...	0.1	...	14.0
Hyamine 3500,[c] 1:500							50.0
1:1000		14.7	24.8	8.2	7.4	...	...
n-Butanol, 5% v/v		8.7	13.9	10.4	1.6	7.2	...
Heated in boiling water bath for 5 min.		8.4	9.1	18.3	14.2	17.2	...
Saline-buffer control		1.9	1.5	2.5	0.9	5.7	4.9
Ethanol (4.2% v/v)-buffer control		2.2	1.8	2.9	1.1	5.6	21.9

[a] The cell concentrations in the reaction mixtures were 8 × 10^9 cells/ml., with the exception of the cells labeled with glutamate-C^{14} which was 3.8 × 10^9 cells/ml. The labeled cells had approximately the following activities (c.p.m./10^9 cells): glucose-C^{14}, 300; acetate-C^{14}, 300; adenine-C^{14}, 2600; sulfate-S^{35}, 1500; phosphate-P^{32}, 40 to 186; glutamate-C^{14}, 520. [b] The radioactivity released in the appropriate control (ethanol-buffer for phenolic disinfectants except phenol and saline-buffer for others) was subtracted to obtain the net leakage. [c] Alkyl (C_{12}–C_{16}) dimethyl benzyl ammonium chloride, Rohm and Haas Co., Philadelphia, Pa.

lipids and proteins (2) of *E. coli*, is also released. The greatest release was found with glutamate-C^{14}-labeled cells and, although it is not clear as to what the glutamate is incorporated into, it is rapidly incorporated into nondiffusible compounds (2) and would not be simply adsorbed to the cells. While it cannot be said that the data in Table I indicate damage to the cell membrane as the basis for the lethal action of phenols, they are compatible with this hypothesis.

It would seem that the source of the radioactivity released from labeled cells must be due either to weakening or destruction of the permeability barrier of the cell, or "uncoupling" of protoplasmic constituents, or a combination of the two. If phenolic disinfectants act by affecting the permeability barriers, they should not cause release of additional radioactivity from cells whose permeability barrier has been destroyed. This can be accomplished by heating the cells, which causes a rapid release of the cellular pool and a certain amount of cytoplasmic degradation (14), or treatment with *n*-butanol, which breaks the osmotic barrier (15).

In Tables II, III, and IV, it can be seen that neither phenol, *p*-chloro-*m*-xylenol (PCMX), or butanol caused significant additional release of radioactivity from heated cells, thus indicating little or no additional cytoplasmic degradation. These results are in agreement with Beckett, *et al.* (14), who studied the release of 260 mμ-absorbing exudate in heat-treated *E. coli*, followed by hexylresorcinol. When labeled cells were first treated with butanol, and then retreated with phenol, *p*-chloro-*m*-xylenol, or butanol, the total counts released never exceeded that obtained with butanol, although the rate of release varied. Beckett, *et al.* (14), concluded from their data that hexylresorcinol caused extensive intracellular uncoupling because much more exudate was released from *E. coli* after preliminary treatment with butanol (which broke the osmotic barrier) than without preliminary treatment of the cells. The data in Tables II, III, and IV seem to be more compatible with the contention that the phenol derivatives considered, like butanol, act by breaking the permeability barrier.

It is interesting to speculate as to whether the alleged damage caused to the cell membrane is quantitatively related to the amount of phenol derivative to which the cell is exposed or whether the effect is such that once small damages are caused, they rapidly enlarge and leakage proceeds continuously. Experimentally, this was approached by treating cells with *p*-chloro-*m*-xylenol, and then exposing these cells to either saline-buffer, butanol, or additional treatments with *p*-chloro-*m*-xylenol. The results obtained are given in Tables V and VI. It would seem that phosphate-P^{32}-labeled cells continued to release some radioactivity once treated with *p*-chloro-*m*-xylenol, and additional exposure to the latter did not markedly affect the release of

TABLE II.—ABILITY OF PHENOL AND *p*-CHLORO-*m*-XYLENOL TO CAUSE RELEASE OF RADIOACTIVITY FROM HEATED OR *n*-BUTANOL-TREATED *Escherichia coli*[a] LABELED BY GROWTH ON SULFATE-S^{35}

Original Treatment of Cells[a]	Extraction Medium	Original Treatment	Radioactivity Released, c.p.m. Extraction No. 1	2	3	Total	Radioactivity Left in Cells, c.p.m.
Heated in boiling water bath for 5 min.	Phenol (12.5 mg./ml.)	4155	126	63	45	4389	15,292
	PCMX (333 mcg./ml.)	4329	105	57	36	4527	15,292
	n-Butanol (5% v/v)	4173	153	66	66	4458	14,683
	Ethanol (4.2% v/v)-buffer	4233	141	27	33	4434	15,042
	Saline-buffer	4188	146	45	33	4412	14,556
n-Butanol (5% v/v)	Phenol (12.5 mg./ml.)	885	1773	966	444	4068	16,983
	PCMX (333 mcg./ml.)	1272	966	765	720	3723	18,144
	n-Butanol (5% v/v)	1012	1257	822	996	4087	17,795
	Ethanol (4.2% v/v)-buffer	1215	852	438	396	2901	18,163
	Saline-buffer	1737	1215	597	423	3972	17,508

[a] The cells had an activity of 1182 c.p.m./10^9 cells.

TABLE III.—ABILITY OF PHENOL AND *p*-CHLORO-*m*-XYLENOL TO CAUSE RELEASE OF RADIOACTIVITY FROM HEATED OR *n*-BUTANOL-TREATED *Escherichia coli*[a] CELLS LABELED BY GROWTH ON ADENINE-C^{14}

Original Treatment of Cells	Extraction Medium	Original Treatment	Radioactivity Released, c.p.m. Extraction No. 1	2	3	Total	Radioactivity Left in Cells, c.p.m.
Heated in boiling water bath for 5 min.	Phenol (12.5 mg./ml.)	16,708	435	314	165	17,622	20,491
	PCMX (333 mcg./ml.)	17,737	434	185	195	18,551	20,491
	n-Butanol (5% v/v)	16,523	434	206	178	17,341	19,675
	Ethanol (4.2% v/v)-buffer	15,686	435	100	101	16,322	20,156
	Saline-buffer	17,515	399	103	147	18,163	19,505
n-Butanol (5% v/v)	Phenol (12.5 mg./ml.)	8,473	3437	8849	374	21,133	22,757
	PCMX (333 mcg./ml.)	8,599	2023	3226	2,285	16,133	24,313
	n-Butanol (5% v/v)	8,422	1971	4299	10,235	24,928	23,845
	Ethanol (4.2% v/v)-buffer	8,086	1742	1461	3,016	14,305	24,338
	Saline-buffer	7,694	1964	4515	6,101	20,279	23,461

[a] The cells had an activity of 2049 c.p.m./10^9 cells.

radioactivity. However, glucose-C^{14}-labeled, *p*-chloro-*m*-xylenol-treated cells did not release additional radioactivity when extracted with saline-buffer or ethanol-buffer. Also additional treatment with *p*-chloro-*m*-xylenol did not cause release of much additional radioactivity.

It is generally assumed that the antimicrobial activity of phenol increases with temperature (16, 17) and one would presume that phenomena associated with killing of bacteria by phenol derivatives should respond similarly. Table VII presents the per cent of radioactivity released from variously labeled cells in the presence of phenol, *p*-chloro-*m*-xylenol, and *n*-butanol at three temperatures. In general, an increase in temperature caused an increase in release of radioactivity. The only exception occurred with sulfate-S^{35}-labeled cells. It would seem that the latter observation coupled with the low release of radioactivity caused in sulfate-S^{35}-labeled cells by butanol would suggest an origin of the radioactivity which might be different from that obtained from cells labeled in other ways. Perhaps the fact that phenol derivatives cause the release of more counts from sulfate-S^{35}-labeled cells than butanol may indicate that some of the counts are coming from surface layers rather than low molecular weight compounds which would be released simply by breaking the osmotic barrier. Joswick (10) described a biphasic release of cell exudate caused by hexachlorophene which is also known to occur with other surface-active detergents (18) and polymyxin (19). He considers the leakage at 0° to be related to membrane damage, and at higher temperatures to be related to autolysis.

TABLE IV.—ABILITY OF PHENOL AND *p*-CHLORO-*m*-XYLENOL TO CAUSE RELEASE OF RADIOACTIVITY FROM HEATED OR *n*-BUTANOL-TREATED *Escherichia coli* CELLS[a] LABELED BY GROWTH ON GLUCOSE-C^{14}

Original Treatment of Cells	Extraction Medium	Original Treatment	Radioactivity Released, c.p.m. Extraction No. 1	2	3	Total	Radioactivity Left in Cells c.p.m.
Heated in boiling water bath for 5 min.	Phenol (12.5 mg./ml.)	877	66	31	71	1045	6512
	PCMX (333 mcg./ml.)	856	46	22	17	940	5773
	n-Butanol (5% v/v)	785	53	40	31	908	5372
	Ethanol (4.2% v/v)-buffer	899	40	23	25	987	5597
	Saline-buffer	936	59	18	30	1043	7170
n-Butanol (5% v/v)	Phenol (12.5 mg./ml.)	623	409	189	37	1258	6027
	PCMX (333 mcg./ml.)	634	352	270	92	1348	6050
	n-Butanol (5% v/v)	491	509	282	79	1361	6378
	Ethanol (4.2% v/v)-buffer	572	292	197	58	1118	7310
	Saline-buffer	555	236	253	47	1091	6490

[a] The cells had an activity of 390 c.p.m./10^9 cells.

TABLE V.—EFFECT OF RE-EXPOSURE TO *p*-CHLORO-*m*-XYLENOL ON RELEASE OF RADIOACTIVITY FROM *Escherichia coli*[a] LABELED BY GROWTH ON PHOSPHATE-P^{32}

Original Treatment of Cells	Extraction Medium	Original Treatment	Radioactivity Released, c.p.m. Extracton No. 1	2	3	Total	Radioactivity Left in Cells c.p.m.
PCMX, 333 mcg./ml.	Saline-buffer	294	424	293	167	1178	1183
	Ethanol (4.2% v/v)-buffer	276	276	179	183	914	1306
	PCMX (333 mcg./ml.)	258	385	349	140	1132	1183
	n-Butanol (5% v/v)	274	340	431	204	1249	1034
Ethanol (4.2% v/v)-control	Ethanol (4.2% v/v)-buffer	166	122	69	47	403	1794

[a] The cells had an activity of 117 c.p.m./10^9 cells.

TABLE VI.—ABILITY OF *p*-CHLORO-*m*-XYLENOL AND *n*-BUTANOL TO CAUSE RELEASE OF RADIOACTIVITY FROM *p*-CHLORO-*m*-XYLENOL-TREATED *Escherichia coli*[a] LABELED BY GROWTH ON GLUCOSE-C^{14}

Original Treatment of Cells	Extraction Medium	Original Treatment	Radioactivity Released, c.p.m. Extraction No. 1	2	3	Total	Radioactivity Left in Cells c.p.m.
PCMX, 333 mcg./ml.	Saline-buffer	423	104	46	46	619	7818
	Ethanol (4.2% v/v)-buffer	405	85	53	65	608	8046
	PCMX (333 mcg./ml.)	425	140	131	162	858	7499
	n-Butanol (5% v/v)	436	224	75	65	800	7118
Ethanol (4.2% v/v)-control	Saline-buffer	225	84	36	42	387	7866
	Ethanol (4.2% v/v)-buffer	204	84	51	64	403	7953
	PCMX (333 mcg./ml.)	243	290	110	141	784	7690
	n-Butanol (5% v/v)	207	332	107	107	753	7370

[a] The cells had an activity of 368 c.p.m./10^9 cells.

However, in the experiment for which results are presented in Table VII, insufficient time would have elapsed for significant autolysis.

Figures 1 and 2 indicate the rates of release of radioactivity from either phosphate-P^{32} or sulfate-S^{35}-labeled cells in constant contact with *p*-chloro-*m*-xylenol. The difference in the shapes of these two curves also suggest the mechanism of release and/or the origin or identity of the radioactive compounds released may be different. The control curves indicate a greater tendency for leakage after 1 to 2 hours, of P^{32}-labeled compounds than of S^{35}-labeled compounds.

In general, the observations presented above and others would suggest that phenols exert their lethal action by physical damage to the permeability barriers. It is quite reasonable to expect that a relatively minor amount of physical damage, such as alteration in permeability without lysis, could be lethal. For example, Strauss (20) reported the formation of a lethal permeability defect in *E. coli* by a mutagenic alkylating agent, ethyl sulfate. On the other hand, Nathan (21) described a permeability change in *Lactobacillus plantarum* caused by chlorpromazine which was nonlethal. It must still be recognized that evidence regarding permeability damage and lethal effects of phenols is circumstantial and, as has been shown by Stedman, *et al.* (22), and Kravitz, *et al.* (23), the total effect of a germicide (a quaternary ammonium compound in their studies) may involve, to different extents, leakage of cellular materials and inhibition of energy yielding reactions. The contribution of each of these effects may depend upon a number of conditions, and varying the latter will vary the influence of a given effect. There are a number of additional approaches to examining the mechanism of the lethal effects of phenolic disinfectants, such as determining the cytological site of uptake of these compounds. If the cell membrane is the site of action of phenolic disinfectants, one should be able to assume that the latter would be bound to the membrane and concentrate there, at least until the integrity of the latter is destroyed and the germicide penetrates into the cell. It would be useful to determine the relative distribution of labeled phenolic disinfectants in the usual fractions obtained in cell fractionation, or even better, among the basic structural fractions, such as the cell wall, cell membrane, and cytoplasm. Beckett, *et al.* (4, 5), have already shown that cell walls from *E. coli* do not bind hexylresorcinol although the whole cell does.

More precise knowledge of the identity of the substances which leak from the cell would be useful for determining to what extent phenol derivatives

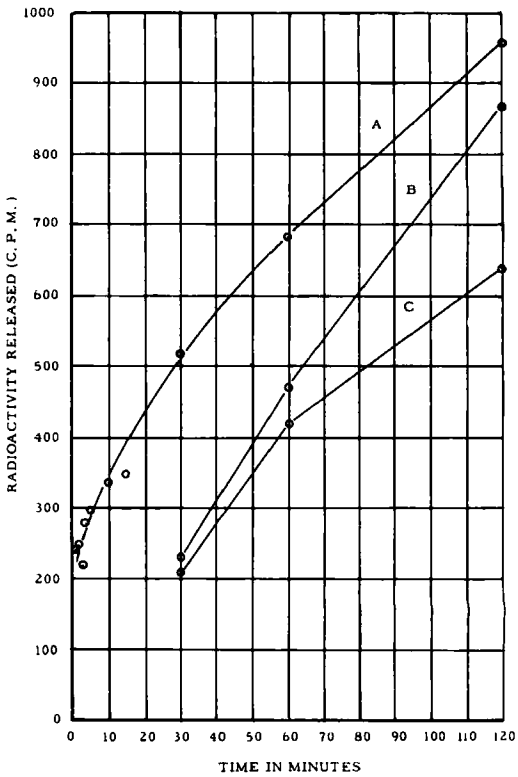

Fig. 1.—Release of radioactivity from *Escherichia coli* labeled by growth on phosphate-P^{32}. The activity of the cells was 116 c.p.m./10^9 cells. A, Cells exposed to 333 mcg./ml. of *p*-chloro-*m*-xylenol; B, saline-buffer control; C, ethanol (4.2% v/v)-buffer control.

TABLE VII.—EFFECT OF TEMPERATURE ON PHENOL, *p*-CHLORO-*m*-XYLENOL, AND *n*-BUTANOL-CAUSED RELEASE OF RADIOACTIVITY FROM LABELED *Escherichia coli* CELLS[a]

Treatment	Temperature, °C.	Radioactivity Released from Cells Labeled by Growth, % Glucose-C^{14}	Adenine-C^{14}	$Na_2S^{35}O_4$	$NaH_2P^{32}O_4$
Phenol, 12.5 mg./ml.	0	2.3	4.4	3.8	6.5
	22	4.8	14.6	11.0	18.4
	40	7.6	48.0	7.6	...
PCMX, 333 mcg./ml.	0	2.2	6.8	5.9	8.9
	22	3.5	7.0	7.1	17.9
	40	4.4	43.2	7.6	...
n-Butanol, 5% v/v	0	2.0	4.2	3.6	...
	22	6.6	8.2	14.2	...
	40	8.4	49.1	9.4	...
Ethanol (4.2% v/v)-buffer control	0	...	2.3	...	4.9
	22	...	3.1	...	5.6
	40	...	7.9	...	...
Saline-buffer control	0	0.7	2.1	0.7	2.5
	22	1.4	2.7	2.1	5.6
	40	1.9	5.8	1.6	...

[a] The labeled cells had approximately the following activities, c.p.m./10^9 cells: glucose-C^{14}, 269; adenine-C^{14}, 2656; sulfate-S^{35}, 1892; and phosphate-P^{35}, 40.

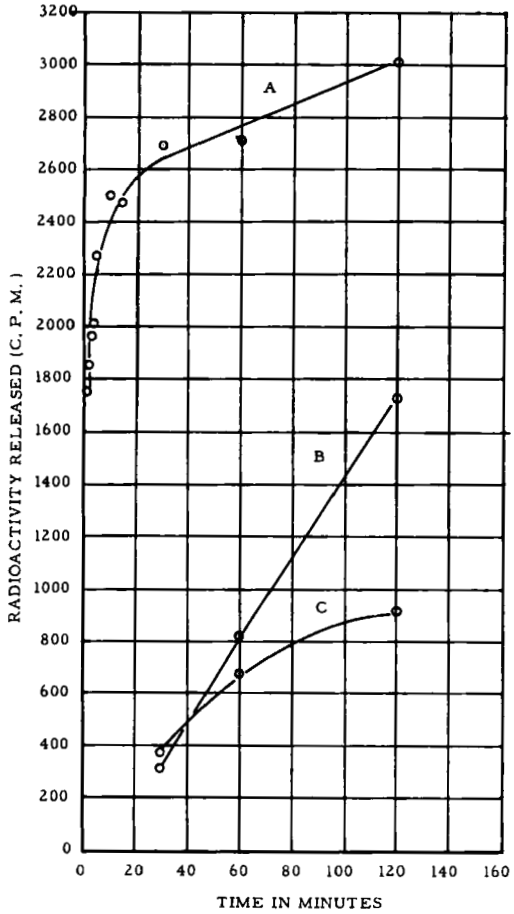

Fig. 2.—Release of radioactivity from *Escherichia coli* labeled by growth on sulfate-S^{35}. The cells had an activity of 1650 c.p.m./10^9 cells. A, Cells exposed to 333 mcg./ml. of *p*-chloro-*m*-xylenol; B, saline-buffer control; C, ethanol (4.2% v/v)-buffer control.

cause cytoplasmic degradation following membrane disruption. Bean and Walters (24) have shown that benzylchlorophenol-induced cell exudate from *E. coli* influenced survival in a bactericidal system and interpreted their data to indicate that the last survivors utilize the released cell constituents as nutrients. Also to be explored are the effects on metabolism of the germicidal derivatives of phenol, and even more challenging, the biochemical and biophysical basis for the relative nontoxicity to animals (25) of certain potent phenolic disinfectants such as *p*-chloro-*m*-xylenol and 2,4-dichloro-*m*-xylenol.

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