des différences structurales profondes de ces carbures, les phénomènes suivants:

1° La substance est solubilisée dans l'eau et son spectre est nettement lisible dans l'UV. sous l'épaisseur de 0,1 mm; la formation d'un complexe hydrosoluble histamine-substance est mise en évidence.

2° Toutes les bandes sont décelables: la fixation se fait donc par molécules entières, hypothèse déjà entrevue par la théorie mathématique[10] et l'expérimentation biologique[11].

3° Ces bandes, comparées à leur position lorsque la substance est observée dans l'eau ou dans l'alcool, sont déplacées vers le visible. L'effet bathochrome traduit une activation de la molécule.

Les substances non cancérigènes de structure analogue à celle du benzopyrène (pérylène et pentacène) ne forment le complexe qu'à l'état de traces difficilement décelables. Les courbes ci-jointes illustrent les résultats acquis.

Le 3,4-benzo-phénanthrène n'est pas excepté du choix de l'histamine.

Les substances stilbéniques étudiées sont: le 4-amino-stilbène et le *p*-diméthyl-amino-stilbène, substances très cancérigènes. Elles conduisent à des résultats analogues.

L'étude des substances azoïques présente une difficulté du fait que le groupement azoïque et que la fonction amine sont déjà susceptibles à eux seuls d'assurer avec des amines la formation de complexes. Aussi, afin de mettre en évidence des particularités d'action de l'histamine sur les substances cancérigènes, l'observation est conduite à la fois sur d'autres amines et sur d'autres substances non cancérigènes qui présentent avec les corps cancérigènes azoïques une parenté structurale.

Les substances azoïques étudiées sont: l'azobenzène, substance non cancérigène, le méthoxy-3-azobenzène, substance non cancérigène, le *p*-amino-azobenzène, substance peu cancérigène, le diméthyl-3,2'-amino-4-azobenzène, substance très cancérigène, le 3'-méthyl-4-diméthyl-aminoazobenzène, substance très cancérigène. On observe que l'effet bathochrome s'accuse dès que le caractère cancérigène apparaît et ce comportement semble spécial à l'histamine qui, dans le cas actuel, a été comparé à l'imidazol et à l'éthylamine.

Il devenait important de montrer que de tels complexes pouvaient se former *in vivo* aux dépens de l'histamine des nerfs. Cette 2e partie a été faite avec le concours de CHAMPY.

Nous avons utilisé la réaction histologique de l'histamine mise au point avec le nitrite mercureux et l'acide osmique[12], qui permet une bonne et fine coloration des filets nerveux notamment des fibres sympathiques dont l'action paraît essentielle dans les phénomènes neurotrophiques.

Nous avons employé la méthode suivante: un peu de substance à éprouver est portée au contact d'une glande et, après un temps tel que la pénétration de la dite substance généralement assez lente à cause de son insolubilité ait pu se faire, nous avons fait des préparations au nitrite mercureux-acide osmique. Nous avons naturellement comparé avec des substances non cancérigènes qui, *in vitro*, ne se complexent pas avec l'histamine[13].

Avec tous les corps cancérigènes étudiés, nous avons vu que la réaction manquait à une certaine profondeur au niveau des points où on avait appliqué la substance sous une épaisseur de 5 à 10 acini, où il ne reste de colorable que de rares gros tronçons de filaments nerveux, ce qui montre que la réaction s'est bien faite. Elle est d'ailleurs très régulière sur l'objet considéré. Nous avons observé très nettement ce phénomène avec le benzopyrène, le 3,4-benzo-phénanthrène, le 4-amino-stilbène, le diméthyl-3,2'-amino-4-azobenzène.

Le 3,4-benzo-phénanthrène, substance peu cancérigène, semble détruire les nerfs moins profondément.

La même expérience faite avec des corps voisins non cancérigènes: le pérylène, l'azobenzène, l'anthracène, le phénanthrène, le pentacène, permet une coloration histaminique des nerfs dès la surface comme dans les tissus normaux.

Il se produit donc *in vivo* comme *in vitro* une fixation de l'histamine par les substances cancérigènes.

On peut rapprocher l'ensemble de ces résultats du fait que l'histamine est absente ou très diminuée dans les tumeurs malignes[14,15] et de cet autre fait que, sous l'action du 48/80, composé qui déplace l'histamine de ses localisations normales et la fait disparaître de la peau, on augmente la tumorogenèse [16–19].

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Summary

Histamine, which is a constituent of neurons shows a particular affinity for cancer-producing substances but not for the non-cancer-producing isomers of such substances.

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Localization of Succinic Oxidase in *Vibrio cholerae*

In view of conflicting evidence on the nature and function of various cell inclusions in bacteria[1] and the importance of 'particulate fractions' in their immunology and chemotherapy, isolation and biochemical characterization of different cell components would be of particular interest in pathogenic organisms[2,3]. Studies have, therefore, been undertaken on *V. cholerae* to localise various enzymes and other important biochemical materials in the cell. The present note describes some preliminary results on the distribution of 'succinic oxidase' in different intracellular fractions obtained from disrupted *V. cholerae* cells by differential centrifugation.

18–20 h growth of *V. cholerae* on meat-papain-digest-agar was harvested with normal saline and washed once by recentrifugation. The cells were disrupted either by

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Table I
Distribution of 'succinic oxidase' activity and nitrogen in different fractions obtained by ultrasonic treatment of *Vibrio cholera* cells

Fractions	Total Nitrogen in mg	% Nitrogen of C. E.	Total activity	% activity of C. E.	Specific activity
C. E.	697·7	—	57420	—	82·2
26·4*p*60	113·8	16·3	28170	49·1	247·4
92*p*120	76·8	11·1	9456	16·8	120·3
92*s*120	437·7	62·6	14758	25·7	33·3
Total recovery	—	90·0	—	91·6	—

ultrasonic treatment or grinding with acid-washed glass powder in a chilled pestle and mortar. A suspension of the disrupted cells in buffered lactose solution (8·5% lactose in *M*/50 phosphate buffer pH 7·0) was centrifuged at 4200 *g* (the '*g*' values refer to the centrifugal force at the bottom of the tube) to settle down the non-ruptured cells and some of the larger cell debris. The supernatant was designated as the crude extract (C. E.). A known volume of this was subjected to differential centrifugation, and the enzyme activities were determined in particulate fractions sedimented up to a gravitational force of 92,000 *g* and in the supernatant obtained at this speed (the latter has been referred to as 'supernatant').

'Succinic oxidase' and cytochrome oxidase were estimated by measurement of oxygen uptake by conventional Warburg's manometric technique (using succinic acid and *p*-phenyline diamine as substrates respectively)[4,5] at pH 8·0 and 37°C. Direct determination of succinic dehydrogenase was made according to the method of SLATER[6]. A balance sheet of the total enzyme activities in different fractions was drawn according to the suggestion of ALEXANDER and WILSON[4].

In case of crude extract obtained by ultrasonic treatment, about 50% of 'succinic oxidase' activity was found to be associated with relatively opaque sediment obtained at 26400 *g* in 1 h (26·4*p*60[7]). The remaining activity was more or less equally shared by the more transparent and red jelly-like particulate material 92*p*120 and the clear yellowish supernatant 92*s*120 (Table I). However, the specific activity (activity per mg nitrogen) of the supernatant was considerably lower than that of the particulate components. Further, the specific activity of 92*p*120 was only about half that of the 26·4*p*60, thus exhibiting a considerably higher concentration of this enzyme system in the fraction that sediments at relatively low speeds.

The results obtained by disrupting the cells with glass powder further demonstrated association of almost entire 'succinic oxidase' activity with the particulate material (Table II). Here again the specific activities of the sediments 7·5*p*30, 25*p*30, and 92*p*75 were found to be in a decreasing order, though the maximum difference among these fractions was not more than 25%. However, their specific activities were 45 to 60 times those of the supernatant, which contained merely about 5% of the total activity of the crude extract, out of a total recovery of about 75%. Direct determination of the 'succinic dehydrogenase and cytochrome oxidase' in different fractions (obtained from glass disrupted cells) further confirmed the localization of succinic oxidase system predominantly in the particulate material. Specific activities of these enzymes were again found to be relatively low in case of particles obtained at higher speeds; the difference being particularly more marked with respect to cytochrome oxidase which also showed a relatively higher recovery in the supernatant.

Table II
Distribution of 'succinic oxidase' and its components in *Vibrio cholerae* disrupted by grinding with glass powder

Fractions	Total Nitrogen mg	Succinic oxidase		Succinic dehydrogenase		Cytochrome oxidase	
		% of C. E.	Specif. activ.	% of C. E.	Specif. activ.	% of C. E.	Specif. activ.
C. E.	181·2	—	59·3	—	13·9	—	127·7
7·5*p*30	6·6	17·8	290·2	25·5	100·1	27·3	998·5
25*p*30	8·5	22·4	281·2	30·5	90·1	33·9	965·1
92*p*75	13·7	27·4	218·2	41·2	75·8	17·2	304·3
92*s*75	149·8	7·0	5·1	3·3	0·56	17·9	28·4
Total recovery	—	74·6	—	100·5	—	96·3	—

Localization of succinic oxidase, as well as a number of other enzyme activities associated with the mitochondria of higher organisms e.g., oxidation of malic acid, DPNH and cytochrome and also synthesis of energy-rich ATP bonds in the particulate material, has been reported in a number of other bacteria[8]. ALEXANDER observed some of these activities (including 'succinic oxidase') in *A. vinelandii* to be much more concentrated in the submicroscopic (60*p* and 144*p*) particles than in the larger 7*p*30 component and therefore suggested that the former might be functioning as the mitochondria in bacteria[4,8,9]. The results with *V. cholerae*, although broadly resembling the observations in *A. vinelandii* with regard to the distribution of nitrogen and cytochrome oxidase and also as to physical appearance of different fractions, do not show a higher concentration of succinic oxidase in the smaller particles; in fact the concentration appears to decrease with the particle size. Observations different from those of ALEXANDER and WILSON with regard to concentration of succinic dehydrogenase in larger and smaller particles have also been reported by DARTER and MILLMAN[10] in *Mycobacterium tuberculosis*.

STANIER[11], on the basis of observations of WEIBULL[12], suggested that the entire cell membrane (termed as the 'ghost') might be carrying out the mitochondrial function in bacteria and the isolated particles (showing the mitochondrial enzyme activities) might merely be the result of mechanical disintegration of this structure. This suggestion has recently been supported by the enzyme studies on the purified 'ghosts' in *B. megaterium*[13]. If the particles, bearing 'succinic oxidase' activity, really result from the fragile cell membrane due to mechanical rupturing of the cells, their size may depend on the extent of disintegration; and the differences between the smaller and larger particles in this respect may be merely arte-

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facts. Thus, a small proportion of the 'succinic oxidase' activity in the supernatant, and the observed decrease in the concentration of this enzyme system in the smaller particles, particularly in the case of ultrasonic treatment, can probably be explained in terms of damage of the membrane structure[14].

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Central Drug Research Institute, Lucknow (India), December 18, 1958.

Zusammenfassung

Verschiedene Fraktionen des mit Ultraschall, bzw. mit mechanischer Behandlung freigesetzten Zellinhaltes von *V. cholerae* wurden auf die Bernsteinsäuredehydrogenase, bzw. die Zytochromoxydasekomponente des Bernsteinsäureoxydase-Enzymkomplexes untersucht. Der letztere ist bei *V. cholerae* an Körnchen mit gewissen Teilchengrössen gebunden, deren Verwandschaft mit Mitochondrien besprochen wird.

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Formaldehyde and Formyl Group Intermediates of the Oxidation of Glyoxylate by Living Yeast and *E. coli* Cells

In a preceding series of experiments, it was established that glyoxylate is an intermediate of the direct oxidation of acetate and glycolate by yeast cells[1]. Further experiments[2,3] have excluded the possibility that glyoxylate might arise, instead of from acetate or glycolate, from malate or isocitrate by the action respectively of the malate synthetase or isocitritase, as was questioned by AJL[4] and UTTER[5]. We have now obtained new proofs on the proper position of glyoxylate in the monocarboxylic acid scheme (MAS) of oxidation of acetate by studying the oxidation of glyoxylate by yeast and *E. coli* cells and isolating its direct intermediates. In fact, from such experiments we were able to isolate formaldehyde and formyl group as was to be expected according to the formulation of the MAS:

acetate → acetyl → glycolate → glyoxylate →
→ formaldehyde → formyl → $H_2O + CO_2$

The present paper will report the results of these experiments. Details of the procedures employed for the preparation of yeast[6] and *E. coli*[7] cells suspensions have been described in previous papers. Formaldehyde was trapped by phenylhydrazine[7,8] or by the simultaneous presence of phenylhydrazine and dimedone[9]. The formyl group was identified as benzaldehyde, which is the result of the formylation of the benzene ring of the phenylhydrazine added to the medium[6,7]. The data of two typical experiments with yeast and *E. coli* cells are assembled in the Table.

The tubes for aeration contained: tube A, 1000 ml of a 30% (w.v.) of starved baker's yeast cells suspension. Tube *B*, 1000 ml of a 12% (w.v.) of starved *E. coli* cells suspension. In both tubes 200 mg of glyoxylic acid (Fluka) were added, while the addition of the phenylhydrazine oxalate (PO) occurred as indicated below.

Time	pH	PO g/l	Formaldehyde mg/l	Formyl group mg/l
A: with yeast cells				
8 h 50 min	5·2	0·50		
10 h 00 min	5·2	0·20		
11 h 20 min	5·3	0·20	0·65	
14 h 25 min	5·3		1·80	
18 h 00 min	5·4		0·20	11·40
B: with *E. coli* cells				
11 h 00 min	6·1	0·40		
12 h 30 min	6·2	0·20		
14 h 15 min	6·3	0·20	1·30	
16 h 00 min	6·4		0·20	
20 h 30 min	6·4		0·00	6·60

The findings of the preceding papers, and those referred to in this report, demonstrate that glyoxylate is an intermediate of the direct oxidation of acetate and glycolate. In effect, glyoxylate has been isolated from the oxidation of the two acids[1] and it cannot arise from malate or isocitrate, because of the inactivity, in our experimental conditions, of the malate synthetase and isocitritase[2,3]. In addition, glyoxylate oxidation leads to the same intermediates (formaldehyde and formyl group) obtained from the oxidation of acetate and glycolate, according to the formulation of the MAS.

In the present experiments, it is of interest to note that glyoxylic acid, also in the presence of phenylhydrazine, was oxidized in the form of its phenylhydrazone, as occurred also in the case of the formaldehyde phenylhydrazone[7,8]. This fact explains the impossibility of trapping large amounts of glyoxylate and formaldehyde during the respiration of acetate in presence of phenylhydrazine. Probably these phenylhydrazones, in dilute solution, are in the addition form[10], a form which is certainly labile and easily oxidizable by separation into its components.

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Riassunto

Dall'ossidazione del gliossilato, tanto con cellule intatte di lievito come con cellule di *E. coli*, sono stati isolati l'aldeide formica e il formile, che sono anche intermedi dell'ossidazione dell'acetato e del glicolato. Il risultato costituisce una nuova prova che il gliossilato è, a sua volta, un intermedio dell'ossidazione dell'acetato e del glicolato, dai quali era anche stato isolato in precedenza.

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