of 5-hydroxylation occurring in the substrate[11]. In order to minimize the quantity of tritiated water present initially in the samples and controls, the total volume of the labeled substrate solution to be used in a particular run was evaporated to dryness in a stream of nitrogen gas immediately before use and reconstituted to the initial volume with deionized water.

In the Figure there is shown the mean rate of hydroxylation as a function of substrate concentration (10^{-2} to $10^{-4}M$); these data exhibit typical Lineweaver-Burke behaviour and the Michaelis-Menton constant is $2 \times 10^{-3}M$ with respect to the substrate. Mean velocity constants at 37 °C were 0.31 mμmole/min (pH 6.0), 0.40 mμmole/min (pH 7.4) and 0.45 mμmole/min (pH 8.0) at a substrate concentration of $5 \times 10^{-4}M$, indicating only a moderate change in the rate with pH. Consequently, all subsequent runs were made at a pH of 7.4. The hydroxylating enzyme appeared to be saturated with respect to the substrate at a concentration of 5×10^{-3} to $10^{-2}M$.

Four substances which are known to exert an inhibitory action on certain oxidative enzymes were examined in our system: (1) aminopterin, which markedly inhibits phenylalanine hydroxylase at a concentration of $5 \times 10^{-4}M$[12] failed to inhibit our enzyme to any appreciable extent, (2) 2-phenylisopropylhydrazine at $10^{-3}M$ brought about a 63% inhibition, (3) sodium ethylenediaminetetraacetate caused 63% inhibition at $10^{-3}M$, and (4) equimolar concentrations of ATP and Mg^{++} together failed to change the reaction velocity.

The ability of enzyme systems present in liver homogenates to hydroxylate catecholamines to 3,4,5-trihydric phenolic amines lends added significance to the psychotomimetic activity of the hallucinogen mescalin. If a catecholamine, such as dopamine or its metabolite 3-methoxytyramine, is hydroxylated in the 5-position through an alternate metabolic pathway instead of being detoxified by the usual mechanisms, a potential psychotogenic substance could be formed. DALY et al.[13] have shown previously that 5-methoxy-3,4-dihydroxy-β-phenethylamine can be converted to 5-hydroxy-3,4-dimethoxy-β-phenethylamine by the action of *O*-methyltransferase; the remaining methylation step to mescalin has not been demonstrated in mammalian tissue. Our enzymatic conversion of 3-methoxytyramine to 3-methoxy-5-hydroxytyramine brings closer the possibility of a biochemical link between phenylalanine and mescalin by the following route:

It is, of course realized that neither 3-methoxy-4,5-dihydroxy-β-phenethylamine nor its *O*-methyl derivatives nor the corresponding oxidative deamination products have been reported as normal or abnormal urinary or other metabolites from mammals. Since the hydroxylation of our substrate occurs at a very low specific rate, it is anticipated that new procedures will be required which will permit the isolation of sufficient 3-methoxy-4,5-dihydroxy-β-phenethylamine for chemical identification[14].

Résumé. Les homogénats de foie de rat et de foie de lapin contiennent un systéme enzymatique capable d'hydroxyler en position 5 la 4-hydroxy-3-méthoxy-phénéthylamine. Ce type d'oxydation pourrait représenter une voie biologique pour la transformation de catécholamines en substances analogues à la mescaline.

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Affinity of Various Cations for *Staphylococcus aureus* Cell-Wall

It has been found that *Staphylococcus aureus* cell-wall can bind metal ions[1]. Its binding capacity is greater for divalent ions (Ca^{++} and Mg^{++}) than for monovalent ones (K^{+} and Na^{+}), it depends on the pH of medium, and it is not affected by the anion to which the cation is bound[1]. Potentiometric titration curves for the cell-wall in the presence of cations show a definite pH shift, greater for Ca^{++} and Mg^{++} than for Na^{+} and K^{+}[1]. It has been postulated that there is a relationship between the binding capacity and the cell-wall poly-ionic structure: specifically it seems that teichoic acid is able to confer this feature to the cell-wall. This paper deals with the association constants of various cations for *S. aureus* isolated cell-wall.

The apparent constants of association enable us to measure the relative affinity of the ions for the cell-wall binding sites and therefore give us information on the cell-wall ion exchange capacity.

Materials and methods. S. aureus cell-wall (strain 22 Istituto Sieroterapico Italiano) was prepared by SALTON's scheme[2] and further purified with 0.1 *M* ethylenediamine tetra-acetic acid (pH 7.5)[1].

The hydrogen ion binding capacity of *S. aureus* cell-wall was studied by suspending 200 mg of lyophilized cell-wall in 50 ml solution of HCl at 0.01–0.000001 *N* or NaOH at 0.001–0.000001 *N*. The binding of metal ions has been studied by a titration method similar to tha reported by GILBERT and MYERS[3].

The potentiometric titration curves of lyophilized cell-wall were carried out using a HCl solution in the presence of metal ions at suitable molar concentrations, so that the medium ionic strength remained at 0.1. After equilibration for 5 h at 25 °C with shaking in a nitrogen atmo-

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sphere, the pH was measured with a Beckman Expandomatic pH-meter (accuracy of pH measurement $\pm$ 0.002 U). The decrease of pH, caused by the metal ions, was taken to be the result of competition between metal ions and hydrogen ions for negative sites on the cell-wall.

The quantity of hydrogen ions bound was then calculated by comparison with the hydrogen ion concentration of the suspension medium without the cell-wall.

Results and discussion. Titration curves of the cell-wall in HCl solution were used to studying the hydrogen ion-binding capacity (Figure). The curve in the Figure has been fully analysed in a previous work[4] and gives 2 quite close pK's in the 3–5 pH range. pK 3.19 was found in more than 90% of the titratable groups, while in the remaining 10% of all dissociable groups the pK was 4.70. Thus, our experiments were performed at pH 3 $\pm$ 0.50 and this to minimize the dissociation of groups dissociable with pK 4.70. The effect of groups dissociable with pK 4.70 was assumed negligible and so the constants studied refer principally to pK 3.19 dissociable groups.

Titration results

Cation	pH	α	α'	α_M	$\log_{10} K_M$
Na^+	2.30	0.884	0.869	0.016	0.04
	2.76	0.750	0.726	0.032	0.06
	3.10	0.546	0.507	0.071	0.04
	3.30	0.438	0.400	0.095	0.02
	3.45	0.346	0.307	0.112	0.05
K^+	2.25	0.900	0.884	0.018	0.05
	2.50	0.838	0.815	0.027	0.08
	2.84	0.700	0.661	0.056	0.01
	3.14	0.515	0.476	0.075	0.08
	3.30	0.442	0.403	0.088	0.07
Zn^{++}	2.30	0.884	0.723	0.182	1.28
	2.55	0.834	0.607	0.272	1.35
	2.70	0.769	0.538	0.300	1.27
	3.04	0.584	0.288	0.506	1.38
	3.50	0.326	0.134	0.588	1.33
Ca^{++}	2.10	0.923	0.815	0.117	1.23
	2.30	0.884	0.757	0.143	1.55
	2.54	0.830	0.615	0.259	1.31
	2.70	0.769	0.500	0.349	1.36
	3.00	0.615	0.323	0.474	1.36
Mg^{++}	2.01	0.938	0.842	0.106	1.32
	2.34	0.886	0.757	0.139	1.14
	2.71	0.769	0.496	0.355	1.37
	3.03	0.580	0.307	0.470	1.32
	3.50	0.326	0.096	0.690	1.51
Mn^{++}	2.01	0.938	0.815	0.131	1.38
	2.35	0.876	0.700	0.200	1.30
	2.70	0.769	0.519	0.325	1.32
	2.85	0.692	0.396	0.427	1.38
	3.06	0.584	0.288	0.566	1.38
Cu^{++}	2.01	0.938	0.819	0.126	1.36
	2.36	0.876	0.692	0.210	1.33
	2.50	0.838	0.623	0.256	1.32
	2.69	0.769	0.576	0.250	1.16
	3.03	0.576	0.280	0.513	1.39
Ce^{+++}	2.07	0.934	0.780	0.164	1.46
	2.41	0.861	0.588	0.317	1.52
	2.56	0.823	0.496	0.397	1.56
	2.76	0.750	0.373	0.502	1.60
	3.11	0.538	0.230	0.572	1.46

It is, further assumed that dissociable acid groups are distributed at random in macromolecular arrays and that they are not competitive. Such approximations allow GILBERT and MYERS's condroitin-sulphate dissociation procedure[3] to be applied in analysing the first section of the dissociation curve

$$\log K = pH + \log \frac{\alpha}{1-\alpha} - A(1-\alpha) \qquad (1)$$

where α is the degree of association of protons with acid groups, K is the intrinsic constant of association and A the electrostatic coefficient. Since the latter is a function of the degree of association and therefore difficult to determine, A has been ignored. Its value, however, is slight. Furthermore, the activity coefficient in the polyelectrolyte phase was taken as being that for cell-wall free solutions. With cations present, the titration curves shifts towards acid values, so indicating the cation's association with acid groups.

The analogue of equation (1) for the cation binding, according to GILBERT and MYERS[3], is:

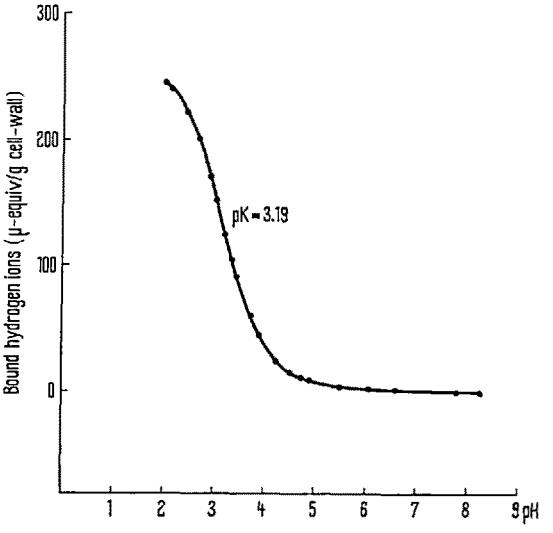
$$\log K_M = -\log C_M + \log \frac{\alpha_M}{1-\alpha'-\alpha_M} - n A (1-\alpha'-n\alpha_M) \qquad (2)$$

where C_M is the concentration of metal ion, corrected for the small amounts of ions bound to the cell-wall, K_M the intrinsic association constant at constant ionic strength, and α' and α_M are the degree of association of H^+ and metal ions respectively for dissociation acid groups. For α_M GILBERT and MYERS's approximate formula was used:

$$M = \frac{\alpha - \alpha'}{\alpha}.$$

The results given in the Table are derived from equation (2) and show an increasing affinity for cell-wall dissociable groups in the order Na^+, $K^+ < Ca^{++}$, Mg^{++}, Zn^{++}, Mn^{++}, $Cu^{++} < Ce^{+++}$.

The Na^+ and K^+ association constants are quite small compared to those for divalent ions. Divalent ions of greater atomic weight show a higher affinity. Cation bind-



Hydrogen ion binding of *S. aureus* cell-wall.

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ing capacity on biological surfaces due to fixed charges and their possible displacement according to their affinity is of considerable importance. Our knowledge of the ion binding capacity for polyelectrolyte structures is recent[5,6]. One highly interesting hypothesis for biological structures is that cations form a mobile monolayer[7,8] around the dissociable groups of polymer chains.

The constants of Na^+ and K^+ found for the cell-wall, are of the order of magnitude for weak interaction. It seems reasonable to regard the monovalent counterions as forming a very highly mobile monolayer on the polymer skeletal surface of cell-wall.

On the other hand the association constants of divalent and trivalent cations are clearly for stronger interactions. This result is consistent with the concept of the counterions closely bound by electrostatic interactions.

Riassunto. Sono state studiate le costanti apparenti di associazione di vari cationi per il cell-wall isolato di cellule di *Staphylococcus aureus*. E' risultato che gli ioni monovalenti presentano una bassa costante di associazione mentre gli ioni bivalenti e trivalenti presentano valori nettamente superiori delle costanti di associazione. Si conclude che mentre i cationi monovalenti sono legati da deboli legami elettrostatici, i cationi di valenza superiore formano con i gruppi dissociabili legami ionici stabili.

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Cell-Like Structures from Simple Molecules under Simulated Primitive Earth Conditions

Over 2 decades ago a group of experiments on the origin of life was performed by HERRERA using ammonium thiocyanate and formaldehyde[1]. This work has become relevant since MILLER's synthesis[2] of formaldehyde in a prebiological system and HEYNS' extension[3] which yielded ammonium thiocyanate.

We chose an experiment[4] in which it was claimed that simple mixing of 7 ml 37% formaldehyde and 3 g ammonium thiocyanate resulted in 'life-like forms'. After mixing the materials, the colorless liquid became slightly red in a few sec, and finally a golden yellow after 1 h. Microscopic examination (without a cover glass) revealed a high density of spheres 1–5 μ in diameter (Figure 1). A drop of water was applied so it slightly overlapped onto the drying mixture (Figure 2). Vigorous streaming of the reaction mixture into the water resulted. The spheres darkened considerably and structures of a greater size and complexity, 10–100 μ in diameter are seen (Figure 1). 0.1 M acetic acid and 0.1 M NaH_2PO_4 gave the same results when used as rehydrants. When the drying spheres were rehydrated with 1% solutions of methylene blue, trypan blue or Ponceau S, they concentrated the stain.

Internal fluid regulation is one of the primary properties of life. The contractile vacuole is used by some unicellular organisms for this purpose[5]. In many cases we observed motion of an internal vacuole-like structure toward the boundary and its subsequent extrusion. The boundary then closed, reformed, and resumed its circular shape (Figure 3).

The yellowing liquid became progressively less abundant as a thin layer of solid formed on its surface and much solid deposited at the bottom. Little supernatant was left after 48 h at room temperature.

UV-radiation is assumed to have been an important energy source for chemical evolution[6]; so we considered its effect on the reaction. We used dilute reactant solutions to test for the catalytic effect of UV-radiation on concentrations more likely to have occurred in a primitive earth environment. A typical starting mixture consisted of equivolumes of 0.5 M NH_4SCN and 1.4 M formaldehyde. Samples were subjected to darkness, room light (fluorescent), and UV-radiation (Ultraviolet Products SC-1). The dark reaction and the second case showed no spheres after 24 h whereas the irradiated sample displayed spheres after 15 min.

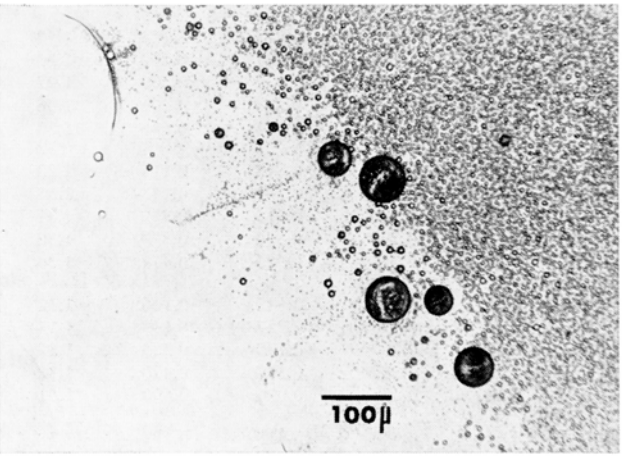

Fig. 1. Multitude of small spheres from a 7 ml 37% formaldehyde, 3 g NH_4SCN mixture. The dark vacuolated spheres were formed by rehydration of the drying drop.

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