the same was found to hold true with bromide when only the spinal fluid concentration was considered; when the degree of permeability, however, was expressed as a ratio (serum bromide/spinal fluid bromide), usually referred to as the permeability quotient or P.Q., the difference in barrier permeability between the various groups at once became apparent. It is probable that similar differences might be shown with the nitrate test were it more precise and were the method applicable to serum analyses.

It appears, then, that even exact determinations of the spinal fluid concentration of any substance used to test barrier function may give little information as to the degree of altered permeability present, except when permeability is so greatly increased that unduly large amounts of the test agent are permitted to pass the barrier. A more precise evaluation of barrier function can be achieved by determining the concentration of the test agent in both blood and cerebrospinal fluid, and expressing the degree of permeability as a quotient (P.Q.).

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Change in Amino Acid Oxidation Following Dissociation of *Staphylococcus aureus.*

M. DOROTHY WEBSTER. (Introduced by C. C. Lieb.)

From the Department of Physiology and Pharmacology, Duke University School of Medicine, Durham, North Carolina.

Using the Methylene Blue technic, Bach[1] has reported that in the presence of *Staphylococcus aureus,* the amino acids with the exception of cysteine and glutamic acid are not active as hydrogen donators. The amino acids in the present study are classed by him as "doubtful or inactive." Soru[2, 3] compared the ability of the dissociated and undissociated *B. coli* to oxidize glucose. He found that the fermentation of glucose, various other substrates, and other sugars is slower with the R form than with the S form. Alanine and serine were so slowly oxidized that no difference in reduction time could be noted. Under similar conditions, using Bacille d'aertrycke, glycine was not attacked, and other substances such as

[1] Bach, D., *Compt. rendu de Soc. Biol.,* 1935, **120**, 608.

[2] Soru, E., *Compt. rendu de Soc. Biol.,* 1936, **121**, 1647.

[3] Soru, E., *Compt. rendu de Soc. Biol.,* 1935, **120**, 232.

glucose, lactate, and succinate were oxidized more slowly by the R than by the S form.

Staphylococcus aureus was isolated from a case of clinical infection. The strain was hemolytic and the colony a bright yellow color. The bacteria were grown on beef extract agar at 37°C and pH 7.4. They were washed off the agar with saline and centrifuged. Washing was repeated 3 times, and the bacteria were then suspended in 0.05 M phosphate buffer, pH 7.8. Bacterial counts were done by means of a hemocytometer and it was found that there were approximately sixteen billion per cc.

Dissociation was brought about by aging the culture. Plates streaked on the 25th day showed many rough white colonies. This rough white dissociate, referred to hereafter as *Staphylococcus albus*, was subcultured and showed no tendency to revert to the smooth yellow form.

One cc of the suspension was used in each Warburg vessel and the volume made up to 2 cc with buffer and solution of the amino acid to be tested. The rate and the amount of the oxygen uptake were then measured. The amount of decarboxylation was determined by tipping in 0.5 M HCl at the end of the experiment. Deamination was measured by vacuum distillation followed by Nesslerization.

Of 13 amino acids tested, only 5, glycine, serine, alanine, proline, and histidine were attacked. Valine, leucine, isoleucine, hydroxyproline, tyrosine, phenylalanine, tryptophane, and methionine were not attacked.

The simpler amino acids, glycine, serine, and alanine were oxidized in the same manner as with *B. proteus*[4] and *B. pyocyaneus*.[5] Glycine was completely oxidized to carbon dioxide, ammonia and water. Both isomers of alanine were oxidized, taking up four atoms of oxygen per molecule of amino acid and producing ammonia and 2 molecules of carbon dioxide. Only the dl-mixture of serine was available, but the ammonia production indicated that both isomers were attacked. Three atoms of oxygen were utilized and one molecule of carbon dioxide was produced per molecule of serine. Dissociation had no apparent effect upon the ability of the bacteria to oxidize any of these simple amino acids.

With proline and histidine the extent of the oxidation was changed upon dissociation of the bacteria. *Staphylococcus aureus* oxidized only the natural isomer of proline, utilizing 8 atoms of oxygen per

[4] Bernheim, F., Bernheim, M. L. C., and Webster, M. D., *J. Biol. Chem.*, 1935, **110**, 165.

[5] Webster, M. D., and Bernheim, F., *J. Biol. Chem.*, 1936, **114**, 265.

TABLE I.

Oxidation, Decarboxylation, and Deamination of Various Amino Acids at 37°C and pH 7.8.

The oxygen uptake and decarboxylation as shown in Columns 4 and 8 are calculated for both isomers, when d-l forms were used. For determining the amount of deamination 1.0 mg was used throughout and the theoretical deamination as shown in Column 11 is calculated on that basis. The oxidation rate is based on the time necessary for half the final oxygen uptake to be reached.

		O_2 Uptake				CO_2 Production			NH_3-N	
(1) Amino acid	(2) Amt. used mg	(3) Exper. mm^3	(4) Theor. mm^3	(5) Atoms	(6) Rate min.	(7) Exper. mm^3	(8) Theor. mm^3	(9) Mol.	(10) Exper. mg	(11) Theor. mg
Glycine										
S. aureus	.25	94	111	3	27	132	140	2	.172	.186
S. albus	.25	103	111	3	30	134	140	2	.176	.186
dl-serine										
S. aureus	.25	73	81	3	40	58	57	1	.126	.133
S. albus	.25	76	81	3	15	46	57	1	.123	.133
dl-alanine										
S. aureus	.25	137	126	4		133	128	2	.147	.158
S. albus	.25	130	126	4		116	128	2	.150	.158
d-alanine										
S. aureus	.25	129	126	4	10	134	128	2	.145	.158
S. albus	.25	133	126	4	15	117	128	2	.146	.158
dl-proline										
S. aureus	.25	101	194	8		77	196	4	.055	.121
S. albus	.25	132	121	5		106	98	2	.098	.121
l-proline										
S. aureus	.25	206	194	8	40	209	196	4	.115	.121
S. albus	.25	121	121	5	48	109	98	2	.098	.121
d-histidine										
S. aureus	.25	18	0	0		8	36	0	.020	.088
S. albus	1.00	4	0	0		0	36	0	.000	.000
l-histidine										
S. aureus	.25	114	116	8	140	29	36	1	.090	.088
S. albus	1.00	62	58	1	120	73*	72*	1	.077	.088

*.5 mg used.

molecule and producing 4 molecules of carbon dioxide. *Staphylococcus albus* attacked both isomers of proline but only 5 atoms of oxygen were utilized and 2 molecules of carbon dioxide produced per molecule of amino acid. The ammonia production indicated that only one isomer was attacked by the original strain and both isomers by the dissociate form.

Histidine in the presence of *Staphylococcus aureus* is oxidized, taking up 8 atoms of oxygen and producing one molecule of free carbon dioxide. Only the natural isomer is attacked. With *Staphylococcus albus* only one atom of oxygen is utilized per molecule of amino acid and one molecule of carbon dioxide produced. In this case also only the natural isomer is attacked.

All the reactions tested were sensitive to 0.005 M KCN.

The course and extent of the oxidation of the simpler amino acids, glycine, serine, and alanine by *Staphylococcus aureus* and *Staphylococcus albus* is the same as with *B. proteus* and *B. pyocyaneus*. It would, therefore, seem likely that there is a common mechanism for the carrying out of these oxidations. A change in the course and extent of the oxidation of proline and histidine takes place when *Staphylococcus aureus* is dissociated. Whether this change is significant in the problem of virulence and toxin production has not yet been determined. The ability to oxidize a given amino acid varies from species to species. Thus, the oxidation of phenylalanine by *B. proteus*[4] utilizes one atom of oxygen, by *B. pyocyaneus*[5] 13 atoms while there is no oxidation by *Staphylococcus aureus* or *Staphylococcus albus*.

Summary. 1. The amino acid oxidation by *Staphylococcus aureus* and the rough white dissociate *Staphylococcus albus* has been studied. 2. Glycine, serine, and alanine are oxidized in the same fashion by both forms. 3. *Staphylococcus aureus* exhibits optical specificity in its oxidation of proline, in that only the natural isomer is attacked. After dissociation, the optical specificity is lost and both isomers are equally oxidized, but the extent is not as great as by the undissociated form. The optical specificity with regard to histidine is maintained after dissociation, but the extent of the oxidation is much reduced.

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