tents and sewage and of its inactivation by current water purification procedures suggests either "superchlorination" or that "ozone treatment might be effective."

The germicidal effects of ozone have been noted in the past but recent quantitative determinations have not been reported and its effect on viruses has not been observed. The current results indicate that ozone acts as a strong virucidal agent and warrant additional experimentation.

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Studies on the Nutrition and Metabolism of *Pasteurella pestis.*

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Although *Pasteurella pestis* has been the subject of a great deal of medical and immunological research, very little work has been done on the physiology of the organism itself. During recent years, attempts have been made to apply technics used in the study of bacterial metabolism to certain pathogenic organisms. The experiments reported in this paper were designed to establish the basic growth requirements of the plague bacillus, and to investigate to some extent the nature of its dissimilatory processes.

Rao[1] claims that 3 amino acids, cystine, phenylalanine, and proline, are essential for the development of *P. pestis,* and that haematin and riboflavin may be considered as accessory growth factors. However, he does not give evidence that the organism can grow with a mixture of these compounds alone, and provides neither a carbon nor a nitrogen source other than amino acids in most of his media. In an excellent study dealing with the nutrition of the members of the genus *Pasteurella,* Berkman[2] could find no effect of 12 nutrilites on the development of their strains of *P. pestis* in a medium containing glucose as well as a mixture of amino acids.

Experiments were carried out in this laboratory and at the Hooper Foundation for Medical Research with a non-pathogenic strain (1122) of *P. pestis,* and with 3 selected pathogenic strains: 337 (from India), and "Yreka" and "Shasta" from California. Since glucose was found to be the best carbon source and ammonium salts an excellent nitrogen source for all organisms tested, the basic medium was prepared with 0.2% glucose, 0.1% NH_4Cl, 0.05% $MgSO_4 \cdot 7H_2O$, 0.005% $FeCl_3$, .001% $CaCl_2$, and M/30 Sorensen KH_2PO_4-Na_2HPO_4 buffer at pH 7.0. Five ml amounts of the basic medium were dispensed in 17 mm test tubes to insure a fair degree of aeration without agitation. All cultures were incubated at 29°C. On the first transfer from blood agar slants, none of the strains tested could grow in the above medium unless small amounts (0.002%) of cystine and phenylalanine were added. Initially large inocula had to be used, and visible turbidity was slow to appear, evidence pointing to a selection of the cells most capable of reproducing in this environment.[3] With subculturing in the same medium, growth became rapid. The addition of 0.002% proline hastened the development of strains 337 and "Shasta" on first transfer, but had no appreciable effect on their subsequent growth. The other strains tested were unaffected by proline. No nutrilite function of haematin, biotin, pantothenic acid, *p*-aminobenzoic acid, riboflavin, nicotinic acid, thiamin, or pyridoxine could be detected for any of the strains.

More detailed studies carried out with strain 1122 showed that:

1. Cystine could be replaced with thiosulfate, sulfite, thioglycollate, or homocystine, but not with methionine.

[1] Rao, M. S., *Indian J. M. Research,* 1939, **27**, 75.

[2] Berkman, S., *J. Infect. Dis.,* 1942, **71**, 201.

[3] Doudoroff, M., *J. Bact.,* 1942, **44**, 451.

2. Phenylalanine normally supported maximum development at a concentration of 0.0002%, but the bacteria could be "weaned" from this requirement to develop at almost their normal rate without it. Strains adapted in this manner could accept glucose as sole carbon source with thiosulfate for source of sulfur. Substitution of tyrosine, and, to a lesser extent, of tryptophane for phenylalanine was found helpful, but not necessary, in effecting the "weaning" (*c.f.* role of homocystine in a similar phenomenon observed with luminous bacteria.[3]) Neither tyrosine nor tryptophane appeared to be as satisfactory to the bacteria as phenylalanine, and it was found impossible to use phenylacetic and indoleacetic acids as substitutes.

3. With cystine and phenylalanine added to the medium, mannitol was found to be almost as good a carbon source as glucose. Pyruvate and lactate were fair, alanine and proline poor, while glycerol, ethanol, succinate, malate, and asparagine were entirely unsatisfactory.

4. Amino acids were in general no more satisfactory as nitrogen sources than were ammonium salts. The addition of glycine to the basic medium had no growth-promoting effect, as claimed by Rao.[1] Although the bacteria could reduce nitrate to nitrite, they appeared to be unable to use the former as a nitrogen source.

5. Certain amino acids were inhibitory to growth when added to simplified media. Thus, the addition of 0.002%-0.01% *l*- or *dl*-leucine to the basic medium with either cystine or thiosulfate caused a prolonged lag in the development of strains adapted to these media without leucine. This effect could be reduced or eliminated by the addition of isoleucine or valine, neither of which appeared to have any growth-promoting value by itself. Methionine was found to be inhibitory when cystine was used as sulfur source, but not when thiosulfate was used. The interaction of amino acids in growth plays havoc with the commonly used "elimination method" of determining minimal requirements, since often the elimination of one constituent prevents development, not because of its indispensability to the organism, but because of its value in counteracting the toxic effect of another substance in the mixture.

6. Very heavy cultures of strain 1122 could be obtained in synthetic media with glucose and small amounts of cystine and phenylalanine if adequate aeration was provided by constant agitation. Pyruvic acid was found to accumulate in the course of development (to about 0.01 N) and to disappear after the sugar had been oxidized. In unagitated cultures, the bacteria died rather rapidly because of the accumulation of pyruvic acid and possibly of fermentation products as well. Pyruvic acid was also produced in cultures of this strain with mannitol as chief carbon source and by all of the other strains of *P. pestis* tested in glucose-containing medium. Pyruvic acid was identified by the preparation of its 2,4-dinitro-phenylhydrazone. (Melting points uncorrected; hydrazone of pyruvic acid, 214.0°C; unknown, 213.25°C; mixed, 213.5°C.) Quantitative estimation was made by the method of Elliot and c.w.[4]

TABLE I.
Fermentation of Glucose by *Pasteurella pestis*.

	Per 100 cc of medium		
	Millimoles	Milliatoms C	Milliatoms "available H" (ref. 5)
Glucose utilized	1.810	10.860	43.44
CO_2	0.247	0.247	—
Formic acid	0.343	0.343	0.69
Acetic acid	1.747	3.494	13.98
Ethanol	1.010	2.020	12.12
Lactic acid	1.027	3.081	12.32
Pyruvic acid	0.025	0.075	0.25
Succinic acid	0.284	1.136	3.98
Products recovered		10.396	43.34
% of recovery		95.8%	99.8%

7. Strain 1122 was capable of development under completely anaerobic conditions in complex media containing glucose. No attempts were made to analyze growth requirements under such conditions. The chief products of glucose fermentation appeared to be lactic and acetic acids and ethyl alcohol. Carbon dioxide, formic acid, succinic acid, and a small amount of pyruvic acid were also found. No hydro-

[4] Elliot, K. A. C., Benoy, M. P., and Baker, Z., *Biochem. J.*, 1935, **29**, 1937.

gen, glycerol, or 2,3-butylene glycol could be detected, and only traces of acetyl methyl carbinol or diacetyl could be shown. A fair carbon and hydrogen balance[5] was obtained for the fermentation of glucose in a medium initially containing 326 mg glucose, 45 mg Difco yeast extract, 3 mg proline, 3 mg phenylalanine, 1.5 mg cystine, 1 mg isoleucine, 1 mg valine, 45 mg NH_4Cl, 45 mg $MgSO_4 \cdot 7\ H_2O$, 2 mg $CaCl_2$, and 1 mg $FeCl_3$ per 100 cc of 0.05 M Sorensen phosphate buffer at pH 7.5. During the fermentation the medium was neutralized at intervals with NaOH, until all of the sugar had disappeared. The results are presented in Table I.

Although the carbon recovery appears to be satisfactory, the ratio of two-carbon to one-carbon derivatives (see 5) is extraordinarily high for an ordinary fermentation, being approximately 3.2. This might indicate that a portion of the one-carbon derivatives remains unaccounted for (as unidentified products in the medium or in the bacterial) or, perhaps that part of the 2-carbon compounds may arise, not through the splitting of a 3-carbon degradation product of sugar, but by some other method such as the reduction of carbon dioxide.

[5] Doudoroff, M., *J. Bact.*, 1942, **44**, 461.

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Resistance of Small Colony Variants (G-Forms) of a Staphylococcus Towards the Bacteriostatic Activity of Penicillin.

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So far, two types of penicillin-resistant staphylococci have been described:

(1) Naturally resistant strains, as reported by Fleming,[1] and by Hobby, Meyer and Chaffee.[2] We, too, found 5 resistant strains of *Staphylococcus aureus* in a group of 12.

(2) Strains rendered drug-resistant by prolonged contact of the organisms with increasing concentrations of penicillin (Abraham and co-workers,[3] Smith and Hay,[4] Rammelkamp and Maxon.[5]) These strains may exhibit slight morphological changes, such as larger size of the coccus[4] or a reduced velocity of growth and enzyme activity.[3]

A third type of penicillin-resistant staphylococci, which has not been described before, is represented by variants occurring when a normal strain of *Staphylococcus albus* was exposed to penicillin. Such strains were studied in our laboratory during recent months. They show the profound cultural changes known as small colony variants (Hoffstadt and Youmans,[6] Swingle,[7] Youmans,[8] Youmans and Delves.[9])

Material and Methods. The penicillin used was produced by growing a strain of *Penicillium notatum* No. 1209* in a modified

[1] Fleming, I., *Lancet*, 1942, **1**, 732.

[2] Hobby, G. L., Meyer, K., and Chaffee, E., PROC. SOC. EXP. BIOL. AND MED., 1942, **50**, 277.

[3] Abraham, E. P., Chain, E., Fletcher, C. M., Florey, H. W., Gardner, A. D., Heatley, N. G., and Jennings, A. M., *Lancet*, 1941, **2**, 177.

[4] Smith, L. D., and Hay, T., *J. Franklin Instit.*, 1942, **233**, 598.

[5] Rammelkamp, C. H., and Maxon, T., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 386.

[6] Hoffstadt, R. E., and Youmans, G. P., *J. Infect. Dis.*, 1932, **51**, 216.

[7] Swingle, E. L., *J. Bact.*, 1935, **29**, 467.

[8] Youmans, G. P., PROC. SOC. EXP. BIOL. AND MED., 1937, **36**, 94.

[9] Youmans, G. P., and Delves, E., *J. Bact.*, 1942, **44**, 127.

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