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Nicotinic Acid and Pantothenic Acid as Essential Growth Factors for *Shigella paradysenteriae* (Flexner).

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Nicotinic acid is an important and, in many cases, indispensable growth factor for *Shigella paradysenteriæ* (Flexner).[1–3] However, in a medium containing ammonia as the only source of nitrogen, only 4 out of 18 strains were able to grow with the addition of nicotinic acid.[3]

We were able to substantially confirm these observations. Besides, we have found that pantothenic acid is an additional growth factor for a not inconsiderable percentage of strains of the Flexner bacillus.

Except in the case of mass cultures mentioned later on, our experiments were made in test tubes containing a total volume of 5 ml of the following medium:

A	Na_2SO_4	12.5 g	
	$MgCl_2 \cdot 6H_2O$	0.05 g	
	Dextrose	12.5 g	
	NH_4Cl	12.5 g	
	Bidistilled water	ad	1000
B	Nicotinic acid in 5 times the required concentration		
C	KH_2PO_4	2.5 g	
	K_2HPO_4	7.5 g	
	Bidistilled water	ad	1000
D	d-Calcium pantothenate in 5 times the required concentration		

Each solution was sterilized separately and the test tubes filled with 2 ml solution A, 1 ml solution B, and 1 ml solution D. After sterilization, 1 ml of solution C was added under sterile precautions.

The phosphate solution had to be added separately because, with higher concentrations of calcium pantothenate, the formation of a precipitate of calcium phosphate was observed after heating. In the case where nicotinic acid or pantothenic acid were omitted, the solutions B or D were replaced by sterile water.

The medium without growth factors—that is, 2.0 ml "A" + 1.0 ml "C" + 2.0 ml H_2O—is hereafter designated as "basic medium."

All tests were made in duplicate tubes, and where growth was observed, 5 passages in the same medium were made, not counting the initial transfer from broth into the synthetic medium.

The sterility of the media was controlled by incubation of uninoculated tubes for 14 days. All tubes were closed with a Parawax cover over the cotton stopper. Inoculations were made each time with three 3-mm loops of culture.

The experiments were arranged in groups which served as controls for each other; for instance, a group on "basic medium" was put on test simultaneously with one containing nicotinic acid alone, one with pantothenate alone, and one with nicotinic acid and pantothenate. All experiments were repeated at least twice. Before a result of "no growth" was accepted as final, inoculations were repeated 4 to 6 times; thus each such statement is supported by 8 to 12 individual inoculations.

Observation for growth was continued for 14 days at 37°C. Altogether 20 strains were investigated whose cultural and serological properties had been carefully established at the start. The strains were identified anew after they had gone through the 5 passages. About half of the strains were old stock strains which, however, had preserved their smoothness. The other half were strains isolated between November, 1942, and April, 1943.*

Results. In confirmation of previous observations, only one of the 20 strains grew regularly and easily in the "basic medium."

[1] Koser, S. A., Dorfman, A., and Saunders, F., Proc. Soc. Exp. Biol. and Med., 1938, **38**, 311.

[2] Kligler, I. J., and Grosowitz, N., *J. Bact.*, 1939, **38**, 309.

[3] Dorfman, A., Koser, S. A., Reames, H. R., Swingle, K. F., and Saunders, F., *J. Inf. Dis.*, 1939, **65**, 163.

* We wish to thank Drs. A. B. Wadsworth, A. V. Hardy, E. Neter, and O. Costa Mandry for providing strains for the present investigation.

Fifteen additional strains grew in a medium containing nicotinic acid. Nicotinic acid and nicotinamide were found to serve interchangeably. As a rule, nicotinic acid was employed in a concentration of 1 μg/ml. We found that 0.1 μg/ml served as well and that, as a rule, growth was still possible but delayed and much weaker with 0.01 μg/ml. These data agree in their order of magnitude with those of Koser and his co-workers.[1]

There remained 4 strains which we were not able to cultivate in our ammonium-nicotinic acid medium. A systematic search of the factor lacking was made. We started with a liver extract which was known to contain, besides nicotinic acid, the following growth factors: thiamine, biotin, folic acid, pantothenic acid, riboflavin, and pyridoxine. Three out of the 4 strains grew easily if 0.5% of this extract was added to the basic medium. The factors just enumerated were then paired with nicotinic acid in the following concentrations: riboflavin 5 μg, thiamine 10μg, biotin 0.02 μg, pantothenic acid 1 μg, folic acid 0.05 μg/ml. As it is known that certain amino acids have an activity like an essential metabolite, tubes with 2 mg of tryptophane, 0.4 mg adenine sulfate, and 0.4 mg of cystine per ml, respectively, were included in this experiment. All 3 strains that grow in the medium enriched with liver extract, multiplied freely in passages when pantothenic acid was added. No other of the substances enumerated above allowed propagation of these strains.

No difference of effect was seen in concentrations of calcium pantothenate between 200 and 0.02 μg. As a rule, 0.2 μg calcium pantothenate was used in our tests.

It appears, therefore, that pantothenic acid is an essential growth factor for at least some of the Flexner strains.† There is no convincing evidence that pantothenic acid influences the rate or abundance of growth in strains for which this substance is not essential.

There remains one strain ("V Boyd") that we have not been able to grow in any one of our synthetic media.‡

From 5 of our strains, single tubes were obtained from which variants could be derived that, after a delayed start, were able to grow more or less abundantly without nicotinic acid. We were not able to find a case where growth depends on the presence of pantothenic acid but not nicotinic acid. In the few cases where multiplication occurred in media containing pantothenic but not nicotinic acid, closer examination revealed each time that these variants could dispense with the pantothenic acid as well; in other words, they were just variants that are able to dispense with nicotinic acid. The question arises whether strains that are able to grow without nicotinic acid would be able to manufacture nicotinic acid from the basic medium, or whether they were able to dispense entirely with this substance (and presumably coenzyme). Definite limiting concentrations of nicotinic acid exist for growth of strains which need this factor.[3] It had been concluded from this fact that no nicotinic acid is formed by the Flexner bacillus. We have in 4 cases cultivated variants, that had been found to do without nicotinic acid, in 500 ml volumes of the "basic medium" (without nicotinic acid), and examined the culture for nicotinic acid by the method of Snell and Wright.[5]§ In each case nicotinic acid was found in amounts of the order of 10^{-2} μg/ml. It appears, therefore, that at least these variants of the Flexner bacillus do manufacture nicotinic acid.‖

† There is a statement in the literature[4] that for *Sh. dysenteriæ* (Shiga) pantothenic acid is not an essential factor. From the data given here it will be apparent that it is hazardous to base generalized statements of this kind on the experience with one single strain.

[4] Krauskopf, E. J., Snell, E. E., and McCoy, E., *Enzymologia*, 1939, **7**, 327.

‡ Since this was written, Dr. S. H. Hutner has investigated this strain. He finds that uracil and tryptophane are to be supplied in the medium in addition to nicotinic acid.

[5] Snell, E. E., and Wright, L. D., *J. Biol. Chem.*, 1941, **139**, 675.

§ We wish to thank Mrs. A. J. Dornbush for making these determinations.

‖ Since this was submitted for publication, similar findings were reported for 2 strains of the Flexner bacillus.[6]

[6] Koser, J. A., and Wright, M. H., *J. Bact.*, 1943, **42**, 239.

From the study of cultures recovered from synthetic media it could be ascertained that variation from "smooth" to "rough" and the appearance of dwarf forms is not markedly influenced as long as a reasonably abundant growth is maintained. In some cases when growth was scant—as was occasionally observed with variants growing without supplementary nicotinic acid—an increased tendency to develop rough and dwarf forms was noticeable. In this case also, however, individual differences are so great that no fixed rule can be given.

Discussion. The distinction between "not exacting" and "exacting" strains has been originally established on the criterion of the ability or inability of a microörganism to satisfy all nitrogen requirements with ammonia nitrogen. In the case of the Flexner bacillus we can, on the basis of our present knowledge, circumscribe the meaning of the term "exacting" somewhat more closely. We know now that there exist at least 3 grades of minimal growth requirements, namely: 1. growth is possible with ammonium salt alone; 2. nicotinic acid has to be provided; and 3. nicotinic acid and pantothenic acid have to be available in the medium.

The term "exacting" can, therefore, be more exactly defined as inability to synthesize nicotinic acid and—in case 3—pantothenic acid. Grade (2) will possibly have to be subdivided according to whether or not an intermediary substance (amino acid) will enable the microorganisms to synthesize nicotinic acid.[3]

Exceptional deficiencies in synthesizing ability do occur.

Summary. 1. Former observations concerning the role of nicotinic acid as an essential growth factor for the Flexner bacillus have been confirmed. Individual strains vary greatly in their requirements and often are able to manufacture this substance themselves or produce variants that have this ability. 2. For certain strains of the Flexner bacillus, pantothenic acid is a second essential growth factor.

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Increased Efficiency of Phenolic Germicides by Addition of Inorganic Salts to Produce Oxidation-Reduction Systems.

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In a previous communication[1] it was shown that inorganic salts of iron, tin, and manganese, when tested individually, exhibited very little or no germicidal activity against *Staphylococcus aureus*. However, when the salts were mixed in certain combinations and proportions to produce oxidation-reduction systems a pronounced germicidal action occurred. The phenomenon was shown to be a function of the positive metallic ions; the negative ions apparently played no part in the reaction.

The addition of an appropriate metallic salt to an inorganic germicide, *e.g.*, ferrous sulfate to mercuric chloride, to produce an oxidation-reduction system, resulted in a greater effectiveness of the latter. The addition of another oxidation-reduction system, such as a mixture of ferrous and ferric sulfates, to the ferrous sulfate-mercuric chloride combination increased still further the efficiency of the mercuric chloride.

The present communication is concerned with the effect of the addition of inorganic metallic salts to the organic phenolic compounds, phenol, cresol, and Hexylresorcinol.

Results from Phenol. An aqueous solu-

[1] Guest, Howard L., and Salle, A. J., PROC. SOC. EXP. BIOL. AND MED., 1942, **51**, 272.