

be said whether or not all of the virus precipitated was in the neutralized state.

These results provide direct evidence for the existence of both soluble and insoluble phases of neutralized virus and for the dissociation of both with dilution when the 2 phases are separated. The data likewise afford an estimate of the distribution of virus between the 2 phases separated in this way by centrifugation. It should be emphasized that the described distribution can be considered only for the special region of serum-virus amounts employed. The reduction in free virus in the supernatant fluid resulting from removal of the insoluble phase, a phenomenon already noted by others in studies with unpurified viral preparations,⁴ could not have been due to sedimentation of free virus since it was not demonstrable in the pellet-suspension. The findings suggest the possibility of a shift in chemical equilibrium as an explanation of the phenomenon.

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Comparative Activity of Nicotinic Acid and Nicotinamide as Growth Factors for Microorganisms.*

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Investigations of the past few years have demonstrated that a number of microorganisms must be supplied with either nicotinic acid or nicotinamide for continued cell multiplication. When these compounds are supplied in excess no difference in activity is noted, but when suboptimum quantities are used a quantitative difference in activity is often encountered.

While studying the nutritive requirements of the Pasteurella group of bacteria, it was found that nicotinamide could *not* be replaced by nicotinic acid in the cultivation of those members of the Pasteurella group which cause hemorrhagic septicaemia in animals. In a basal medium of hydrolyzed purified gelatin, supplementary amino acids, glucose and inorganic salts, growth of many strains of the hemorrhagic septicaemia Pasteurellae occurs readily when both

⁴ Friedewald, W. F., and Kidd, J. G., *J. Exp. Med.*, 1940, **72**, 531.

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nicotinamide and pantothenic acid are added.¹ On substitution of nicotinic acid for nicotinamide growth of cultures does not occur. This observation has been repeated a number of times with 16 *Pasteurella* cultures. Two different samples of both nicotinic acid and nicotinamide were used. One-tenth microgram of nicotinamide per cc of medium is sufficient for prompt growth of cultures whereas amounts of 0.1, 1.0, 10.0 and 100 μg of nicotinic acid per cc, in the presence of pantothenic acid, did not cause growth. The same samples of nicotinic acid supported growth of dysentery bacilli and *Proteus*.

The inability of the hemorrhagic septicaemia *Pasteurellae* to utilize nicotinic acid was shown further by metabolic studies of a typical *Pasteurella* culture. The culture used for these experiments was grown in the basal medium of hydrolyzed gelatin previously described¹ supplemented with 0.004 μg per cc of nicotinamide and 0.1 μg per cc of calcium pantothenate. The amount of nicotinamide used was suboptimum but permits the development of a crop of cells sufficient for the tests. Cells from 24-hour cultures at 37°C were centrifuged, washed with *M/20* phosphate buffer at pH 7.4, recentrifuged and suspended in the phosphate buffer.

Comparative data are given in Tables I and II. The figures shown are typical of other tests. Both diphosphopyridine nucleotide and nicotinamide stimulated respiration of *Pasteurella suisseptica* grown in a medium deficient in nicotinamide, while nicotinic acid produced no such stimulation. Other tests have shown that pantothenic acid together with either nicotinamide or nicotinic acid gave results equivalent to those secured with the amide or the acid alone. These results confirm the growth tests in showing that certain members of the *Pasteurella* group are able to utilize nicotinamide but not nicotinic acid.

TABLE I.
Effect of Nicotinamide, Nicotinic Acid and Diphosphopyridine Nucleotide on Methylene Blue Reduction by *Pasteurella suisseptica*.

	Time required for reduction,* min
Control	>60
Nicotinamide (20 γ)	20
Nicotinic acid (20 γ)	>60
Diphosphopyridine nucleotide (50 γ)	17

*Each Thunberg tube received 0.3 cc of 2% glucose and 2 cc of a suspension of washed cells in phosphate buffer. After 20 minutes' equilibration at 38°C 50 γ of methylene blue were tipped into each tube.

¹ Berkman, S., Saunders, F., and Koser, S. A., *PROC. SOC. EXP. BIOL. AND MED.*, 1940, 44, 68.

TABLE II.
Effect of Nicotinamide, Nicotinic Acid and Diphosphopyridine Nucleotide on
Respiration of *Pasteurella suisseptica* as Measured by the Direct Warburg Method.

	Oxygen consumed in 2 hr,* mm ³
Control	13.0
Nicotinamide (20 γ)	69.0
Nicotinic acid (20 γ)	16.3
Diphosphopyridine nucleotide (50 γ)	161.7

*Each vessel received 0.3 cc of a 2% solution of glucose and 2 cc of a suspension of washed cells in phosphate buffer. After 10 minutes' equilibration at 38°C the substance being tested was tipped into the vessel from the sidearm. The total volume in each vessel was 2.7 cc.

In Table III the relative growth-promoting effects of nicotinamide and nicotinic acid for several different microorganisms have been tabulated. The reports are arranged in the order of increasing effectiveness of the amide. The results of our present study are included in the last line of the table.

This arrangement brings out strikingly the relative effectiveness of the amide and acid in promoting growth of those microorganisms for which these compounds must be supplied. The finding for the diphtheria bacillus, showing a distinctly greater growth-promoting effect for the acid, is quite different from that of other microorganisms thus far reported. *Proteus* can apparently make use of the acid about as effectively as the amide, while the staphylococcus

TABLE III.
Relative Growth-promoting Effect of Nicotinamide and Nicotinic Acid.

Organism	Ratio of activity, amide to acid*
Diphtheria bacillus ²	1:10
<i>Proteus</i> ³	1:1
<i>Proteus</i> ⁴	1:1
Staphylococcus ⁵	5:1
Dysentery bacillus ⁶	10:1
Certain <i>Pasteurellae</i>	∞ (acid ineffective)

*The results given in the literature are usually on the basis of growth at the first 24 or 48 hours after inoculation. In at least one instance, however (dysentery bacillus), it has been shown that on continued incubation of cultures for 7 to 14 days, slow growth occurs in the presence of successively smaller amounts of nicotinic acid, so that eventually the results with the two compounds are much the same. In this instance, the results at 24 hours are significant from the standpoint of bringing out differences in growth-promoting effect.

² Mueller, J. H., *J. Bact.*, 1937, **34**, 429.

³ Fildes, P., *Brit. J. Exp. Path.*, 1938, **19**, 239.

⁴ Breslove, B. B., unpublished results from this laboratory.

⁵ Knight, B. C. J. G., *Biochem. J.*, 1937, **31**, 731.

⁶ Dorfman, A., Koser, S. A., Reames, H. R., Swingle, K. F., and Saunders, F., *J. Infect. Dis.*, 1939, **65**, 163.

and dysentery bacilli evidently experience more difficulty in the utilization of the acid. While due allowance must be made for variation of individual strains the general trend is quite evident.

When nicotinic acid is supplied in place of the amide, it is often assumed that in the course of cell metabolism the acid is first converted to the amide before the compound is utilized further in cell respiration, although the results with the diphtheria bacillus would seem to be an exception to this interpretation. From the data presented it appears that microorganisms differ markedly in their ability to bring about this conversion. In view of these differences it seems reasonable to expect the existence of microorganisms which are completely unable to bring about this conversion and thus require the amide without being able to utilize the acid. The hemorrhagic septicaemia Pasteurellae appear to fit in this category and represent the widest divergence in ability to utilize these two compounds.

A further summary relating to the entire enzyme-coenzyme complex is of interest in showing gradations in synthetic properties among microorganisms. *Bacterium coli* may be taken as an example of those organisms which can synthesize the entire diphosphopyridine nucleotide molecule. Most strains of dysentery bacilli, *Proteus* and *Staphylococcus* are unable to synthesize the pyridine moiety of the nucleotide. These organisms, when supplied nicotinic acid, differ quantitatively in their ability to convert the acid to the amide but nevertheless are all able to perform this reaction and are also able to synthesize the coenzyme. Certain Pasteurellae are unable to convert the acid to the amide, but are able to synthesize the coenzyme when the amide is supplied, while *Hemophilus influenzae* must be supplied with the whole coenzyme.[†] It is possible that some of the intracellular parasites represent a further step in this series, and must be supplied with the enzyme as well as the coenzyme.

Summary. Nicotinic acid and nicotinamide do not always produce equivalent growth-promoting effects upon microorganisms. A series of microorganisms is presented showing a ratio of activity of amide to acid varying from one-tenth to infinity. The last case is represented by certain organisms of the Pasteurella group whose growth and respiration is promoted by nicotinamide, but not by nicotinic acid. A further summary is presented of synthetic abilities of different microorganisms with respect to the entire enzyme-coenzyme complex.

[†] While it is commonly assumed that the whole coenzyme molecule is needed by *H. influenzae*, it should be pointed out that the pyridine-ribose-phosphate portion of this molecule has never been tested for V factor activity. See Bass *et al.*, *J. Infect. Dis.*, 1941, **68**, 175.