Table 1

Vitamin required (either alone or with others)	Vitamin-requiring bacteria		
	No. of isolates	Percentage of total isolates	Approximate number per gm. soil
Thiamine (B_1)	96	19·2	10,100,000
Biotin	81	16·2	8,500,000
Vitamin B_{12}	35	7·0	3,700,000
Pantothenic acid	23	4·6	2,400,000
Folic acid	15	3·0	1,600,000
Nicotinic acid	10	2·0	1,000,000
Riboflavin	3	0·6	300,000

Of a total of 499 isolates, 135 or 27·1 per cent (corresponding to 14·1 millions per gm. of soil) required one or more vitamins for growth. The vitamins found to be needed, either alone or with others, are shown in Table 1 in order of frequency. With the great majority of the strains more than one vitamin was required; only in the case of thiamine, biotin and riboflavin were organisms found that needed but a single vitamin. In all, seventeen different 'patterns' were noted for vitamin requirements. For none of the isolates was pyridoxin, *p*-aminobenzoic acid, choline or inositol found to be essential, though in some cases these factors provided stimulation of growth.

Microbial growth-promoting substances and organisms requiring or synthesizing them have received less attention than the subject of microbial antagonisms in soil; yet growth-factor effects could well be as important as antibiotic activity in affecting the microbial equilibrium. The findings reported underline the importance of including growth-promoting substances in any consideration of the microbial economy of soils, whether related to problems of fertility or to those of the interrelationships of the normal soil microflora, plant disease organisms and the plant itself. The results also emphasize the wide diversity of the soil microflora in its nutritional aspects and point to the soil as an important habitat of vitamin-requiring bacteria.

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Action of Pepsin on Synthetic Substrates

It has recently been suggested[1] that the apparent first-order kinetics observed in the hydrolysis of certain synthetic substrates (for example, acetyl-L-phenylalanyl-L-tyrosine) by pepsin could be explained if one of the hydrolysis products (acetyl-L-phenylalanine) were only slowly released from the enzyme, as indicated in the following scheme:

$$E + AB \underset{k_2}{\overset{k_1}{\rightleftharpoons}} E.AB \xrightarrow{k_3} EA + B$$

$$e \quad s \quad p \quad q$$
(total)

$$EA \xrightarrow{k_4} E + A$$

where $k_4 < k_3$. (A, acetyl-L-phenylalanine; B, L-tyrosine. The small letters will be used as symbols for the concentrations of the various species, in the equations which follow.) The reaction-rate was measured by the rate of production of B (L-tyrosine amino nitrogen). This scheme would not, however, explain the observed results.

Making the usual steady-state assumptions and putting $K_m = \frac{k_2 + k_3}{k_1}$ we have:

$$\frac{ds}{dt} = k_3 p = \frac{k_3(e - q)s}{K_m + s}$$

This equation cannot be integrated directly (contrast ref. 1, equation 22) since q is a function of t. However, in the steady state $k_3 p = k_4 q$. That is, dq/dt, like dp/dt, is so much smaller than ds/dt that it can be neglected except in the initial stages of the reaction.

Therefore, $$q = \frac{k_3 es}{K_m k_4 + (k_3 + k_4)s}$$

and $$p = \frac{k_4 es}{K_m k_4 + (k_3 + k_4)s} = \frac{es}{K_m + \left(1 + \frac{k_3}{k_4}\right)s}$$

When $k_3 > k_4$, first-order kinetics will only be observed when $s \ll K_m$. This mechanism cannot explain the observed results, since s was of the same order of magnitude as K_m. It is clear, from the equation for p, that the proposed mechanism will lead to Michaelis–Menten kinetics with apparent k_3 and K_m values that are $k_4/(k_3 + k_4)$ times their true values.

The succeeding communication describes further experiments which provide the correct explanation of the first-order kinetics.

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In a previous communication[1] regarding the action of pepsin on two of its synthetic substrates, acetyl-L-phenylalanyl-L-tyrosine and N-acetyl-L-tyrosyl-L-tyrosine, results were obtained which seemed to indicate that the products of the hydrolysis were released from the enzyme-substrate complex at different rates, and that it was this phenomenon which accounted for the logarithmic course of the reaction. A communication afterwards received from Dr. N. M. Green showed that the release of the second product at a very slow rate, according to the hypothesis presented, would not lead to a logarithmic course (see accompanying communication by Dr. N. M. Green). A further study of the hydrolysis of acetyl-L-phenylalanyl-L-tyrosine by pepsin was therefore made, and has shown the conclusions that were previously drawn to be incorrect.

Since Haldane[2] has shown that a hydrolysis will follow a logarithmic course when the affinity of one of the products for the enzyme is the same as that of the substrate, experiments on the inhibiting effects of the products were again made. In these experiments it was found that concentrations of acetyl-L-phenylalanine and of the combined products of the hydrolysis which had previously inhibited the hydrolysis of acetyl-L-phenylalanyl-L-tyrosine only 6 per cent now caused 22 per cent inhibition. A search for any differences in technique that might account for these divergent results revealed that in the