contents are fundamentally part of the genotype or part of the phenotype is of interest.

The answer to this question also bears on whether protein or nucleic acid is the heart of the genetic apparatus. Evidence can be cited for the importance of either type of structure (16,17). It is, therefore, possible that either protein or nucleic acid or both may function as ultimate determinants of biological specificity, or both may be manifestations of a more fundamental structure, or perhaps, interactions of structures. The present results, like those of Knight's study on tobacco mosaic virus proteins(16), indicate that amino acid-containing structures play a central role in some of such determinations. On the other hand, the limited diversity of types found in cereal seeds(7) by these criteria, as well as statistical analyses of amino acid composition(18), suggest that no very elaborate structural code would be required for the nucleic acids to determine types of protein.

Summary. Wheat, rye, and a wheat-rye hybrid have been analyzed for leucine, lysine, methionine, and phenylalanine. The assays have included total, N-terminal, N-penultimate, and C-terminal proportions of these amino acids. In all cases, only lysine is N-terminal. C-Terminal assays permit the conclusion that this lysine is all terminal, and not free. The proportions of total lysine and N-lysine in the hybrid are intermediate between the values in the parent strains. The comparable result for leucine is not intermediate.

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Latent Viral Infection of Cells in Tissue Culture. III. Role of Certain Amino Acids.* (22526)

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Previous investigations have demonstrated that tissue cultures of minced chick embryos, maintained for 11 to 14 days in a simple medium of inorganic salts and glucose, can be infected readily with psittacosis virus but do not support the multiplication of this agent (1,2). Following inoculation, such a latent infection can be maintained successfully for periods up to 15 days, the duration of cell survival under such conditions(2). Moreover, it has been established that viral multiplication can be induced at any time during this period by the addition of embryo extracts or

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by a synthetic tissue culture medium(1-3). The finding that the initiation of viral multiplication can be accomplished by the addition of a completely synthetic medium made it possible to attempt the definition of the specific stimulatory principles involved. Further work has established that a modification of Parker's synthetic tissue culture medium #199, *i.e.*, medium #635 (equivalent to #199 except for the elimination of the purines, pyrimidines, pentose sugars, adenosine, adenylic acid, and ATP; and the addition of increased amounts of cysteine, glutathione, and ascorbic acid) was as potent as medium #199 in stimulating viral multiplication(3). Essentially, medium #635 consists of amino acids, water and fat soluble vitamins, plus inorganic salts and glucose. Partial fractionation of this mixture was performed and the important stimulatory groups therein were found to be the amino acids and the water soluble vitamins(3).

The studies to be described were directed toward determining which of the component amino acids of medium #635 were important in initiating the multiplication of latent psittacosis virus in chick embryo cells.

Materials and methods. The virus studied was the 6BC strain of psittacosis virus, obtained originally from Dr. K. F. Meyer, and since passed repeatedly in eggs via the yolk sac route. The preparation of a uniform virus suspension has been described(2). A dilution of the standard virus suspension in Hanks's(4) balanced salt solution (BSS) to a concentration of $10^{2.0}$ to $10^{3.0}$ LD_{50} per ml was used to infect the tissue cultures.

Tissue cultures. As described previously (1), 10-day chick embryos were harvested and minced, and this minced tissue was washed 4 times with BSS containing 0.125 ml of a 1.4% solution of sodium bicarbonate per ml. Then aliquots of tissue were transferred to 10 ml Erlenmeyer flasks each containing a disc of perforated cellophane and 1.8 ml of BSS with 0.025 ml of 1.4% sodium bicarbonate. All cultures were incubated at 36°C. Culture fluids were changed completely after the first 24 hours and every 4 days thereafter. On the 13th day of incubation, the cultures were inoculated with psittacosis virus, and the virus inoculum was titrated(1). On the 14th and 18th days culture fluids were removed completely for titration and were replaced with equal amounts of a test medium, or in the control flasks with the complete stimulating medium or BSS. Fluids were removed and titrated a third time at the end of the experiment on the 22nd day. Virus titers in the culture fluids were calculated as $\log_{10}$ of the LD_{50} for 7 day embryonated eggs, according to the method of Golub(5). After removal of the culture fluids at the conclusion of each experiment, the remaining cells were fixed by the addition of a 1:1 ether-alcohol mixture, and the cellophane discs from various representative flasks were removed and stained by a modification of the Papanicolaou technic (6). The stained cellophane discs were examined microscopically to ascertain the quality and quantity of the cell population and to establish the presence or absence of psittacosis inclusion bodies.

Materials tested. All media tested were variations of Parker's medium #199(7) and were prepared according to the directions supplied by Dr. Parker, with the exception of cysteine which was used in a concentration of 100 mg per ml. The basic test medium, designated hereafter as the complete medium, contained the following constituents of Parker's medium #199: the amino acid and water soluble vitamin fractions, cysteine, inorganic salts and glucose. All fractions were prepared in stock solutions which were stored in the cold with the exception of tyrosine and cysteine which were stored at room temperature. The test media, each deficient in one amino acid, were prepared by appropriate combination of the stock solutions, and were sterilized by filtration through a sintered glass filter immediately before use. The sterile solutions were stored under refrigeration.

Experimental. These investigations were begun with the information that the water soluble vitamins and amino acids of Parker's synthetic medium #635 were capable of stimulating the growth of psittacosis virus in chick embryo tissue culture(3). Since it had previously been noted that the amino acid ana-

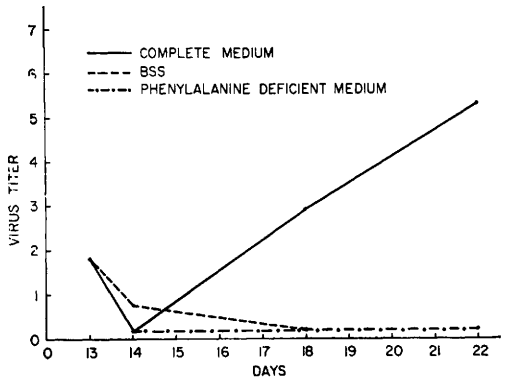

FIG. 1. Effect of phenylalanine deficiency on stimulation of growth of psittacosis virus (6BC) in chick embryo tissue cultures cultivated in BSS for 13 days.

logues, B-2-thienylalanine and 6-methyltryptophane, inhibited the growth of psittacosis virus in tissue culture(8), it was decided to test the effect of the omission of single amino acids on the stimulatory capacity of the complete medium. Accordingly, media were prepared which were identical to the complete medium in every respect except for the exclusion of one amino acid. In a series of experiments the stimulatory capacity of each of these deficient media was compared with that of the complete medium and with Hanks's BSS. It was found on repeated observations that phenylalanine and tryptophane were essential to stimulation of viral multiplication. Representative experiments are illustrated graphically in Fig. 1 and 2. Aspartic acid, hydroxyproline and lysine, on the other hand, were found not to be essential (Table I). At the conclusion of each experiment the cellophane discs from representative flasks were stained and examined as a check on cellular growth. In all cases, including those cultures grown in deficient media, viable cells were seen.

Discussion. The present studies confirm the finding that chick embryo cells grown *in vitro* in a deficient medium lose the capacity to support proliferation of psittacosis virus, but that this capacity can be restored by addition of certain nutrient substances to the culture fluids(1,2). It had already been shown that water soluble vitamins and amino acids alone could produce this same effect(3) and now it has been found that at least 2 amino acids are essential to stimulation of viral proliferation, while at least 3 are not. The finding that phenylalanine and tryptophane are essential agrees with the previous observation(8) that the corresponding amino acid analogues, B-2-thienylalanine and 6-methyl-tryptophane, inhibit the growth of psittacosis virus in tissue culture without showing evidence of toxicity for the host cells. Furthermore, it is of interest to note that these 2 amino acids are among those found by Eagle to be essential for the cultivation of mammalian cells *in vitro*(9).

The data presented lend additional support to the concept that the capacity of a cell to support the multiplication of a virus is directly related to the availability of essential nutrient substances to such a cell. Moreover, it would appear that viral multiplication is dependent not only upon an adequate supply of essential metabolites to the host cell, but that indeed a cell must be capable of a high level of metabolism if it is to support the proliferation of a virus. The mere viability of a cell by no means insures viral growth, for, as shown in the studies described herein, such growth never occurred in the absence of phenylalanine or tryptophane, although in every case viable cells were available to the virus. It is probable that the cell, even in this depleted state, has available sufficient quantities of essential metabolites to remain viable, but insufficient in amount to permit shunting of

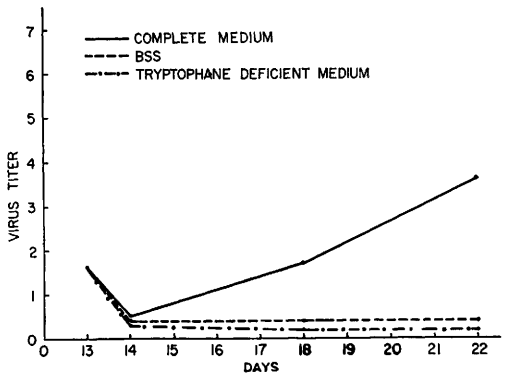

FIG. 2. Effect of tryptophane deficiency on stimulation of growth of psittacosis virus (6BC) in chick embryo tissue cultures cultivated in BSS for 13 days.

TABLE I. Effect of Various Amino Acid Deficiencies on Stimulation of Growth of Psittacosis Virus (6BC) in Chick Embryo Tissue Cultures Cultivated in BSS for 13 Days.

Amino acid deficiency		Virus titers* 13th day	14th day	18th day	22nd day
Aspartic acid		1.9	.1	4.7	5.0
Controls	Complete medium	1.9	.1	3.0	5.3
	BSS	1.9	.9	.1	.1
Hydroxyproline		3.1	.5	1.9	4.2
Controls	Complete medium	3.1	.1	2.1	4.2
	BSS	3.1	.1	.1	.1
Lysine		1.9	.1	3.9	5.4
Controls	Complete medium	1.9	.1	3.0	5.4
	BSS	1.9	.9	.1	.1

* Log of LD_{50}.

cellular metabolism into the reduplication of virus particles. Hence, when the virus enters a deficient cell, it remains present in the latent state in the non-infectious phase(2) until such time as the necessary metabolites become available, or until the death of the cell. In psittacosis, a disease characterized by long periods of latent infection in birds, this concept is of special interest, as a variety of changes in the host may lead to the activation of infection. As indicated by these studies, changes in cell nutrition as it relates to the amino acid may be important determinants in such activation.

Summary. Studies on the amino acid requirements for the activation of latent infection with psittacosis virus of chick embryo tissues cultivated *in vitro* revealed that phenylalanine and tryptophane are essential to the proliferation of psittacosis virus, while aspartic acid, hydroxyproline, and lysine are not. The importance of host cell metabolism to viral infection is discussed with special reference to the amino acids.

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Tungstate Antagonism of Molybdate in *Aspergillus niger*.* (22527)

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A requirement for molybdenum by *Aspergillus niger* has been well established(1), particularly when nitrate is the sole source of nitrogen(2). Mo is specifically required in the nutrient medium for the enzymatic reduction of nitrate(3), and Nicholas and Nason (4) showed that it is part of the prosthetic group of the enzyme nitrate reductase. With Mo present in the growth medium, no nitrogen compounds other than the nitrate ion are

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