Presumably, the casein basal diet contained enough vit. B_{12} as an impurity in the casein so that the additional supplement of 3 μg/100 g was sufficient for protection against the CCl_4. Growth of rats on the casein basal diet was not significantly improved by supplementation with vit. B_{12} alone. The apparent growth improvement seen in the data in Table II was not nearly so evident when comparison was made with controls from the specific experiment. Dilution of these control values with large numbers of control values from other experiments was done to emphasize the lethal influence of CCl_4 since this was not affected by growth or body size of the rats.

Goyco(6) recently reported on the beneficial effect of vit. B_{12}, vit. E and methionine on utilization of yeast protein by rats. His results were similar in many respects to the data obtained with the soybean meal protein diet and herein reported.

Summary. Vit. E and vit. B_{12} can replace each other in promoting protein utilization and in protecting against acute carbon tetrachloride toxicity in young rats reared on 10% protein diets deficient in these factors. The vit. B_{12} requirement for protection against CCl_4 is considerably higher than for growth. Folacin supplements, alone, had no effect, but, in the presence of vit. B_{12} or vit. E, this factor markedly improved protein utilization and growth. L-Cystine protected against the acute toxicity but a synergism between cystine and vit. E was not noted.

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Inhibition of Influenza and Mumps Viruses in Tissue Culture by Basic Amino Acids.* (18829)

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Recent studies(1-4) have indicated that modification of the host-cell metabolism by a variety of substances may interfere with the growth of animal viruses in tissue culture. Emphasis has been placed on inhibition of viruses by metabolic antagonists such as methoxinine and malonic acid(1-3) but an excess of amino acids may also inhibit virus multiplication as exemplified by the effects of lysine and tryptophane on a neurotropic virus in mouse brain tissue cultures(1). In the present study inhibition of the growth of influenza and mumps viruses by arginine and other amino acids was observed. Investigations on the effects of these substances on the viruses and tissues themselves are reported.

Methods. Tissue cultures of minced chorioallantoic membrane of 10 to 12 day old chick embryos in balanced salt solution with glucose and sodium bicarbonate were prepared according to methods previously described(4-5). Allantoic passage strains of mumps and influenza (PR8 and Lee) were inoculated at dilutions of 10^{-2} into tissue cultures containing the substances to be tested. Cultures containing mumps and the PR8 strain of influenza were incubated at 35°C, those with the Lee strain at 31°C. Virus multiplication was measured by hemagglutination titrations with chicken erythrocytes

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TABLE I. Effect of Amino Acids on Growth of Influenza B in Tissue Cultures.

Substance	Conc., mg/ml	6 day hemagglutination titers Test	Control	Ratio control/test
l-arginine	5	12	60	5
	5*	2	100	50
	2	15	39	2.6
dl-lysine	5	3	65	22
	2	6	45	7.6
	2*	2	70	35
dl-ornithine	10	<1	100	>100
	2	80	100	1.3
Guanidoacetate	10	12	100	8.3
	2	32	100	3.1
dl-histidine	5	38	77	2
Creatine	5	56	34	.6
Protamine	1	16	45	3

* Buffered at pH 7.8-8.0. In remaining experiments a drop in pH to 7.0 or 6.8 occurred over a period of 6 days.

and in some instances by infectivity titrations.

Results. The results of experiments with the Lee strain of influenza B are summarized in Table I. L-arginine and dl-lysine have been studied most extensively and were found to produce some inhibition at concentrations down to 1 or 2 mg/ml. Inhibition of formation of viral hemagglutinins was marked with ornithine and moderate with guanidoacetate at 10 mg/ml. Histidine produced only slight effects while results with creatine at the concentrations tested were negative. Small effects similar to those with histidine were seen with other amino acids such as glycine, and also with asparagine. Protamine at the maximum non-toxic concentration of 1 mg/ml did not markedly effect the growth of virus. L-lysine was found to be approximately twice as active as the dl-form. Lysine and arginine at concentrations of 10 mg/ml inhibited the growth of influenza A when this was inoculated at dilutions of 10^{-3} but had little effect against larger inocula. With influenza B addition of 5 mg/ml of arginine 24 hours after the virus produced the same degree of inhibition as when added immediately, but no effect was observed when the period was increased to 48 hours. Infectivity titrations in representative experiments indicated that arginine and lysine reduced the infective property of influenza B in tissue cultures to about the same extent as hemagglutination.

Inhibition of the Lee strain by lysine and arginine was favored by using cotton stoppered flasks and adding enough bicarbonate buffer to the medium to maintain the pH between 7.8 and 8.0 (Table I), also by omission of phosphate from the medium. No reversal of the effect of arginine was obtained with ribose nucleic acid, adenylic acid, methionine or creatine, but preliminary experiments suggest that phospholipids may have some reversing activity since virus grows normally in cultures containing 5 mg/ml of arginine and 10% of egg yolk.

Extension of these observations to mumps virus which grows more slowly gave a better insight into the kinetics as will be seen in Fig. 1. Although a single addition of 10 mg of arginine at the beginning of the experiment was without effect, changing the fluids so as to add fresh amounts of arginine at 4 days resulted in inhibition as did delay in the addition of arginine until the 4th day after inoculation. In the cultures containing arginine some growth of mumps virus was observed between the 12th and 20th day and removal of the arginine by changing the fluids on the 8th day to balanced salt solution without arginine resulted in more active growth of virus by the 16th day. The loss of effect with time does not seem to be due to disappearance of the arginine because no marked diminution could be detected by assays with an arginine requiring mutant of *E. coli.*

No direct inactivating effect of arginine on influenza virus *in vitro* could be demonstrated. When dilutions of virus in balanced salt solution were incubated at 31°C with arginine at a concentration of 20 mg/ml infectivity disappeared less rapidly than in controls without arginine. Similar concentrations of arginine when added to dilutions of mumps or influenza virus plus chicken erythrocytes did not alter the hemagglutination end point. Arginine at concentrations of 5 mg/ml did not prevent the adsorption of virus to chorioallantoic membrane either at 37°C or at 4°C.†

Exposure of fragments of chorioallantoic membrane for periods up to 16 days to ar-

† We are indebted to Mr. Robert Gohd for these determinations.

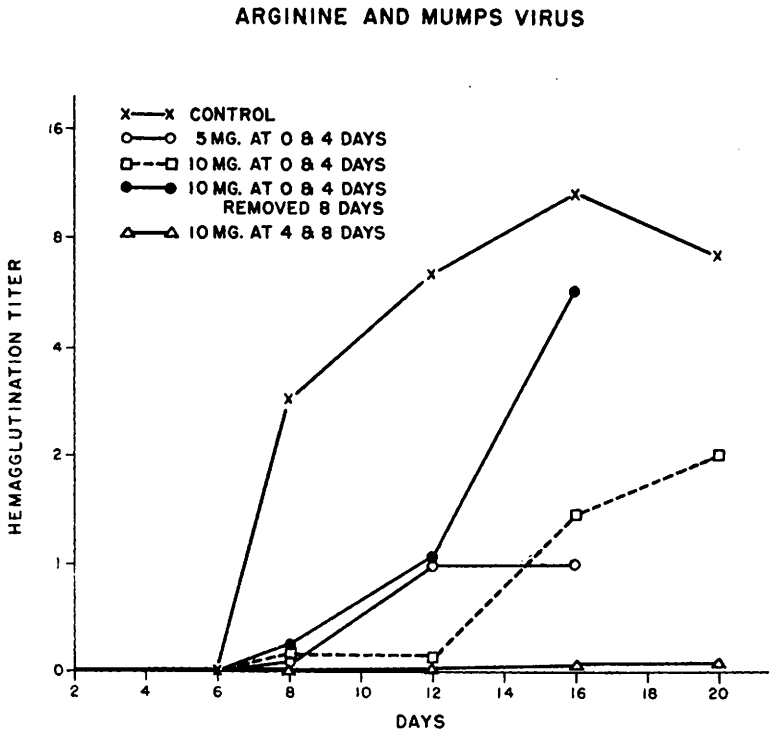

FIG. 1. Open circles and squares; balanced salt solution containing arginine changed to same solution at 4 days. Solid circles; same but additional change to balanced salt solution without arginine at 8 days. Triangles; started with balanced salt solution only and changed twice to arginine 10 mg/ml at 4 and 8 days. Control curve represents avg titers of series with and without change of solution.

ginine at concentrations of 10 mg/ml or lysine at 5 mg/ml did not reduce the ability of the cells to grow after explant to a fibrin clot medium in roller tube cultures. In such fragments the outgrowth of epithelial tissue and fibroblasts occurred in the same manner as in the controls. Concentrations of arginine and lysine of 20 mg/ml were lethal to tissue. When the amino acids were added directly to the roller tube cultures retarded growth of tissue was observed with lysine at 10 mg/ml but was less evident with arginine.

Since some inhibitors of virus growth were shown to reduce tissue respiration(2) the effect of arginine on oxygen uptake of the chorioallantoic membrane suspended in buffered salt solution was determined in the conventional Warburg apparatus. Respiration was found to proceed at the same rate in the presence of arginine at a concentration of 5 mg/ml as in controls without arginine. Exposure to arginine in virustatic concentrations for as long as 6 days did not affect the rate of oxygen uptake. Similarly the rate of disappearance of glucose in tissue cultures measured over a period of 6 days was identical in the presence and absence of arginine. In contrast to some other inhibitors, arginine does not seem to exert its virustatic action by affecting the respiratory activities of the host cell.

In preliminary experiments no effect of arginine in virustatic concentrations on the growth of *A. aerogenes* in a minimal medium containing glucose could be found, and the capacity of this organism to adapt to growth on mesoinositol was not affected(6).

Discussion. Although several different amino acids will reduce the rate of viral multiplication in tissue cultures those containing 2 amino groups appear to be the most active. Arginine which has been studied in most detail

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probably inhibits the growth of influenza and mumps viruses by some effect on the host cell or on the interaction of host cell and virus. This effect is reversible by removing the amino acid and is brought about in the absence of demonstrable changes in the oxygen consumption, glucose utilization, or growth potential of the tissue. The reversal of the effect of arginine by egg yolk suggests the possibility of interaction with lipids in the cell membrane. The augmenting effect of an alkaline reaction (pH 8.0) is similar to that seen with basic antibacterial substances such as streptomycin and diamidines(7). Synthetic lysine polypeptides have been reported to inhibit the growth of influenza virus(8), but these cause aggregation of virus particles, bacteria and red cells while arginine and lysine apparently have no direct effect either on the infective or hemagglutinating properties of mumps and influenza viruses.

Summary. Arginine, lysine, ornithine, and to a lesser extent some other amino acids retard the growth of influenza and mumps viruses in tissue cultures when added at concentrations of 1 to 10 mg/ml. The virustatic effect of arginine and lysine could not be attributed to toxicity or to direct action of these amino acids on the viruses.

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Failure of an Ergot Preparation to Shorten Coagulation Time in Hemophilia.* (18830)

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Vodopivec(1) describes a striking acceleration of the clotting of the blood of hemophilics by administration of a mixture of equal parts of 3 hydrogenated ergot alkaloids, namely dihydroergocristine, dihydroergocornine, and dihydroergokryptine. This preparation, known also as Hydergine or CCK-179, was reported to be effective orally in dosage of 1 mg every 3 hours and parenterally after a single injection of 0.6 mg. The clotting time fell to normal 2 to 3 hours after oral, 1 to 2 hours after subcutaneous, and 30 minutes after intravenous administration. The effect persisted for a day after the drug was given orally, and for several hours after parenteral treatment. Although we are ignorant of other observations on the use of ergot derivatives in hemophilia, several papers(2-4) discuss the adrenergic blockade effected by Hydergine and its several components.

Because great clinical interest attaches to any pharmacologic agent able to accelerate coagulation in hemophilia, we have examined the effects of Hydergine† in 4 hemophilics.

Methods. Coagulation time in glass at 37.5°C was determined by the method of Pohle and Taylor(5). One-stage prothrombin levels were measured as described by Rosenfield and Tuft(6), and 2-stage determinations by the method of Ware and See-

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† Kindly supplied by Mr. Kenneth Ericson of Sandoz Pharmaceuticals, New York City.

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