ing high EA ratios are associated with low percentages of nitrogen (except preparation 13). The index of each of the hyaluronic acid preparations was plotted on arithmetic graph paper as the ordinate (Fig. 1). Laboratory preparations 7 and 13 provided the maximum deviations from the curve. Preparation 13 gave an unusually low turbidity reading after having been acted on by 5 turbidity reducing units of bull testis enzyme. It is doubtful whether preparation 7 actually was a hyaluronate. Certain actions of hyaluronidases of varying origin on these substrates will be reported at length.

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18 November 1954

Foot-and-Mouth Disease Virus: Its Growth and Cytopathogenicity in Tissue Culture

We wish to report that foot-and-mouth virus (FMV) reproduces and causes cytopathogenic changes in both swine and bovine kidney tissue cultures. Practical techniques have been developed for the assay of the virus and its antibodies as well as for the production of virus on a relatively large scale.

Swine or bovine kidney from young animals was cut into pieces weighing approximately 0.5 g and placed in phosphate buffer solution at *p*H 7.3 containing penicillin and streptomycin. The tissue was washed repeatedly in fresh changes of buffer solution and then stirred gently in a 0.25-percent trypsin buffer solution. The liberated cells were decanted at 10-minute intervals through cheesecloth, centrifuged at low speed, washed twice, and diluted 1 to 200 with culture medium. The medium consisted of Hanks' salt solution containing 2.0 percent bovine serum, 0.5 percent lactalbumin hydrolyzate, penicillin, and streptomycin. Culture tubes (16 mm in diameter by 150 mm) and Roux flasks were seeded with the cellular suspensions and incubated stationary at 37°C. The swine and bovine cultures developed confluent outgrowths of mixed epithelial and fibroblastic cells within 3 and 6 days, respectively.

Table 1. Cytopathogenicity and growth of foot-and-mouth disease virus type A in tissue culture.

Passage		Total dilution* (neg. log)	Tissue culture ID_{50}/ml		Generalized FMV disease in
No.	Method†		TP expt. in swine kidney (log)	Titrations in bovine kidney (log)	
1	CP	2.0			
2	CP	3.0			
3	CP	4.0			
4	TP	5.0	5.80		
5	TP	9.0	5.98	6.80	Guinea pigs
6	TP	13.0	5.68	5.68	
7	TP	16.0	‡	5.98	
8	TP	19.0	4.80		
9	TP	21.0	6.15		
10	TP	25.0	5.93		
11	TP	29.0	5.68		
12	TP	31.0	5.98	5.47	
13	TP	39.0	5.80		Steer
14	TP	35.0	6.13		

* Effective dilution of original mouse infectious tissue (neg. log). † CP, conventional passage method; TP, titration-passage technique. ‡ Culture acidity interfered with reading.

Foot-and-mouth virus type A was passed 14 consecutive times in swine cultures (Table 1). The first four passages were made by conventional methods. The virus inoculum for the first passage was of bovine origin and was passed once in suckling mice just prior to its use (*1*). When it was inoculated into cultures, cytopathogenic changes were produced that were similar to those reported for cultures infected with vesicular exanthema and vesicular stomatitis viruses (*2, 3*). Isolated plaques of pyknotic cells were formed in less than 15 hours. Passages 5 to 14 were made by a titration-passage technique, which had been used previously with vesicular stomatitis virus, in which each passage is made as a complete titration (*3*).

Five cultures were inoculated with each dilution passed. After 20 hours' incubation, the cultures were examined microscopically. The pooled fluid from the highest dilution in which all cultures exhibited extensive cytopathogenic damage was serially diluted and used as inocula for the next titration passage. In most instances, this procedure effected a 10^{-3} or 10^{-4} interpassage dilution of the cytopathogenic agent (Table 1, column 3). The inocula used for serial passages 5 to 7 and 12 in swine cultures were also assayed in bovine cultures. Table 1 shows that titration-passage inocula usually contained about $10^{6.0}$ ID_{50} per milliliter for both kinds of cultures. After a few passages in swine cultures, it was evident that infectivity of the original virus inoculum had been diluted out and that the cytopathogenicity that persisted was probably owing to the propagation of FMV.

This presumption was confirmed by animal inoculations and serum neutralization tests. Passage fluid No. 5 (10^{-9} dilution of the original mouse tissue) was inoculated intradermally into the foot pads of guinea pigs. Passage fluid No. 13 was inoculated intradermolingually into a Hereford steer. Both animal species developed extensive local and generalized lesions of foot-and-mouth disease.

In the neutralization tests, the capacities of normal guinea pig serum and of two antiserum samples (*1*) to neutralize the infectious culture fluid from passage No. 5 were determined. The antiserums used were from guinea pigs that had been hyperimmunized to vesicular stomatitis virus (New Jersey type) and to FMV (type A). Tenfold serial dilutions of the infectious fluid were made and aliquots of each were mixed in equal parts with a 1 to 5 dilution of each serum type. The virus and virus-serum mixtures were incubated for 1 hour at 37°C and then assayed in bovine kidney cultures. The virus fluid alone was found to contain $10^{5.83}$ tissue culture ID_{50} per 0.2 ml, but in the presence of the type A antiserum, it retained less than 10 such units of infectivity. The normal and heterologous serums had no detectable neutralizing activity.

It is estimated that the kidneys from one calf will supply enough cells to prepare 50,000 cultures in tubes or more than 300 in Roux flasks. The cultures in tubes are now being used regularly for assays of virus taken directly from in-

fected bovine tongue tissue. The Roux flask cultures are used for the production of FMV on a relatively large scale. Each flask yields 75.0 ml of fluid containing approximately $10^{7.0}$ tissue culture ID_{50} per milliliter.

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1 August 1955

Ability of Sodium Sulfate to Stimulate Growth of the Chicken

Reports demonstrating that only a trace of $Na_2S^{35}O_4$ is incorporated into sulfur amino acids (*1, 2*), that larger fractions are incorporated into taurine (*1, 3–5*), but that the largest uptake of radiosulfate occurs in the chondroitin sulfate matrix of cartilage (*2, 3*) led us to study the nutritional significance of these findings (*6*).

Chickens were fed rations containing casein, 15 percent; gelatin, 10 percent; corn oil, 4 percent; salts "A" (*7*), 6 percent; all essential vitamins in excess of their requirements (*8*); and glucose to make 100 percent. The protein furnished about 0.08 percent cystine and 0.51 percent methionine.

Sulfur or Na_2SO_4 added to this diet did not improve growth; however, when the sulfates of magnesium, manganese, and copper present in salts "A" were replaced with equimolar levels of the corresponding oxides or chlorides (new basal diet: LC2MS), it was found that sodium sulfate improved growth and feed efficiency. Typical data are given in Table 1. Dietary sulfate, in addition, appears to be capable of stimulating normal feather development, even though the sulfur amino acid content was too low to support optimal growth.

Feathers from birds that received $Na_2S^{35}O_4$ for 10 to 14 days in amounts sufficient to maintain blood levels of 0.01 μc/ml of plasma were quite radioactive (0.5 to 1.0 percent of the total isotope dose), but feather cystine and methionine accounted for less than 5 percent of this activity. Sulfate isolated after feather hydrolysis accounted for 60 percent, and about 30 percent was contributed by a nonsulfur amino acid, nonsulfate fraction that is still unidentified. This unidentified fraction also accounted for about 30 percent of the total feather activity when methionine-S^{35} was fed in a similar fashion even though the sulfate fraction then accounted for less than 5 to 10 percent.

Sulfur amino acids isolated from hydrolyzates of other tissues from birds that had received $Na_2S^{35}O_4$ for the period of 10 to 14 days incorporated only small amounts of S^{35}. We feel that the low levels of activity found in tissue sulfur amino acids merely reflect bacterial synthesis in the alimentary tract.

Taurine accounted for approximately 15 to 25 percent of liver activity; sulfates (after acid treatment) accounted for about 60 percent. Machlin (*5*) reported that about 20 percent of the sulfur from $Na_2S^{35}O_4$ that is retained by chickens is incorporated into body taurine. We found that, regardless of the ration, chickens retained initially 50 to 75 percent of a given oral dose of $Na_2S^{35}O_4$, and by 10 to 14 days, 15 to 30 percent had not been excreted. We also confirmed in a preliminary way the reported (*2, 3*) rapid uptake of radio sulfate into the mucopolysaccharide-mucoprotein "organic" sulfate fractions isolated from connective tissues.

Our work indicates that the chicken can satisfy part of its total sulfur requirement with inorganic sulfate. The sulfur amino acids meet much larger fractions of this sulfur requirement. However, large quantities of either methionine or methionine hydroxy analog did not appear completely able to satisfy the total sulfur requirement when they were administered to rapidly growing chickens that were fed a cystine-low, inorganic-sulfur-free diet. Cystine, added to such diets (provided that methionine was not limiting), did satisfy the requirement under these conditions when it was added at sufficiently high levels. Inorganic sulfate cannot replace dietary cystine or methionine for protein synthesis, and studies with $Na_2S^{35}O_4$ appear to confirm these results, although sulfate apparently will "spare" dietary sulfur amino acids for protein synthesis. The effect of elemental sulfur and inorganic compounds of sulfur, the metabolism of S^{35} and $Na_2S^{35}O_4$, the effect of sulfate on methionine-deficient diets, as well as a more detailed account of the work reported here, is in preparation.

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6. We wish to express our appreciation to G. M. Briggs, National Institutes of Health, and L. J.

Table 1. Effect of Na_2SO_4 and graded levels of sulfur amino acids and analogs on growth of chickens.

Ration and supplements	Gain (g)	Standard error*	Feed efficiency†	Feather score‡
Five weeks				
Basal (LC2MS)§	371.5	24.5	2.84	1.2
Basal + 0.5% Na_2SO_4	488.1	24.6	2.70	3.6
Basal + 0.22% DL-methionine	516.7	23.8	2.43	1.8
Basal + 0.22% methionine hydroxy analog‖	521.0	19.7	2.41	2.1
Basal + 0.22% analog + 0.5% Na_2SO_4	617.0	16.4	2.38	3.8
Three weeks				
Basal (LC2MS)#	119		4.01	
Basal + 0.5% Na_2SO_4	214		2.54	
Basal + 0.22% DL-methionine	163		2.26	
Basal + 0.22% DL-methionine + 0.5% Na_2SO_4	301		2.15	
Basal + 0.44% DL-methionine	259		2.46	
Basal + 0.44% methionine hydroxy analog	263		2.36	
Basal + 0.49% analog	274		2.14	
Basal + 0.44% analog + 0.5% Na_2SO_4	308		1.71	
Basal + 0.4% L-cystine	311		1.75	

* Standard error for one group of 10 to 12 New Hampshire cockerels raised in starting batteries of conventional type with raised wire screen floors.
† Feed consumed divided by weight gained. Under these conditions, an improvement (less feed consumed per unit gain) in excess of 0.10 was found to be significant.
‡ Average of scoring by two persons of each group; a difference in excess of 0.5 was found to be significant on a scale of 0 to 4.0.
§ 4.1 g $MgCl_2$, 0.37 g $MnCl_2$, and 0.03 g $CuCl_2$ used instead of corresponding sulfates in salts "A."
‖ Calcium DL-2-hydroxy, 4-methylthiobutyrate.
1.1 g MgO, 0.32 g MnO_2, and 0.03 g $CuCl_2$ used instead of corresponding sulfates in salts "A."