in contrast to the findings in normal subjects is possibly because of quicker synthesis of nicotinic acid. The high initial urinary nicotinic acid and its greater output after tryptophan feeding in schizophrenia supports this hypothesis.

Summary. 1. Urinary excretion of 5-hydroxyindolylacetic acid and 3-indoleacetic acid was estimated in 10 patients suffering from schizophrenia both before and for 3 days after feeding of tryptophan. Similar studies were made on 10 normal subjects also. 2. Urinary excretion of 3-indoleacetic acid increased both in normal and schizophrenic subjects after administration of tryptophan. Tryptophan also gave rise to increase in urinary excretion of 5-hydroxyindolylacetic acid in schizophrenia but no significant rise was detected in normal subjects. 3. Urinary excretions of nicotinic acid, quinolinic acid, NMN, 6-pyridone, tryptophan, kynurenin, anthranilic acid and 3-hydroxyanthranilic acid were estimated in 10 schizophrenic subjects before and for 3 days after feeding of tryptophan. 4. Nicotinic acid, quinolinic acid, NMN and 6-pyridone were present in urine initially and they were excreted in greater quantities after tryptophan feeding. Tryptophan which was initially absent also appeared in urine after its administration. Kynurenin, anthranilic acid and 3-hydroxyanthranilic acid could not be detected in urine both before and after administration of tryptophan.

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Purification and Electron Microscopy of Foot-and-Mouth Disease Virus. (23838)

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The present study concerns purification of foot-and-mouth disease virus (FMDV) from infectious tissue culture fluid, as well as identification of the virus particle in the electron microscope by the sensitive method of direct particle counting(1,2) and by aggregation of virus particles by antiviral serum(3). The method of analytical electron microscopy(1) was used to determine the concentration of virus particles in purified virus concentrates and to estimate the number of such particles which constitute one plaque-forming unit (PFU) of infectivity for bovine-kidney monolayer cultures(4).

Materials and methods. Virus was cattle-adapted FMDV, type A, strain 119, obtained from the Research Institute, Pirbright, England. It was subsequently transferred serially 70 or more times in bovine-kidney cultures in Roux flasks, according to procedures previously outlined(5). Twenty to 40 such cultures were used per serial transfer. Each

flask yielded approximately 75 ml of infectious fluid, ranging from $10^{6.5}$ to $10^{8.3}$ PFU per ml. Fluids were harvested, pooled, adjusted to pH 7.5, and stored in 750-ml amounts in paraffin-sealed bottles at −40°C. Smaller volumes for infection of subsequent cultures were placed in flame-sealed ampules. Frozen virus, as needed, was thawed to 0°C. Virus treated in this manner maintained a constant plaque-forming activity for several months. *Infectivity measurements.* Infectious culture fluids and purified viral fractions therefrom were assayed in bovine kidney monolayer cultures by a plaque procedure described in detail elsewhere(4). Twenty to 80 PFU were almost always recorded for one of the dilutions inoculated. In this range twice the standard deviation of plaque counts varies from the mean by less than 31%(4). *Nitrogen analyses.* A digestion and nesslerization procedure with a reproducibility of at least 5% in the range of 5 to 16 μg N per sample was used to determine the protein-nitrogen content of tissue culture fluids and purified virus fractions(1). *Analytical electron microscopy.* Correlation of counts of characteristic physical particles with infectivity and antigenic tests makes unequivocally possible identification of a virus particle in preparations containing only a few particle species (1,3). In these experiments 8 parts of purified virus concentrate in ammonium acetate or ammonium bicarbonate at pH 7.5 were mixed with 2 parts of a 1:1 mixture of 1% bovine albumin (Armour) and uniform-sized polystyrene latex (PSL) spheres (88 mμ and 6.5×10^{10} per ml). The final mixture was deposited on collodion-coated electron microscope specimen grids as microdroplets from the low-velocity spray of a glass nebulizer (6). These droplets after drying, in air and *in vacuo,* to remove volatile and sublimable substances were shadowed with uranium and photographed in the electron microscope. The circular-shaped microdroplet residues were imaged in their entirety at magnifications of 5,000 or greater, sufficient to permit inspection and accurate enumeration of the characteristic particles present. Particle sizes and concentrations were determined by comparison with the known values for PSL spheres. The ratio of physical particles to plaque-forming activity of the purified virus concentrates was then calculated. The critical experiments for identifying the FMDV particle were similar to those of Eckert *et al.*(3) for the identification of myeloblastosis virus particles in which degrees of virus neutralization and precipitation of virus adenosine triphosphatase activity by specific immune serum were correlated with aggregation of particles in the electron microscope. In the present experiments steers were used as the homologous host for preparation of immune sera. After recovery, their neutralizing antibodies were enhanced by suitably-spaced booster injections of virus. In preparation for the particle aggregation tests, sera and purified virus concentrates were mixed as follows: Bovine albumin, usually employed for spraying with virus concentrates and PSL particles, was replaced wholly or in part by serial dilutions of immune serum in the tests and by normal serum in controls. The mixtures were incubated at 37°C for one hour, and portions were then used for electron micrography to determine degree of aggregation of the characteristic physical particles. A measure of aggregation was obtained from the difference between particle concentration in normal serum and concentration of unclumped particles in the presence of antibody. *Virus purification.* Purification and concentration sufficient for imaging and identification of FMDV in the electron microscope were accomplished by successive application of chemical and physical procedures not detrimental to the unstable infective property of this virus(7). Degrees of purification at each stage were established by infectivity and protein-nitrogen assays. The purification and concentration procedure is summarized as follows: Frozen infective tissue culture fluid (320 to 2,400 ml) at pH 7.5 was thawed to 0°C, and the virus, as well as considerable amounts of nonviral protein, was aggregated at −3°C by slow addition of methyl alcohol to a concentration of 20%. The suspension was stored overnight or longer at −6°C and centrifuged at 2,000 rpm for one-half hour in an International refrigerated PR-1 centrifuge. The sedimented precipitate was suspended at pH 7.5 to one-fourth the original

TABLE I. Recovery of Infectivity and Degree of Purification of Type A Foot-and-Mouth Disease Virus.

Purification step No.	Fraction	Recovery of infectivity (%) Tissue culture pool No. 1	2	3	4	5	6	7	8	9	10	avg*	Purification* μg PN/ml	Increase in specific infectivity†
	Original TC fluid											100	230	
1	Soluble part of methanol ppt.	80	69	100	92	92	71	97	100	87	88	88	60	3.4-fold
2	Aqueous phase after extraction with organic solvent (s)	54	69	107	106	116	89,80‡	55	112§	87	95	88	9	22.8-fold
3	Purified virus concentrates after differential centrifugation	27	22	36	62	62	27	30	75	15	82	44	1.5	68.0-fold

* Avg of 10 experiments; μg PN of the fractions were corrected to unconcentrated basis.
† See text for definition.
‡ BuOH-$CHCl_3$ mixture followed by trichlorotrifluoroethane.
§ Trichlorotrifluoroethane instead of BuOH-$CHCl_3$.

volume in phosphate-buffered saline (0.11 M NaCl in 0.01 M Na_2HPO_4) or 0.15 M ammonium acetate. Two volumes of a 50:50 or 85:15 mixture of *n*-butanol and chloroform or, in some experiments, of trichlorotrifluoroethane were introduced with stirring. Mixing was accomplished over a one-half hour period in the former case by intermittent shaking and in the latter case by a Servall Omnimixer operating at top speed with its cup immersed in an ice bath. The organic aqueous emulsion which resulted was broken by centrifugation at 2,000 rpm for one-half hour. The separated aqueous phase contained the virus. Further purification and concentration were accomplished by one cycle of high-and-low-speed centrifugation in a Spinco Model-L centrifuge. The virus was sedimented from the aqueous phase during a 135- or a 150-minute run in a No. 30 rotor at 30,000 rpm (78,410 g). The resulting virus pellet was suspended by vigorous pipetting in a small volume of phosphate-buffered saline, ammonium acetate, or ammonium bicarbonate at pH 7.5 and finally cleared of large insoluble aggregates by centrifugation in a No. 40.2 rotor at 10,000 rpm (6,370 g) for 30 minutes. The supernatant fluid from this run represented the purified virus concentrate and, when in ammonium acetate or bicarbonate, was ready for electron microscopy. Purified concentrates in phosphate-buffered saline were suitable for protein-nitrogen analyses, but for use in electron microscopy were first dialyzed against ammonium acetate. The degree of concentration achieved varied between 50- and 400-fold depending upon the initial volume of infectious tissue culture fluid and upon fluid volumes used to resuspend high-speed pellets. The temperature was maintained at 4°C except where otherwise stated.

Results. Recovery and purification of virus infectivity in 10 experiments are summarized in Table I. The average recovery of virus after successively applied purification steps of alcohol precipitation, extraction with organic solvents, and differential centrifugation was 88, 88, and 44%, respectively. Infectious tissue culture fluids averaged 230 μg of protein nitrogen per ml (μg PN/ml). Corrected to an unconcentrated basis, the successive fractions contained on the average 60, 9, and 1.5 μg PN/ml. Therefore, the increase in specific infectivity of the virus for each step, *i.e.*, equivalent to increases in infectivity to protein-nitrogen ratios, was 3.4-, 6.7-, and 3.0-fold, respectively, the overall increase being 68-fold. If, however, infectivity was lost without concomitant disruption of virus particles, then virus-particle purification would be 153-fold. It is known that this can occur since 22-mμ particle counts (see below) of purified virus concentrates stored for several weeks at 4°C remained constant, while

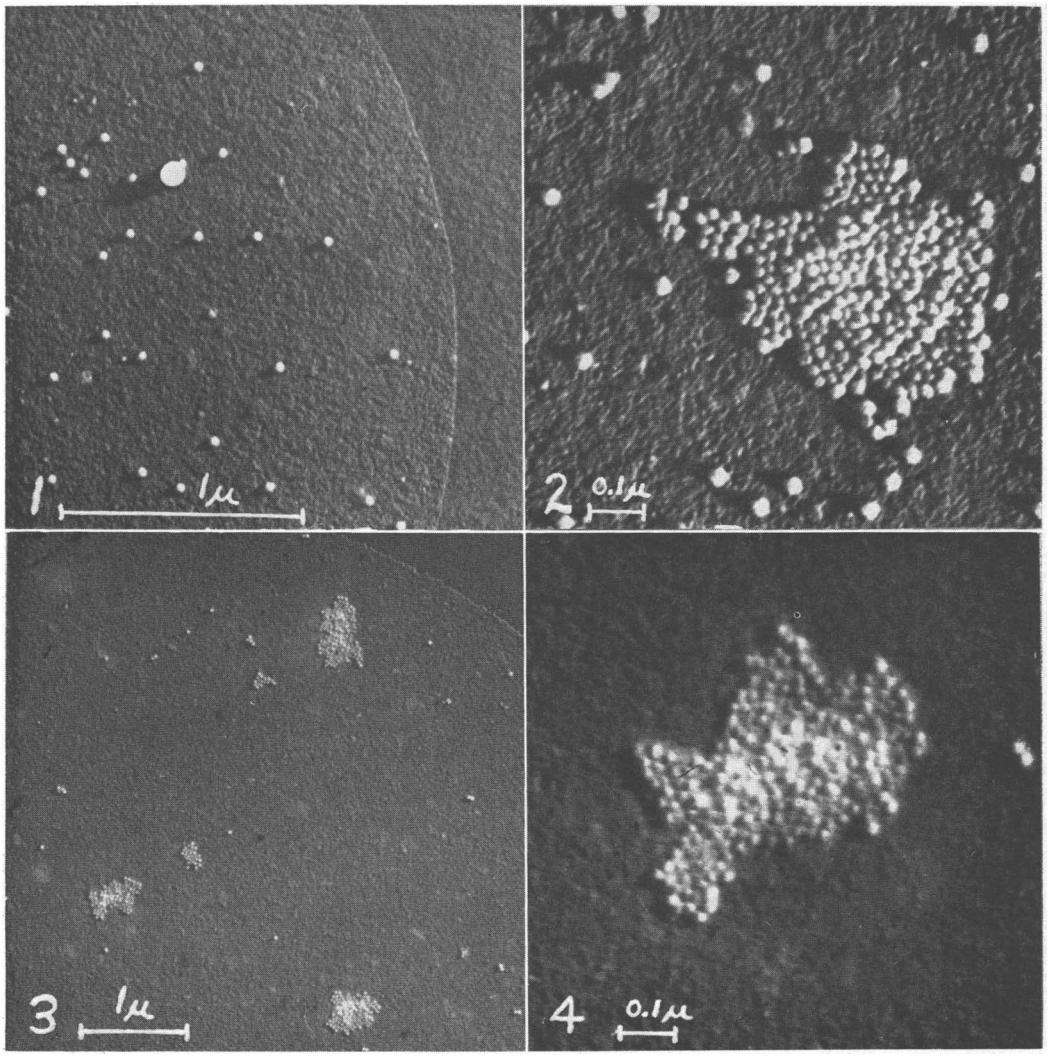

FIG. 1. Section of spray droplet with 22-mμ FMDV particles, normal bovine serum albumin, and a single 88-mμ polystyrene latex sphere, × 31,200.

FIG. 2. Cluster of 22-mμ FMDV particles without bovine serum albumin, × 65,000.

FIG. 3. Section of spray droplet showing 22-mμ FMDV particles specifically aggregated in the presence of immune bovine serum, × 13,000.

FIG. 4. FMDV aggregated with immune bovine serum, × 65,000. Here and in Fig. 3 the particles, while still distinguishable as the 22-mμ type, appear enmeshed in matrices derived from immune bovine serum components.

infectivity decreased to about 0.01% of its initial value.

Electron micrographs of air-dried spray-droplet patterns derived from purified virus concentrates revealed a uniform-sized. discrete particle in large numbers (Fig. 1) not found in control concentrates prepared from the fluids and extracts of comminuted cells of uninfected tissue cultures. When measured individually, this particle had an apparent width of about 28 mμ, but in linear groupings of clusters which sometimes form in the absence of albumin the average width approaches a minimum value of 22 mμ (Fig. 2). The latter value may approximate the true dimension of this particle since the 28 mμ diameter of poliovirus in close-packed arrays is consistent with its diameter in frozen-dried preparations where flattening due to surface-tension forces does not occur(6). The term

"width" rather than "diameter" is used since contours of air-dried 22-mμ particles and their shadows suggest a roughly spherical particle with angular surface. Whether the observed angularity resides in the particle itself or is an artifact due to asymmetrical flattening or accretion of foreign material remains to be determined on frozen-dried specimens. For example, Kaesberg(8) and Williams(9) have shown by precise analysis of particle and shadow contours that certain so-called spherical plant and insect viruses are actually icosahedrons.

The 22-mμ particle content and infectivity of purified virus concentrates were correlated. The two kinds of measurement were made with nearly the same precision. In plaque assays of FMDV infectivity the standard deviation is about 15% when 47 to 66 plaques are counted(4). A comparable standard deviation in the ratio of 22-mμ particles to PSL spheres, *i.e.*, 14%, is obtained by counting as few as 50 of the PSL spheres randomly mixed with a large excess of 22-mμ particles(1). Four times the minimum number of PSL particles were counted, and the 22-mμ counts usually exceeded the PSL by 5- to 50-fold. All particles of both types in a droplet pattern were counted. Indication of random mixing at the time of spraying was obtained from ratios in undiluted, 2-fold, and 4-fold dilutions of a purified virus concentrate. The respective ratios of 22-mμ particles to PSL spheres were 5.3:1, 2.4:1, and 1.15:1. The 22-mμ particles could be counted at concentrations as low as $10^{9.7}$ per ml by examining many droplet patterns; counting was easier when concentrations were 10^{11} or higher. Depending upon individual infectivities of tissue-culture-virus pools, which ranged from $10^{6.5}$ to $10^{8.3}$ PFU per ml, and loss of particles and infectivity during purification and concentration, the average 22-mμ particle/PFU ratio for 11 purified virus concentrates was 690 (Table II). The spread was wide, ranging from 140 to 1,600 physical particles per PFU in experiments where the *n*-butanol-chloroform mixture or trichlorotrifluoroethane was used alone. In one case where both were used successively this ratio was only 33. Therefore, presumptive but not conclusive

TABLE II. Recovery of Infectivity, 22-mμ Particles, and Ratios of Particles to Plaque-Forming Activity in Purified Concentrates of Type A Foot-and-Mouth Disease Virus.

Purified virus concentrate No.	Recovery of infectivity (%)	22-mμ particles/ml	PFU/ml	22-mμ particles/PFU
5	62	9.7×10^{10}	8.0×10^{7}	1200
6*	27	6.5×10^{9}	2.0×10^{8}	33
7	30	2.5×10^{11}	$3.1 \times$ "	800
8†	75	5.4×10^{10}	7.5×10^{7}	720
9	15	5.2×10^{9}	$2.7 \times$ "	190
10	82	2.5×10^{11}	7.0×10^{8}	360
11	71	8.5×10^{10}	$3.5 \times$ "	240
12	30	$2.6 \times$ "	3.2×10^{7}	810
13	55	$8.4 \times$ "	$5.5 \times$ "	1500
14	40	4.8×10^{11}	3.0×10^{8}	1600
15	11	1.2×10^{10}	8.5×10^{7}	140
			Avg	690

* BuOH-$CHCl_3$ mixture followed by trichlorotrifluoroethane.

† Trichlorotrifluoroethane instead of BuOH-$CHCl_3$.

evidence based on particle counting alone was obtained for identity of FMDV with the 22-mμ particle. Convincing evidence would be a relationship of one physical particle per minimum infectivity unit.

Since this 1:1 relationship was not found and because another particle approximately 8 mμ in diameter was also seen in electron micrographs of some purified virus concentrates, evidence of an unequivocal nature for the identity of the 22-mμ particle uniquely present in purified virus concentrates was sought and obtained. It was found by analytical electron microscopy that the 22-mμ particles aggregated into clumps when treated with serum from steers immunized with FMDV derived from bovine-tongue epithelium (Fig. 3 and 4). Aggregation was specifically due to the antiviral serum and did not occur in the presence of normal bovine serum. Moreover, diminution of particle aggregation corresponded approximately to the degree of dilution of the immune bovine serum. Unlike the individually resolved particles of Fig. 2 prepared without added serum or serum albumin, particles within aggregates in the presence of immune serum were poorly delineated probably because of accretion of antibody molecules. The occasional clustering of some of the 22-mμ particles which oc-

curred in the absence of added serum albumin (Fig. 2) differs both in kind and degree from the aggregation brought about by immune bovine serum.

Discussion. In contrast with earlier unconvincing attempts by von Ardenne and Pyl (10), Bernard *et al.*(11), and Epstein *et al.* (12), results of the present studies clearly establish that the 22-mμ particles are the physical agents of foot-and-mouth disease and that, depending upon viability, the number of virus particles constituting one PFU for bovine-kidney monolayer cultures varies from 33 to 1,600. This wide variation may be attributed in part to the lability of FMDV infectivity(7) and also to possible insensitivities of the plaque assay in bovine-kidney cultures. Physical particle to infectivity ratios lower than 33:1 for FMDV may be difficult to obtain, especially since this value approximates the minimum ratio of 36:1 found for the more stable poliovirus on sensitive human amnion cells(13,14).

The 8-mμ particle can be eliminated as being virus on the basis that it is not always present in purified concentrates, and also that it cannot be associated with a sedimentation constant, s_{20}, of 137 S found for FMDV by Bachrach(15) and later confirmed at 142 S and 140 S by Randrup(16) and Pyl(17), respectively. An s_{20} value of this magnitude, while completely inconsistent with an 8-mμ particle, would be expected for a 22-mμ virus nucleoprotein in which the nucleic acid is an appreciable portion of the total mass of the particle, possibly as high as one-third of the mass as is the case with poliovirus(18). It has not been the purpose of the present study to identify the 8-mμ particle. It may be the extraviral complement-fixing antigen first observed in centrifugation experiments by Traub and Pyl(19) and later by Bachrach(20) and Bradish *et al.*(21).

Several methods of virus purification were used without success. Physical adsorption methods, protamine-sulfate clearing, and alternate freezings and thawings added little to the degree of virus purification, while enzyme treatments with ficin, papain, trypsin, ribonuclease, and desoxyribonuclease destroyed FMDV infectivity.

On the basis of the 22-mμ size and concentration of FMDV as determined by electron microscopy, the amount of protein and nucleic acid nitrogen attributable to virus may be calculated, assuming that about one-sixth of the particle weight is nitrogen. When this value is compared with protein-nitrogen values determined by digestion and nesslerization of purified virus concentrates, it is found that the 22-mμ particles constitute no more than 1% of the total protein of purified concentrates. This residuum of protein of low molecular weight is being effectively eliminated by zone centrifugation in density gradients and will be the subject of another paper.

Summary. Foot-and-mouth disease virus (FMDV), type A, from bovine-kidney tissue cultures has been purified successively by methanol precipitation, extraction with organic solvents, and differential centrifugation. The FMDV particle in the resulting purified virus concentrates has been identified by analytical electron microscopy in air-dried specimens as a uniform-sized 22-mμ particle. This identification was made through correlative experiments relating particle counts to infectivity and to aggregation of the particles with antiviral bovine serum. While an average of 690 virus particles was present in one plaque-forming unit for bovine-kidney monolayer cultures, a value as low as 33 was obtained.

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Influence of Vit. B_{12} and Sulfur Amino Acids on Liver Glutathione Level in Mice. (23839)

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Several recent reports suggest the existence of a relation between Vit. B_{12} and sulfhydryl metabolism. Dubnoff showed this vitamin to play a role in the reduction of S-S groups(1). Ling and Chow(2) found low non-protein sulfhydryl values in rat erythrocytes deficient in cobalamin, while Register(3) reported low levels in both erythrocytes and livers in B_{12}-deficient rats as compared to the corresponding control animals. In previous studies, we observed low values of reduced glutathione (GSH) in erythrocytes of rats, raised on a diet deficient in Vit. B_{12}(4); nonetheless, liver levels in both rats and mice kept under these conditions were normal(5,6). In Sanguinetti and Marchetti's experiments, the liver GSH in B_{12}-deficient rats was even higher than in the controls(12).

In the present paper the effect of Vit. B_{12} and sulfur amino acids on the GSH level of livers of mice is further studied.

Materials and methods. 300 adult albino mice of our stock were used. Those used for B_{12}-supplemented groups had been kept on complete commercial rat stock ration containing about 3 μg/100 g of this vitamin. Those deficient in Vit. B_{12} had been raised for several generations on a soybean-corn ration (diet 1, Table I). The performance of this deficient stock has been studied in detail(7). All animals used were between 10 and 14 weeks old when the respective experiments were started. At this age, the large weight differences existing at weaning between the B_{12}-deficient and normal mice have practically disappeared, all weighing between 22-25 g. The mean weight at weaning at 28 days of the deficient animals was 12 g, while that of the controls was 17 g. Care was taken that all B_{12}-deficient mice had a weaning weight between 11 and 13 g and continued on the deficient diet until the start of the experi-

TABLE I. Composition of Experimental Diets.†

No.	Soy meal	Bean meal	Corn meal	Starch	Sesame oil	Salts USP XII No. 2	Vit. mixture*
1	46		46		5	2	1
2		50		42	5	2	1
3				92	5	2	1

* Containing/100 g of diet: Thiamine-HCl, 0.3 mg; riboflavin, 0.3 mg; pyridoxine-HCl, 0.2 mg; Ca-pantothenate, 0.2 mg; niacin, 2 mg; folic acid, 0.025 mg; biotin, 0.01 mg; p-aminobenzoic acid, 25 mg; inositol, 10 mg; choline-HCl, 100 mg.

† 0.2% of percomorphum oil and 0.2% of wheat germ oil added.