TABLE II. Distribution Ratios of Test Amino Acids Among Fluids and Tissues in the Pregnant Guinea Pig. The recorded tissue levels are calculated per g cell water, relative to concentration in the appropriate plasma; *i.e.*, as the quantity $(F - mP)/nP$, where F represents epm per g fresh tissue, P represents epm per ml of plasma, and m and n are the number of g respectively of extracellular and cellular water per g of fresh tissue.

Amino acid	No. of animals	Fetal/maternal		Liver relative to its plasma	Muscle relative to its plasma
AIB	6	6.4 + 3.9	Maternal	47 ± 18	7.8 ± 7.6
			Fetal	2.1 ± 1.2	3.5 ± 0.4
1-Aminocyclopentanecarboxylic acid	6	8.7 + 2.1	Maternal	51 ± 35	8.0 ± 4.4
			Fetal	1.18 ± 0.08	1.7 ± 0.3
Fluoro-AIB	1	6.5	Maternal	22	6.7
			Fetal	1.18	1.44

appears not to have a strong effect on its distribution in the pregnant guinea pig (Table II). A CF_3 group, however, in place of one of the CH_3 groups of α-aminoisobutyric acid, largely abolishes the uphill transport. This result is not surprising since the structural change lowers the pK_a values to 0.5 and 5.9 for the carboxyl and amino groups respectively (to be published), thereby causing this amino acid to be an anion at neutral pH values. Comparatively slow transport is also seen for it in all other systems so far studied.

Summary. Three model amino acids resistant to metabolic alteration are, like normal amino acids, strongly concentrated across the placental barrier in the guinea pig. Accordingly these substances are suitable for study of this active transport. Although maternal tissues accumulated these 3 amino acids vigorously, the fetal tissues reached only moderately higher levels of the models than did the fetal plasma. Two fluorine-containing amino acids showed no advantages as models; one of them was only slightly concentrated by the placenta.

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Synergistic Effect of Molybdenum and Fluoride on Dental Caries in Rat.* (27313)

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Adler has reported that the presence of molybdenum in the drinking water is associated with a decreased incidence of dental caries in humans(1). He has also reported a significant reduction in incidence of caries in rats which received 0.1 ppm molybdenum in drinking water(2). Other studies have shown that the presence of molybdenum in drinking water is associated with an increased skeletal fluoride retention in the rat(3), and that rate of fluoride absorption from the gastrointestinal tract of the rat is increased in the presence of molybdenum(4). This study was performed to learn whether fluoride and

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TABLE I. Dental Caries Incidence.

Group*	Mean No. Lesions	% Reduction	"p"
1	9.6 ± .7†	—	—
2	7.9 ± .6	17.7	.05
3	6.5 ± .9	32.3	.02
4	4.6 ± .7	52.1	.001

* 1 = controls; 2 = 25 ppm molybdenum; 3 = 25 ppm fluoride; 4 = 25 ppm molybdenum + 25 ppm fluoride.
† Standard deviation.

molybdenum administered together have a synergistic effect upon the incidence of dental caries in the rat.

Methods. A total of 160 weanling albino Sprague-Dawley strain rats were divided by sex and weight into 4 groups. Group 1 served as a control and received redistilled fluoride-free water *ad libitum*. The second group received 25 ppm molybdenum (as ammonium molybdate) while the third group received 25 ppm fluoride (as sodium fluoride). Group 4 received drinking water containing both 25 ppm molybdenum and 25 ppm fluoride. All animals were housed in pairs in raised wire cages in an air-conditioned room, and received *ad libitum* the same cariogenic diet.† The animals were weighed once each week throughout the study of 130 days, after which period all animals were sacrificed by ether inhalation and their heads removed and prepared for dental caries evaluation(5). Both femurs were removed from each carcass and prepared for fluoride analysis(6). Molybdenum determinations were performed according to the colorimetric procedure of Marmoy using only the ashed carcass samples due to the low molybdenum concentrations in the incisors and femurs(7).

Results. The average weight gain of the animals indicated that none of the supplements adversely affected body weight gain under the conditions of this study. The control males gained 267 g and the control females 158 g. All other groups gained comparable or more weight.

The effect of fluoride and molybdenum on dental caries is shown in Table I. The controls had an average caries score of 9.6 lesions per animal. When 25 ppm molybdenum was placed in the drinking water an average caries score of 7.9 lesions per animal was obtained, or a decrease in incidence of 18%. When the drinking water contained 25 ppm fluoride an average caries score of 6.5 lesions per animal was found, or a reduction of 32 per cent. The animals which received both molybdenum and fluoride were found to have an average score of 4.6 lesions per animal. Comparison of this value with the control shows that the combination of fluoride and molybdenum decreased the incidence of caries by 52%. These data indicate that the combination of molybdenum and fluoride was nearly 3 times more effective in reducing incidence of caries than molybdenum alone, and 62% more effective than fluoride alone.

The fluoride and molybdenum results are shown in Table II. The femurs from the control animals contained 218 ppm fluoride and a total amount of 126 μg fluoride. The femurs from the animals which received 25 ppm molybdenum but no supplemental fluoride contained 205 ppm fluoride and a total of 140 μg of fluoride. Addition of 25 ppm fluoride to the drinking water resulted in an average femur fluoride concentration of 3770 ppm and an average total of 2.54 mg of fluoride. When both molybdenum and fluoride were present in the drinking water, average concentration of fluoride in the femur was 4060 ppm with an average total of 2.90 mg. Comparison of these latter 2 groups indicates that the presence of molybdenum in the drinking water in addition to fluoride is associated with a 14.2% increase in total amount of fluoride in the femur.

The carcasses from the control animals contained an average of 1.11 mg of fluoride while carcasses of the animals which received only supplemental molybdenum (Group II) contained 1.19 mg of fluoride. The carcasses from Group III which received only supplemental fluoride contained an average of 19.21 mg of fluoride while those from Group IV which received both fluoride and molybdenum contained 23.20 mg fluoride.

The concentration of fluoride found in the

† The composition of this diet, in per cent, was as follows: yellow corn grits, 46.0; powdered whole milk, 28.5; sucrose, 19.5; alfalfa meal, 4.8; sodium chloride, 1.0; and irradiated yeast, 0.2.

TABLE II. Amount of Fluoride in Femur and Carcass.

Group*	Femur fluoride Concentration (ppm)	Femur fluoride Total (mg)	Incisor Fluoride (ppm)	Carcass fluoride Concentration (ppm)	Carcass fluoride Total (mg)	Carcass Mo (ppm) Mo
1	218 ± 31†	.126 ± .02	51.8	143.6 ± 19.8	1.110 ± .14	8.19 ± .64
2	205 ± 31	.140 ± .04	46.3	139.4 ± 26.4	1.190 ± .16	233.5 ± 22.1
3	3770 ± 210	2.54 ± .09	1490	2540 ± 480	19.210 ± .312	7.89 ± 1.04
4	4060 ± 160	2.90 ± .14	1890	2840 ± 450	23.200 ± .353	231.8 ± 33.8

* 1 = controls; 2 = 25 ppm molybdenum; 3 = 25 ppm fluoride; 4 = 25 ppm molybdenum + 25 ppm fluoride.
† Standard deviation.

ash of the pooled incisor samples showed similar results. The incisors of Groups I and II contained 51.8 and 46.3 ppm fluoride, respectively. In those groups receiving supplemental fluoride (Groups III and IV) concentrations of 1490 and 1890 ppm fluoride were found in the absence and presence of molybdenum, respectively.

The results obtained from the molybdenum analyses are also shown in Table II. The carcasses of those animals which received no supplemental molybdenum (Groups I and III) contained comparable amounts of molybdenum with values of 8.19 and 7.89 ppm. The carcasses of the animals which received supplemental molybdenum (Groups II and IV) also contained comparable concentrations of molybdenum with values of 233.5 and 231.8 ppm.

Discussion. An increased skeletal retention of fluoride in the presence of molybdenum has been reported(3). This earlier study indicated that presence of 50 ppm molybdenum (as ammonium molybdate) in the drinking water of rats for a period of 30 days resulted in increases of fluoride retention of 19.3 and 16.3% in the whole carcass and femur, respectively. In the present study, the presence of molybdenum in the absence of added dietary fluoride was found to increase the retention of fluoride in the femurs by 11.1%. When fluoride was added to the drinking water the presence of molybdenum resulted in a 14.2% increase in fluoride retention in the femurs. Similar trends were found in carcasses and incisors. When supplemental fluoride was administered the presence of molybdenum increased the concentration of fluoride by 26.8 and 11.8% in incisors and carcasses, respectively. Expressed in terms of total amount of fluoride retained, the presence of molybdenum resulted in a 20.9% increase. These data confirm earlier reports that the presence of molybdenum is associated with an increased skeletal retention of fluoride.

The data obtained from dental caries examination indicate that drinking water fortified with 25 ppm molybdenum reduced caries incidence by 17.7% ($p = 0.05$). Even though elevated levels of molybdenum supplementation have been employed in this study, a caries reduction of this magnitude tends to confirm the earlier reports by Adler. However, an even more dramatic action of molybdenum is seen in the presence of supplemental fluoride. Addition of 25 ppm fluoride to the drinking water resulted in a 32.3% reduction in incidence of caries, significant at the 0.02 probability level. When the animals were given drinking water containing both 25 ppm fluoride and 25 ppm molybdenum a caries reduction of 52.1% was obtained, significant at the 0.001 probability level. This suggests that the combination of molybdenum and fluoride was 61.4% more effective in reducing the incidence of dental caries than was the same level of fluoride in absence of molybdenum, this comparison being significant at the 0.05 probability level. It may be that one explanation for the anticariogenic effect of molybdenum is that its presence in the water increases the absorption or skeletal retention of fluoride.

The data obtained from analysis of the carcasses for molybdenum give additional information about the molybdenum-fluoride interrelationship. In the absence of supplemental molybdenum (Groups I and III) comparable values for molybdenum were found in the

carcass, indicating that the presence of supplemental fluoride (Group III) did not alter the levels of molybdenum retained. When molybdenum was present in the drinking water (Groups II and IV) comparable concentrations of molybdenum were also found in the carcass, again indicating that the presence of supplemental fluoride (Group IV) did not alter the skeletal retention of molybdenum. These data indicate that the increased anticariogenic effectiveness and increased skeletal retention of fluoride in the presence of molybdenum may be due to the ability of molybdenum to increase the metabolic availability of the fluoride ion.

Summary. A study was conducted to determine the effect of molybdenum and fluoride upon dental caries in the albino rat. Addition of 25 ppm molybdenum to the drinking water decreased the incidence of dental caries by 18% while addition of 25 ppm fluoride decreased the caries incidence by 32%. Addition of both 25 ppm molybdenum and 25 ppm fluoride resulted in a greater effect, with the caries incidence reduced by 52%. Fluoride analysis performed upon the femurs, incisors, and whole carcasses confirmed earlier reports that the presence of molybdenum is associated with an increased skeletal retention of fluoride. The analysis of the carcasses for molybdenum indicated comparable concentrations of molybdenum in the presence and absence of fluoride suggesting that the increased anticariogenic effectiveness and skeletal retention of fluoride in the presence of molybdenum may be due to the ability of molybdenum to increase the metabolic availability of the fluoride ion.

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A Cell Culture System on Agar Permitting Direct Investigation of Viral Antigens by Immunodiffusion.* (27314)

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Since the report of Lewis and Lewis(1), investigators have used agar in studies on cell migration *in vitro* and in the field of organ culture. Zinsser *et al.*(2) obtained multiplication of rickettsiae in surviving tissue fragments on an agar substrate, and subsequently several viruses have been so propagated. Recently, Wallace and Hanks(3) and Keefe *et al.*(4) reported growth of cell lines on agar. The recognized usefulness of the gel-diffusion method for the demonstration of antigen-antibody reactions suggested its direct application to the problem of detection and identification of viruses replicating in cells grown on agar, provided that an appropriate agar-tissue culture system could be devised. The present report describes a technic that permits growth of cells in wells made in nutrient agar, the propagation of viruses therein, and the demonstration of specific diffusable viral components or products by addition of appropriate antisera to adjacent wells.

Materials and methods. Maintenance of cell line. Chang's conjunctival cells were grown in "milk dilution bottles" in Eagle's basal medium (Microbiological Assoc., Inc.) plus 5% inactivated horse serum, 0.042% so-

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