tecting changes occurring in their metabolism. 5. The possible association of vit. A and carotene changes with changes in lipids during pregnancy was considered.

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Adenovirus Plaque Formation in Grivet Monkey Kidney Cells. (27377)

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The plaque technic developed by Dulbecco (1) provides a method which is suitable for quantitative analysis of infectivity of virus preparations, isolation of pure lines of virus, and differentiation of virus strains and types (2,3). Plaque formation by adenovirus type 2 in stable cell lines was demonstrated by Bonifas and Schlesinger(4) and types 4 and 5 by Kjellén(5). This report describes the adaptation of the plaque technic to adenovirus types 1 through 7 in monolayers of grivet monkey kidney cell cultures (*Cercopithecus aethiops*).

Materials and methods. Tissue culture. Grivet monkey kidney cell cultures were prepared in Smith bottles by a method previously described(6).

Viruses. The strains of adenovirus types 1 through 7 used were of human origin. They were isolated in human embryonic cell cultures and subsequently adapted to monkey kidney cells and are strains employed routinely in this laboratory. All of the virus samples described here were obtained from adenovirus infected fluids of grivet monkey kidney cell cultures.

Agar overlay medium A was prepared as described previously(6), with 2 exceptions. The neutral red was omitted from the overlay and a solution of *protamine sulfate* was added to yield a final concentration of approximately 400 μg/ml.

Agar overlay medium B was prepared in the same manner as agar overlay medium A, except that neutral red was added to yield a final concentration of 1:25,000.

Virus seeding. The medium was removed from each of the cell culture bottles. The cell sheets were washed once with Hanks' balanced salt solution adjusted to pH 8.0. Bottles were seeded with appropriate dilutions of virus at 0.5 ml per bottle. Hanks' balanced salt solution containing 0.1% *bovine serum albumin* $\left(\frac{W}{V}\right)$ was used as the virus diluent. The bottles were placed in the 36°C incubator for 1.5 hours. At the end of the incubation period, the cell sheets were overlaid with 15 ml of the agar medium (A, above). After the agar had hardened, the bottles were incubated at 36°C in an inverted position. At the end

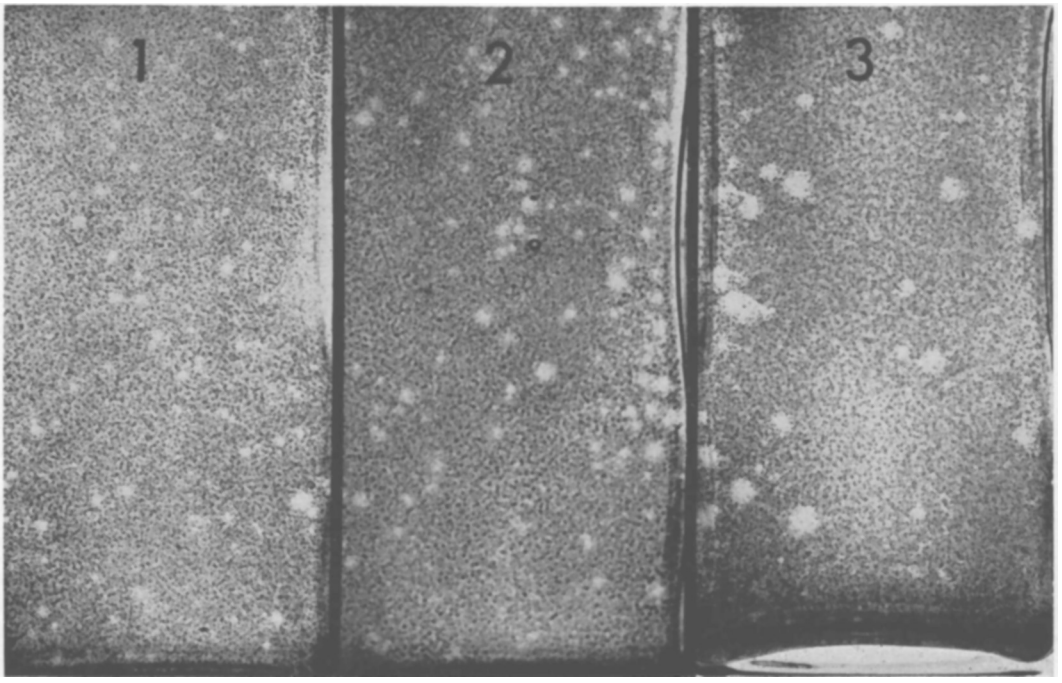


FIG. 1. Typical plaques of adenovirus types 1, 2, and 3 on grivet monkey kidney cell cultures.

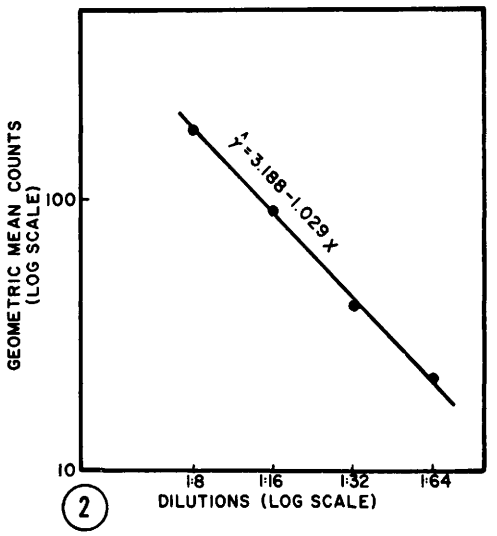
of 10 days incubation, 10 ml of the neutral red agar overlay (B, above) was added to each bottle. The bottles were then incubated for 24-48 hours, at which time the plaques were read.

Results and discussion. Incubation time. By the 10th day of incubation plaques were easily discernible. The number of plaques per bottle usually increased by the 12th day and the plaques grew significantly in size. After the 12th day, there was no significant increase in plaque numbers. Typical plaques obtained with adenovirus types 1, 2 and 3 are shown in Fig. 1.

Proportionality with dilution. An evaluation of the proportionality obtained with dilution of virus samples was analyzed statistically and a typical analysis is shown in Fig. 2. The plot of the regression of the logarithm of the count *vs* the logarithm of the dilution indicated a linear plot which did not differ significantly from expectation (*i.e.*, expected slope of 1.0). Extrapolation from the regression line gave an estimated plaque count of 1540 for the starting log 3 dilution of the original preparation with 95% confidence limits of 1340 and 1780. From analysis of the data obtained in various assays, the most precise log counts were obtained at dilutions that yield between 20 and 200 plaque forming units per bottle.

Role of protamine. It has been found that many lots of grivet monkey kidney cell cultures undergo severe degradation when placed under an agar overlay. Liebhaber and Takemoto(7) indicated superior plaque formation with certain viruses when protamine or other basic substances were incorporated in the agar overlay. According to these authors, the protamine binds acidic polysaccharides present in the agar. In the experiments described, the protamine sulfate prevented the degradation of grivet monkey kidney cell cultures when placed under an agar overlay. Apparently, protamine overcame the toxicity of the agar to the cell cultures.

Bovine serum albumin in the diluent. In certain instances when extremely high dilutions of viruses were necessary, it was found that there was greater recovery of plaque forming units when bovine serum albumin was added to the diluent. The loss of plaque forming units on dilution in salt solution may have been due to adsorption of virus particles



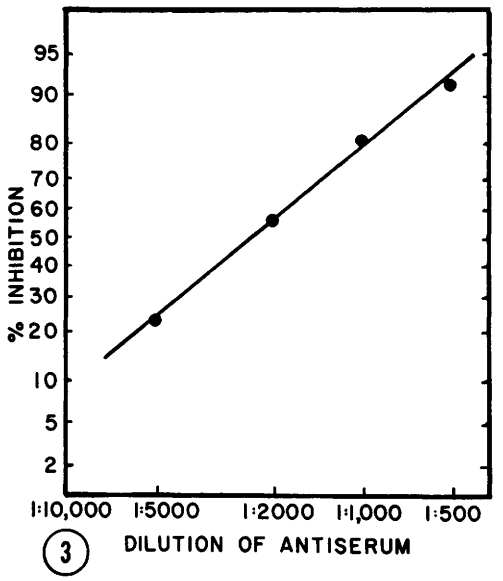


FIG. 2. Demonstration of proportionality of plaque formation with dilution by adenovirus type 2.

FIG. 3. Probit analysis of plaque inhibition with dilution of antiserum, adenovirus type 2.

to the glass of the container. Bovine serum albumin is an excellent desorbing agent(8).

Titration of immune serum. Several experiments were performed to attempt the titration of immune serum by the plaque method. The results of a typical experiment are plotted in Fig. 3. This plot was based on probit analysis as described by Finney(9). The χ^2 (chi-square) test showed good agreement with the expected variance about parallel bottles assuming a Poisson distribution (10). There was excellent agreement between the observed and the estimated per cent inhibition. The titer (ID_{50}) of this serum was estimated to be 1:2360 with 95% confidence limits of 1:1850 and 1:3010. A second experiment with the same immune serum yielded a titer of 1:2540 with 95% confidence limits of 1:2080 and 1:3100. The challenge dose in both experiments approximated 90-100 PFU. Three bottles were used at each dilution for estimation of per cent inhibition.

Summary. A method has been described for quantitative assay of infectivity of adenovirus types 1 through 7 in grivet monkey kidney monolayer cell cultures. Protamine sulfate has been found to aid in prevention of degradation of the cell cultures when placed under agar for maintenance during formation of plaques. Presumptive evidence has been obtained that the method may be useful for quantitative assay of adenovirus antibody in immune serum by plaque inhibition.

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