tors in 6 patients with chronic pancreatitis, in 6 dogs with incomplete obstruction of the pancreatic ducts and in 13 normal untreated dogs are reported. The coagulation factors were determined quantitatively on plasma fractions obtained by a low temperature procedure. Amylase determinations were done on all specimens.

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A Simple Technic for Demonstrating Plaque Formation with Virus of Vaccinia.* (20378)

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A technic for the demonstration of focal necrotic areas (plaques) by animal viruses would provide a more accurate means of titration. It would also be a valuable tool for studying their growth characteristics and genetic properties. In addition, it might be helpful in isolating mutant viruses and in studying antiviral substances. There has been publication on such a technic. Gey and Bang(1) stated briefly that they had been able to produce focal necrosis in static slide cultures of a rat sarcoma with the virus of Eastern equine encephalomyelitis. Recently Dulbecco(2) reported in detail on plaque formation in chick embryo tissue cultures by the viruses of Western equine encephalomyelitis and Newcastle Disease. The present paper describes a simple, readily reproducible technic whereby plaque formation has been consistently obtained with the virus of vaccinia.

Materials and methods. The cell suspension. Five, 11-day-old chick embryos were decapitated, washed twice with Gey's saline (GS) and pressed into 15 ml of GS through a wire mesh strainer, having 15 strands per cm. This material was centrifuged at 110 x G for 30 seconds and the resulting supernatant was discarded. The sediment was resuspended, pipetted rapidly 20 times with a 10 ml pipette and centrifuged at 110 x G for 15 seconds. The supernatant was then removed and allowed to stand for 20 minutes, during which time the large particles sedimented into the bottom 1 ml. This was discarded and the resulting preparation consisted, for the most part, of a suspension of single cells. The cell concentration of the suspension was then determined in a hemocytometer and an amount added to the nutrient fluid which resulted in a final cell concentration of 8 x 10^6 cells per ml. This usually required about 10 ml of the cell

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TABLE I. Relation between Number of Plaques Formed in Culture and Concentration of Vaccinia Virus. Virus sample was .1 ml except where noted.

Exp.	Virus dilution 10^{-3}	10^{-4}	10^{-5}	10^{-6}
1	Confluent	237	21, 21, 23	4
2	"	115	9	2
3	"	125, 112	32 (.4 ml)	—
4	"	475	32	—
5	—	—	28, 24 (.2 ml), 65 (.4 ml)	—
6	—	252 (.2 ml)	27, 24 (.2 ml)	3 (.2 ml)
Avg		198	16.5	2.5

suspension per 20 ml of nutrient fluid. *The nutrient fluid.* This was composed of 40% horse serum heated to 56° for 30 minutes; 20% embryo extract (1:1 in GS) prepared from 10-day-old embryos; and 40% GS. Fifty units of penicillin and 50 μg of streptomycin were added per ml. The initial pH of the nutrient fluid was 7.6 ± .1. *Cultures.* Cultures were set up in regular 100 x 15 mm test tubes, in 50 mm Carrel flasks, or in 50 mm Petri dishes (incubated in an atmosphere of 5% CO_2). The flask preparations had the advantage of very uniform cell sheets while the Petri dish preparations had the advantage of accessibility. Cultures were also prepared on small cover slips (5 x 20 mm) for microscopic examination of stained preparations. It was found that the cells attached themselves more firmly and formed a more homogeneous sheet when they were allowed to settle onto a glass surface which had been coated with a thin clot of chicken plasma. This consisted of .4 ml of plasma (heparin 1:50000) and .1 ml of embryo extract (1:1 in GS) for the flask and dish preparations. After the clot had hardened 3 ml of nutrient fluid containing 8 x 10^6 cells per ml were added per flask or dish and the cultures incubated overnight at 37°. After overnight incubation a fairly uniform sheet of cells had formed, and the virus sample was added at that time. *Vaccinia virus.* Three different strains were used: 1) smallpox vaccine obtained from Lederle Laboratories (Lot No. 2095-205B and Lot No. 2095-206A), 2) a calf lymph preparation that had been passed 4 times in the rabbit's testicle and twice on the chorioallantoic membrane (CAM) of the chick embryo, 3) a strain of Levaditi's neurovaccinia that had been passed 23 times in the rabbit's testicle and twice on the CAM. The latter two strains were obtained from Duran-Reynals. All virus stocks were cultured to make certain that they were free from bacteria. After removing the nutrient fluid and washing the tissue cultures with 2 ml of GS, the virus inoculum was added and the flasks incubated at 35° for one hour. A thin clot of chicken plasma was then placed over the cell sheet, since this resulted in a finer, more uniform sheet and more discrete lesions. Plaques were readily produced even without addition of this plasma clot. After the clot hardened 3 ml of nutrient fluid were added. The pH of this fluid dropped rapidly and it was necessary to change it on the third day after adding the virus. *Vital staining of the cultures.* Since it was difficult to visualize the plaques in unstained preparations, neutral red (National Aniline) in GS was added to the nutrient fluid (at a pH of 6.8-7.0) to give a final concentration of 1:5000.

Results. Plaques were consistently produced with all of the strains of vaccinia virus and were readily visible upon staining with neutral red as early as 48 hours after the addition of virus. These appeared as round, white, opaque areas of destroyed cells against a background of pinkly stained living cells. The plaques reached a maximum diameter of about 1 mm on the fifth day. Microscopic examination of fixed and stained preparations of plaques formed in coverslip cultures, showed the plaques to be composed of cell debris, surrounded by healthy fibroblast-type cells. In the cover-slip cultures, typical eosinophilic, cytoplasmic, inclusion bodies of vaccinia were seen in the cells surrounding the focal lesions after staining with hematoxylin and eosin. The plaques were always absent when the

Plaque formation by the virus of vaccinia in chick embryo tissue cultures. These cultures were stained with neutral red.

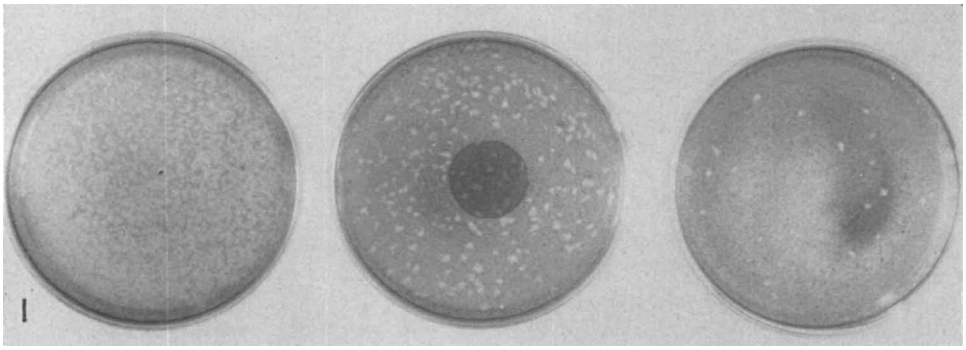

FIG. 1. Petri dish preparation showing effect of virus dilution on number of plaques. Virus dilution left to right: 10^{-3}, 10^{-4}, 10^{-5}. (Filter disc in center of middle culture was used in an unsuccessful attempt to demonstrate inhibition by an antimetabolite.)

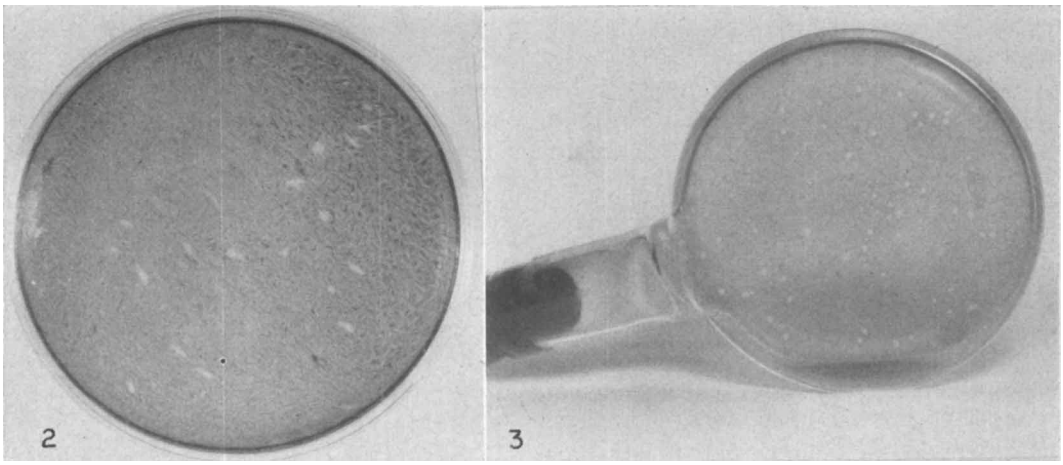

FIG. 2. Petri dish preparation without upper plasma clot showing less discrete plaques and less homogeneous cell sheet. Virus dilution: 10^{-5}.

FIG. 3. Carrel flask preparation showing uniform cell sheet and discrete lesions. Virus dilution: 10^{-5}.

virus was absent and their number increased in proportion to the amount of virus added, as illustrated in Table I. In the lower dilutions (10^{-2}, 10^{-3}) practically all the cells were destroyed, with release of the dye into solution, and in the higher dilutions (10^{-4}, 10^{-5}, 10^{-6}) discrete, separated plaques were obtained. This is illustrated in Fig. 1, 2. A comparative study with the chick embryo demonstrated that the number of pox produced on the CAM by the virus of vaccinia correlates with the number of plaques produced in culture, although the titer was almost 6-fold higher *in vivo*. For example, at 10^{-6} dilution the averages were 2.5 plaques and 14 pox. In a number of different experiments vaccinia virus was incubated with rabbit antivaccinial serum for one hour at 37°. The number of pox formed on the CAM and the number of plaques formed in culture were consequently reduced 95% (Table II). Normal serum under the same conditions had no effect on the number of plaques formed in culture. In several recent experiments the virus inoculum was added in 2 ml of GS and this was replaced with 3 ml of nutrient fluid after 24 hours. At 48 hours the cell sheet was covered with a plasma clot and 3 ml of

TABLE II. Effect of Antivaccinial Rabbit Serum on Plaque Formation in Culture and Pock Production on Chorioallantoic Membrane of the Chick Embryo. An equal amount of serum was added to virus dilution (10^{-5}) and mixture incubated at 37° for one hour.

	Exp.	Control	Antiserum	% reduction
Plaques	1	28, 24 (.2 ml), 65 (.4 ml)	1, 2 (.4 ml)	95
	3	27, 24 (.2 ml)	10 (.4 ml)*	61
Pox	1	32, 22, 58, 61 (.05 ml)	2, 1, 1, 2 (.1 ml)	96
	2	24, 42, 40, 44, 38 (.05 ml)	3, 1, 1, 3, 1, 2 (.1 ml)	95

* Serum frozen-thawed 4×.

fresh nutrient fluid added. Using this modified technic a plaque to pock ratio close to 1:1 was obtained.

Discussion and summary. A simple technic is described whereby the production of plaques in chick embryo tissue cultures has been consistently obtained with vaccinia virus. The plaques are probably due to viral activity. This conclusion is supported by the fact that 1) the plaques are absent when the virus is absent and their number increases with the concentration of the virus, 2) the number of plaques produced in tissue culture correlates with the number of pox produced on the CAM of the developing chick embryo and 3) specific antibody reduces both the number of plaques formed in tissue culture and the number of pox formed on the CAM by 95%.

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Characteristic Individual Electrophoretic Patterns in Humans.* (20379)

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The electrophoretic analysis of serum and plasma proteins in normal individuals and in patients with a variety of diseases has been repeatedly described(1,2). Six to 9 different protein components can generally be recognized in the plasma at pH 8.6. It is customary to characterize these protein components by their electrophoretic mobility and to determine their relative amounts by measuring the surface area of the electrophoretic patterns. From the data thus obtained and from the value for total plasma protein, determined by chemical analysis, the absolute concentrations of the plasma protein components can then be computed. Consequently, the electrophoresis of human plasma is generally considered to be a method for quantitative determination of some of the protein constituents of blood. Many attempts have been made to correlate the values obtained by electrophoresis for the various protein components with disease, age, sex, and other conditions of the blood donor. In most instances the plasma protein composition appears to be rather independent of these factors. Only in a few cases are the electrophoretic plasma protein values more or less specific for a disease, such as p.e. in multiple myeloma. In the course of studies on plasma proteins in patients from 3 separate hospitals and in a number of normal individuals, 1250 electrophoretic plasma analyses of 440 individuals have been carried out. In many cases, analyses have been performed on the same

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