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Response to Nephrotoxic Serum in the Newborn Rat. (28604)

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Intravenous administration of rabbit-anti-rat nephrotoxic serum to newborn rats at 0-3 hours following birth failed to demonstrate at 3 weeks of age or older the typical clinical and biochemical response observed in adult rats(1,2). The response to nephrotoxic serum in adult rats was accompanied by the attachment of the injected heterologous gamma globulin to the kidney antigen in the glomerulus(3). It was also demonstrated that rabbit gamma globulin was attached to the newborn rat glomerulus within 24 hours after intravenous administration of rabbit-anti-rat serum(4). Hammer *et al.*(5) have shown that acute proliferative glomerular changes, associated with proteinuria, occur early in newborn rats following injection with nephrotoxic serum, and that this glomeruuar damage is self-limited by 4 to 6 weeks.

The present study was designed to obtain data not included in our earlier studies during the first and second week periods following administration of nephrotoxic serum in the newborn rat (0 to 3 hours of age). Distinct nephrotoxic effects were found in the kidneys of rats examined at the end of the first week of life with less marked changes at 2 and 3 weeks of age. At 4 weeks and later there were no significant pathologic findings. The plasma biochemical findings at the time when the renal lesion was present did not vary from normal.

Methods. Rabbit-anti-rat nephrotoxic serum was prepared by the method of Smadel (6), modified by Heymann(7). Seventy-one percent of the nephrotoxic serum dose for adult rats was administered intravenously to 27 newborn rats (Wistar strain) within 3 hours after birth, and these animals were sacrificed at 3, 4, 13, 15 and 18 weeks of age. Thirty-seven additional newborn rats were given on a weight basis 4 times the dosage of nephrotoxic serum capable of inducing experimental nephritis in the adult rat and were sacrificed at 1, 2 and 3 weeks of age. One hundred and eighty-six control rats received normal rabbit serum and were sacrificed at 1, 2, 3, 4, 5 and 6 weeks of age. The day before sacrifice, each animal 3 weeks and older was placed in a metabolic cage with water *ad libitum* and timed urine specimens were collected for protein analysis.

Sera obtained from rats of the same age and experimental design were pooled and biochemical analyses were done in duplicate. Renal tissue obtained at autopsy was fixed in formaldehyde and paraffin sections were stained with hematoxyin and eosin and with the PAS reagent. Urea nitrogen was determined by the micro-diffusion method of Conway(8), and cholesterol by the method of Bloor(9). Serum protein electrophoresis was analyzed with a Spinco apparatus. Urine proteins were determined by the method of Hiller, McIntosh and Van Slyke(10), and serum proteins by the method of Gornall, Bardawill and David(11).

Results. 1. *Weight:* The data are presented in Tables I, II and III. Animals sacrificed at 1 week of age did not show significant difference in mean weight between controls and those injected with 4 times the nephrotoxic dose, 10.6 ± 1.2 g *vs* 11 ± 2.8

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TABLE I. Control Rats. Mean values ± 2 SD.

No. of rats	Age, wk	Wt, g	Urine prot., mg/hr	BUN, mg %	T.P., g %	Alb., g %	Glob., g %	A/G	Choles., mg %	Alb., %	Tot. glob. %	A_1 glob. %	A_2 glob. %	β glob. %	γ glob. %
28	Newborn	5.4 ± .6	—	—	—	—	—	—	—	—	—	—	—	—	—
40	1	10.6 ±1.2	.00	27.0 ±18.1	4.7 ±2.3	1.8 ±.8	2.9 ±1.2	.7 ±.4	164 ±88	39.1 ±14.5	61.8 ±17.2	9.2 ±10.3	15.3 ±8.6	25.5 ±5.3	10.8 ±5.7
45	2	22.9 ±3.9		29.0 ±6.8	4.5 ±1.5	1.8 ±.7	2.7 ±1.1	.7 ±.3	182 ±35	40.3 ±10.0	59.9 ±10.7	9.0 ±9.6	14.9 ±3.5	23.9 ±7.0	11.6 ±5.9
31	3	33.8 ±12.8	.02 ±.04	17.0 ±5.1	4.3 ±.5	2.5 ±.9	1.8 ±1.0	1.5 ±1.2	83 ±35	58.2 ±20.8	32.9 ±7.2	9.9 ±5.0	14.6 ±5.8	17.8 ±5.0	7.9 ±3.9
27	4	49.5 ±21.2	.12 ±.07	19.0 ±10.6	5.1 ±.3	2.8 ±.6	2.3 ±.5	1.2 ±.4	109 ±34	55.4 ±11.3	35.1 ±3.1	10.6 ±2.8	12.0 ±2.9	18.6 ±4.7	5.7 ±2.2
11	5	85.1 ±12.6	.09 ±.06	20.0 ±5.6	5.4 ±1.0	3.0 ±1.1	2.4 ±.7	1.3 ±.7	56 ±28	55.0 ±13.3	37.8 ±5.6	13.5 ±4.7	12.3 ±4.5	17.7 ±7.9	4.6 ±3.2
4	6	—	—	29.0 ±10.6	5.2 ±.4	2.8 ±.6	2.4 ±1.0	1.3 ±.5	99 ±32	56.3 ±10.5	—	11.6 ±5.2	11.3 ±4.1	16.5 ±1.0	4.4 ±3.4

TABLE II. Response of Newborn Rats to Nephrotoxic Serum. Mean values ± 2 SD. 2.0 ml/100 g 1:2 I.V. (71% of NTD).

No. rats	No. serum		Sacr. age, wk	Wt, g	Urine prot., mg/hr	BUN, mg %	T.P., g %	Alb., g %	Glob., g %	A/G	Choles., mg %	Alb., %	Tot. glob. %	A_1 glob. %	A_2 glob. %	β glob. %	γ glob. %
4	2	Mean ± 2 SD	1	—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	2	Mean ± 2 SD		—	—	—	—	—	—	—	—	—	—	—	—	—	—
4	2	Mean ± 2 SD	3			15.0	4.6 ±1.6	2.3 ±.6	2.2 ±2.1	1.2 ±1.4	168 ±80	52.2 ±30.0	36.6	9.1	11.1	28.2	10.7
5	2	Mean ± 2 SD	4			17.0 ±6.0	4.7 ±1.9	3.0 ±.7	1.7 ±1.3	2.0 ±1.3	102 ±26	65.4 ±13.6	34.6 ±13.6				
3	2	Mean ± 2 SD	13	303 ±105	.4 ±.8	18.3 ±9.0	4.7 ±1.3	1.9 ±1.4	2.7 ±1.4	.7 ±.5	86 ±29	40.9 ±15.4	—	24.0 ±11.0	9.4 ±9.1	18.7 ±4.0	7.0 ±2.6
3	503	Mean ± 2 SD	15			21.0 ±9.0	4.4 ±.8	2.2 ±.7	2.2 ±.7	1.1 ±.5	97 ±33	49.3 ±15.6	—	14.0 ±2.0	10.2 ±4.2	14.5 ±1.8	10.9 ±6.6
4	503	Mean ± 2 SD	18	167 ±22	.0	28.0 ±7.0	6.3 ±1.5	2.9 ±1.0	3.4 ±.5	.9 ±.3	114 ±42	46.0 ±5.7	—	15.5 ±1.8	11.3 ±3.6	20.3 ±3.8	7.0 ±4.9

NTD—Nephrotoxic dose.

NTD 1.4 ml/100 g rat tissue PS #2 and #503.

TABLE III. Response of Newborn Rats to Nephrotoxic Serum. Mean values ± 2 SD. 2.0 ml/100 g 1:1 I.V. (4 times the NTD).

No. rats	No. serum		Sacr. age, wk	Wt, g	Urine prot., mg/hr	BUN, mg %	T.P., g %	Alb., g %	Glob., g %	A/G	Choles., mg %	Alb., %	Tot. glob.	A_1 glob.	A_2 glob.	β glob.	γ glob.
													%				
5	2	Mean ± 2 SD	1 day	—	—	—	—	—	—	—	—	—	—	—	—	—	—
21	4	Mean	1	11	.2	24.0	4.6	1.4	2.9	.5	165	33.0		13.7	17.3	27.4	8.5
		± 2 SD		±2.8		±5.2	±1.8	±.5	±.7	±.2	±105	±10.7		±8.6	±7.6	±7.5	±7.8
7	4	Mean	2	18	.5	30.6	4.7	1.7	3.0	.6	166	36.8		12.5	14.1	28.2	8.5
		± 2 SD		±6.9		±3.3	±.4	±.2	±.6	±.2	±24	±6.5		±2.9	±3.0	±5.7	±1.3
4	4	Mean	3	46		17.5	5.1	2.6	2.6	1.0	94	50.1		9.9	13.4	20.0	7.1
		± 2 SD		±11.9		±4.2	±.5	±.3	±.5	±.3	±81	±7.2		±2.6	±6.3	±6.7	±5.3

NTD—Nephrotoxic dose. NTD 1.4 ml/100 g rat tissue PS #2. NTD .5 ml/100 g rat tissue PS #4.

g. Edema was difficult to evaluate in these small rats.

At 2 weeks of age, mean weight for control rats was 22.9 ± 3.9 g as against 18 ± 6.9 g in the 4 times the nephrotoxic dose group.

At 3 weeks of age, the mean weight for the control rats was 33.8 ± 12.8 g; in the 4 times the nephrotoxic dose group, it was 46 ± 11.9 g. Since the mean weight in the experimental group was derived from 4 rats of the same litter, it would be difficult to interpret the significance of this difference.

2. *Biochemical:* Data is presented in Tables I, II and III. *B.U.N.* There is no significant difference at all ages tested (1, 2 and 3 weeks), in the mean B.U.N. value between controls and experimental rats given 4 times the adult nephrotoxic dosage.

Serum proteins: No significant difference was found in total serum protein, albumin and globulin fractions between controls and the experimental rats given 4 times the nephrotoxic dose.

Urinary proteins: No significant proteinuria (>1.0 mg/hr) was obtained in rats tested at 1 and 2 weeks of age. Two rats given 4 times the nephrotoxic dose by weight showed some proteinuria, 0.2 and 0.5 mg/hr but this was not comparable to proteinuria obtained in adult rats given nephrotoxic serum.

Serum cholesterol: 1 week of age. Mean serum cholesterol value in control animals was comparable to the mean value obtained in the rats given 4 times the nephrotoxic adult dose, 164 *vs* 165 mg%.

2 weeks of age. Mean serum cholesterol values were comparable in the control group and the 4 times the nephrotoxic dose group, 186 ± 35 *vs* 166 ± 24 mg%.

3 weeks of age. Mean serum cholesterol value showed values comparable for the control and 4 times the nephrotoxic dose group. Serum cholesterol was elevated in the 71% nephrotoxic dose group, 83 ± 35 *vs* 168 ± 80 mg%.

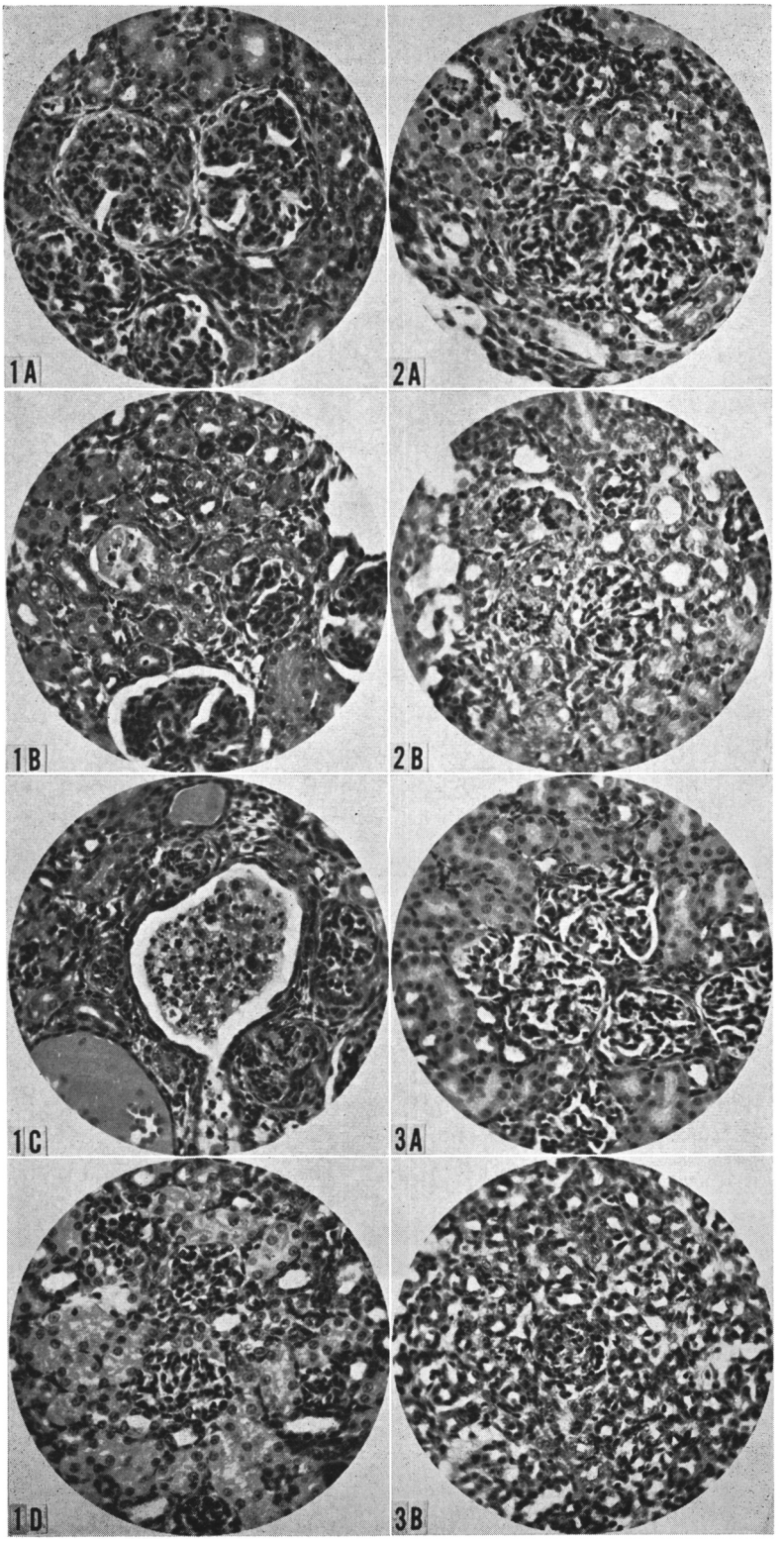
Renal Pathology. 1 week old rats. The histologic changes in the kidneys of the neonatal rats given 4 times the nephrotoxic dose and sacrificed at 1 week of age are shown in

Fig. 1A, 1B and 1C. The microscopic findings of recent renal damage are distinct. Acute glomerulitis is clearly observed underneath the nephrogenic zone in the inner cortex. There is enlargement of many glomeruli due to hypercellularity. Numerous leukocytes are seen in the loops of these swollen glomeruli (Fig. 1A). The capsular space of Bowman is obscured. Some of the glomeruli closer to the surface are not affected. There is conspicuous cast material of leukocytes in the lumina of some convoluted tubules in the cortex, along with some necrotic cellular debris (Fig. 1B). In the kidney of one animal in particular, there is considerable cast material in the collecting tubules of the medulla, including masses of erythrocytes with leukocytes and hyaline casts (Fig. 1C). A kidney section of a normal control from a rat one week of age is shown in Fig. 1D. In the neonatal rats, given only 71% of the adult rat nephrotoxic dose and sacrificed at one week of age, the histologic renal changes are less marked. In one of the animals, only a few glomeruli in the junctional zone of the cortex and medulla were slightly affected. In other animals, several glomeruli showed changes of acute glomerulitis in addition to a few leukocytes and erythrocytes in some cortical tubules (Fig. 2A and B).

Animals sacrificed at end of second week: In the animals given 4 times the nephrotoxic dose, only a few glomeruli within the innermost cortical zone appear slightly hypercellular and larger than the glomeruli in the outer zone (Fig. 3A). In addition, there are rare casts containing leukocytes and necrotic debris in a few convoluted and collecting tubules of the cortex and medulla (Fig. 3B). In the animals of this series, which had received 71% of the neprotoxic dose, the positive findings were even less marked, consisting of a few hypercellular glomeruli in the inner cortical zone, a rare hyaline cast and a few erythrocytes in a tubule within this zone.

Animals sacrificed at end of third week: In these animals, all of which had received 4 times the nephrotoxic dose, there was only a rare enlarged glomerulus in the inner cortical zone showing a thickened capsule, prominence of the basement membrane or a few synechiae. The remainder of the glomeruli throughout the mid and outer cortex appeared normal. There were a few leukocytes in nearby tubules and a few interstitial leukocytes surrounding a few PAS positive protein casts in the junctional zone. The prominent basement membrane in the enlarged glomeruli (usually not more than one in each section), entirely within the inner cortical zone, was very conspicuous (Fig. 4). In the kidneys of the group (4 animals) receiving 71% of the nephrotoxic dose, there were no pathologic findings. Kidneys of the rats sacrificed at 4, 15 and 18 weeks respectively did not reveal significant pathologic changes; some had received 4 times the nephrotoxic dose, others, 71% of this dose.

Discussion. Experimental renal disease in adult rats induced with nephrotoxic serum simulates in many ways the nephrotic syndrome observed in infants and children. Marked edema, ascites associated with hypoproteinemia and hyperlipemia was almost always observed in adult rats studied by Heymann and Lund(7). The hypoproteinemia, usually severe at first, slowly regressed and, with improvement, the serum proteins were restored to normal values after several weeks or months. When, however, chronic renal disease had developed, these findings lasted without remission for as long as a year. When the newborn rat was given a dosage of nephrotoxic serum capable of inducing experimental nephrosis in the adult rat, it was apparent that the response observed during the first 3 weeks of age (and older) was different; there was neither massive ascites nor severe hypoproteinemia and hyperlipemia(1, 2). The presence of rabbit gamma globulin, attached to the glomerulus of the newborn rat, as revealed by indirect fluorescent staining technique(4), in the absence of the biochemical alterations, emphasizes a difference in the response of the newborn rat from the adult rat. Histologic examination of the kidneys of the newborn rats, 7 days after administration of nephrotoxic serum, revealed distinct glomerular damage, particularly in the inner cortex. This is in contrast to the findings in rats injected in the newborn period and sacrificed at 3 weeks of age and


1A
2A
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1D
3B

FIG. 1 A. Hematoxylin-Eosin. 304 ×. 4 times the nephrotoxic dose capable of producing experimental nephrosis in adult rat on a weight basis was administered intravenously in these newborn rats. Rats were sacrificed at 1 wk of age. Note enlarged hypercellular glomeruli, especially 2 in the center of the field with fairly numerous leukocytes in capillaries.

FIG. 1 B. PAS. 304 ×. 4 times the nephrotoxic dose capable of producing experimental nephrosis in adult rat on a weight basis was administered intravenously in these newborn rats. Rats were sacrificed at 1 wk of age. Note moderately enlarged and distinctly hypercellular glomeruli; in center, a proximal convolution containing leukocytes and necrotic epithelial debris; at right, a hyaline cast in one tubule.

FIG. 1 C. Hematoxylin-Eosin. 304 ×. 4 times the nephrotoxic dose capable of producing experimental nephrosis in adult rat on a weight basis was administered intravenously in these newborn rats. Rats sacrificed at 1 wk of age. Note numerous erythrocytic and hyaline casts in distended collecting tubules of medulla.

FIG. 1 D. Normal control. Hematoxylin-Eosin. 304 ×. Normal adult rabbit serum was injected intravenously for controls. Rats sacrificed at 1 wk of age.

FIG. 2 A. PAS. 304 ×. 71% of nephrotoxic dose capable of producing experimental nephrosis in the adult rat on a weight basis was administered intravenously in these newborn rats. Rats sacrificed at 1 wk of age. Note hypercellular glomeruli and leukocytic exudate in lumen of a tubule at upper left.

FIG. 2 B. Hematoxylin-Eosin. 304 ×. 71% of nephrotoxic dose capable of producing experimental nephrosis in the adult rat on a weight basis was administered intravenously in these newborn rats. Rats sacrificed at 1 wk of age. Note cellular debris with leukocytes in lumens of 2 proximal convolutions.

FIG. 3 A. Hematoxylin-Eosin. 304 ×. 4 times the nephrotoxic dose capable of producing experimental nephrosis in adult rat on a weight basis was administered intravenously in these newborn rats. Rats sacrificed at 2 wk of age. Note hypercellular glomeruli within innermost cortical zone.

FIG. 3 B. Hematoxylin-Eosin. 304 ×. 4 times the nephrotoxic dose capable of producing experimental nephrosis in adult rat on a weight basis was administered intravenously in these newborn rats. Rats sacrificed at 2 wk of age. In center of picture, there are scattered leukocytes in lumina of 2 tubules of medulla.

older; their renal lesions were comparatively slight or entirely missing, suggesting healing of the earlier lesion and development of newly formed glomeruli in the nephrogenic zone. The absence of biochemical alterations as found in the very young rat despite the pathologic changes in the glomeruli shows a difference in response from older rats.

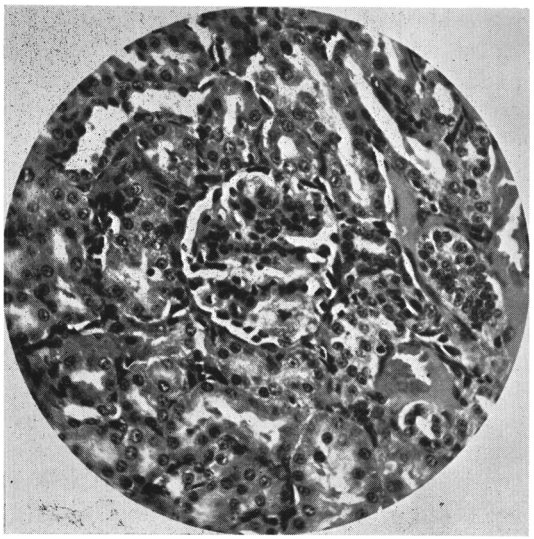

FIG. 4. Hematoxylin-Eosin. 304 ×. 4 times the nephrotoxic dose capable of producing experimental nephrosis in adult rat on a weight basis was administered intravenously in these newborn rats. Rats sacrificed at 3 wk of age. Note a single, slightly enlarged glomerulus with prominent basement membrane and some synechiae with Bowman's capsule.

Summary. 1. Nephrotoxic serum administered to newborn rats 0 to 3 hours of age produces glomerular damage, particularly in the inner cortex, but fails to produce the same clinical or biochemical response observed in the adult rat. 2. Rats injected in the newborn period and sacrificed at 3 weeks of age and later show only minimal histologic lesions in the inner cortical zone. 3. The developing glomeruli in the cortical zone appear to be spared the nephrotoxic effects of kidney antiserum. 4. These newborn rats injected with nephrotoxic serum did not develop chronic renal disease.

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Characteristics of a Plaque Method for the Murine Salivary Gland Virus.* (28605)

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The murine salivary gland virus (MSGV) belongs to a group of unclassified mammalian viruses (cytomegaloviruses(1) characterized by unique cytopathic alterations(2), which may be harmlessly confined to the salivary glands, or widely and lethally distributed in other viscera. The murine(3), human(4) and cavian(5) strains have been serially propagated in tissue culture, but quantitative studies have not been reported. The objectives of this report are to describe a rapid, precise, quantitative method for studying MSGV, and to present certain data obtained by its use.

Materials and methods. Cell cultures. Monolayers of mouse embryonic cells, prepared by the method of Youngner(6) from NLW Swiss mice, were grown in 1-liter Blake culture bottles, using 50 ml of a growth medium (GM) composed of 90% Earle's balanced salt solution (BSS), Eagle's amino acid and vitamin mixture(7) in double strength (BME×2), 10% calf serum, phenol red, 100 u/ml penicillin, 100 μg/ml streptomycin and 25 u/ml Mycostatin. Cells were trypsinized and subcultured every 3 or 4 days for 4-8 weeks; then arbitrarily discarded and replaced by new lines. After 2 subcultures, the cells became uniformly fibroblastic in appearance. All cultures were incubated at 37°C without CO_2.

Virus cultivation. Latently infected mice were obtained through the courtesy of Dr. Margaret G. Smith. Using a procedure similar to that used by Smith(3), the above described monolayer cultures were readily infected, nearly all of the cells showing cytopathic effect (CPE) in 2-4 days, in primary as well as subsequent cultures. Serial passage of the virus was made every 3-5 days by transferring 10 ml of fluid from an infected to an uninfected bottle culture. After 38 such passages in 1-liter bottle cultures, always with rapid and generalized CPE because of the high input level, plaque studies were undertaken.

Procedure for plaque formation. Uninfected cells were obtained by trypsinizing densely populated bottle cultures, centrifuging at 500 RMP for 5 minutes, decanting the trypsin, and resuspending the cells in the GM described, with addition of 0.005 M Tris. Five ml of this medium, containing approximately 1.8×10^6 cells, was pipetted into each of a series of flat-bottom 40 mm Petri dishes. Confluent monolayers formed within 24 hours, at which time the medium was removed, and the cells washed with 2 ml of

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