concentration between these animals and their saline-injected controls was observed.

Chronic growth hormone administration (3 mg/rat/day for 3 days) to hypophysectomized rats also increased rate of NEFA release ($P<.01$) from their epididymal fat bodies (Table I). Under these circumstances plasma NEFA and blood glucose concentrations were unchanged by the growth hormone treatment.

Discussion. The results of this study clearly indicate that hypophysectomy inhibits and growth hormone stimulates release of NEFA from adipose tissue and validate the conclusion that the increase in plasma NEFA concentration observed following growth hormone administration signifies increased NEFA mobilization from fat stores. The mode of action of growth hormone in this regard, however, is not clear. That growth hormone does not have a direct lipolytic action on adipose tissue has been suggested by the experiments of White and Engel(9) in which growth hormone was added to adipose tissue *in vitro*.

It has been suggested(10) that the regulation of NEFA mobilization is mediated by availability of glucose for cellular oxidation. If the action of growth hormone is explicable on this basis, *i.e.* by inhibiting glucose oxidation, it was not reflected by alterations in blood glucose concentrations as measured in the present experiments.

Summary. The release of nonesterified fatty acids (NEFA) from epididymal fat bodies removed from normal, hypophysectomized and hypophysectomized growth hormone treated rats has been investigated. Hypophysectomy inhibits and growth hormone administration stimulates NEFA release from adipose tissue *in vitro*. These findings are taken as direct evidence for the concept that growth hormone treatment causes NEFA mobilization from fat stores.

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Quantitative Determination of Infectious Units of Measles Virus by Counts of Immunofluorescent Foci.* (24915)

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Enumeration of infectious animal virus units depends either upon determination of dilution endpoint or upon counting foci of virus growth(1). The latter approach, modeled after bacterial colony count technic, has been utilized to count proliferative and necrotic pocks on animal skin and cornea, and on chorioallantois of embryonic chick. It is now widely employed for counting plaques in cultures of animal cells(2). Many viruses that multiply in animal cells do not form plaques. Demonstration and enumeration of

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foci of virus antigen in such cells can perform the same function as plaque counts. Dulbecco and Vogt(3) suggested that the fluorescent antibody technic would serve this purpose. Noyes(4) counted fluorescent foci obtained with West Nile virus by a method that enabled him to compare relative concentrations of virus; and Hotchin(5) reported that methyl cellulose, which does not fluoresce, could be used as substitute for agar in an overlay designed to prevent secondary plaque formation. We found that counts of fluorescent foci can be used to measure amount of infectious virus in suspensions of a measles strain that fails to form well-defined plaques in the cell-virus system. The technic should be useful for quantitative investigation of other viruses in cells that support virus growth but in which plaques are not recognized.

Materials and methods. The Edmonston strain of measles virus(6)‡ was employed. The virus had undergone 26 passages in human kidney cells, 8 passages in human amnion cells, and 29 passages in human epidermoid carcinoma No. 2§ (H. Ep.-2) cells. In H. Ep.-2 cells under fluid, the virus induces formation of giant cells and of long cellular processes, and also causes necrosis, but as mentioned above, countable plaques were not observed under agar overlays. H. Ep.-2 cells were grown on 12 mm round coverslips in 60 mm petri plates, 6 coverslips/plate. Four drops (approximately 0.2 ml) of a suspension containing 100,000 H. Ep.-2 cells/ml were placed on each coverslip and allowed to attach to the glass for approximately 3 hours at room temperature. The cells were then flooded with 4 ml of nutrient fluid consisting of 80% Eagle's basal medium,‖ 15% pooled horse serum, and 5% calf serum.‖ Penicillin (100 units/ml), streptomycin (100 μg/ml) and nystatin (Mycostatin, Squibb, 100 units/ml) had been added to the fluid. The pH was then adjusted to 7.5 with $NaHCO_3$. The petri plates were put unsealed into standard desiccator together with a lighted candle. The desiccator was tightly closed, sealed, and incubated at approximately 37°C. After 3 days, good sheets of cells had usually formed, and the coverslips were transferred to dry petri dishes 0-2 days later. For virus assays, cell sheets were inoculated with 0.05 ml of appropriate virus dilution. Each virus dilution was tested on 4 or more coverslips. The virus was permitted to adsorb for 2 hours at room temperature before an overlay was added. The overlay, consisting of 2% methyl cellulose and 20% horse serum in Eagle's basal medium, was prepared as follows: the methyl cellulose (4000 centipoises) was repeatedly washed with absolute ethanol and with ether, then air dried. Two g of the powder were suspended in 40 ml of boiling distilled water, autoclaved, and cooled to approximately 40°C. Forty ml of double strength Eagle's basal medium with antibiotics and 20 ml of horse serum were then added. The suspension was mixed well and stored at 4°C as a viscous sol. Inoculated cells in plates were reincubated for 3-6 days in the candle desiccator at 37°C. At this temperature the viscous methyl cellulose almost gels. After 3-6 days, plates were refrigerated at 4°C for approximately one hour, which reliquefies the methyl cellulose. Although the overlays were still viscous, they could then be decanted, following which the coverslips were washed repeatedly with chilled, phosphate-buffered saline. The cells were fixed with acetone for 10 minutes at room temperature, and prepared for immunofluorescent microscopy by indirect technic(7,8). Convalescent-phase serum from measles patients was used as antimeasles antibody. Fluorescein-labeled antihuman globulin horse pseudoglobulin had previously been absorbed twice with mouse liver powder to remove nonspecific staining properties(7,9). Controls consisted of infected cells treated with acute-phase serum from measles patients, and uninfected cells treated with convalescent-phase serum; both sets were then reacted with labeled antihuman pseudoglobulin. Coverslips were mounted in phosphate buffered glycerol (pH 7.0) on glass slides. All counts were done on coded specimens. They were made with the aid of eyepiece grid and mechanical

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§ Dr. Alice Moore, Sloan-Kettering Inst. for Cancer Research, N. Y., kindly supplied H.Ep.-2 cells.

‖ Microbiological Assoc.

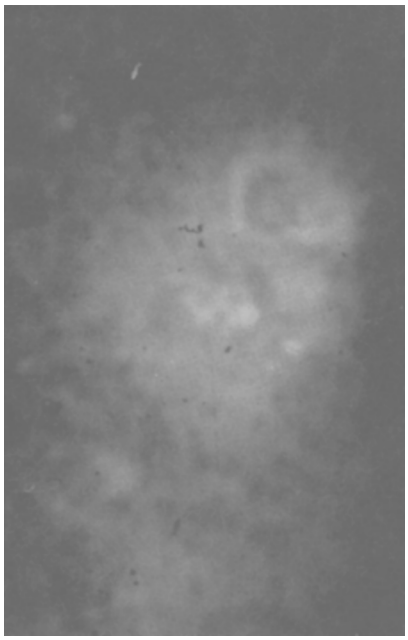

FIG. 1. Immunofluorescent focus of measles antigen in H.Ep.-2 cells after 5 days of development under methyl cellulose overlay. 530×.

stage under low power (100X) of the fluorescence microscope, similar to the one previously described(10). Photomicrographs were taken with camera coupled to Leitz Micro-Ibso attachment. Anscochrome film was exposed 8 minutes and processed as recommended by the manufacturer, although it was found advantageous to develop at ASA 125. Black and white reproductions were prepared from color slides. Concurrent infectivity titrations of the virus were carried out in 16 x 125 mm screw cap tubes containing monolayers of H. Ep.-2 cells. Our experiments involved 24 tubes/dilution; each tube received 0.05 ml of inoculum. Tubes were incubated in stationary racks at 37°C and the cells inspected for cytopathogenic changes for 14 days following inoculation. Fluid changes were carefully performed to prevent cross-contamination; separate pipettes were used for each tube.

Results. Fluorescent foci appear in H. Ep.-2 cells infected with Edmonston strain of measles virus as early as 12 hours after inoculation(11), and in the absence of an overlay a few foci consisting of single cells or small groups of cells can regularly be distinguished one day after inoculation. Multicellular foci appear within 3 days, but single-cell or small foci are also present at this time. This polymorphism is probably the result of secondary spread of infection.

Most fluorescent foci seen after 3 days incubation under methyl cellulose consisted of 1-2 cells. Five day foci were multicellular, approximately circular, relatively uniform in size, and were readily countable at 100X magnification (Fig. 1). They appeared to be randomly distributed on coverslips.

Five aliquots of stock measles virus suspension were assayed separately on different days and the results compared. Three assays were done by means of fluorescent focus counts in which methyl cellulose overlays were employed, while the fourth and fifth were infectivity titration in tubes. H. Ep.-2 cells were used for all 5 experiments, and the volume of inoculum was 0.05 ml for each coverslip preparation or tube. Inocula were stored sealed in dry ice box until diluted for use.

A fluorescent focus count assay that was read after 5 days of incubation will be presented first (Table I, solid circles in Fig. 2). There was good correlation between relative amount of virus in the inoculum and corresponding mean count. In the range of 125-8.3 mean counts/dilution, 2-fold dilutions of virus yielded slightly greater than 2-fold de-

TABLE I. Counts of Immunofluorescent Foci on Coverslips 5 Days after Inoculation of 2-Fold Dilutions of An Aliquot of Stock Measles Virus.

	Immunofluorescent focus counts			
Dil. of virus	Each coverslip	Mean ± S.D. of mean	Variance	Fold decrement
1:25	173, 114, 84, 135, 139, 119, 117, 119	125.0 ± 9.0	648	
1:50	17, 56, 55, 39, 59, 58, 47, 53	48.0 ± 5.0	200	2.6
1:100	23, 17, 20, 22, 19, 18	19.8 ± .94	5.37	2.4
1:200	8, 12, 11, 1, 7, 11	8.3 ± 1.67	16.7	2.4
1:400	5, 3, 1, 10, 4, 7	4.7 ± 1.23	9.07	1.8

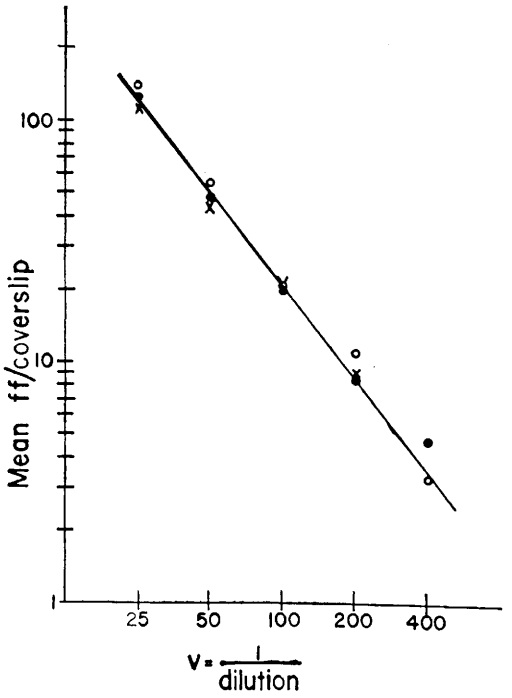

FIG. 2. Relationship between virus in inoculum (V) and counts of immunofluorescent foci (ff) on coverslips 3, 5, and 6 days after inoculation of 3 different aliquots of stock measles virus on different days. ○ = 3 day foci; ● = 5 day foci; and × = 6 day foci.

crements in number of foci counted.

The 5 day counts were confirmed by fluorescent focus count assays of 2 other aliquots of stock virus suspension (Fig. 2). Average counts at end of 3 days (open circles) and 6 days (crosses) incubation are plotted, together with data from the assay just discussed. On the whole the 3 assays agree. Since the counts were made after differing incubation periods, these results, in addition to indicating the degree to which the procedure is reproducible, demonstrate that formation of secondary foci probably occurs to a negligible extent under the overlay, between the time that foci first appear (3 days) and the time that multicellular foci have formed (5 and 6 days).

To estimate the virus content of the inoculum by an independent method, half-log dilutions respectively of a portion of the aliquot used for the 5 day counts, and of another aliquot of the same stock virus suspension, were inoculated into tubes of H. Ep.-2 cells, 24 tubes/dilution. The percentage of tubes exhibiting cytopathogenicity (Table II) fits the Poisson distribution for the hypothesis that one infectious particle can initiate infection. Such an all-or-none method as tube titration is inherently less precise than a plaque or focus counting technic unless more than 24 tubes/dilution are employed. When calculations are based on mean fluorescent focus counts in the range of 20 foci/coverslip or less, there appeared to be approximately 3 to 4 times as many fluorescent foci/unit volume of stock virus suspension than would be expected from the results of tube titration. While the conclusion that 3-4 fluorescent focus units are equal to a unit capable of giving rise to cytopathogenicity is not warranted from this experiment because of technical differences in the 2 types of assays, it is a possibility that deserves further study.

Discussion. The procedure described has marked advantages for several types of investigation. First, it can be employed, as in this study, to determine quantitatively the amount

TABLE II. Distribution of Endpoint Cytopathogenic Changes in Tubes Containing H. Ep.-2 Cell Monolayers 14 Days after Inoculation of $10^{-0.5}$-fold Dilutions of An Aliquot of Stock Measles Virus.

Dil. of virus (−log)	No. of tubes		% positive tubes	
	Inoculated	Positive*	Observed	Expected†
2.0	24	22	92	99
2.5	"	19	79	76
3.0	"	5	21	36
3.5	"	3	13	13
4.0	"	2	8	6
4.5	"	0	0	1
5.0	"	0	0	<1
1.5	"	24	100	99
2.0	"	24	100	99
2.5	"	21	88	86
3.0	"	12	50	47
3.5	"	5	21	18
4.0	"	1	4	6

* Tubes showing cytopathogenicity were scored "positive."

† On assumption that one infectious particle is capable of initiating infection, and that such particles are distributed by the Poisson distribution, the expected number of particles/tube was taken as arithmetic mean of Poisson estimates for dilutions in which both "positive" and "negative" tubes occurred. Expected percentages of "positive" tubes were calculated from expected number of particles/tube.

of virus that will be productive of infection in a cell system that does not form plaques under conditions employed. Second, the method might be useful for assay of non-plaque forming variants in a cell-virus system in which plaques are formed. Third, the technic might be practicable for study of latently infected cells. Use of the method as assay procedure to detect foci of incomplete virus multiplication, in which virus antigen is produced, might be listed in any of these 3 categories.

The procedure is time-consuming and the technic is demanding, so that assay by fluorescent focus counts cannot be recommended as convenient. The chief practical difficulties seem to occur at the time the infected coverslips are prepared for fluorescence microscopy. Sometimes it is difficult to wash methyl cellulose completely from the infected coverslips, and if this is the case, the reaction of virus antigen with antibody is probably blocked, and quantitative results cannot be obtained. A second practical difficulty that has been encountered is due to the variability of different batches of fluorescein-labeled antibody with respect to antigen-antibody combination. With respect to another potential source of error, it is essential that a measured volume of virus suspension be adsorbed to a cell sheet having defined limits. Confinement of an inoculum to the surface of a coverslip culture can be achieved by transfer of coverslip to a dry surface before addition of inoculum.

The conclusion that the methyl cellulose overlay was effective in preventing formation of secondary foci is supported by other evidence in addition to the data (Fig. 2) already presented. Foci spread concentrically from the few cells observed at end of third day of incubation. The regular, circular multicellular areas containing virus antigen that had formed at the end of 5 days incubation were relatively uniform in size. Furthermore, foci were apparently distributed at random on the coverslips; and finally, on some coverslips, there was only one focus.

Counts were approximately proportional to amount of virus added. This result is consistent with the hypothesis that each focus is the consequence of infection with a single infectious particle of measles virus. Additional evidence for a one particle hypothesis was obtained by tube infectivity titrations since the proportion of tubes exhibiting cytopathogenic changes closely followed the expected Poisson distribution.

Summary. 1) The number of infectious units of measles virus in a suspension were assayed by counting microscopically the number of immunofluorescent foci in infected coverslip tissue cultures, after they were incubated under methyl cellulose overlay. The overlay prevents secondary spread of virus and is removed before the infected cell sheets are treated with antimeasles serum and fluorescein-labeled antiglobulin for detection of virus antigen by the indirect fluorescent antibody procedures. 2) Aliquots of stock virus suspension were serially diluted and assayed separately by this procedure. Results of independent assays agreed well. Ratios between fluorescent focus counts on successive dilutions approximated the reciprocal of dilution factor and there appeared to be a linear relation between amount of virus inoculated and count. In comparison with a virus titration in monolayers of same cell in tubes read for endpoint cytopathogenicity, fluorescent focus count assay of same stock virus had the same advantage as plaque technic. Data were consistent with the hypothesis that one infectious particle of measles virus is sufficient to initiate infection. 3) The method seems useful for investigation of latent infection, incomplete virus multiplication, non-plaque-forming variants of plaque-forming viruses, or other cell-virus systems in which virus antigen is produced but plaques are absent.

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Distribution Kinetics of Intravenous Epinephrine. (24916)

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Epinephrine given intravenously rapidly disappears from plasma(1). Guyton and Gillespie found that infusion of .0025 mg/kg/min into a dog sustained blood pressure at 200 mm Hg(2). Using constancy of pressure as criterion they calculated that rate of destruction was directly proportional to quantity in the body; the amount destroyed/minute being .631 times that quantity. The following studies were carried out to analyze, by means of fluorometrically determined plasma levels, distribution kinetics and disappearance rate of intravenously injected epinephrine.

Methods. Mongrel dogs of both sexes weighing 8 to 20 kg were anesthetized with intravenous pentobarbital. Six were given rapidly into femoral vein a single injection of 40 μg epinephrine/kg as l-epinephrine bitartrate. Five were similarly injected, then immediately given a constant intravenous infusion of epinephrine to 75 minutes duration. Blood pressures were recorded continuously from a cannula in femoral artery. Heparinized blood was drawn from opposite femoral artery immediately prior to, in some instances during, and at frequent intervals following administration of epinephrine. Plasma epinephrine contents were determined fluorometrically by modification of the method of Lund(1,3).

Results. Immediately following injection of 40 μg epinephrine/kg blood pressures rose to 300-350/200-250 mm Hg, remained for approximately one minute, fell during the next minute to 160-180/100-120 mm Hg, then slowly returned toward pre-injection levels over subsequent 4 minutes. During continuous infusions, blood pressures were maintained at about 200/150 mm Hg, then at cessation fell to 50-70/30-50 mm Hg within 2 minutes.

When epinephrine is given rapidly intravenously, arterial plasma levels reach a peak within several seconds and fall to pre-injection values within 4 to 6 minutes (Fig. 1). Mean half-time for disappearance from the plasma compartment of epinephrine, which is in excess of pre-injection level, is 23 seconds. Mean calculated disappearance rate constant $(k_1)^\dagger$ is −1.77. By extrapolation to zero time the calculated volume of distribution of injected epinephrine averages 45 ml/kg. This approaches the plasma volume of the dog(4).

Five experiments were carried out in which epinephrine was infused at a constant rate following rapid injection of 40 μg/kg. When given at a rate of 11 or 12 μg/kg/min, plasma levels continued to rise at 2 μg/l/min. When administered at a rate of 6 or 7 μg/kg/min more nearly constant levels of about 100 μg/l

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† k_1 (disappearance rate constant for rapid injection) and k_3 (disappearance rate constant for slow phase after prolonged infusion) $= \frac{2.303}{T_1 - T_2} \times \log \frac{C_{T_1} - C_0}{C_{T_2} - C_0}$; where C_0 is pre-injection plasma epinephrine concentration and C_{T_1} and C_{T_2} are concentrations at T_1 and T_2 minutes, respectively. k_2 (disappearance rate constant for rapid phase after prolonged infusion) $= \frac{2.303}{T_1 - T_2} \times \log \frac{C_{T_1} - C_{Eq}}{C_{T_2} - C_{Eq}}$; where C_{Eq} is plasma epinephrine concentration (21 μg/l) at beginning of slow disappearance phase. Regression lines calculated by method of least squares.