the neoantigen, the "enhancer" and the oncogenic factor which produces ependymomas are identical or different.

Although there is no evidence that the Gomen strain of adenovirus 7 can produce ependymomas, we have observed the animals for only 120 days and observation is continuing. We have not produced ependymomas in newborn hamsters inoculated with large doses of adenovirus 12 and observed for 2 years. The remote possibility remains that the E46 strain could produce ependymomas without the portion of the SV40 genome within its capsid. To eliminate this possibility, strains of E46 without the hybridized portion of SV40 must be shown to be incapable of inducing ependymomas. Such experiments are now in progress.

Summary. Strain E46 of adenovirus 7 produced ependymomas when inoculated intracerebrally in newborn hamsters. These tumors were morphologically identical to those produced by intracerebral inoculation of SV40 and unlike any tumors produced in hamsters by oncogenic adenoviruses. It has been previously shown that strain E46 is a "hybrid" virus containing the portion of the SV40 genome responsible for production of SV40 neoantigen within an adenovirus 7 capsid. The present results indicate that the "hybrid" contains that portion of the SV40 genome responsible for induction of ependymomas.

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An Evaluation of the Variability in Measurement of Some Body Composition Parameters.* (30569)

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The measurement of body composition in gross biochemical terms has become increasingly important as an aid to clinical diagnosis and treatment(1). As better and simpler methods based on non-destructive dilution techniques or physical measurements have become available, these methods have been applied as routine procedures for the measurement of such parameters as total body water, extracellular water, blood volume, total circulating proteins, total exchangeable potassium and sodium and total body fat(2).

Survey of the literature has revealed very little information concerning the accuracy and reproducibility of the methods used. In studies of populations of well and sick subjects, the variations among individuals and

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among health states are likely to be of much greater importance than the inherent methodologic experimental error and observed differences between populations. Differences in body composition measurements repeated at intervals in the same individual, however, are composed of experimental error, variation around the true value of the individual, and real changes, if any, in his body composition. To determine whether real changes have occurred, estimates of experimental error and variation are necessary.

Even with the relatively old technique of using radio-iodinated serum albumin as a tracer in measurement of plasma volume, few observations on the reproducibility of measurements have been made. Inkley *et al* (3) made duplicate measurements 2 weeks apart on 10 subjects and found variations of 77-805 ml in plasma volume with a standard deviation of the differences of 289 ml.

Kaltreider *et al*(4) performed duplicate measurements of sodium space on 6 subjects, found a mean difference of 0.98 liter or 4.9% between estimations and concluded that in order for a difference in 2 successive observations in the same subject to be considered a real change the difference must exceed 1.38 liters.

In the development of the antipyrine dilution method for total body water, Steele *et al* (5) repeated observations on 3 subjects 4 times at spacings of 48-72 hours and reported reproducibility "within $\pm$ 8%" of the mean antipyrine space.

The present report is concerned with determining the variability between pairs of estimations of some body compartments made one week apart in order to interpret whether differences observed in the body composition of individual paraplegic patients represented real changes or the normal variation of observations at different times.

Methods. Selection of patients. The 26 patients used in this study were selected from the population of a hospital for the chronically ill; 8 had diagnoses of stroke, 5 paraplegia, 6 multiple sclerosis, 5 hip fracture, and 2 rheumatoid arthritis. The body composition of some of these patients has been reported elsewhere(9) as part of a study comparing "poorly nourished" and "well nourished" patients.

Patients were admitted to a Metabolic Ward for an 8-day period. The group of observations described below were done on the day after admission and repeated in identical manner 7 days later. The spacing of 7 days was selected (a) to allow time for isotope clearance from the body and (b) to conform to the availability of sodium^{24}, the shipment of which was limited to one day a week. During the 8-day period the patients' pattern of activities and dietary intake prior to admission to the Metabolic Ward were maintained as closely as possible.

Experimental schedule. At about 7 A.M. on the test day the patient was weighed and a 15-ml blood sample was taken (5 ml clotted for serum protein; 10 ml oxalated for hematocrit, hemoglobin and plasma blanks of antipyrine and sodium space measurements). Through the same needle, 50 ml saline containing 1 g antipyrine and 35 μc sodium^{24} were injected slowly from a preweighed syringe. Exact injection volume was determined from the weight of the empty syringe and the specific gravity of the injection fluid.

A light, fat-free breakfast was allowed after injection, and 100 ml water given every hour to compensate approximately for body losses. Blood samples (10 ml, oxalated, for antipyrine and sodium^{24} assays) were taken at 3, 4, 5 and 6 hours after injection.

Immediately following the 6-hour sample, 5 μc of I^{131}-radioiodinated serum albumin (RISA-Squibb) in 20 ml saline were injected using the same technique as above. A final blood sample (10 ml, for plasma volume) was taken 10 minutes after this second injection. Lunch was delayed until after the final blood sample.

Skinfold measurements were done at a convenient time during the day.

Body composition techniques. a. Total body water. Antipyrine was measured in plasma and in a 1:5000 dilution of the injection fluid by the nitrosylation method of Davidson and MacIntyre(6). A least squares method was used to extrapolate the plasma concentration at 3, 4, 5 and 6 hours after injection back to the injection time, assum-

ing an exponential rate of disappearance of antipyrine from plasma from the 3rd to 6th hour. Concentrations of antipyrine in all samples were corrected for the apparent pre-injection concentration; the maximum correction was 2%.† Total body water was then calculated from the formula:

$$\text{Total body water, liters} = \frac{\text{Concn. antipyrine in injection fluid} \times \text{injected vol in liters}}{\text{Concn. antipyrine in plasma, extrapolated to injection time}}$$

b. Sodium space. Sodium^{24} was counted in a crystal scintillation well-counter as total counts above background in plasma and a 1/1000 dilution of injection fluid. These same samples were later used for antipyrine assay. Sodium space was calculated from the formula:

$$\text{Sodium space, liters} = \frac{0.93 \times \text{cpm per ml injection fluid} \times \text{injected vol liter}}{\text{cpm per ml plasma (mean of four plasmas)}}$$

This formula assumes that the plasma sample counted for Na^{24} is 93% water and 7% solids.

c. Blood and plasma volume. Blood and plasma volumes were calculated independently from the difference in counts of blood and plasma samples taken before and 10 minutes after RISA injection. This method assumes that the background of residual Na^{24} activity is constant during this time.

$$\text{Blood or plasma vol, ml} = \frac{\text{cpm per ml RISA injection fluid} \times \text{injected vol, ml}}{\text{cpm per ml blood or plasma due to RISA}}$$

d. Hemoglobin and serum proteins. Hemoglobin concentrations were measured colorimetrically by the acid hematin method (calibrated against iron determinations). Total serum protein concentrations were measured by micro-Kjeldahl nitrogen estimation on a tungstic acid precipitate. Serum albumin was measured by the same method after separation from globulins with 19 volumes of 28% sodium sulfate.

e. Skinfold measurements. Large skinfold calipers with a surface area of 30 mm^2 and a constant pressure of 10 g/mm^2 were used to measure in mm the thickness of a pinched up double thickness of skin at 2 sites—(1) the posterior upper arm at a point midway between the acromial process and the olecranon, and (2) just below the inferior angle of the scapula.

Derived body composition parameters. Body fat was derived from the following relationship that assumes fat free tissue to be 72% water(7):

% body fat = 100% — (1.39 × % total body water).

Lean body mass was taken as body weight less body fat (as defined above).

Intracellular water was taken as total body water less sodium space. It is realized, however, that sodium space is significantly higher than extracellular water, so that this derived parameter does not really represent all the water in body cells. Total circulating hemoglobin and proteins were derived by multiplying blood or plasma volume by the corresponding blood or serum protein concentrations.

Statistical analysis. The following model serves as a basis for the analysis of results:

It was assumed that the observation of each of the body composition parameters could be characterized as being composed of specific and random components:

$$X_{ij} = \mu + a_i + \epsilon_{ij}$$

where i denotes the individual patient and j denotes the first or second observation, X_{ij} is the jth observation on the ith individual, μ is the population mean. $\mu + a_i$ represents the basic average value for the ith individual about which the parameter varies from time to time. ϵ_{ij} represents the deviation of an individual's parameter from its basic level at any time i plus the error of measurement of the i-j observation.

It was also assumed that the ϵ_{ij} follow a normal frequency distribution with mean 0 and standard deviation $\sigma\omega$.

† We observed that Gantrisin (sulfisoxazole) interferes with the spectrophotometric antipyrine assay and it was not given during the studies reported here.

TABLE I. Variability of Body Composition Parameters in Paired Observations on the Same Individual.

Parameter	Pairs of observations*	Mean of first observations	Mean difference (1st—2nd)	S.D. of difference
Measured				
Body wt, kg	26	57.04	.09	1.04
Body water, l	25	33.29	.95	3.07
" " % body wt	25	58.90	1.47	5.19
Sodium space, l	26	18.66	.16	.69
" " % body wt	26	33.25	.22	1.04
Plasma vol, l	22	2.88	.00	.34
" " % body wt	22	4.75	−.03	.59
Blood vol, l	21	4.43	.00	.60
" " % body wt	21	7.88	−.02	1.07
Hematocrit, %	25	40.10	.30	1.46
Hemoglobin, g/100 ml blood	26	12.44	.13	1.16
Serum protein, g/100 ml serum	26	6.95	.00	.34
" albumin, " " "	26	3.29	.04	.29
Arm skinfold, mm	20	7.70	.13	1.25
Scapula skinfold, mm	20	9.50	.15	.77
Derived				
Body fat, kg	25	10.77	−1.20	4.59
" " % body wt	25	18.13	−2.04	7.21
"Intracellular water," l	25	14.63	.75	3.15
" " % body wt	25	26.65	1.19	5.13
Total hemoglobin, g	21	552	9.10	99
Total serum protein, g	22	185	− .20	24
" " albumin, g	22	87	1.30	12

* This value is less than 26 when a pair of observations was not completed or a value was excluded for known technical reasons on one or more patients.

The differences between the pairs of observations one week apart would then be expected to follow a normal distribution with mean 0 and standard deviation $\sqrt{2}\,\sigma\omega$, if the individual did not change during the week.

Results and discussion. Variations in each of the observed body composition parameters are summarized in Table I as the mean difference between 2 observations one week apart and the standard deviation of the differences. The mean value of the difference between first and second observations was not significantly different from zero in any of the measured and derived parameters.

The observations of O'Toole *et al*(8) indicate that the variability of observations one week apart is considerably greater than that expected from experimental error alone. They analyzed the variability arising from various sources in the estimations of total exchangeable sodium, potassium and chloride. Coefficients of variation for 34 pairs of estimations (on duplicate samples after a single injection) were 2.5% for exchangeable sodium, but this variation increased to 5.4% when separate estimations were carried out one week apart on 43 patients.

The variations reported here therefore will include biological fluctuations in addition to experimental error, but it is assumed that one week is too short for significant trends in body composition to occur under the conditions of this study.

To test the effect of the type of patient on the body composition variability noted above, they were placed in one of the following 3 sub-groups determined by the original selection(9): a. 4 paraplegic patients; b. 13 patients judged to be "poorly nourished"; c. 9 "well nourished" patients.

The standard deviations of the difference between the observations were calculated for each sub-group separately. For most parameters, the 3 standard deviations were not significantly different, so that the whole group of 26 patients was considered homogeneous from this point of view. However, the parameters of serum protein and albumin and arm skinfold thickness give different deviations on the 3 sub-groups ($P<.05$ in Barlett Chi-Squared test)(10), suggesting that the experimental error in observation of these parame-

TABLE II. Significance of Body Composition Changes in 2 Paraplegic Patients.

Parameters	Subject R. G. Initial	Increase in 3 mo		Subject A. H. Initial	Increase in 3 mo	
Weight, kg	62.9	+ 3.0	P <.01	88.7	— 5.8	P <.001
Body water, l	29.6	+ 6.5	P <.05	37.4	+ 2.5	NS
" " % body wt	47.1	+ 7.7	NS	42.2	+ 5.9	NS
Sodium space, l	19.7	— .9	NS	24.4	— 2.7	P <.001
" " % body wt	31.3	— 2.8	P <.01	27.5	— 1.4	NS
Plasma vol, l	3.12	— .33	NS	2.79	+ .53	NS
Blood ", l	5.06	— .13	NS	5.04	+ .51	NS
Hematocrit, %	37.0	+ 7.0	P <.001	43.0	— 1.0	NS
Hemoglobin, g/100 ml blood	12.1	+ 1.9	NS	13.7	— .7	NS
Serum protein, g/100 ml	6.44	— .39	NS	7.03	— .04	NS
Serum albumin, " "	3.10	— .06	NS	3.63	+ .17	NS
Body fat, kg	21.8	— 6.1	NS	36.7	— 9.2	P <.05
" " % body wt	34.6	—10.8	NS	41.3	— 8.2	NS
"Intracellular water," l	10.0	+ 7.3	P <.05	13.0	+ 5.2	NS
" " % body wt	15.8	+10.5	P <.05	14.7	+ 7.3	NS
Total hemoglobin, g	612.	+78.	NS	690.	+31.	NS
Total serum protein, g	201.	—32.	NS	196.	+36.	NS
" " albumin, g	97.	— 9.	NS	101.	+25.	P <.05
Skinfold, mm, arm	11.	+ 2.	NS	18.5	— 6.0	P <.001
scapula	9.5	+ 1.	NS	22.0	—10.0	P <.001

NS = Not significant (P >.05).

ters (at an interval of 7 days) is dependent on the type of patient.

The standard deviations in Table I allow assessment of the significance of changes in body composition observed in one subject over a period of time, provided the measuring procedures remain identical to those used in acquisition of the data in Table I. Such an application is illustrated in Table II which shows the changes observed in 2 paraplegic patients over a period of 3 months during metabolic studies. Subject R. G. had a weight gain of 3.0 kg, whereas the standard deviation of week-to-week differences is 1.04 kg. Thus his weight change was greater than would be expected from weekly variation. The gain was associated with increase in body water, apparently of the "intracellular" type. On the other hand, Subject A. H. lost weight and his sodium space and body fat were lowered during the studies.

These typical illustrations serve to illustrate the value of considering observed changes in body composition in terms of the variance of the methods used. For example, although a 28% decrease in the body fat of Subject R. G. was not significant, it is apparent that a smaller decrease of 11% in the sodium space of Subject A. H. is highly significant due to the lower variability inherent in the measurement.

To avoid erroneous conclusions concerning body composition changes in individuals or differences between individuals, it is therefore highly recommended that estimates of the variance of observations be used as set forth in this report. Ideally, these estimates should be made by each laboratory for their own standardized techniques and methodology of measuring body composition parameters. A "second best" approach would be to use the estimate of variance given in this report and elsewhere in the literature where applicable. The importance of carrying out and publishing such estimates of variance must be emphasized, for only with these aids to interpretation can the measurement of body composition move from the area of pure research into medical practice.

Summary. The variability in observation of some parameters of gross body composition was assessed by paired observations one week apart in 26 chronically ill subjects. The results of this study permit statistical evaluation of the significance of changes in these parameters observed in other subjects, using the same experimental techniques.

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Rat Epiphyseal Cartilage: II. Sulfate-35 Incorporation into Proteinpolysaccharide, *in vitro*.* (30570)

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The isolation of a crude proteinpolysaccharide (PP) from epiphyseal cartilage in relatively large yields has been reported(1). This communication reports our observations on the incorporation of media radiosulfate into the crude PP of rat epiphyseal cartilage employing standard *in vitro* technique.

Methods and materials. Animal and tissue preparation. Male weanling rats, 21 days old (40-45 g) of the Sprague-Dawley strain (Charles River Labs.) were fed on Purina chow and water *ad libitum* until experimentation. The animals were anesthetized and killed by cervical dislocation and the lower extremities rapidly dissected free. The proximal tibial and distal femoral epiphyses after exposure were separated from their shafts by a slice through the metaphyseal junction of the epiphyseal plate. The epiphyses were further cleaned of adherent tissue and 3 longitudinal slices were made producing 4 segments approximately 0.5 to 1.0 mm in thickness. The slices were kept on an iced petri dish until all dissections were completed, and then placed in tared 25 cc incubation flasks containing 3.0 cc of incubation media. The tissue was proportioned in essentially 2 designs so that the tissue from one animal was present in each flask or the tissue of one half of one animal was present in each of two paired flasks. All incubations were begun less than 30 minutes after the animals were killed. Usually 6 to 12 animals were used in each experiment. They were 22 to 25 days old.

Media and incubation. Krebs-Ringer bicarbonate (KRB) was used in all experiments containing one-half the suggested calcium concentration and fortified with glucose at a concentration of 100 mg% unless otherwise specified. The total volume in each flask was 3.0 cc and contained 3 microcuries of $Na_2S^{35}O_4$ per flask (18.2 curies/mole-specific activity New England Nuclear Co.). After the cartilage was added to the tared flasks the wet weight of the cartilage segments was determined. The flasks were then covered with serum stoppers and incubated at 37°C in a metabolic shaker (70-75 cycles/minute) following 10 minutes of aeration with 95% O_2, 5% CO_2 mixture.

Isolation of proteinpolysaccharide (PP). The cartilage segments were rapidly removed from the media, washed with 100 cc of distilled water and quickly frozen. Preliminary attempts to stop the reaction by addition of

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