the activity of Ra, expected in amounts of 0.5×10^{-10} c (*8*), could not be measured in its presence despite the fact that the probable average deviation of a 2-hour reading of background was equivalent to only 4×10^{-11} g of Ra in the body.

Subsequent observations on members of our staff, a few visitors from various parts of the country and from overseas, local medical students, and foreign student members of the reactor school have disclosed the presence of a photopeak around 660 kev (*9*) in the scintillation spectra of all our test subjects (see Fig. 1) and its gradual increase in three individuals who were available for long-term study. No correlation was noted between net photopeak height and geographic origin of the subject. This type of radiation was detected also in various samples of urine, where the presence of the suspected element, Cs^{137}, was confirmed through chemical separation (*10*) of the activity by the addition of carrier to the ashed urine and double precipitations of cesium silicowolframate and perchlorate (*11*). The amounts involved *in vivo* ($\sim 10^{-9}$ c) are several orders of magnitude lower than accepted permissible levels (*12*).

In order to reconcile the persistence of the photopeak with the relatively short biological life of cesium (*13*), a few tests were performed to identify the contaminating sources. Of the foods, some, but not all, of the meats and milk powders were positive, whereas drinking water, vegetables, and sea scallops proved to be negative. Filter-collected dust of laboratory air and sweepings from house carpets from Chicago, Cleveland, and Tucson failed to disclose the Cs^{137} photopeak in the presence of overriding activities at 150, 500, and 750 kev. These energies indicate the presence of other fission products in the atmosphere—namely Ce, Zr-Nb, and Rh-Ru—and their absence from the spectra of human beings and cattle products is suggestive of low retention on the part of the intact mammal. In general, these findings are consistent with the known abundance of fission products of nuclear detonations and with their metabolic properties (*13*). Moreover, they suggest that Cs^{137} might be gaining access into the human body by its continual deposition on grazing lands and following thereafter much the same pathways described in the case of Sr^{90} (*14*).

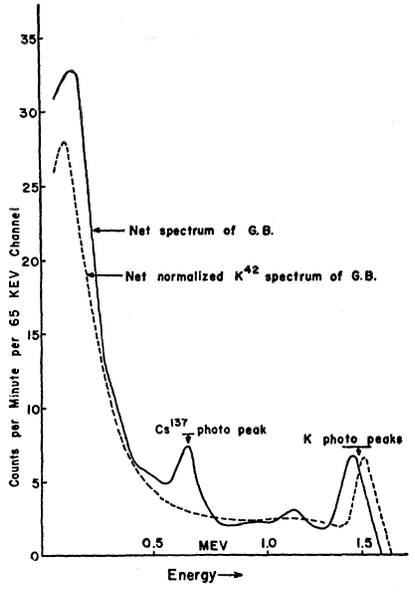

Fig. 1. (Solid curve) Typical scintillation spectrum of a human being (1955). (Dashed curve) Scintillation spectrum of the same individual after administration of K^{42} with its own natural spectrum subtracted. Ordinates are normalized to equal photopeak values.

Despite these difficulties, estimates of total body potassium remained feasible by restricting analysis to the 700-kev-to-1.6 Mev pulse-height band. For 12 male subjects (22 to 34 years old) who were studied, the average amount of potassium as percentage of body weight was 0.188 ± 0.006; for three women (22 to 29 years old), it was 0.154 ± 0.003.

Our figures appear somewhat lower than those based on body activity measurements and in better agreement with those obtained by radioisotope dilution techniques (*15*). Although the averages quoted here possess statistical uncertainties considerably lower than those of previous reports, they cannot be assumed to demonstrate either the existence or absence of nonexchangeable potassium in human beings of the much larger variations in individual potassium content. Clarification of the issue will require application of both techniques to the same subject, with accuracies higher than those reported heretofore (*16*) and with due attention paid to the existence of exogenous contaminants (*17*).

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Mitochondrial Self-Duplication Observed in vitro

Two mechanisms of genesis of mitochondria have been suggested. Lindberg and Ernster (*1*) have concluded that the microsomal fraction of cytoplasm is gradually and continually converted into mitochondria by retention of synthesized protein. On the other hand, Ephrussi (*2*) and others advocate cytoplasmic genetic continuity, thus requiring autoduplication of these cytoplasmic elements. Such a process was reported for mitochondria in 1910 by Faure-Fremiet (*3*). In the course of some studies we performed on the morphology of mitochondria, this phenomenon of self-duplication was also found to occur *in vitro*.

In these experiments (*4*) mitochondria from fasted rat liver were used (Long-Evans, 200 g). They were prepared and examined in either 0.25*M* or 0.60*M* sucrose (*5*).

The mitochondria in the "fluffy layer," which is usually discarded, was found to contain a high percentage of limbus or club forms (*6*). Examination with the phase microscope (× 1455) at room temperature showed that many of this type had a slight constriction in the transverse plane. The larger rod forms found in the fluffy layer were also similarly constricted. If these types were watched over a period of time, they were seen to constrict actively, thus eventually forming two fragments. At least one of these

fragments was able to form the typical "crescent" which is a degenerating form of normal mitochondria (*6*). Those observed in 0.6*M* sucrose retained normal morphology for a longer period. The time for the complete process varied from 15 to 35 minutes. The actual division took about 8 minutes.

In general it was noted that there was a gradation of response to the unnatural *in vitro* conditions which appeared to be correlated with the size of the mitochondria. The larger mitochondria formed crescents of a large size in extremely short intervals, whereas the smallest freshly isolated ones took somewhere between 5 and 20 hours to form typical but diminutive crescents. It would appear therefore that this group of small mitochondria were newly formed by unequal division of larger mitochondria and had not yet elaborated intramitochondrial protein.

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Dual Requirement of Walker Carcinosarcoma 256 in vitro for Asparagine and Glutamine

A routine procedure for the cultivation of the Walker carcinosarcoma 256 in tissue culture recently was developed in this laboratory (*1*). The technique involved the use of cell suspensions, which were prepared from the freshly excised tumor, as the initial inoculum. The medium consisted of 5-percent rat or horse serum, an amino acid and vitamin mixture, and 0.05-percent Bacto yeast extract with Earle's balanced salt solution (*2*) as the diluting fluid. Both serum and yeast extract were essential for satisfactory growth. The cells adhered to the surface of T-15 flasks and increased several fold within 72 to 96 hours.

Although the medium was adequate for good growth of the Walker tumor, the available amino acids and other factors in the serum and yeast extract precluded the determination of specific nutritive requirements of the Walker tumor *in vitro*. Thus, further simplification of the medium was desirable. The present report describes a simplified medium that permits the study of essential components for the Walker tumor. Furthermore, a dual requirement of the tumor for asparagine and glutamine is demonstrated.

Initial studies consisted of observing the growth-promoting activity of yeast extract after it had undergone treatments or fractionation. The activity was lost upon autoclaving in 2*N* HCl for 1 hour at 15 lb pressure but was retained upon autoclaving in a simple aqueous solution. Decolorizing a solution of yeast extract with Nuchar would not remove the activity, but the active portion was adsorbed on columns of IRA 400 (hydroxyl form) or on Dowex 50 (hydrogen form). Thus, the growth factors appeared to be amphoteric in nature and labile under acid conditions of hydrolysis.

An active fraction was isolated from the Dowex-50 column by elution with 1.5*N* HCl in a chromatographic procedure similar to that of Stein and Moore (*3*). This fraction was found to contain asparagine, by chromatography on paper buffered at *p*H 6.2 according to the procedure of McFarren (*4*). Upon replacement of yeast extract with 0.05–10m*M* L-asparagine (5), the cells proliferated as rapidly in 72-hour test periods as they had previously on whole-yeast extract. Without L-asparagine or yeast extract, the cells did not survive. D-Asparagine (*5*), was inactive, although it did not inhibit growth appreciably when it was added at 20m*M* in the presence of 1.5m*M* L-asparagine.

Following this work, the use of dialyzed serum was studied. Human serum was dialyzed for 24 hours at 4°C in a slow-moving shaker against 100 vol of Earle's solution. This preparation adequately replaced the requirement of whole serum, whether rat, horse, or human. The vitamin complement of the medium was altered to insure fortification for prolonged growth. The composition of this new simplified medium (herein referred to as medium 2) is shown in Table 1. Medium 2 was found to support subcultures indefinitely.

The biological and chemical similarity of glutamine to asparagine, as well as the essentiality of glutamine to other mammalian cells in tissue culture (*6, 7*), prompted the investigation of the glutamine in addition to the asparagine requirement of the Walker-256 cells. The cultures were established as was previously described (*1*) and grown for 48 hours in 2 ml of medium, which was then replaced by 3 ml of fresh medium. Growth was determined by whole-cell counts in a hemocytometer with an overall accuracy of ± 10 percent.

The response of the Walker-tumor cells to aspartic acid and glutamic acid and their amides is shown in Table 2. One can observe the nonessential nature of L-aspartic acid and L-glutamic acid to the Walker cells. L-Aspartic acid in higher concentrations did not appear to be exceptionally toxic. However, L-glutamic acid exhibited a striking toxicity to the cells in concentrations above 2m*M*. This is in contrast with the nontoxicity of L-glutamic acid reported for other mammalian cells under tissue-culture conditions (*7*).

Evidently, the Walker-256 cells have a dual requirement for asparagine and glutamine, since the cells failed to become established and died rapidly upon omission of either compound from the medium. It was also interesting to note that the requirement for glutamine was several times as great as the requirement

Table 1. Composition of medium 2.

Component	Concentration of final medium (μg/ml)	Percent
DL-Tryptophan	6.1	
DL-Phenylalanine	24.8	
L-Tyrosine	8.2	
L-Arginine · HCl	15.8	
L-Histidine · HCl · H_2O	6.3	
L-Lysine · HCl	27.4	
L-Cysteine · HCl · H_2O	7.9	
DL-Methionine	13.4	
L-Isoleucine	18.8	
L-Leucine	18.8	
DL-Valine	35.2	
DL-Threonine	35.7	
Glycine	11.3	
DL-Alanine	27.7	
L-Proline	16.8	
Hydroxy-L-proline	18.1	
DL-Serine	31.8	
L-Aspartic acid	20.0	
L-Glutamic acid	22.1	
L-Glutamine	219.2	
L-Asparagine · H_2O	30.0	
Thiamine · HCl	0.2	
Riboflavin	0.2	
Pyridoxine · HCl	0.5	
Pyridoxal · HCl	0.5	
Nicotinic acid	0.5	
Nicotinamide	0.5	
Ca pantethenate	0.2	
Biotin	0.2	
Folic acid	0.2	
Choline chloride	5.0	
Inositol	1.0	
PABA	1.0	
Ascorbic acid	0.5	
Glutathione	0.5	
B_{12}	7.5×10^{-5}	
Na penicillin G	50.0	
Streptomycin sulfate	50.0	
Phenol red	2.5	
NaCl		0.65
KCl		0.04
$MgSO_4 \cdot 7H_2O$		0.02
$NaH_2PO_4 \cdot H_2O$		0.014
$NaHCO_3$		0.22
Glucose		0.30
Dialyzed human serum		5.0