Discussion. The incidence of liver tumors produced by a potent carcinogen such as butter yellow is but little changed by variations of dietary protein levels. In contrast, with a weak hepatic carcinogen such as Safrole, only one tumor occurred among 10 animals receiving a low-protein diet. When 30% of protein was fed, 7 out of 10 animals had liver tumors. Thus, a conclusive demonstration of carcinogenicity can be made with animals fed high-protein diets because of their extended lifespan, even though some hepatomas occurred in the 30% protein controls. However, an inconclusive result is obtained when using a low-protein diet.

From a practical point of view, the safety testing of intentionally low-caloric diet foods on diets covering a wide range of protein content is especially important because the increasing use of sweeteners(7)§ in place of sugar results in low-carbohydrate, relatively high-protein diets, since persons accustomed to consuming large amounts of carbohydrates today are switching in large numbers to the low-caloric products.

The varying response to different modifications of the diet in rats receiving Safrole or butter yellow suggests that these substances may achieve similar end results such as shortening life and increasing tumor incidence by different metabolic mechanisms. The clarification of this question, however, requires additional experiments having more exact control of food intake than that required by conventional chronic oral toxicity feeding techniques.

§ For example, cyclamate consumption alone was reported as being 7 million pounds in 1964, up from 0.5 million pounds in 1958.

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Glucose Uptake Related to Proliferation of Animal Cells *in vitro*. (30389)

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The literature on glucose consumption by animal cells *in vitro* is voluminous. The most consistent conclusion has been the lack of any correlation among glucose uptake, cell numbers, and rates of proliferation (*e.g.*, 1-8). This report presents data which clarifies this apparent anomaly by demonstrating that these factors are directly related when animal cells are provided a suitable environment *in vitro*; also, the magnitude of uptake may be fundamentally different in cells of fibroblast- *vs* epithelial-like morphology. To our knowledge, neither of these aspects has been demonstrated previously in animal cell culture systems.†

Methods. Animal cells of diverse origins and morphology were employed. Experiments were conducted in a perfusion system for replicate T-60 flask cell cultures(9), which has several advantages compared with conventional culture systems. These include provisions for pH control, maintenance of nutrient levels, elimination of "waste" accumulations, and production of unusually dense cell populations.

Jensen rat sarcoma and Walker rat car-

† A relationship between glucose utilization and proliferation of WI-38 cells has recently been reported by Cristofalo and Kritchevsky (Proc. Soc. Exp. Biol. and Med., 1965, v118, 1109).

TABLE I. Environmental Control and Proliferation of WISH Human Amnion Cells in Perfused T-60 Flask Cultures.

Day	Cell No.*	pH	Perfusion rate†	Effluent glucose conc‡
0	4.51	7.12	0	—
1	8.89	7.09	6	268
2	13.9	7.11	8	267
3	21.7§	7.12	16	263
4	29.7	7.07	33	250
5	43.6	7.13	72	255
6	43.5	7.04	86	249
7	51.6	7.04	121	249
8	67.4	7.11	133	256

* $\times 10^{-6}$/T-60 flask.
† ml/day/T-60.
‡ mg %; conc in influent medium was 280.
§ Glass surface became covered with cells at *ca* 20×10^6.

cinosarcoma 256 cultures were initiated from freshly excised tumors carried in female Holtzman rats. WISH human amnion and HEp-2 human carcinoma cells were purchased from American Type Culture Collection. WI-38 human diploid (lung) cells were supplied by Dr. L. Hayflick, Wistar Institute, Philadelphia, Pa. The cells were cultured in the perfusion system for 7 to 8 days in Medium 7a(10,11) supplemented with 5% dialyzed human serum (Jensen and Walker) or 10% whole calf serum. After an initial period of 24 hours perfusion, cultures were terminated at daily intervals. Whole cell counts were made in a hemocytometer, and glucose concentrations (as reducing sugars) in the influent and effluent media were determined according to Somogyi(12). Rates of glucose uptake were calculated as μmoles $\times$ 10^{-9}/hr/cell(13).

Results and discussion. The data in Table I illustrate control of pH, perfusion rate, glucose concentrations, and proliferation in 8-day perfusion experiment with WISH cells. Since *ca* 20×10^6 WISH cells cover the floor of a T-60 flask, the 8th day population of 67.4×10^6 (Table I) was equivalent to *ca* 3 layers, *i.e.*, monolayer equivalent = M.E. = 3.*

The data summarized in Fig. 1 show daily rates of glucose uptake superimposed on the proliferation curves to facilitate visual comparison of the two. In some instances proliferation virtually ceased for 1 to 2 days; and this presumably was due to an initial reaction of some cells to "overcrowding," usually after reaching the equivalent of 1 to 2 cell layers in density. Marked changes in rates of proliferation have been observed in over 40 perfusion experiments to date. Because of the perfusion conditions, it is unlikely that they occurred because of limitations of nutrient supplies. They were due, perhaps, to mitotic inhibitions caused by physical confinement and cell-cell interaction. Appreciable amounts of glucose were consumed during these periods (Fig. 1), but at much slower rates than during proliferation. The most striking correlation of glucose uptake with rate of proliferation was seen in the experiment with WI-38 cells where each

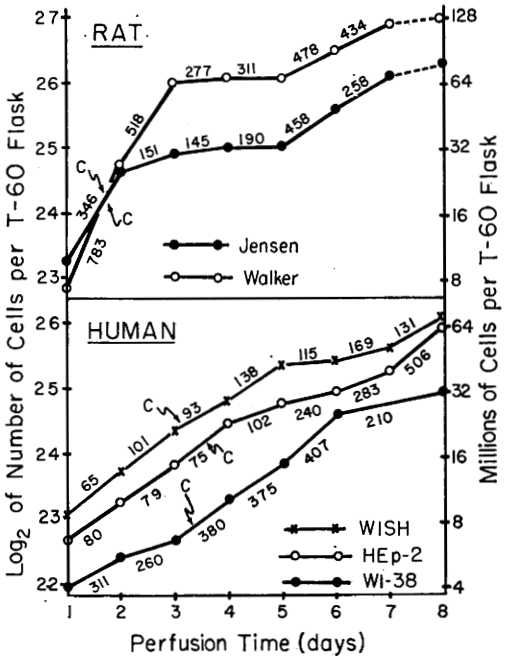

FIG. 1. Illustrating the correlations of rates of glucose uptake in μmoles $\times$ 10^{-9}/hr/cell with rates of proliferation in perfused cultures of Jensen rat sarcoma, Walker rat carcinosarcoma 256, WISH human amnion, HEp-2 human carcinoma, and WI-38 human diploid (lung) cells and the change in magnitude of uptake before and after approximate confluency (C) of epithelial-like cell populations (WISH and HEp-2). Duplicate analyses gave data with average variation of $\pm$ 10%.

* The significance of monolayer equivalents, M.E., is discussed further in another paper, J. Cell. Biol., in press. M.E.'s as high as 17 have been obtained in perfused hamster cell cultures (250 x 10^6 cells/T-60).

change in proliferation rate, however slight, was accompanied with a proportionate change in uptake. A discrepancy appears in the Jensen experiment at the 5th to 6th day when the rate of uptake was larger than during the 1st to 2nd day period. Otherwise, the changes were in proportion to the proliferation.

Two of the human cell types, HEp-2 and WISH, have pronounced epithelial-like morphology, and the magnitude of glucose uptake in pre-confluent populations of these was much less than in the other cell types. During the period of formation of multilayers, 4th to 8th day, glucose uptake increased, especially in the HEp-2 cells derived from malignant tissue, but retained, nevertheless, a degree of correlation with rate of proliferation.

Although this report specifically illustrates the relationship between glucose uptake and proliferation (and possibly morphology), it also stresses the use of perfusion systems in many types of animal cell culture studies. Nutritional and metabolic investigations, in particular, may be facilitated when they are conducted (a) in controlled *in vitro* environments, (b) within well-defined phases of population development, and (c) in dense as well as dilute populations.

Summary. Perfused animal cell cultures with controlled pH and glucose concentrations exhibited direct correlations of rates of glucose uptake with rates of proliferation and population density. Further, rapidly proliferating cells of fibroblast-like morphology consumed glucose at *ca* 4-fold the rates observed in cells of epithelial morphology increasing at comparable rates.

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Complement Dependence of Human Serum Toxicity for Melanoma S91 Cells.* (30390)

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Toxicity of normal human serum to cultured mammalian cells(1,2) and to the ascites cells of various mouse tumors(3-5) has been extensively studied. Inability to produce suspensions of single cells of sufficiently high viability from solid tumors has hindered a study of the effects of serum on this type of cell. In view of the observation by Abramoff *et al*(6) that the S91 melanoma contains antigenically distinctive components it is pertinent to determine if normal human serum is cytotoxic to cells of this solid tumor. For these reasons it was considered of interest to

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