Reports

Metabolic Differences between Hepatic Parenchymal Cells and a Cultured Cell Line from Liver

A number of human and animal cells capable of being grown in serial culture are now available in several laboratories. These cell lines have arisen from many different organs and from both normal and malignant tissue. Included are cell cultures from normal liver, intestinal epithelium, kidney, tonsil, skin, conjunctiva, appendix, and a number of neoplasms. If the cells from normal tissues are truly representative of primary functional cells of the tissues of origin, they could have many important uses—for example, in the study of the biochemistry, physiology, and pharmacology of these tissues as well as in the cultivation of fastidious viruses and other intracellular microorganisms. Therefore, it is important that methods be sought for identifying these cell strains as typical of the labeled tissues of origin.

Among normal tissues, certain ones may be characterized by examination of their enzymatic activities. The liver parenchymal cell has been studied extensively and has been found to have a unique enzymatic pattern, which distinguishes it from other cells. For example, among the glycolytic enzymes in liver are found both glucose-6-phosphatase (*1*) and fructose diphosphatase (*2*), which are absent from most other tissues. Liver contains an active hexose-monophosphate "shunt" and also possesses the enzyme fructokinase, which is specific for ketoses (*3*). Another distinctive feature is the fact that these enzymatic activities have been found in a relatively constant ratio (*4*).

Since serially cultured hepatic parenchymal cells would provide a particularly useful research tool, we have examined (*5*) the enzymatic activities of one of the culture strains of human liver. This cell line, originally isolated by Chang, in 1954 (*6*), has been carried through more than 100 transfers in several different media. In this laboratory, the cells were grown in bottle cultures in Eagle's basal medium (*7*), containing 20 percent serum. After growth in this medium, the cells were scraped from the glass, centrifuged at low speed, and washed three times in 0.15*M* KCl. The lightly packed cells were then suspended in nine volumes of ice-cold 0.15*M* KCl and homogenized in a Potter-Elvehjem homogenizer. Enzymatic assays for glucose-6-phosphatase (*1*), fructose diphosphatase (*8*), fructokinase (*9*), and glucokinase (*10*) were performed with this homogenate. An aliquot of the homogenate was centrifuged at 18,000*g* for 10 minutes, and the supernatant fraction was employed in the assay of glucose-6-phosphate dehydrogenase (*11*).

The only activity found was that of the enzyme glucose-6-phosphate dehydrogenase. The other enzymes were not detected by methods capable of measuring activities as low as 0.2 μmole of substrate reacting per minute per gram of cells. On one occasion, after growth in Eagle's basal medium containing 5 millimolar glucose and undialyzed horse serum, the activity of the glucose-6-phosphate dehydrogenase was 0.9 μmole of substrate reacting per minute per gram of cells (assuming that 1 ml of packed cells weighs 1 g). This activity was substantially unchanged (0.8 μmole of substrate reacting per minute per gram) after the cells were carried through four subcultures in Eagle's basal medium, containing 5 millimolar fructose and 20 percent undialyzed human serum. It was noted that these cells grew very well in this medium, with fructose rather than glucose as the major carbohydrate, after an initial lag period on the first subculture.

Because of difficulty in obtaining fresh material, little information is available on the activity of these enzymes in human liver. Glucose-6-phosphatase activities of normal human livers have been reported (*11a*) as 1.4 to 3.9 μmole of substrate reacting per minute, per gram of liver.

Studies carried out in this laboratory (*4*), in which normal rat livers were employed, revealed the average activities (in micromoles of substrate reacting per minute per gram of liver) of these enzymes to be glucose-6-phosphatase, 6.5; fructose diphosphatase, 5.9; fructokinase, 5.0; and glucose-6-phosphate dehydrogenase, 0.97. It appears to be significant that the only enzymatic activity detected in the Chang liver cell line is that of glucose-6-phosphate dehydrogenase, which is the least active of these enzymes in normal rat liver.

The foregoing observations indicate that these cells do not behave, metabolically, like hepatic parenchymal cells. The true nature of the cells, therefore, remains in doubt. Several alternative explanations present themselves: (i) These cells may not be the progeny of parenchymal cells but, rather, of bile duct epithelium, vascular endothelium, and so forth. In the usual initiation of a serially cultured cell line, explants or trypsin dispersions are made from pieces of tissue that are obtained at autopsy or biopsy. Initially, these cultures may contain many cell types, and the type which survives and multiplies is not necessarily the major functional cell of the tissue from which it is derived but, rather, that cell which is most adaptable to the cultural environment. Of course, even after many subcultures, these cell lines may consist of a number of different cell types. (ii) These cells may represent mutants of hepatic parenchymal cells which have arisen during the course of many generations in tissue culture and may, therefore, be quite different, metabolically, from the originally isolated cell. There are several recorded observations of suspected mutations of cells in tissue culture, including changes of an apparently malignant nature (*12*). It has, in fact, been noted in another laboratory that the Chang liver cell has undergone changes suggestive of neoplasia (*13*). In this regard it is of interest to note that the enzymatic observations reported here bear similarities to those reported for the Novikoff hepatoma and dimethylaminoazobenzene (DAB)-induced hepatomas (*14*). (iii) Perhaps these cells are true parenchymal cells which, in the tissue-culture environment, have undergone adaptive changes as a result of the absence of hormonal or morphogenetic regulation. Three of the hepatic enzymes that are studied here [glucose-6-phosphatase (*15*), fructose diphosphatase (*16*), and glucose-6-phosphate dehydrogenase (*11*)] are known to respond markedly *in vivo* to hormonal changes. Such adaptive changes might well be in the direction of a less differentiated or more primitive metabolism. In this regard, it seems possible that many cell types, when maintained over long periods in a tissue-culture environment (such as is

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presently available) may revert to types that employ similar basic metabolic pathways.

Regardless of the explanation for these observations, they indicate a severe limitation in the use of these cells for many kinds of investigations. Although other cell lines may exist which do actually represent and behave as the primary functional cell of the tissue of origin, this remains to be established for each culture strain. Enzymatic methods similar to those employed here should be of value in performing such identifications.

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Strontium-90 Hazard: Relationship between Maximum Permissible Concentration and Population Mean

Recent discussions of radiation fallout (*1, 2*) have related the population mean Sr^{90} body burden to the maximum permissible concentration, or MPC. Libby (*1*) has introduced the "MPC unit" (1 μc of Sr^{90} per kilogram of calcium) and has used it to express concentrations of Sr^{90} in milk, plants, soil, and so forth. The direct comparison of any mean with MPC, of course, implies that the two different terms are expressed in comparable units. Unfortunately, this is not true. The MPC unit represents the maximum permissible concentration of Sr^{90} within the body that may be considered safe for any individual—and the individual is assumed (*3*) to be a professional isotope worker, probably male, 45 or more years of age, who is exposed to the isotope only under rigidly controlled laboratory conditions. A population mean, on the other hand, does not represent a maximum value, but rather a value which, by definition, will be exceeded by 50 percent of a population.

How much of a quantitative difference results from this inequality of units of expression? One example may suffice. In discussing the Sr^{90} burden in a general human population, at least three additional factors must be taken into consideration: S, a safety factor; C, an allowance for children; and H, a heterogeneity factor. Thus,

$$MPC_{pop.} = \frac{MPC_{oc.}}{SCH}$$

where $MPC_{pop.}$ is the maximum permissible concentration that would be safe for a population mean, and $MPC_{oc.}$ is the maximum permissible concentration for occupational isotope workers (1 μc of Sr^{90} per kilogram of calcium, *3*).

Because of the uncertainty in the figures for maximum permissible concentration, it is usually suggested that members of the general public should not be exposed to more than one-tenth of the radiation hazard that is permitted for occupational workers (*4*). The value of S is therefore taken to be 10.

Kulp, Eckelmann, and Schulert (*2*) showed that children (0 to 4 years old) accumulate Sr^{90} more rapidly than do adults (40 to 60 age group). The concentration of Sr^{90} in the bones of children averaged 4 to 5 times that found in the general population (and 10 percent of the children exceeded this figure by a factor of 20-fold or more). Added to this differential accumulation factor is the factor of the increased vulnerability of this age group. This factor is difficult to evaluate without direct experiments. It can be pointed out, however, that children in this age group have over twice the expected life-span ahead of them in which to develop neoplastic or bone calcification changes in response to radiation exposure. For the moment, C is taken to be 5×2, or 10.

Within the 10 to 80 age group, there is a substantial variation in body burden of Sr^{90} for people in one area, who are presumably exposed to the same environmental Sr^{90} concentration. This may be related to differences in food habits, idiosyncrasies of calcium metabolism, and other factors. From the data of Kulp, Eckelmann, and Schulert (*2*) one can estimate that 6.8 percent of this population group will have a body burden of Sr^{90} which exceeds by at least fivefold the population mean value for that age and area, 1 percent will exceed its population mean by about tenfold, and 0.2 percent will exceed its own population mean by more than 50-fold. It is evident, therefore, that H must exceed 10. Combining these terms, one would estimate that:

$$MPC_{pop.} \leq \frac{1\ \mu c\ Sr^{90}/kg\ Ca}{10 \times 10 \times 10}$$

$$= 1\ m\mu c\ Sr^{90}/kg\ Ca$$

A comparison of this figure with estimates (*1, 2*) of the Sr^{90} concentration in man leads to the conclusion that the Sr^{90} burden in the general population in 1955 was at least 10 percent of the $MPC_{pop.}$, and that if the predictions (*1, 2*) concerning the next 10 to 15 years are correct, the population mean value will shortly reach and exceed the maximum level compatible with public health and safety. This view gives a very different picture of the probable safety situation from the one obtained by a direct comparison of $MPC_{oc.}$ and the population mean (*1, 2*) and points to the hazards of introducing novel units of expression without first considering their fundamental nature.

Meanwhile, if any simple safety measures are available, advantage should be taken of these without delay. Since some 80 percent of the dietary calcium (and thus Sr^{90}) that enters the body comes from milk and milk products (*5*) it would seem possible to decrease the intake of Sr^{90} by decalcifying milk. Such a procedure is reportedly effective (*6*). The technology and economics of this process are essentially those used currently in the production of low-sodium milk (*7*) and in a process for soft-curd milk (*8*). Milk calcium could then be replaced by calcium derived from ancient sources (limestone, for example) which would have a much lower Sr^{90} content. This procedure would not seriously disturb the calcium balance in the general population, and it could provide an immediate four- to fivefold reduction in the intake of Sr^{90}.

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