

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" ($1 \mu\text{c}$ 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 dif-

ferent 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,

$$\text{MPC}_{\text{pop.}} = \frac{\text{MPC}_{\text{oc.}}}{SCH}$$

where $\text{MPC}_{\text{pop.}}$ is the maximum permissible concentration that would be safe for a population mean, and $\text{MPC}_{\text{oc.}}$ is the maximum permissible concentration for occupational isotope workers ($1 \mu\text{c}$ 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 popula-

tion 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:

$$\begin{aligned} \text{MPC}_{\text{pop.}} &\leq \frac{1 \mu\text{c Sr}^{90}/\text{kg Ca}}{10 \times 10 \times 10} \\ &= 1 \text{ m}\mu\text{c Sr}^{90}/\text{kg Ca} \end{aligned}$$

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 $\text{MPC}_{\text{pop.}}$, 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 $\text{MPC}_{\text{oc.}}$ 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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Amylase in Electrophoretic and Ultracentrifugal Patterns of Human Parotid Saliva

The action of human saliva on starch was reported for the first time by Leuchs in 1831, but the active material, an α -amylase, was not crystallized from saliva until 1948, by Kurt Meyer and associates (1). The crystallization was confirmed in 1953 by Muus (2), who reported electrophoretic and ultracentrifugal analyses of her crystalline preparation. Kinnersly (3) demonstrated the presence of amylase on an electropherogram of whole saliva on paper but did not relate it to the three protein components which he observed.

The present work describes the location of amylase in ultracentrifugal and electrophoretic patterns of the secretion of the human parotid gland. It is a part of a more extensive investigation of the composition of human parotid and submaxillary gland secretions by electrophoretic and ultracentrifugal methods (4). The parotid gland secretion was found (4) to contain a maximum of 12 electrophoretically separable components and three or four ultracentrifugally separable components. The electrophoretic components have been numbered, with the most positively charged at pH 8.6 as component 1 (4).

The parotid gland secretion, stimulated by chewing paraffin, was collected by means of a parotid cup (5) similar to that described originally by Carlson and Crittenden and by Lashley. The tubes from the cup emptied directly into receivers supported in an ice bath. The saliva was concentrated to approximately one-fourth of its original volume by dialysis overnight at 4°C, against a 25 percent solution of polyvinylpyrrolidone. It was then dialyzed in the cold for from 65 to 70 hours against the buffer to be used for electrophoresis. The Miller-Golder buffer (6) of 0.1 ionic strength and pH 8.5 was employed. It contained 0.02M Veronal and 0.08M sodium chloride. After dialysis, the sample was centrifuged in the ordinary laboratory centrifuge at 2° to 4° for 20 minutes. Electrophoretic, ultracentrifugal, and amylase determinations were performed on the supernatant liquid.

Electrophoresis was performed in a 6-ml cell in the Perkin-Elmer model 38 Tiselius electrophoresis apparatus. Ultracentrifugal analyses were performed in the Spinco model E ultracentrifuge at about 26°C. Amylase activity was determined by the method of Myers, Free, and Rosinski, as adapted for work with saliva by Schneyer (7).

In order to locate the position of the amylase boundary in the electrophoretic pattern, both ascending and descending limbs of the cell were sampled at the time of maximum resolution. Fine glass capillaries were inserted into each limb to the center of each successive boundary, as seen by the Longworth optical system. Five samples of about 0.1 ml were usually taken from each limb, beginning with the uppermost boundary. At the center of each boundary, the concentration of the component forming the boundary should be one-half of the original concentration. In succeeding samples taken from the same limb, the concentration of the first component should be greater than 50 percent of the initial concentration.

Amylase activity was found at the boundary of either component 6 or 7, in both ascending and descending limbs, in about 50 percent of the initial concentration. Since no amylase occurred in the descending limb in either components 4 or 5, all of the components which had appeared up to that point (1, 2, 3, 4, and 5) were eliminated. By similar reasoning, since no amylase appeared in component 8 in the ascending limb, all of the components which had appeared up to that point (12, 11, 10, 9, and 8) were eliminated. Component 6 had an average mobility ($\pm$ standard deviation) of $-1.1 \pm 0.20 \times 10^{-5}$ cm²/volt-sec, whereas component 7 had an average mobility of $-1.7 \pm 0.05 \times 10^{-5}$ cm²/volt-sec. The closeness of the mobilities of these two components made further identification impossible, particu-

larly since both components showed variation in individual samples and were not always distinguishable. The average mobility of the component containing the amylolytic activity was $-1.5 \pm 0.25 \times 10^{-5}$ cm²/volt-sec. in the Miller-Golder buffer of 0.1 ionic strength and pH 8.5. In some samples this activity was contained in component 6 and in others, in component 7. No estimate of the homogeneity of the component which contains the amylase is available. The amylase component is marked with vertical arrows on typical patterns in Fig. 1.

The crystalline human amylase studied by Muus (2) had a somewhat different mobility. From the relationship of mobility to pH, as given by Muus (2), the mobility of her component A, under the conditions used in this study, should be -2.3×10^{-5} cm²/volt-sec. This value is considerably higher than the mobility, -1.4×10^{-5} cm²/volt-sec, found in the present work for the amylase in the parotid gland secretion. Presumably this difference in mobility arises from the presence of other components in the parotid gland secretion.

A partition cell was used to help locate the ultracentrifugally separable component which contained the amylase. In the general investigation (4), parotid saliva was shown to contain two fast moving components and either one or two slowly moving ones. In alternate experiments the ultracentrifuge was allowed to run with a force of about 250,000g until either the fastest or the two fastest components had sedimented past the partition. Samples were withdrawn from both the top and bottom chambers of the cell after the rotor had stopped. The amylase content of the samples was then determined.

The amylase in the parotid gland secretion sediments with an average rate of 4.1 Svedberg units (S). This rate agrees well with the average found in the general study (4) for one of the components, 4.2 S (4). When some of the 4.1 S component remained in the top chamber of the cell, appreciable amylase activity (27 to 61 percent of the original activity) appeared in the sample withdrawn from the top chamber of the cell. In the experiments in which the 4.1 S component was allowed to sediment completely to the bottom chamber, the sample withdrawn from the top chamber contained little or no amylase activity (0-8 percent). It seems definitely established, therefore, that the 4.1 S component contains the amylase. This component is marked with an arrow in Fig. 1, which shows a typical sedimentation pattern of the parotid gland secretion. As with the electrophoretic components, the proportion of the component which is pure amylase has not been determined.

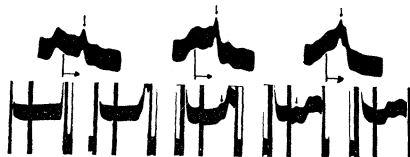


Fig. 1. (Upper patterns) Electrophoretic patterns of the descending limb of parotid gland secretion from three individuals. The horizontal arrows mark the starting boundary. Field strength, 4.4 v/cm; time, 192 minutes. (Lower patterns) Ultracentrifugal patterns of a parotid gland secretion at 0, 24, 48, 96, and 136 minutes. Centrifugal force, about 250,000g. The components with amylolytic activity are marked. The experiments were performed in the Miller-Golder, barbital-NaCl buffer, pH, 8.5; $\Gamma/2$, 0.1.