may lead to selective isolation of certain cell types. As an example, our knowledge of the Paneth cells and argentaffine cells would be significantly advanced if these types could be isolated for study. Whatever the outcome of these considerations, the science and practice of nutrition will benefit as techniques develop so that the properties of gastrointestinal tissues become more completely understood at the cellular level.

CARBOHYDRATE METABOLISM OF RATS CONSUMING 450 P.P.M. FLUORIDE

Carbohydrate metabolism is altered in rats fed 450 p.p.m. fluoride. This may be an indirect effect of these large amounts of fluoride related to growth retardation and depressed food consumption.

The pros and cons of adding fluoride to water supplies have been discussed rather vehemently in popular articles in recent years. Articles of more scientific interest, dealing with the effect of fluorides on biochemical pathways, have recently appeared. Impairments in lipid metabolism, lowered fatty acid oxidase (*Nutrition Reviews* **18,** *79 (1960)*), have been discussed, as well as enhancement of fluoride toxicity by high levels of dietary fat.

E. J. Zebrowski and J. W. Suttie (*J. Nutrition* **88,** *267 (1966)*) have recently investigated the influence of large amounts of fluoride ingestion by rats on the metabolism of glucose labeled with C^{14}.

Holtzman (female) weanling rats were fed either a diet without fluoride or one containing 450 p.p.m. fluoride ad libitum for 30 to 35 days (preliminary) before administration of the labeled glucose. This large amount of fluoride caused a depression in growth during the preliminary period. The authors report 10,000 to 16,000 p.p.m. in the femur ash of animals fed fluoride as compared with 400 to 800 p.p.m. in the control rats. Prior to administering the labeled glucose, the animals were fasted for 12 hours and then allowed food ad libitum for three hours.

Each rat was given 2.5 μc. of carrier-free glucose-UL-C^{14} (12 μc. per μmole) intraperitoneally. Carbon dioxide was collected at 20 minute intervals for the first hour, 30 minute intervals for the second hour, then at 165 and 210 minutes.

For the determination of liver glycogen, the animals were stunned and exsanguinated, and the livers immediately excised and frozen in dry ice until glycogen was isolated.

In the liver slice studies, livers were excised, rinsed in Hastings medium I, and stored in the ice-cold medium until sliced and incubated. The authors state that slices from five animals (control) were pooled and mixed, as were those from animals fed fluoride before slices were taken for incubation. The reaction was stopped with NaOH.

In the glycogenolysis studies, liver slices were obtained from unfasted rats.

The results on expired carbon dioxide indicate that there was no difference between the control rats and those fed fluoride, although the specific activity of the CO_2 of the rats fed fluoride was significantly higher ($P < 0.01$). Zebrowski and Suttie suggest "... that the higher specific activity of the carbon dioxide expired by the fluoride-fed rats was the result of a lower expiration of carbon dioxide by the smaller fluoride-fed rats and not of an increased utilization of glucose for carbon dioxide production."

In order to follow the rate of incorporation of uniformly labeled glucose-C^{14} by ten fluoride and ten control rats, a time study was performed. Each rat was given 1.25 μc. of labeled glucose intraperitoneally;

animals were sacrificed at one, three, and five hours post-injection. The results show that the rate of incorporation for control rats was more than twice that for those given fluoride after one hour ($P < 0.01$). The reverse was true after five hours; those fed fluoride had the higher activity ($P < 0.05$). The authors state that "Although these data show a relative retardation or inhibition of glucose-UL-^{14}C incorporation into liver glycogen by the fluoride-fed rats, they do not, however, exclude the possibility that the retardation may be due to an inhibition of absorptive processes."

Glycogen metabolism was investigated further by *in vitro* incorporation of labeled glucose into glycogen by liver slices. As in the whole animal studies, the incorporation was significantly higher after one hour ($P < 0.05$), but no differences were observed after three and five hours. This suggests to the authors that liver glycogen was in a more dynamic state in the control rats.

The relative rates of glycogenolysis in liver slices from unfasted control animals and those fed fluoride were investigated. Zebrowski and Suttie report a 20 per cent greater degradation of glycogen from the liver slices of control rats. They state that this is further evidence of an altered glycogen metabolism in the animals fed fluoride. They say, "...no indication was given, however, whether the observed impairment was due to a reduced enzyme concentration in the tissue or to a direct inhibition of the enzymes by tissue fluoride."

To eliminate the possibility of enough fluoride in soft tissues of rats fed fluoride to inhibit glycogenolysis, the authors report that they added fluoride to the liver slices obtained from the control animals. They state that no significant differences were found between the control samples and those with added fluoride. This suggests to the authors that, if fluoride does alter the turnover of liver glycogen, its action is not by direct enzyme inhibition.

Zebrowski and Suttie conclude that the effect of fluoride on enzyme inhibition must be indirect, and suggest that the indirect effect on glycogen metabolism may be due to the alteration in food consumption (depressed) for rats receiving 450 p.p.m. fluoride in the diet, thus affecting liver enzyme concentration.

The results of this study indicate a defect in the carbohydrate metabolism of rats fed these large quantities of fluoride. Whether this defect is directly related to fluoride, or a secondary effect related to food consumption, remains to be proven. Zebrowski and Suttie state that the evidence indicates that the defect is probably secondary to a more direct effect of fluoride on some other area of metabolism. They cite the work of D. F. Steiner, V. Rauda, and R. H. Williams (*J. Biol. Chem.* **236,** *299 (1961)*) and state that they "...demonstrated that several of the enzymes concerned with glycogen metabolism in the liver can be varied by regulating food intake." This would suggest that, in future research, investigators interested in studying the effect of a nutrient or nutrients on biochemical pathways should consider controlling the food intake of their animals.

REGULATION OF GLUCONEOGENESIS

Activities of the two key enzymes in gluconeogenesis, pyruvate carboxylase and phosphoenolpyruvate carboxykinase, increase in liver preparations in conditions where gluconeogenesis is increased. Production of oxaloacetate is also increased.

The localization and separation of enzymes, substrates, and cofactors within the different structural units of the cell appears to be an important factor in the potential regulation of metabolic pathways. Such a situation appears to exist in the reactions