

Studies on the Localization of Tagged Methionine Within the Pancreas.*

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Recent studies of intermediary metabolism with isotopic compounds^{1,2} have amply confirmed the concept of Schoenheimer³ that tissue proteins are in a dynamic state of equilibrium with amino acids of exogenous source. It has been shown that different tissues show different activities in this respect,⁴ and that intestinal mucosa is particularly active. Friedberg⁵ postulated that the secretion of digestive enzymes and muco-proteins, which are rapidly lost from the tissue, would account for this rapid metabolic rate. On this basis he predicted that pancreas would show high activity, a fact actually shown earlier by Tarver and Schmidt.⁶

In balance studies, Gordon⁷ showed that when labelled methionine, prepared with isotopic, radioactive sulphur (S^{35}), was given orally to animals, a high percentage of radioactive sulphur was recovered from pancreas, small intestine and liver. It was thought that such concentrations represent rapid incorporation of methionine into digestive enzymes; however, a possible role in the synthesis of

hormones, particularly of insulin, cannot be excluded by ordinary balance studies. Attempts to demonstrate more accurate localization of isotopic compounds within tissues by radioautography have thus far been unsatisfactory in our hands. The present experiments attempt to localize the site of early methionine metabolism in the pancreas.

Experimental. Radioactive sulphur (S^{35}) was incorporated in methionine[†] starting with soluble sulphate. The specific activity of the final product (crystalline dl-methionine) approximated one microcurie per milligram (on date of receipt), based on a radioassay by another laboratory. In all cases this was administered intravenously in 2 ml water, and Table I shows the dosages given.

Preparation of and findings in the 4 cats in Table I were as follows:

No. 1 A normal cat. *Autopsy*: Pancreas was grossly normal; no sections were made.

No. 2 The pancreatic duct was ligated at the duodenum. Postoperative course was uneventful, and the animal gradually gained weight. Methionine was given 104 days after duct ligation. *Autopsy*: Grossly, there was complete late atrophy of the duodenal portion of the pancreas, partial atrophy of the tail of the pancreas (it is believed that an accessory duct preserved this portion). No sections were made.

No. 3 A ligature was placed around the body of the pancreas in its midportion. Postoperative course was uneventful, and weight was regained by the time of administration of labelled methionine 118 days after ligation. *Autopsy*: The pancreas appeared grossly normal proximal to the ligature, but there was apparent complete atrophy distal to the

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1 Kamen, M. D., *Radioactive Tracers in Biology*, New York, Academic Press, Inc., 1947.

2 Symposium on Radioactive Isotopes, University of Wisconsin, 1947.

3 Schoenheimer, R., *The Dynamic State of the Body Constituents*, Harvard University Press, 1941.

4 Tarver, H., and Morse, L. M., *J. Biol. Chem.*, 1948, **173**, 53.

5 Friedberg, F., *Science*, 1947, **105**, 314.

6 Tarver, H., and Schmidt, C. L. A., *J. Biol. Chem.*, 1942, **146**, 69.

7 Gordon, E., personal communication.

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TABLE I.
Doses of Labelled Methionine Given and Content* of Radioactive Sulphur Found in the Pancreas.

Cat No.	Wt, kg	Methionine given, microcuries, per kg	Normal pancreas		Atrophic pancreas 8 hr
			4 hr	8 hr	
1	2.1	2.4	2970	3000	
2	2.9	1.7			1560†
3	3.1	2.4		2610	101
4	3.6	2.1		1500	8.1

* Standard specific activity ($\times 10^4$).

† From portion of pancreas which showed incomplete atrophy.

ligature. Sections showed normal pancreas proximal to the ligature; in the ligated portion, as seen in Fig. 1, there was patchy and incomplete atrophy of acinar tissue with occasional islets. The preservation of portions of acinar tissue is attributed to incomplete ligation.

No. 4 A ligature was placed around the body of the pancreas in its midportion. Again, postoperative course was uneventful, and weight was regained by the time of administration of labelled methionine 125 days after ligation. *Autopsy*: The pancreas appeared grossly normal proximal to the ligature, but there was apparent complete atrophy distal to the ligature. Sections showed normal pancreas proximal to the ligature. In the ligated portion, as seen in Fig. 2, there was marked atrophy of acinar tissue with intact islets, surrounded by fibrous tissue and lymphocytes.

All animals showed normal blood sugar concentrations after these operative procedures. In all cases methionine was given under light nembutal anesthesia. In Cats No. 1 and No. 2 pancreatic and liver biopsies were performed by laparotomy 4 hours after administration of methionine. All animals were sacrificed 8 hours after administration of methionine, and the organs and tissues were weighed, frozen and subsequently assayed.

The tissues were oxidized by digestion in Kjeldahl flasks containing Pirie reagent (perchloric acid-nitric acid-copper nitrate mixture), and soluble sulphate was precipitated with benzidine dihydrochloride. Sulphur content was determined by alkali titration, and sulphate was subsequently precipitated as barium sulphate. This precipitate was filtered with standardized apparatus on weighed paper, and radioactivity was directly measured with a thin-window, bell-type Geiger

counter, the sensitivity of which was established by assay of a sample of known radioactivity. All dosages and counts for this laboratory are based on this standard. Assayed counts were corrected to a standard weight of barium sulphate, and were corrected for decay, based on the original assay. All results are based on duplicate analyses.

The radioactivity of samples, expressed as Standard Specific Activity (S.S.A.),⁴ was calculated as follows, and is shown in Table II:

$$\text{S.S.A.} = \frac{100 \times \text{assayed counts} \times \text{weight of animal (kg)}}{\text{mEq sulphur in sample} \times \text{counts in dose}}$$

Discussion. It has been known since the work of Laguesse that the islet cells of the duct-ligated portion of a pancreas are physiologically intact, a fact which is supported in these animals by the morphological appearance of the pancreas. It would appear that the low concentration of radioactive sulphur found in tissue from the ligated portion, as compared with that from normal pancreas (Table I), is due to loss of acinar tissue. On this basis it appeared that the rapid early turnover of methionine by the pancreas is due to metabolic activity of the exocrine portion of the gland rather than to the needs of the islets. Some labelled methionine may be converted into cystine, which may then be incorporated in insulin by islet tissue, but, if so, this represents a small percentage of the unexpectedly high concentration of activity found in normal pancreas. The high concentrations found in liver, duodenum and jejunum (Table II) confirm the results of other investigators.

Summary. Methionine labelled with radioactive sulphur (S^{35}) was given intravenously to 4 cats, one normal, 3 treated so as to pro-

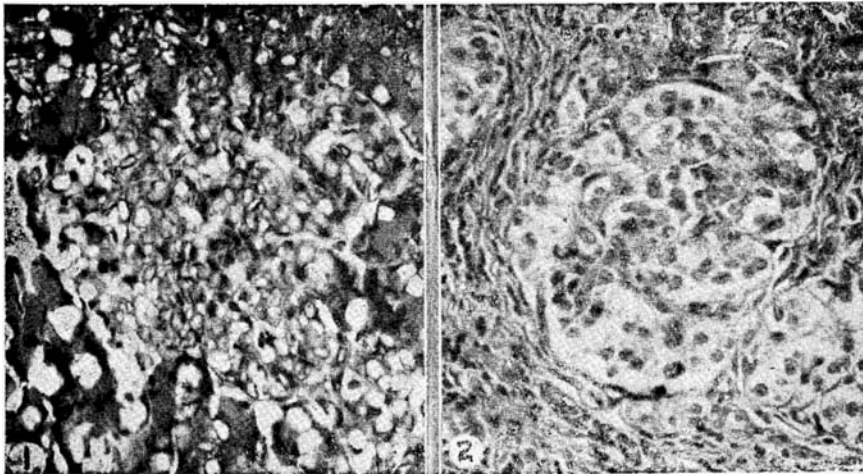


FIG. 1. Island of Cat 3. The cells of the island show some vacuolation and degranulation. Partial atrophy of the acinar tissue about the island is shown by the vacuolation and distortion of these cells.

FIG. 2. Island of Cat 4. The normal island is surrounded by fibrous tissue and lymphocytes. Atrophy of acinar tissue was complete in this portion of the animal's pancreas.

TABLE II.
Concentration of Radioactive Sulphur in Various Tissues.*

Cat No.	Duodenum	Jejunum	Liver	Bile	Kidney	Ovary	Testis
1	2185		1430		721		
2	1120		1540		684	77.1	
3	1440	882	1370		471	170	
4	840	672	848	528	699		

* Expressed as standard specific activity ($\times 10^4$)

duce varying degrees of atrophy of the acinar tissue of the pancreas. The animals were sacrificed 8 hours after methionine was given, and various tissues were assayed for radioactive sulphur.

In all animals high activity was found in pancreas, small intestine and liver. In the atrophic portion of the pancreas where a ligature had been placed around the body of that gland, low activity was found as contrasted

with high activity found in the normal part of the gland. Where atrophy was incomplete, the activity found was intermediate between that of normal and atrophic tissue.

It is concluded that the high activity of radioactive sulphur found in the pancreas of normal cats after administration of labelled methionine represents principally the metabolic activity of the exocrine portion of the pancreas.