TABLE II.
Effect of Secretin on Pancreatic Secretion in Man.

Subject	Fasting duodenal content, 20-min. sample		Following secretin					
			20 min.		40 min.		60 min.	
	Vol., ml	Trypsin, K × 10^{-3}	Vol., ml	Trypsin, K × 10^{-3}	Vol., ml	Trypsin, K × 10^{-3}	Vol., ml	Trypsin, K × 10^{-3}
Student	7	60	82	110	61	25	50	10
Psycho-neurosis	4	20	51	162	42	70	35	52
Chronic ulcerative colitis	—	120	60	120	32	75	14	50
Duodenal ulcer	14	45	111	100	48	68	50	20
Chronic pancreatitis	15	0	90	0	51	0	43	0
Chronic pancreatitis advanced	4	0	10	0	10	0	4	0

but only at a tremendous and uneconomical loss in yield. In view of the recent work of Greengard, Wolfrom and Ness(17), showing that crystalline secretin picrolonate is indistinguishable by crystallographic methods from crystalline pyridine picrolonate, it is questionable whether attempts at crystallization of secretin by similar procedures would prove profitable.

Secretin made as described here proved suitable for intravenous administration in man. It was free of vasodepressor and pyrogenic substances and apparently non-antigenic. Cholecystokinin, enterocrinin, pancreaozymin, and hypoglycemic substances were not present in measurable amounts. It is of importance to note that the procedure is relatively simple and that reproducible results have been obtained consistently by the authors in this laboratory as well as by investigators in another institution(18).

Summary. A method for the preparation of a potent secretin suitable for intravenous administration in man is described.

17. Greengard, H., Wolfrom, M. L., and Ness, R. K., *Fed. Proc.*, 1947, v6, 115.

18. Bernhart, W. F., and Alburn, H., personal communications.

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The *In Vitro* Conversion of C^{14}-Labeled Glucose to Fatty Acids.* (17679)

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Although the importance of lipogenesis in carbohydrate utilization has been recognized for some time, it was Stetten and Boxer who first called attention to the quantitative significance of this conversion in the normal animal(1). With the aid of deuterium as a labeling agent, they showed that, in the rat fed a high carbohydrate diet, at least 10 times as much of the dietary glucose is used to synthesize fatty acids as is used in the synthesis of glycogen. More recently, lipogenesis was studied with C^{14}-labeled glucose by Masoro *et al.*(2). In mice that had been maintained for some time on a high carbohydrate diet, 10-15 per cent of the ingested glucose-C^{14} was recovered as fatty acids in 24 hours, and 12 and 16% in 48 hours. These recoveries were observed in mice whose weights had remained constant for a 2-week

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1. Stetten, D., and Boxer, G. E., *J. Biol. Chem.*, 1944, v155, 231.

2. Masoro, E. J., Chaikoff, I. L., and Dauben, W. G., *J. Biol. Chem.*, 1949, v179, 1117.

TABLE I.

The Conversion of C^{14}-Glucose to Fatty Acids by Surviving Liver, Kidney, and Diaphragm Prepared from Rats Fed a High Carbohydrate Diet.

Each incubation flask contained 350-430 mg of tissue, 4.5 cc of Krebs-bicarbonate buffer, and 0.5 cc of a saline solution containing C^{14}-labeled glucose. The period of incubation was 3 hours at 37.5°. The contents of 2 or more flasks were pooled for analysis.

Exp.	Tissue	Avg amt of tissue per flask, mg.	Initial glucose conc. of medium,§ mg %	No. of flasks pooled for analysis	% of added glucose-C^{14} recovered as	
					CO_2 per g tissue	Fatty acids, per g tissue
1*	Liver	313	422	3		.40
	Kidney	340	350	9		.04
	Diaphragm	370	350	4		.05
2†	Liver	310	432	5		.44
	Kidney	314	432	5		.04
	Diaphragm	337	432	5		.02
3‡	Liver	513	418	2	5.8	.56
	Diaphragm	405	418	2	2.5	.04
	Skeletal muscle	765	418	2	1.2	.01
4‡	Liver	496	392	2	4.7	.81
	Diaphragm	510	392	2	3.0	.17

* Liver slices were prepared from a single rat, whereas the amounts of kidney and diaphragm slices recorded above were obtained from 3 rats.

† The amount of each tissue incubated was obtained from a pool containing slices from 4 rats.

‡ All tissues used in this experiment were obtained from a single rat.

§ Glucose concentration of medium before addition of slices.

period. There can no longer be any doubt, from these findings as well as from the observations of Stetten, that lipogenesis from carbohydrate does occur in rats and mice maintained in the steady state.

C^{14}-labeled glucose has been used in the present investigation to study lipogenesis by surviving slices of liver, kidney, and muscle that were prepared from rats that had been fed a high carbohydrate diet. When these slices were incubated in a Ringer-bicarbonate medium containing 0.4 per cent of C^{14}-labeled glucose, significant amounts of the C^{14} were converted to fatty acids.

Experimental. Animals and tissues. Rats were fed, for 2 or more weeks, a high carbohydrate diet containing 60% dextrose, 22% casein, 6% cellu-flour, 6% brewer's yeast, and 6% salt mixture(3). The rats also received, twice weekly, several drops of a fish oil containing 400 A.O.A.C. chick units of vitamin D and 3000 U.S.P. units of vitamin A per g. The rats, which weighed from 150 to 250 g, were sacrificed by fracture of the cervical vertebrae, and the required tissues were rapidly removed. Slices of liver and kidney were prepared freehand with a thin razor blade. The diaphragm was trimmed to remove the central tendon and extraneous tissue. The tissues were collected in a Petri dish containing cold Ringer-bicarbonate solution. Before their transfer to the incubation flasks, they were blotted free of excess moisture, and weighed.

Radioactive Glucose. The radioactive glucose was prepared photosynthetically according to the method of Putnam *et al.*(4). It was shown to be uniformly labeled with C^{14}(5). We are indebted to Dr. W. Z. Hassid and Mr. S. Abraham for the sample of labeled glucose used in this study.

Incubation procedure. The Ringer-bicarbonate solution was prepared according to Krebs and Henseleit(6), and adjusted to pH

3. Hawk, P. B., Oser, B. L., and Summerson, W. H., *Practical Physiological Chemistry*, 12th edition, Blakiston, Philadelphia, 1947.

4. Putnam, E. W., Hassid, W. Z., Krotkov, G., and Barker, H. A., *J. Biol. Chem.*, 1948, v173, 785.

5. Hassid, W. Z., personal communication.

6. Krebs, H. A., and Henseleit, K., *Z. physiol. Chem.*, 1932, v210, 33.

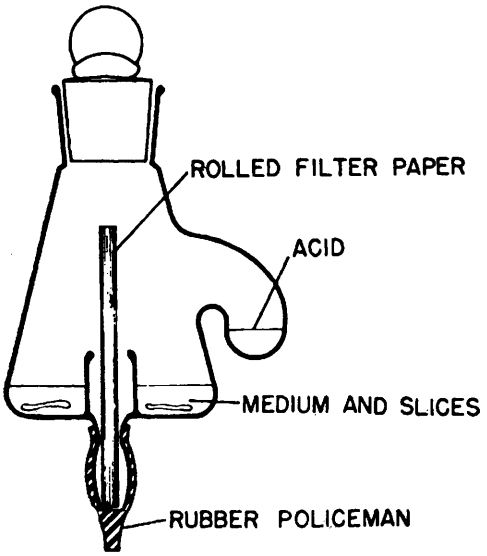

FIG. 1.
Incubation flask.

7.4 just before use. To each flask was added 4.5 cc of this buffer and 0.5 cc of a saline solution containing 4% labeled glucose. The specific activity of the added glucose was 1 x 10^4 counts per minute per mg of glucose.

The flasks were gassed with a mixture consisting of 95% O_2 and 5% CO_2. The flasks were then placed in a constant temperature water bath maintained at 37.5°, and gently shaken during the 3 hours of incubation.

Collection of $C^{14}O_2$. In the first 2 experiments (Table I), 50-cc, glass-stoppered Erlenmeyer flasks were used for incubation. At the end of the experiment the stopper was removed and 1 cc of 1N HCl was added to stop the reaction. This procedure did not, of course, permit collection of the CO_2 within the flask.

In experiment 3 and 4, incubation of the tissue slices was carried out in Erlenmeyer flasks modified, as shown in Fig. 1, to permit collection of the CO_2. An amount of 5N H_2SO_4 (usually 0.2 cc) sufficient to stop the reaction and to liberate the CO_2 from the medium was placed in the sidearm. The center well extended through the bottom of the flask and was sealed with a rubber policeman. A roll of No. 50 Whatman filter paper was placed in the center well. Five minutes before the end of the experiment, approximately one cc of 30% KOH was injected through the rubber policeman into the well with the aid of a syringe. The alkali was rapidly taken up by the filter paper. At the end of the experiment the flask was tilted so as to transfer the acid from the sidearm to the medium. The flask was allowed to stand for 30 minutes to permit absorption of the CO_2 by the alkali. The filter paper was then removed from the center well and placed in a 50-ml volumetric flask. The center well was washed 5 times with distilled water and the washings were transferred to the volumetric flask. Aliquots were removed for the determination of C^{14} according to the method of Entenman *et al.*(7). The efficiency of this method for collecting CO_2 was tested as follows. Five cc of a solution of Na_2CO_3 labeled with $Na_2C^{14}O_3$ were placed in the main compartment of the flask, and 0.2 cc of 5N H_2SO_4 in the sidearm. One cc of 30% KOH was then injected into the center well, the flask was tilted, and the acid mixed with the carbonate solution. The flask was allowed to stand for 30 minutes. In several experiments of this type, 97-99% of the added C^{14} was recovered.

Analysis of Flask Contents. After incubation, the acidified contents of several flasks were combined for analysis. The combined contents were filtered. The tissue on the filter paper was then thoroughly washed several times with distilled water, and these washings were added to the filtrate. The aqueous phase† was then transferred to a volumetric flask and made to volume. *This fraction is referred to in Table II as fraction A.* Its C^{14} content was determined as described below. Lipids were extracted from the residue on the filter paper by a modification of the method described by Sperry *et al.*(8). The residue was transferred, by means of hot

7. Entenman, C., Lerner, S. R., Chaikoff, I. L., and Dauben, W. G., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 364.

† In several experiments the aqueous phase was thoroughly extracted with petroleum ether and the petroleum ether solution tested for C_{14} content. None was found.

8. Sperry, W. M., Waelsch, H., and Stoyanoff, V. A., *J. Biol. Chem.*, 1940, v135, 281.

TABLE II.

Showing the Extent to Which the Added C^{14} Was Recovered in the Various Fractions in Experiments with Liver Slices.

Each incubation flask contained 350-500 mg of slices, 4.5 cc of Krebs-bicarbonate buffer, and 0.5 cc of a saline solution containing C^{14}-labeled glucose. The period of incubation was 3 hours at 37.5°. The contents of 2 or 3 flasks (containing a total of about 1 g of slices) were pooled for analysis. All values refer to percentages of the added C^{14}.

			Fraction B		
Exp.	$C^{14}O_2$	C^{14} in Fraction A	Fatty acid, C^{14}	Rest, C^{14}	Total C^{14} accounted for
5	4.5	94	.7	1.3	101
6	3.1	97	.5	*	101
7	4.4	94	.7	*	99
8	4.2	94	.8	*	99
9	5.0	89	*	*	94
10	5.8	88	1.6	*	95
11	6.0	86	.8	*	93

* Not determined.

alcohol, to an Erlenmeyer flask and to this was added one cc of 90% KOH. The final alcohol concentration was adjusted to 50 per cent with water (the total volume at this point was about 10 cc), and the mixture was heated on a steam bath for several hours. *This alkaline hydrolysate is referred to as fraction B.* The alcohol was first allowed to evaporate, and enough 95% ethyl alcohol was now added to restore the concentration of alcohol in the mixture to 50%. The unsaponifiable material was removed by 3 extractions (each time with about 75 cc) of petroleum ether. The combined petroleum ether extracts were thoroughly washed, first with 2N KOH and then with distilled water after the manner of Sperry *et al.*(8). These washings were returned to fraction B.

The petroleum ether extract containing the unsaponifiable material was analysed for its C^{14} activity. The C^{14} counts recovered in this fraction were negligible under the conditions studied here. Fraction B was heated on a steam bath to free it of alcohol and then was made acid to bromcresol-green and the acidified mixture allowed to stand for at least one hour at 4° to permit the fatty acids to form aggregates. The precipitated fatty acids were separated by filtering the mixture through No. I Whatman paper. The filtrate was found to contain C^{14} (referred to as rest C^{14} in Table II) which was not extractable with petroleum ether. The fatty acids were dissolved with acetone and the filter paper was boiled with acetone to ensure complete recovery of the fatty acids. The combined acetone solutions were evaporated on a steam bath, and the last traces of this solvent were removed under a stream of CO_2. The fatty acids were redissolved in hot petroleum ether and the petroleum ether extract was washed 3 times with distilled water. Flasks equipped with sidearms were used for this purpose. The petroleum ether extract was transferred to a flask containing about 5 g of anhydrous sodium sulfate. The dried petroleum ether solution was made to volume and aliquots were removed for the determination of fatty acid-C^{14}.

Determination of C^{14}. C^{14}-containing materials were oxidized to CO_2 by the method of Van Slyke and Folch(9). The CO_2 evolved was collected as $BaCO_3$ and the C^{14} content of the $BaCO_3$ was determined by the method of Entenman *et al.*(7).

Results. In the first 2 experiments (Table I), the conversion of the added glucose-C^{14} to fatty acids was studied. In the next 2 experiments, the incorporation of the C^{14} into CO_2 as well as fatty acids was measured. The recoveries of the C^{14} in these fractions are expressed as percentages of C^{14} added to the medium.

Liver. From 0.4 to 0.8% of the C^{14} was recovered as fatty acids per g of tissue, and approximately 5% was converted to CO_2.

Kidney. Only a small fraction of the

9. Van Slyke, D. D., and Foch, J., *J. Biol. Chem.*, 1940, v136, 509.

added C^{14} was recovered as fatty acid in the experiments with kidney slices.

Muscle. Data are presented for both diaphragm and skeletal muscle obtained from the rat's thigh. The thigh muscles were sliced along their longitudinal axes.

The diaphragm sheets oxidized 2.5-3% of the C^{14}-glucose to CO_2. From 0.02 to 0.2% was recovered as fatty acids. Less of the C^{14} was recovered as fatty acids and CO_2 in the experiments with skeletal muscle.

Recovery of the Glucose-C^{14} in Various Fractions. Typical results of the C^{14} recoveries in the various fractions separated in the course of experiments carried out with approximately one gram amounts of liver slices are recorded in Table II. At the end of the run, the largest amount of the C^{14} was found in the aqueous phase designated fraction A. Osazone-forming compounds, which we have assumed to be mainly glucose, accounted for about 80 per cent of the C^{14} contained in fraction A.

From 3 to 4.5% of the added C^{14} was converted to CO_2. About 2% was found in fraction B, less than half of which was in the form of lipids. The C^{14} recovered in these various fractions accounted for practically all of the glucose-C^{14} added to the medium.

Comment. Conclusive evidence that the conversion of glucose to fatty acids can occur in tissues other than the liver was provided in an earlier report from this laboratory(2). Rats that had been maintained on a high carbohydrate diet for 2 or more weeks were deprived of their livers and gastrointestinal tracts by evisceration and were then injected with C^{14}-labeled glucose. Palmitic acid containing C^{14} was isolated from these eviscerated preparations. The conclusion based on the eviscerated animal is fully supported by the demonstration that kidney slices and diaphragm convert glucose to fatty acids. An indication of the relative significance of lipogenesis in glucose utilization by isolated liver and diaphragm, can be obtained by comparing for each, the recovery of C^{14} as CO_2 with recovery as fatty acids. In the case of the animal used in experiment 4, the ratios were 6 and 18, respectively. Since fatty acids are degraded more rapidly in the liver than in extrahepatic tissues(10), these ratios suggest that fatty acid formation plays a more important role in glucose utilization per gram of liver than per gram of muscle.

Summary. 1. The conversion of C^{14}-labeled glucose to fatty acids by surviving slices of liver, kidney, and diaphragm is demonstrated.

2. The recoveries of fatty acid-C^{14} in the experiments carried out with liver slices were about 10 times as great as those observed with diaphragm or kidney.

3. The glucose-C^{14} recovered as fatty acid-C^{14} in the experiments with liver slices was 1/6 to 1/10 of that which had been converted to CO_2.

4. An incubation flask is described that is suitable for the collection of CO_2 evolved in tissue slice experiments from C^{14}-labeled compounds.

10. Goldman, D. S., unpublished observations.

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