TABLE III.
Dog Fetal Endocrine Tissues Transplanted to the Anterior Chamber of the Eye of Normal Dogs and Puppies.

(A)	9 gonads were transplanted:
	1 grew for approximately 60 days and regressed.
	2 abscesses.
	6 initial failures.
(B)	11 adrenal transplants:
	4 "Takes"—2 were growing and surviving between 7 to 8 weeks.
	5 initial failures.
(C)	8 thyroid transplants:
	2 growing and surviving at the end of 30 days.
	6 initial failures.

tained colloid. The fetal ovaries after transplantation show maturity and follicular development, and in the case of the rabbit, the transplant will give a positive Friedman reaction. Rabbit fetal testes removed eighty-five days after transplantation show spermatids. There is increased differentiation of the various zones of the adrenal cortex as well as cellular growth and multiplication of cells. The medulla does not survive. Pancreas transplants removed 30 to 120 days later reveal well formed ducts lined with low cuboidal epithelium, but the Islets of Langerhans have all regressed. We are investigating methods of increasing the percentage of survival by applying Halstead's Law of Endocrine Deficiency either at the time of transplantation or soon thereafter. We may state that the percentage of "takes" of the ovary and testes has increased from 60% to 90%. This observation will be reported at a later date upon a larger series which is now under investigation. In the case of the adrenal and thyroids, our best growths and survivals have been in those animals which had the corresponding endocrine organs removed. In our laboratory thyroidectomized guinea pigs have homologous fetal thyroid transplants which have been growing for over 10 months. We have also been investigating sites for transplantation other than the anterior chamber of the eye in order to transplant fetal endocrine tissue clinically. In only a few cases have we been successful, this site being the kidney, with the adrenal transplant.

Summary. (1) Embryonic adrenal, ovary, thyroid and testes have been homologously transplanted to the anterior chamber of the eye of normal dogs, guinea pigs and rabbits with a 50 to 60% survival beyond six weeks in the latter 2 species of normal animals. (2) In transplants of pancreas, only the ducts survived. (3) The best growth occurred in those animals in which the corresponding endocrine organ was removed.

Received January 3, 1950, P.S.E.B.M., 1950, v73.

The Preparation of Secretin. (17678)

M. H. F. FRIEDMAN AND J. E. THOMAS.
From the Department of Physiology, Jefferson Medical College, Philadelphia, Pa.

Several methods have been described for extraction and purification of secretin but unfortunately these have in general proved to be cumbersome, time-consuming, or unsuitable for producing secretin economically in more than small quantities. The present report describes a simplified procedure for preparation of a potent secretin which has been given intravenously to more than 150 human subjects without evidence of untoward reactions. The potency of various fractions was tested on dogs by an assay procedure described elsewhere(1) and expressed in terms of the clinical unit established by Hammersten and coworkers(2) which, as was shown by Greengard and Stein(3), is identical with the Ivy

1. Friedman, M. H. F., and Thomas, J. E., *J. Lab. Clin. Med.*, 1950, v35, 366.

2. Hammersten, E., Agren, G., and Lagerlof, H., *Acta Med. Scand.*, 1937, v92, 256.

3. Greengard, H., and Stein, I. F., PROC. SOC. EXP. BIOL. AND MED., 1941, v46, 149.

dog threshold unit(4). Tests have shown the following substances to be either absent from the secretin preparations or present in barely detectable concentrations: vaso-depressor substances (blood pressure method on atropinized cat); pancreozymin (determinations of concentration of trypsin(5) and amylase(6) of the pancreatic juice); cholecystokinin (unanesthetized dog method(7)); hepatocrinin (unanesthetized cholecystectomized dog method(8)); enterocrinin* (procedure of Nasset (9)) and hypoglycemic substances (blood sugar determinations by Folin-Wu method).

Preparation of Secretin. The most suitable starting point was found to be preparation of a salt cake as described by Voegtlin, Greengard and Ivy(10). This consists of soaking for 30 minutes the everted upper 6 feet of hog intestine in ice-cold 0.13 N HCl, 200 ml per meter of intestine. Constant stirring is avoided since this results in extraction of large amounts of inert material from which the secretin is subsequently separated with difficulty. The intestine is removed and sodium chloride is added with constant stirring to the acid solution to 32% concentration. Following 15 to 24 hours storage in the refrigerator (5°C) the supernatant fluid is decanted and the precipitate is partially dried by *pressure filtration* until it contains not more than 40% moisture. The filtrate contains vasodepressor substances and drying the salt cake by evaporation only reduces the moisture content without removing them. To the salt cake are added 5 parts by weight of absolute acetone-free methanol. After storage at 5°C for 12 hours the methanol is separated by filtration. The residue is reextracted for several hours in the cold with 2.5 parts of methanol and the methanol filtrates combined if studies on distribution and yield of secretin are undertaken. Large quantities of secretin however may be prepared most economically by only a single methanol extraction. To the methanol filtrates are added exactly 3 volumes of absolute acetone. Addition of more acetone than this yields a greater precipitate but the additional material so obtained is inert as a pancreatic stimulant. After not more than 24 hours storage in the refrigerator the acetone precipitate is collected by filtration and dried with acetone and ether. The precipitate is agitated in cold saturated sodium chloride solution, 50 ml per g, and the material remaining insoluble after two hours is recovered and dried with acetone and ether.

The precipitate is suspended without agitation in approximately 100 times its weight of distilled water which has been acidified with 1 part of glacial acetic acid. After storage for 12 hours in the refrigerator the insoluble material (often found floating on the surface) is removed by filtration in the cold. To the cold filtrate enough cold solution of 100% trichloracetic acid is added to make a final concentration of 10%. Maximum precipitation occurs during storage in the refrigerator for 24 to 72 hours. The fine precipitate is collected by centrifugation at low speed and washed 5 times with absolute acetone, 3 times with absolute ethyl ether, and dried over calcium chloride. The dry precipitate is agitated for 6 hours in normal butanol, 100 ml per g precipitate, in a water bath at 40°C. The insoluble material obtained by centrifugation is retained and washed 3 times with absolute acetone and twice with absolute ethyl ether.

After determining the potency as described elsewhere(1), the active material is dissolved in distilled water to a concentration of 20 clinical units per ml. The aqueous solution is measured into vials or ampules of 5 ml capacity and reduced to the dry state by freezing and lyophilization in the usual manner.† The vials, each containing a stand-

4. Greengard, H., and Ivy, A. C., *Am. J. Physiol.*, 1938, v124, 427.

5. Friedman, M. H. F., *Exp. Med. Surg.*, 1948, v6, 149.

6. Myers, V. C., Free, A. M., and Rosinski, E. E., *J. Biol. Chem.*, 1944, v154, 39.

7. Snape, W. J., Friedman, M. H. F., and Thomas, J. E., *Gastroenterology*, 1948, v10, 496.

8. Friedman, M. H. F., and Snape, W. J., *Fed. Proc.*, 1945, v4, 21.

9. Nasset, E. S., *Rev. Gastroenterol.*, 1942, v9, 188.

* We are indebted to Professor E. S. Nasset who kindly determined the effect of certain preparations on the secretion of the small intestine.

10. Voegtlin, W. L., Greengard, H., and Ivy, A. C., *Am. J. Physiol.*, 1934, v110, 198.

TABLE I.
Effect of Intravenous Injection of Secretin at 10-minute Intervals on the Pancreatic Secretion in Unanesthetized Dog (Temporary Intubation of Pancreatic Duct *in Situ* via Duodenal Fistula).

Dog No.	Date, 1946	No. of samples	Vol. of 10-min. samples			N, mg/cc	Trypsin, K × 10^{-3}
			Max, ml	Min, ml	Avg, ml		
1-44	1/31	4	4.8	4.2	4.55	1.6	100
	2/18	4	4.7	2.4	3.7	1.9	100
	7/17	4	3.3	2.5	3.0	1.6	80
1-45	1/25	2	6.0	5.7	5.85	1.5	117
	2/13	2	5.0	4.7	4.85	1.0	97
	3/21	3	5.4	5.0	5.2	1.6	125
1-46	2/14	3	3.0	2.5	2.8	2.0	120
	5/ 1	3	6.6	5.1	5.7	1.5	120
	14	6	8.6	1.5	5.75	1.5	105
2-46	7/12	3	4.0	2.8	3.6	1.6	77
	18	4	6.4	4.6	5.5	1.7	112
	22	4	6.2	3.1	4.8	1.3	90

ard amount of secretin, are then sealed and stored. The lyophilized secretin is readily soluble in distilled water. No detectable loss in potency of the material sealed in the ampules has been found after one year's storage at room temperature.

Single, multiple(11) and continuous(12) injections of this preparation of secretin in doses up to 18 units per kilo body weight have been made in unanesthetized dogs without untoward reactions. Single, double and triple hourly injections of this secretin in 1.1 units per kilo body weight have been made in over 150 human subjects without evidence of cardiovascular, respiratory, or anaphylactoid reactions(13).

In Tables I and II are summarized typical results obtained in the dog and man following intravenous administration of secretin prepared as described above. The pancreatic function tests in man were carried out by the use of a double lumen tube with constant aspiration of the gastric and duodenal contents (as described by Lagerlof(14)). Pancreatic juice in the dog was obtained by direct intubation of the pancreatic duct in the unanesthetized animal as described by Hart and Thomas(15). The low concentrations of enzymes and nitrogen in the pancreatic juice secreted in response to secretin injection offer evidence of the absence of pancreozymin or other ecbolic agents from the secretin preparation.

Discussion. The most potent secretin preparation hitherto described is the secretin picrolonate of Hammersten, Agren *et al.*(16). Its potency of 1 cat unit per .004 mg would correspond to 16 clinical units per mg. Our best preparations assayed 12 clinical units per mg but the usual product ranged from 6 to 8 units. Since in the picrolonate the secretin appears to be combined with variable amounts of picrolonic acid, it is impossible to make an exact comparison between the two preparations. Further fractionation of our preparations was found to increase the potency

† Lyophilization for the purpose of increasing solubility was suggested by Dr. F. W. Bernhart to whom we are greatly indebted for carrying out this stage in the procedure and for much invaluable assistance. Financial assistance from Wyeth Incorporated is acknowledged.

11. Friedman, M. H. F., unpublished data, 1943-47.

12. Thomas, J. E., and Crider, J. O., PROC. SOC. EXP. BIOL. AND MED., 1947, v64, 27.

13. Friedman, M. H. F., and Snape, W. J., PROC. SOC. EXP. BIOL. AND MED., 1949, v70, 280.

14. Lagerlof, H., *Pancreatic Function and Pancreatic Disease Studied by Means of Secretin*, New York, Macmillan, 1942.

15. Hart, W. M., and Thomas, J. E., *Gastroenterology*, 1945, v4, 409.

16. Hammersten, E., Agren, G., Hammersten, H., and Wilancer, O., *Biochem.*, 1933, v264, 275

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.

Received January 9, 1950. P.S.E.B.M., 1950, v73.

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

S. S. CHERNICK, E. J. MASORO, AND I. L. CHAIKOFF.
From the Division of Physiology, University of California Medical School, Berkeley.

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

* Aided by grant from the American Cancer Society as recommended by the Committee on Growth of the National Research Council.

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.