4. Dinning, J. S., Neatrour, R., Day, P. L., *ibid.*, 1954, v209, 717.
5. Weimer, H. E., Moshin, J. R., *Ann. Rev. Tuberc.*, 1953, v68, 594.
6. Winzler, R. J., *Method of Biochem. Analysis*, 1955, v2, 279.
7. Sobel, A. E., Mayer, A. M., *J. Biol. Chem.*, 1945, v157, 255.
8. Fiske, C. H., SubbaRow, *ibid.*, 1925, v66, 375.
9. Youngburg, G. E., Youngburg, M. V., *J. Lab. Clin. Med.*, 1930, v16, 158.
10. Bragdon, J. H., *J. Biol. Chem.*, 1951, v90, 513.
11. Banerjee, S., Chatterjee, K. P., PROC. SOC. EXP. BIOL. AND MED., 1957, v94, 474.
12. Koiw, E., Gronwall, A., *Scand. J. Clin. Lab. Invest.*, 1952, v4, 245.
13. Roboz, E., Hess, W. C., Forster, F. M., *Arch. Neurol. Psych.*, 1955, v73, 536.
14. Hotchkiss, R. D., *Arch. Biochem.*, 1948, v16, 131.
15. McDonald, H. J., Bermes, E. W., *Biochim. Biophys. Acta*, 1955, v17, 290.
16. Swahn, B., *Scand. J. Clin. Lab. Invest.*, 1953, v5, Suppl. 9.
17. Sohar, E., Bossak, E. T., Adlersberg, D., PROC. SOC. EXP. BIOL. AND MED., 1955, v90, 305.
18. Rosenman, R. H., Friedman, M., Byers, S. O., Smith, M. K., *J. Clin. Invest.*, 1956, v35, 522.
19. Totter, J. R., Kelley, B., Day, P. L., Edwards, R. R., *Fed. Proc.*, 1950, v9, 238.
20. Plant, G. W. E., Belheil, J. G., Lardy, H. A., *Abst. Am. Chem. Soc.*, Atlantic City, 1949, 65C.
21. Gordon, M., Ravel, J. M., Eakin, R. E., Shive, W., *J. Am. Chem. Soc.*, 1948, v70, 878.
22. Welch, A. D., Sakami, W., *Fed. Proc.*, 1950, v9, 245.
23. Adlersberg, D., Bossak, E. T., Sher, I. H., Sobotka, H., *Clin. Chem.*, 1955, v1, 18.
24. Oncley, J. L., Gurd, F. R. N., Melin, M., *J. Am. Chem. Soc.*, 1950, v72, 458.

Received September 14, 1959. P.S.E.B.M., 1959, v102.

Serum Amylase Changes Due to Antipancreas Serum. (25390)

P. F. POKORNY, J. F. RANSDELL AND J. T. BARRETT
(Introduced by Frank B. Engley, Jr.)
Depts. of Microbiology and Pathology, School of Medicine, University of Missouri, Columbia

Revival of interest in effects of antiorgan serum on normal animals has been directed largely toward the heart, kidney and thyroid. An additional endocrine organ, the pancreas, has been the subject of 2 conflicting reports. In one study murine pancreas demonstrated no serologic activity(1), in the other(2) it was shown that rat pancreas stimulates production of precipitating antisera in rabbits. The latter report suggested structural peculiarities of rat pancreas were in part responsible for its poor antigenicity, and the poor concentration of radiolabeled antibody when administered passively. The work presented here shows the discrete guinea pig pancreas is antigenic as determined by *in vitro* and *in vivo* serologic reactions and that its antiserum produces pancreas pathology and alters serum amylase levels.

Materials and methods. After removal of the pancreas from several ether-anesthetized guinea pigs, a 10% w/v homogenate was prepared in cold saline in Waring blendor. Immunization of male rabbits was performed according to Estes(3) with the complete homogenate. When the precipitating titer of undiluted immune serum reached 1:500 (supernatant of 10% suspension diluted 1:50), the rabbits were bled and serum collected. In view of ultimate use of antisera, a biologic assay seemed desirable and the passive cutaneous anaphylactic (PCA) method of Ovary (4) was chosen. Four hours after dermal sensitization with 0.1 ml of different antisera, 1 ml of a 50-50 mixture of 1% Evans blue and supernatant from the 10% pancreas extract was given intracardially. Reactive sites were measured after 45 minutes. To determine if injections of antiserum into normal guinea pigs would induce pathologic or enzyme changes, 2 intraperitoneal injections of 5 ml of pooled antiserum were given, one the same day and one the 4th day after base level serum amylase determinations were completed(5). On 5th day, serum amylase was again determined, the animals sacrificed, and the pan-

TABLE I. Serologic Reactions of Guinea Pig Pancreas with Its Antiserum.

Rabbit	Precipitin titer dilution of antigen	PCA, mm × mm
APS-2	1:500	17.5 × 21.5
-3	1:1000	24 × 32
-4	"	not tested
-5	"	20 × 22
-6	"	15 × 17.5
NRS-1	<1:10	1 × 1.5
-2	"	1 × 2
-3	"	1 × 1

APS—Immunized with pancreas antigen.
NRS—Normal rabbit serum.
PCA figures are avg of 2 animals.

fold the NRS group. These figures include 2 animals in the APS treated group that were not examined histologically. An additional 4 animals given antiheart serum revealed an average increase of 366 units of serum amylase, essentially the figure for normal controls.

Discussion. Anti-endocrine organ sera seem to have either of 2 biologic effects depending upon antigenicity of the endocrine hormones. If the hormones are antigenic, the antiserum will contain antibodies which neutralize the hormone on passive immunization of normal animals. This seems to be the situation with antipituitary and antithyroid sera

TABLE II. Effects of Antipancreatic Serum on Normal Recipient Guinea Pigs.

	Interstitial pancreatitis	Pancreatic serositis	Serum amylase changes — Increase	Range
NRS	1/7*	2/7	384 units	−600 to +1600
APS	4/7	7/7	931 " †	−250 to +2900

* No. positive/No. tested. † 9 animals.

creas removed for histopathologic examination. For precipitin, PCA, and passive immunizations parallel experiments with normal rabbit serum were used as controls.

Results. Serologic reactions of the antipancreas (APS) and normal rabbit sera (NRS) are recorded in Table I. There seems to be no question that an antibody does exist since both tests are positive in every instance. Pre-immunization titers of experimental rabbits were less than 1:10 or the same as the normals listed. The antipancreas sera were also tested for guinea pig erythrocyte agglutinins and lysins, as well as serum and heart extract precipitins, all of which were negative. The positive PCA reactions were a deep blue in color with a small central ring characteristic of intense reactions.

Examination of hematoxylin, eosin stained sections of pancreas from the APS and NRS treated animals revealed the former to have definite pancreatic serositis in every instance and an acute interstitial pancreatitis in 4 of 7 animals examined (Table II). Although one animal of the NRS group is stated as having pancreatitis, it was not remarkable; serositis was present in 2 controls. Post-injection serum amylase increased in both groups; however, the APS treated animals increased 2.4

which depress growth rates(6) and O_2 consumption(7) respectively. Other endocrine antisera lacking antihormones cause tissue damage and excessive release of the hormone with typical changes in physiology of the animal(2).

In our instance the pancreas was damaged sufficiently to cause release of amylase which was not neutralized by any anti-amylase component of the serum sufficiently to depress blood levels and so simulates the second condition above. This increase in a serum enzyme due to immunologic tissue damage by a specific anti-organ serum is a finding which may be extendible to other organs known to be the source of specific enzymes.

Summary. As determined by an *in vitro* and an *in vivo* test, guinea pig pancreas has been found to be antigenic. Its antiserum produced pancreas pathology and serum amylase increases when administered passively to normal guinea pigs.

1. Henle, W., Chambers, L. A., Groupe, V., *J. Exp. Med.*, 1941, v74, 495.
2. Anigstein, L., *et al., Tex. Rep. on Biol. and Med.*, 1954, v12, 945.
3. Estes, H. R., *Arch. Path.*, 1949, v47, 399.
4. Ovary, Z., *et al.*, PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 397.

5. King, E. J., *Micro-Analysis,* 1956, Grune and Stratton, N. Y.

6. Anigstein, L., Whitney, D. M., Rennels, E. G., *Tex. Rep. on Biol. and Med.,* 1958, v16, 303.

7. Anigstein, L., Eklund, G. W., Whitney, D., *ibid.,* 1957, v15, 205.

Received September 17, 1959. P.S.E.B.M., 1959, v102.

Release of Inorganic Iodine from Thyroid Gland in Initial Phase of TSH Action. (25391)

SHIGENOBU NAGATAKI, KAZUO SHIZUME, KUNIO MATSUDA AND JUN ISHII
(Introduced by R. L. Swank)
Prof. Okinaka's Medical Clinic, University of Tokyo Medical School, Hongo, Tokyo, Japan

It has been well established that TSH accelerates release of thyroid hormone from the thyroid gland and increases uptake of inorganic iodine by the thyroid gland(1,2,3,4). While the acceleration of the hormone release takes place within several minutes after injection of TSH, there is a latent period of several hours before the thyroid gland starts to increase its uptake of inorganic iodine(1,5,6,8) and few studies have been reported on the behavior of the thyroid gland in handling inorganic iodine shortly after injection of TSH. Our observation that TSH releases the inorganic iodine from the thyroid in the early phase of its effect prompted this report.

Materials and methods. Seventeen male dogs weighing about 10 kg and maintained on a low iodine diet for 2 weeks were utilized. In 13 dogs, 3 to 7 days before the experiments, 0.5 to 2 mc of carrier free I^{131} were injected intramuscularly, while in the other 4 dogs, a similar amount of I^{131} was injected 20 hours before the experiments. Under morphine anesthesia (100 mg), a midline incision was made in the neck to dissect out the thyroid gland and its vessels. The thyroid venous blood samples were collected from polyethylene catheters inserted into the inferior thyroid veins. A catheter was placed in the femoral artery to collect arterial blood samples and to measure arterial blood pressure. During the experimental period, about 10,000 units of heparin in saline were infused intravenously. Blood transfusion was used if necessary, to maintain constant blood pressure.

Method. In 14 dogs, after control period of 30 minutes to 2 hours, one USP unit of TSH in 10 ml saline was injected intravenously, and the effect of TSH observed for 2 to 10 hours thereafter. In 2 dogs, observation was started 8 hours after administration of TSH to determine duration of its action. At intervals of 15 to 30 minutes during control and experimental periods, blood samples were collected from catheters in the thyroid veins and femoral artery, and blood pressure at each time was recorded. Blood flow of the thyroid vein was estimated by volume of blood collected during each interval. Measurement of PBI^{131}: Samples from thyroid veins and femoral artery were centrifuged to separate 1 ml of plasma. Radioactivity of the plasma, measured in well type scintillation counter for 5 minutes, represented total plasma radioactive iodine. Five ml of 20% trichloroacetic acid was added in the same tube to precipitate the protein bound iodine, and the precipitate was washed twice with 5 ml of 10% trichloroacetic acid. The activity of the precipitate, redissolved with 0.5 ml of 2 N sodium hydroxide, which was measured in a well type scintillation counter for 5 minutes, represented the PBI^{131} concentration in 1 ml of plasma. Concentration of inorganic iodine was expressed by the difference between the activity of total and PBI^{131}. Radiopaperchromatographic technic: 3 to 5 ml of plasma was acidified to pH 2-3 with 5 N HCl, extracted 3 times with 0.1 N HCl saturated butanol after addition of carriers, neutralized with 4 N NH_4OH and evaporated to dryness in a 50°C water bath. The residue was dissolved in 0.2 ml of butanol and placed on Toyo filter