in the 2 fractions of liver cells of laying hens were not those induced by residual serum proteins in liver homogenates. The antigenic properties of phosphoproteins found in microsomal and supernatant fractions of laying hens and estrogenized chickens appear to be specific for the 2 fractions of their liver cells. These findings give immunological support to the idea that the liver is the locus of phosphoprotein production in the laying hen. Microsomal and supernatant fractions of liver cells of laying hens had both specific antigenic properties of S-I and S-III, in which antigenicity of S-I in the microsomal fraction was weak. The reason for this weakness is not clear. The results permit the assumption that microsomes play an important role in phosphoprotein syntheses in liver cells or that they produce the S-I fraction (low density lipoprotein) and S-III fraction (phosphoprotein) in liver cells of laying hens and secrete them into blood stream.

Summary. Microsomal and supernatant fractions of liver cells of laying hens and estrogenized chickens showed immunologically specific antigenic properties similar to those of phosphoproteins found in the sera of laying hens. However, nuclear and mitochondrial fractions derived from liver cells of laying hens, 4 fractions from spleen cells of laying hens and 4 fractions from liver cells of cocks, did not show any such specific antigenic properties. Microsomal and supernatant fractions of liver cells of laying hens and estrogenized chickens had both specific properties of S-I and S-III fractions. Microsomal and supernatant fractions absorbed well anti-S-III and anti-S-I antibodies, respectively.

1. Laskowski, M., *Biochem. Z.*, 1935, v278, 345.
2. Common, R. H., Chapman, D. G., Maw, W. A., *Canad. J. Zool.*, 1951, v29, 265.
3. Hosoda, T., *Igaku to Seibutsugaku, (Med. and Biol.)*, 1952, v23, 9.
4. Hosoda, T., Kaneko, T., Mogi, K., Abe, T., *Poul. Sci.*, 1955, v34, 9.
5. Hosoda, T., *Igaku to Seibutsugaku, (Med. and Biol.)*, 1952, v25, 237.
6. Francis, G. E., *Biochem. J.*, 1952, v51, 715.
7. Francis, G. E., Wormall, A., *ibid.*, 1950, v47, 380.
8. Abe, T., Tanabe, Y., Kaneko, T., Mogi, K., Hosoda, T., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 703.
9. Peter, T., Anfinsen, C. B., *J. Biol. Chem.*, 1950, v186, 805.
10. Higashi, T., *J. Jap. Biochem. Soc.*, 1959, v30, 1037.
11. Hogeboom, G. H., *Methods in Enzymology*, Academic Press, 1955, v1, 16.
12. Tanabe, Y., Abe, T., Hosoda, T., PROC. SOC. EXP. BIOL. AND MED., 1961, v106, 506.
13. Peter, T., Jr., *ibid.*, 1957, v229, 659.
14. Hoagland, H. B., Stephenson, M. L., Scott, J. F., Hecht, L. I., Zamecnik, P. C., *J. Biol. Chem.*, 1958, v231, 241.

Received July 6, 1961. P.S.E.B.M., 1961, v108.

Elevation of Proteolytic Activity in the Pancreas of Hypophysectomized Rats by Hormonal Therapy.* (26902)

B. L. BAKER, H. W. CLAPP, C. R. ANNABLE AND M. M. DEWEY
Department of Anatomy, University of Michigan Medical School, Ann Arbor

Following hypophysectomy a severe reduction in weight of the pancreas occurs in the rat(1) and dog(2). In the rat most acini are partially involuted with loss of cellular size, zymogenic granules and cytoplasmic RNA(3,4,5). Concurrently, the amount of amylase(6) and proteolytic activity(7) is reduced. Experiments aimed at clarifying the hormonal pathway by which the hypophysis exerts its influence over the pancreas have entailed both ablation of other endocrine glands and hormonal replacement therapy of endocrine-deficient animals. These studies reveal that thyroid hormone plays a key role(3,8,9,10,11,12) in maintaining the

* Supported in part by research grants from U.S.P.H.S., Inst. Res. Grant, Am. Cancer Soc., and Upjohn Co.

normal structure of pancreatic acini. Hypophyseal somatotropin(13,14) and adrenocorticosteroids(15,16,17,18) are also implicated. Indeed, administration of combined somatotropin, corticosterone and l-thyroxine to *ad libitum*-fed hypophysectomized rats restores pancreatic weight and histology to an approximately normal condition(19).

Few attempts have been made to restore to normal the content of digestive enzymes in the pancreas of hypophysectomized animals by hormonal therapy. Nishikawara *et al.*(20) found feeding of thyroid tissue to be effective with respect to amylase. This report is concerned with the capacity of combined administration of somatotropin, corticosterone and l-thyroxine (STC) to elevate proteolytic activity (PA) in the pancreas of hypophysectomized rats.

Procedures. Young adult, female Sprague-Dawley rats were fed *ad libitum* a diet consisting of Purina Laboratory Chow supplemented with greens and cod liver oil. After hypophysectomy, a mean of 16 to 25 days elapsed before initiation of hormonal therapy (Table I). The rats were divided into the following groups: (a) hypophysectomized, vehicle - treated, (b) hypophysectomized, hormone-treated, and (c) nonhypophysectomized, vehicle-treated. Somatotropin† was dissolved in distilled water (1.25 mg/ml); corticosterone was suspended in 0.9% NaCl (150 μg/ml) with one drop of Tween 80 being added/10 ml of saline; and sodium l-thyroxine pentahydrate was dissolved in distilled water (60 μg/ml) with one drop of N/10 NaOH per 50 ml of solution being used to facilitate solution of the hormone. Twice daily, 125 μg of the somatotropin, 15 μg of corticosterone and 3 μg of thyroxine were administered together in one injection. One-half unit of insulin was added in Experiment 1 to the treatment of Group 3. Nonhypophysectomized rats received an equal volume of 0.9% NaCl, with Tween 80 and N/10 NaOH being added in the amounts used for dissolving the hormones. Treatment was continued for 14 to 43 days at which time food, but not water, was denied the animals for 24 hours in Exp. 1 and 18 hours in Exp. 2 and 3 prior to termination of experiment. The pancreas was removed while the rats were under sodium amytal anesthesia and dissected free of extraneous fat and connective tissue. Completeness of hypophysectomy was verified by microscopic examination of sections of the pituitary area in all hypophysectomized rats treated with hormones and in the hypophysectomized, vehicle-treated rats which exhibited a questionable loss in body weight.

The procedure for determination of PA (activated trypsinogen, chymotrypsinogen) was described previously(7), and is based on release from a substrate containing hemoglobin(21) of tyrosine-like compounds by pancreatic proteolytic enzymes. The color developed following addition of the Folin-Ciocalteu reagent was determined in a Klett-Summerson colorimeter and PA expressed as mg of tyrosine released per minute at 37°C by reference to a standard curve previously worked out with the pure amino acid. Our previously published procedure was altered in the following ways. The original pancreatic homogenate was diluted to a final concentration of 1 g of pancreas in 1.25 L of physiological saline. The homogenate was brought to pH 5 by addition of 0.1 M phosphate buffer in Exp. 1 and 2 and by addition of acetate buffer in Exp. 3. Five ml aliquots of the homogenate were activated by addition of 0.1 ml (10 μg) of trypsin and warming at 37°C for 24 hours. Trypsin was found to effect complete activation much more consistently than was true of the enterokinase. Nitrogen content of the homogenate was determined by the micro-Kjeldahl method(22) and expressed in relation to wet weight of the gland.

Results. Treatment with STC increased the body weight of hypophysectomized rats to a level comparable with that of the nonhypophysectomized controls (Table I). Similarly, the weight of the pancreas was in-

† We wish to express our appreciation to the Endocrinology Study Section, U.S.P.H.S., for somatotropin (Armour's Somar), to Merck and Co., Inc., for corticosterone, and to Smith, Kline and French Laboratories for sodium l-thyroxine pentahydrate (Elthrin Sodium).

TABLE I. Data on Treatment and Body Weights.

Group	Treatment	No. of rats	Mean days hyp. to treatment	Mean days of treatment	Mean body wt (g) At hyp.	At inj.	Final	Mean wt pancreas (mg)
Exp. 1								
1	Hyp.–veh.	11	24 ± 2*	29 ± 3	174 ± 6	159 ± 3	164 ± 3	516 ± 35
2	Hyp.–STC	9	27 ± 3	31 ± 4	174 ± 5	157 ± 6	239 ± 7	920 ± 43
3	Hyp.–STC–ins.	3	25 ± 5	29 ± 1	188 ± 5	164 ± 6	250 ± 9	1052 ± 19
4	Nonhyp.–veh.	13	—	—	182 ± 4	212 ± 4	240 ± 5	1128 ± 44
							1 *vs* 2 <.001†	1 *vs* 2 <.001
							2 *vs* 4 >.05	2 *vs* 4 <.01
							1 *vs* 4 <.001	1 *vs* 4 <.001
Exp. 2								
5	Hyp.–veh.	4	16 ± 0	21 ± 2	197 ± 3	175 ± 1	174 ± 2	603 ± 21
6	Hyp.–STC	5	16 ± 0	21 ± 2	197 ± 2	183 ± 3	235 ± 1	924 ± 22
7	Nonhyp.–veh.	5	—	—	198 ± 2	214 ± 2	229 ± 4	1049 ± 43
								5 *vs* 6 <.001
								6 *vs* 7 >.05
Exp. 3								5 *vs* 7 <.001
8	Hyp.–veh.	13	22 ± 1	17 ± 1	172 ± 3	153 ± 3	150 ± 2	454 ± 23
9	Hyp.–STC	14	22 ± 1	17 ± 1	172 ± 2	148 ± 3	215 ± 2	840 ± 18
10	Nonhyp.–veh.	14	—	—	170 ± 1	200 ± 2	214 ± 3	930 ± 23
							8 *vs* 9 <.001	8 *vs* 9 <.001
							9 *vs* 10 >.05	9 *vs* 10 <.01
							8 *vs* 10 <.001	8 *vs* 10 <.001

* Stand. error of mean. † Student Fisher "t"; probability of error.

Hyp. = hypophysectomized; STC = somatotropin, corticosterone, thyroxine; veh. = vehicle; ins. = insulin.

creased in hypophysectomized rats but not to non-operated control levels.

The amount of nitrogen per g of pancreas (Table II) was reduced by hypophysectomy in Exp. 3; in Exp. 2 change in the same direction was indicated. Hormone therapy failed to alter the concentration of nitrogen. However, total nitrogen content was reduced by hypophysectomy and increased by hormone treatment.

Total PA was reduced by hypophysectomy and increased significantly by STC over that in hypophysectomized rats although not up to the level observed in nonhypophysectomized rats (Exp. 1 and 3). However, the influence of hypophysectomy and of treatment with STC on concentration of PA varied from experiment to experiment. PA per g of pancreas was reduced by hypophysectomy in Exp. 1 and was increased by STC. When related to nitrogen per g of pancreas, PA was increased by hypophysectomy, these values being restored to the level of those in nonhypophysectomized rats by hormone therapy. This trend was also evident in Exp. 2 and 3 when PA was related to the wet weight of the pancreas.

Addition of insulin (Exp. 1) to the hormone therapy appeared to increase the effectiveness of the treatment in every respect.

Discussion. In a previous study, in which the pancreatic homogenate was activated with enterokinase(7) a reduction in concentration of PA was demonstrated consistently after hypophysectomy, although with the exception of those animals studied 3 days after operation, this decline was not great. Haist(23) observed variable changes in PA after pituitary ablation. In the present study, a reduction was observed only in Exp. 1 when the significance of the difference between the activity in hypophysectomized and nonhypophysectomized rats appeared to arise chiefly from the high values for the latter group. The high level in the controls probably occurred because these animals were deprived of food for a longer time before being killed than was true for the rats in Exp. 2 and 3. Thus, a greater build-up of stored enzyme was permitted in the nonhypophysectomized rats.

The response of pancreatic PA to hypophysectomy differs somewhat from that of amylase. Although total content of both is low-

TABLE II. Effect of Hormone Treatment on Proteolytic Activity and Nitrogen.

Group	Treatment	Nitrogen (mg/g pancreas)	Total nitrogen (mg)	Proteolytic activity (mg tyrosine) Per g pancreas	Total	Per mg nitrogen* × 100
Exp. 1						
1	Hyp.–veh.			8.8 ± .5†	4.6 ± .5	
2	Hyp.–STC			11.6 ± .8	10.6 ± .8	
3	Hyp.–STC–ins.			13.3 ± 1.6	14.1 ±2.0	
4	Nonhyp.–veh.			10.8 ± .5	12.3 ± .8	
	1 *vs* 2			<.01‡	<.001	
	2 *vs* 4			>.05	>.05	
	1 *vs* 4			<.01	<.001	
Exp. 2						
5	Hyp.–veh.	34.4 ± 1.0	20.8 ± 1.0	10.9 ± 1.2	6.5 ±1.0	31.4 ± .3
6	Hyp.–STC	36.7 ± 1.2	34.4 ± 1.6	8.6 ± 2.0	7.9 ± .7	23.4 ± 1.6
7	Nonhyp.–veh.	36.2 ± 1.2	37.7 ± 1.8	8.4 ± .6	8.7 ± .7	23.1 ± 1.4
	5 *vs* 6	>.05	<.001	>.05	>.05	>.05
	6 *vs* 7	>.05	>.05	>.05	>.05	>.05
	5 *vs* 7	>.05	<.001	>.05	>.05	=.05
Exp. 3						
8	Hyp.–veh.	30.6 ± .8	13.8 ± .8	8.4 ± .4	3.8 ± .3	27.5 ± 1.4
9	Hyp.–STC	30.5 ± .8	25.5 ± .8	6.9 ± .5	5.8 ± .5	22.1 ± 1.7
10	Nonhyp.–veh.	34.0 ± .7	31.6 ± 1.1	7.3 ± .5	6.9 ± .5	21.1 ± 1.3
	8 *vs* 9	>.05	<.001	<.05	<.01	<.05
	9 *vs* 10	<.01	<.001	>.05	>.05	>.05
	8 *vs* 10	<.01	<.001	>.05	<.001	<.01

* $\frac{\text{mg tyrosine/g pancreas}}{\text{mg nitrogen/g pancreas}} \times 100.$

† Stand. error of mean.

‡ Student Fisher "t"; probability of error.

Hyp. = hypophysectomized; STC = somatotropin, corticosterone, thyroxine; veh. = vehicle; ins. = insulin.

ered, the concentration of only amylase is reduced(6). Correlated with this evidence of depressed protein synthesis are the depletion of RNA as revealed by basic staining(3) and biochemical analysis(5) and the reduced quantity of zymogenic granules(4).

It is clear that a large measure of restoration of total PA can be effected by administration of somatotropin, corticosterone and thyroxine if the period of treatment is sufficiently prolonged (Exp. 1). Addition of insulin facilitates the effect. It is probable that other hormones, especially from the hypophysis, would augment the restoration further.

Summary. Hypophysectomy of the rat failed to reduce consistently the concentration of PA in the pancreas when computed on the basis of wet weight. When related to mg of nitrogen per g of pancreas the amount of PA was increased. Treatment with STC restored body weight, increased pancreatic weight and total PA. Except when insulin was added to the hormonal regimen, PA did not reach the level observed in the nonhypophysectomized controls.

1. Bryans, F. E., Kinash, B., Ashworth, M. A., Haist, R. E., *Diabetes,* 1952, v1, 358.

2. Koster, S., *Pflüger's Arch. ges. Physiol.,* 1950, v224, 212.

3. Baker, B. L., Pliske, E. C., *Sym. Soc. Exp. Biol.,* The University Press, Cambridge, England, 1957, No. 11, 329.

4. Baker, B. L., *Am. J. Clin. Nutr.,* 1957, v5, 445.

5. Sesso, A., Tramezzani, J. H., Valeri, V., Migliorini, R. H., *Compt. rend. Acad. Sci.,* 1955, v241, 775.

6. Barrett, J. L., Nishikawara, M. T., Haist, R. E., *Am. J. Physiol.,* 1955, v182, 35.

7. Baker, B. L., Reid, N. L., Thoms, N., *ibid.,* 1956, v186, 57.

8. Kojima, M., *Quart. J. Exp. Physiol.,* 1917, v11, 255.

9. Hashimoto, H., *Am. J. Physiol.,* 1922, v60, 357, 365.

10. Hammett, F. S., *Endocrinology,* 1926, v10, 248.

11. Kinash, B., Haist, R. E., *Canad. J. Biochem.,* 1955, v33, 380.

12. Townsend, S. F., Baker, B. L., PROC. SOC. EXP. BIOL. AND MED., 1959, v100, 33.

13. Kinash, B., MacDougall, I., Evans, M. A., Bryans, F. E., Haist, R. E., *Diabetes*, 1953, v2, 112.

14. Sesso, A., Valeri, V., *Exp. Cell Res.*, 1958, v14, 201.

15. Baker, B. L., Abrams, G. D., *The Hypophyseal Growth Hormone, Nature and Actions*, ed.: R. W. Smith, Jr., O. H. Gaebler, C. N. H. Long, Blakiston, N. Y., 1955, 107.

16. Kinash, B., Haist, R. E., *Am. J. Physiol.*, 1954, v178, 441.

17. Sesso, A., Migliorini, R., *Acta Physiol. Latino-Am.*, 1959, v9, 5.

18. Guelzow, M., Trettin, H. J., Diwok, K., *Z. ges. exp. Med.*, 1960, v134, 95.

19. Baker, B. L., *Anat. Rec.*, 1958, v131, 389.

20. Nishikawara, M., Barrett, J., Maykut, M., Sprague, L., Haist, R. E., *Fed. Proc.*, 1954, v13, 105.

21. Glass, G. B. J., Pugh, B. L., Wolf, S., *Rev. Gastroenterol.*, 1951, v18, 670.

22. Hawk, P. B., Oser, B. L., Summerson, W. H., *Practical Physiological Chemistry*, Blakiston, N. Y., 1954, p876.

23. Haist, R. E., *The Hypophyseal Growth Hormone, Nature and Actions*, ed.: R. W. Smith, Jr., O. H. Gaebler and C. N. H. Long, Blakiston, N. Y., 1955, 437.

Received July 7, 1961. P.S.E.B.M., 1961, v108.

Predictability of the Changes in Hematocrit which Follow Repeated Withdrawal of Blood.* (26903)

TAKANORI ARAMAKI AND HAROLD S. WEISS (Introduced by Paul Griminger)
Department of Poultry Science, Rutgers, The State University, New Brunswick, N. J.

A formula was derived for determining hematocrits (Hcts) of successive blood samples on the basis that sampling losses were being exactly replaced by extravascular fluid. When applied to experimental data, good agreement between measured and theoretical Hcts were encountered in several series of tests, as described herein.

Procedure. With the assumption that total blood volume remains constant through replacement of lost cells and plasma by extravascular fluid, it can be shown that the hematocrit of any particular blood sample (Hct_n) in a consecutive series of samples of constant size, is given by the exponential equation:

$$Hct_n = Hct_o \left(1 - \frac{s}{BV} \right)^{n-1}$$

Where:

Hct_o = hematocrit of the initial sample in a series
s = sample size (constant over the series)
BV = total blood volume
n = number of blood samples taken

Hematocrits so calculated were compared to Hcts measured during experiments which required repeated handling and multiple blood samplings in the chicken.

All studies were conducted on unanesthetized adult male chickens of the White Leghorn breed, 12-24 months of age. Hematocrits were measured on heparinized blood samples centrifuged in graduated tubes for 20 minutes at approximately 1500 × g. As the blood volume (BV) required by the formula had not been determined on the birds for which Hcts were available, and as literature values for BV of adult male chickens varies from 9-10%(1) to 5.6 ml/100 g body weight(2), it was necessary to measure this parameter in a group of comparable animals. The method used was basically the T-1824 dilution technic(3), except that the calculations were made on a single sample of wing vein blood taken 2 min. after injection of dye.

Results and discussion. Blood volume. Total BV of 8 chickens weighing 2491 ± 48 g (SE) was determined to be 6.6 ± 0.25 ml (SE) per 100 g body weight (BW), and

* Paper of the Journal Series, N. J. Agric. Exp. Station, Rutgers, The State University, New Brunswick. Supported in part by grants from Commercial Solvents Corp., American Cyanamid Co., and PHS.