by isologous anti-seminal plasma immune serum. 3) Normal rabbit sera fix complement with rabbit spermatozoa. 4) No degenerative damage to rabbit testis or epididymis could be produced by injections of spermatozoa or seminal plasma in Freund's adjuvant.

1. Henle, W., *J. Immunol.*, 1938, v34, 325.
2. Katsh, S., *Am. J. Obstet. & Gyn.*, 1959, v77, 946.
3. Freund, J., Thompson, G. E., Lipton, M. M., *J. Exp. Med.*, 1955, v101, 591.
4. Ruemke, P., *Auto-antilichamen tegen spermatozoen als oorzak van onvruchtbaarheid bij de man.* 1959, Uitgeverij Excelsior S' Gravenhage, Holland.
5. Wilson, L., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 652.
6. ———, *Fert. a. Steril.*, 1956, v7, 262.
7. Ruemke, P., *Vox Sanguinis*, 1954, v4, 135.
8. ———, *Immunopathology, 1st Internat. Sympos., Basel/Seelisberg*, 1958, B. Schwabe & Co., Basel, Switzerland, p145.
9. Rao, S. S., *Conference on Physiol. Mechanism Concerned with Conception*, West Point, N. Y., July, 1959.
10. Weil, A. J., Kotsevalov, O., Wilson, L., PROC. SOC. EXP. BIOL. AND MED., 1956, v92, 606.
11. Pernot, E., *Bull. Soc. Chim. Biol.*, 1956, v38, 1041.
12. Weil, A. J., Finkler, A. E., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 794.
13. Pernot, E., Szumowski, P., *Bull. Soc. Chim. Biol.*, 1958, v40, 1423.
14. Macirone, C., Walton, A., *J. Agric. Soc.*, 1938, v28, 122.
15. Gregoire, A. T., Bratton, R. W., Foote, R. H., *J. Animal Sci.*, 1959, v17, 243.
16. Bishop, D. W., Katsh, S., personal communication.
17. Voisin, G., Delaunay, A., Barber, M., *Ann. Inst. Pasteur*, 1951, v81, 48.
18. Freund, J., Lipton, M. M., Thompson, G. E., PROC. SOC. EXP. BIOL. AND MED., 1954, v87, 408.

Received August 24, 1959. P.S.E.B.M., 1959, v102.

Biological Effects of Hypoglycin A in Hydrocortisone Treated Adrenalectomized Rats.* (25340)

I. RINGLER, L. BORTLE, E. HEYDER, A. MONTEFORTE, J. PERRINE AND E. ROSS
(Introduced by Earl H. Dearborn)
Experimental Therapeutics Research Section, Lederle Labs., Pearl River, N. Y.

Hassall and Reyle(1,2) succeeded in isolating 2 peptides, hypoglycin A and B, from fruit seeds of the tree, *Blighia sapida*, and reported them to cause hypoglycemia in rodents. Subsequent structural analyses revealed hypoglycin A to be an amino acid: cyclopropanepropionic acid, α-amino-2-methylene(3,4,5,6). Several authors reported on pharmacological and biochemical actions of hypoglycin A(7,8, 9,10). Attention in this report is directed to action(s) of hypoglycin A on several parameters of hydrocortisone-induced gluconeogenesis.

Methods. Adult male Sherman rats (140-170 g) were bilaterally adrenalectomized and maintained with 0.9% sodium chloride solution, along with high protein diet *ad libitum*. On fourth post-operative day the food was removed. Sixteen hours later the rats were injected subcutaneously with 1 mg of hydrocortisone suspended in 0.2 ml of a carboxymethylcellulose vehicle. Hypoglycin A was dissolved in water and 0.2 ml of the solution was injected intraperitoneally at time of steroid administration. Control animals received injections of the vehicles. No food or water was allowed during test period. Thirty minutes after initiating treatment, rats were injected intraperitoneally with 5 ml of 0.9% sodium chloride, and 2 rats placed in each metabolism cage. Four hours after steroid or drug administration animals were anesthetized with sodium pentobarbital and livers were excised and hydrolyzed immediately in 30% potassium hydroxide. During removal of liver, blood was drawn from the portal vein and analyzed for glucose by the Nelson method(11). Liver glycogen was determined by the anthrone method of Seifter, *et al.*(12).

* This work was performed in the Pharmacological Research Dept., Dr. Earl H. Dearborn, Head.

TABLE I. Effects of Hypoglycin A on Liver Glycogen, Blood Glucose and Urinary Glucose and Nitrogen in Fasted Adrenalectomized Rats Treated with Hydrocortisone: 4 Hour Assay.

Dose, mg						
Hydrocortisone	Hypoglycin A	No. rats	Liver glycogen, mg/g liver	Blood glucose, mg %	Urine glucose,* mg %	Total urinary nitrogen, mg
		4	2.6 (2.1– 3.3)†	76 (64– 84)	73 (68– 78)	21 (14–29)
1.0		4	8.1‡ (7.0–11.3)	98‡ (88–106)	39 (38– 40)	48 (38–58)
1.0	.125	4	8.1 (7.8– 8.9)	106‡ (98–116)	57 (50– 64)	51 (19–82)
1.0	.25	4	6.2 (3.1– 8.1)	108‡ (102–116)	59 (54– 64)	27 (17–37)
1.0	.50	4	6.0 (4.7– 7.7)	106‡ (102–112)	62 (50– 74)	39 (30–48)
1.0	1.0	4	5.1§ (4.5– 5.6)	105‡ (92–116)	48 (44– 52)	45 (35–55)
1.0	2.0	4	5.4§ (4.7– 6.3)	106‡ (102–112)	56 (50– 62)	49 (44–54)
1.0	4.0	4	10.8 (9.0–14.1)	103‡ (98–106)	61 (52– 70)	47 (42–51)
1.0	8.0	4	2.9§ (1.8– 4.4)	93‡ (84– 98)	91 (62–120)	56 (42–70)
1.0	16.0	4	3.2§ (2.6– 3.9)	72 (60– 82)	70	26

* Total reducing substances as measured by Nelson method(11).
† Mean (range).
‡ $P < .05$ when compared to untreated controls.
§ $P < .05$ " " " hydrocortisone-treated controls.
No gross hypoglycemic symptoms observed.

Glucose was assayed in aliquots of urine by the Nelson method(11), and nitrogen was analyzed by the micromethod of Shepherd, *et al.*(13). Liver glycogen deposition and blood glucose data were statistically analyzed by unpaired replicate ranking procedure of Wilcoxon(14). Analysis of urinary glucose and nitrogen excretion figures was prohibited since there were only 2 observations/group for each parameter.

Results. Rats injected with hydrocortisone alone exhibited a significant increase in liver glycogen ($P<0.05$), blood glucose ($P<0.05$) and urinary nitrogen excretion as compared to untreated controls (Table I). There also was a suggested decrease in urinary glucose levels. Hydrocortisone-stimulated liver glycogen deposition was reduced in animals receiving 1, 2, 8 and 16 mg of hypoglycin A. The aberrant glycogen value recorded for the group injected with 4 mg was unexplainable. Blood glucose concentrations were reduced in rats treated with 16 mg of hypoglycin A. These data suggest that depletion of liver glycogen precedes reduction of blood glucose. Urinary glucose values were apparently unaffected by hypoglycin A. Urinary nitrogen excretion also appeared unaltered.

Discussion. Feng and Patrick(8) reported that the most outstanding change produced by hypoglycin A was a delayed hypoglycemia, preceded by exhaustion of liver glycogen. Hypoglycemic activity also has been demonstrated in adrenalectomized animals(7,9). The present evidence indicates that hypoglycin A is capable of antagonizing the action of hydrocortisone as evidenced by decreases in both liver glycogen deposition and hyperglycemia. It is suggested that this antagonism is not mediated through: 1) a "phlorhizin-like action" since no increase in urinary glucose was observed, or 2) interference with conversion of protein to carbohydrate intermediates inasmuch as urinary nitrogen excretion was, in general, unaltered. These results are consistent with the hypothesis(8) that the primary action of hypoglycin A is the interference with hepatic glycogen production.

Summary. Administration of hypoglycin A (cyclopropanepropionic acid, α-amino-2-methylene) to adrenalectomized male rats reduced the degree of hydrocortisone-induced liver glycogen deposition and hyperglycemia, without altering either urinary glucose or nitrogen excretion.

The authors express their thanks to Dr. R. S. DeRopp and associates of the Biochemistry Dept. for supplies of hypoglycin A, and also to W. Cekleniak for aid in conducting glucose and nitrogen analyses.

1. Hassall, C. H., Reyle, K., *Biochem. J.*, 1955a, v60, 334.
2. ———, *W. Ind. Med. J.*, 1955b, v4, 83.
3. DeRopp, R. S., Van Meter, J. C., DeRenzo, E. C., McKerns, K. W., Pidacks, C., Bell, P. H., Ullman, E. F., Safir, S. R., Fanshawe, W. J., Davis, S. B., *J. Am. Chem. Soc.*, 1958, v80, 1004.

4. Holt v., C., Leppla, W., *Angew. Chem.*, 1958, v70, 25.

5. Wilkinson, S., *Chem. Ind.* (Rev.), 1958, 17.

6. Carbon, J. A., Martin, W. B., Swett, L. R., *J. Am. Chem. Soc.*, 1958, v80, 1002.

7. Chen, K. K., Anderson, R. C., McCowen, M. C., Harris, P. N., *J. Pharm. & Exp. Therap.*, 1957, v121, 272.

8. Feng, P. C., Patrick, S. J., *Brit. J. Pharm.*, 1958, v13, 125.

9. Holt v., C., Leppla, W., *Bull. Soc. Chim. Belg.*, 1956, v65, 113.

10. DeRenzo, E. C., McKerns, K. W., Bird, H. H., Cekleniak, W. P., Coulomb, B., Kaleita, E., *Biochem. Pharm.*, 1958, v1, 236.

11. Nelson, N., *J. Biol. Chem.*, 1944, v153, 375.

12. Seifter, S., Dayton, S., Noric, B., Muntwyler, E., *Arch. Biochem.*, 1950, v25, 191.

13. Shepherd, R. G., Howard, K. S., Bell, P. H., Cacciola, A. R., Child, R. G., Davies, M. C., English, J. P., Finn, B. M., Meisenhelder, J. H., Moyer, A. W., vander Scheer, J., *J. Am. Chem. Soc.*, 1956, v78, 5051.

14. Wilcoxon, F., Some Rapid Approximate Statistical Procedures, Am. Cyanamid Co., Pearl River, N. Y., 1949.

Received August 24, 1959. P.S.E.B.M., 1959, v102.

Adrenal Corticoid Activities of Δ^1, 9α fluoro-hydrocortisone 16α, 17α isopropylidenedioxy.*† (25341)

I. RINGLER, L. BORTLE, E. HEYDER, A. MONTEFORTE, J. PERRINE AND E. ROSS
(Introduced by Earl H. Dearborn)
Experimental Therapeutics Research Section, Lederle Labs., Pearl River, N. Y.

Important chemical transformations which augmented the mineralo- and/or glucocorticoid actions of hydrocortisone include: halogenation at C-6(1), C-9(2) and C-12(3); introduction of a double bond between C-1 and C-2(4); and methylation at C-2(5), C-6 (6) and C-16(7). Bernstein and associates (8-12) dissociated the undesirable sodium-retentive activity of certain potent glucocorticoids by inclusion of 16α hydroxyl group. Fried, *et al.*(13) recently reported that 16α, 17α diol groups of Δ^1, 16α hydroxy, 9α fluoro-hydrocortisone reacted with various aldehydes and ketones to form cyclic acetals and ketals, which exhibit greater glucocorticoid activity than the parent steroid. This report details the biological activities of Δ^1, 9α fluoro-hydrocortisone, 16α, 17α isopropylidenedioxy (triamcinolone acetonide).

Materials and methods. A 3-day bioassay was employed in which both liver glycogen deposition and thymus involution were determined in each of a group of adrenalectomized immature male rats. Urine volume and electrolyte excretion also were measured. Immature male rats of Sherman strain, weighing 40-60 g, were bilaterally adrenalectomized. Animals were maintained with Purina Lab. Chow and 1% NaCl drinking fluid *ad lib.* Twenty-four hours after adrenalectomy the rats were injected subcutaneously or given by gavage graded doses of steroid suspended in 0.2 ml of a modified carboxymethylcellulose vehicle (14). Control animals were injected with vehicle only. One-half hour after administration of the steroid the rats were injected intraperitoneally with 3 ml of 0.9% saline and 3 rats placed in each metabolism cage without food or water. Five hours later urine volume was measured and the cages rinsed with distilled water. The animals were then removed from the metabolism cage and returned to holding racks containing the stock diet and saline drinking fluid. Total milliequivalents of sodium and potassium in urine samples were determined by flame photometry. Steroid was administered daily for the next 2 days. The

* The generic name of this steroid is triamcinolone acetonide. It is currently being marketed as a topical anti-inflammatory agent under trademarks Aristocort® Triamcinolone Acetonide Cream (Am. Cyanamid Co.) and Kenalog® (E. R. Squibb & Sons).

† The authors are indebted to Dr. S. Bernstein and associates for generous supplies of steroids, and J. D. Haynes for statistical analyses.