the lipotropic effect of ethinyl estradiol *plus* methionine appeared to be more pronounced in Fischer rats than seen before[1,2] with rats of genetically different and less homogeneous strains. For instance, in one experiment (Table I, Exp. 24), the average fat content of the liver in the control animals was found to be 33.1%, in the group treated with methionine, 18.7%, whereas in rats treated with methionine *plus* ethinyl estradiol, the perfectly normal figure of 5.7% was reached. It should be kept in mind that in these experiments the animals were pair-fed.

In histological examination§ the testes of rats receiving estrogen showed signs of degeneration with consecutive atrophy. In the control groups the testes exhibited essentially normal histological findings. In female rats receiving estrogens constant estrus was observed in contrast to the control rats and to the animals treated with methionine only, which showed essentially normal estrous cycles.

The exceptionally marked response of rats of the Fischer strain to an alipotropic diet is in good accord with the recently published behavior of the same strain with regard to methionine uptake by liver slices.[5] Compared with an inbred Wistar strain, liver slices obtained from Fischer rats showed a very low uptake of methionine. These observations together with the results here presented should reemphasize the importance of genetic variations in stock laboratory animals when used in this type of experiment.

Conclusions. 1. The fat infiltration of the liver in response to an alipotropic diet is more uniform and more intensive in rats of the inbred Fischer strain than that seen in the past in genetically less homogeneous strains. 2. Estrogen, in particular ethinyl estradiol, when given in combination with methionine, exerts a very marked lipotropic effect with a corresponding reduction of the liver fat to normal values.

§ We are indebted to Dr. Harry Goldblatt (Institute for Medical Research, Cedars of Lebanon Hospital, Los Angeles, Calif.) for the histological examination of the testes.

[5] Rutman, R., Dempster, E., and Tarver, H., *J. Biol. Chem.*, 1949, **177**, 491.

Received July 22, 1949. P.S.E.B.M., 1949, **71**.

17254. Effects of Pituitary Adrenocorticotropic Hormone (ACTH) in Children with Non-Addisonian Hypoglycemia.

IRVINE McQUARRIE, E. G. BAUER, M. R. ZIEGLER, AND W. S. WRIGHT.
From the Department of Pediatrics, University of Minnesota Medical School, Minneapolis

Severe chronic hypoglycemia of the non-Addisonian type presents a difficult etiologic and therapeutic problem, unless by good fortune it is found on direct examination of the pancreas to be due to a removable adenoma of the islet cells in that organ, a comparatively rare pathological condition. Dietary control of the blood sugar level, which may be quite successful in some cases of mild "functional" or "recurrent" hypoglycemia, is ineffectual in the severe persistent type. Surgical removal of as much as 80 to 85% of the histologically normal pancreas, a therapeutic procedure of last resort, is likely to give temporary relief only, as in two members of the series of patients included in the present investigation. Cautious use of alloxan in one severe case of idiopathic hypoglycemia appeared to be successful.[1] Despite its hepatotoxic action, this agent deserves further trial but only under the strictest supervision.

Repeated attempts on our part to force the gluconeogenetic action of cortin to a degree sufficient to control non-Addisonion hypoglycemia, while midly encouraging in a few instances, have been unsuccessful in the main. However, the isolation of pituitary adreno-

[1] Talbot, N. B., Crawford, J. D., and Bailey, C. C., *Pediatrics*, 1948, **1**, 337.

corticotropin (ACTH) in purified form by Li, Evans and Simpson[2] and by Sayers, White and Long[3] and the demonstration of its diabetogenic effects in normal adult subjects by Browne[4] and Conn, Louis and Wheeler[5] encouraged us to resume our earlier attempts to counteract abnormal hypoglycemic reactions by the administration of a long-acting hormonal agent having a physiological action antagonistic to that of insulin.

The investigation on the metabolic and clinical effects of ACTH reported here was carried out first on 2 severely afflicted children (B.G., age 1 year, and her brother J. G., age 4½ years) and subsequently on 3 additional cases, (brothers, R.R., age 1 year, D.R., age 2½ years, and Dern. R., 4½ years of age) of the less severe "recurrent" or "functional" type. The infant, B.G., had manifested such frequent and severe hypoglycemic reactions, including numerous convulsions, that she had been subjected to partial pancreatectomy when the use of a variety of therapeutic measures, including frequent feedings of either a high-protein, low-carbohydrate, or a high-carbohydrate diet, had failed to alleviate her condition. An estimated 80 to 85% of her histologically normal pancreas had been extirpated. Although this radical procedure had resulted in restoration of the fasting blood sugar to normal values with complete relief from hypoglycemic symptoms for about three weeks, the hypoglycemic state had recurred shortly thereafter in a degree of severity almost as marked as that observed before the operation. The older brother, J.G., who had followed a similar metabolic and clinical course over a period of 3 years was only slightly better off than B.G., despite the surgical removal in two successive operations of even a greater proportion of his apparently normal pancreatic tissue. The other three patients had likewise had severe hypoglycemic symptoms including convulsions when for any reason their food intake was reduced.

Methods. In order to insure quantitative collections of all urine and feces as well as accurate accounting of the amounts of the special dietary formula consumed by each patient, special nurses were assigned to the small metabolic ward where the experimental subjects were kept on specially constructed collection frames. The standard semi-liquid diet, calculated to be nutritionally adequate in every respect, contained 42 g of protein, 45 g of fat and 118 g of carbohydrate in each kg. The daily allowance, adjusted to the caloric needs of the individual subject, was given in three equal meals shortly after 7:00 A.M., 1:00 P.M., and 7:00 P.M.

Fasting blood samples for glucose and eosinophil cell counts were obtained routinely at 7:00 A.M. every day or every second day throughout the entire period of study. The potassium and inorganic phosphorus of the serum were determined at the end of each major period. The 24-hour urine samples were analysed while fresh for total nitrogen, uric acid, creatinine, phosphorus, chloride, sodium and potassium throughout the entire period of study and for 11-oxycorticosteroids and 17-ketosteroids during the last two days of the control and ACTH periods. When successfully collected, stools for each major period were analysed for N, P, Cl, Na and K. Standard analytical methods were employed for the various substances named. The "true" blood glucose was determined by Nelson's[6] modification of the Shaffer-Somogyi[7] method, according to which values below 50 mg/100 ml are regarded as hypoglycemic.

In each experiment ACTH* was administered intramuscularly in equal doses (either 9 or 10 mg equivalent to Armour standard preparation LA-1-A) every six hours over a period of 4 days. This test period was preceded by a control- or pre-period of several days length and was followed by a post-

[2] Li, C. H., Simpson, M. E., and Evans, H. M., *J. Biol. Chem.*, 1943, **149**, 413.

[3] Sayers, G., White, A., and Long, C. N. H., *J. Biol. Chem.*, 1943, **149**, 425.

[4] Browne, J. S. L., Josiah Macy Jr. Foundation Report, New York, June, 1943.

[5] Conn, J. W., Louis, L. H., and Wheeler, C. E., *J. Lab. Clin. Med.*, 1948, **33**, 651.

[6] Nelson, N., *J. Biol. Chem.*, 1944, **153**, 375.

[7] Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 695.

* Supplied by the Armour Laboratories through the courtesy of Dr. John R. Mote, Medical Director.

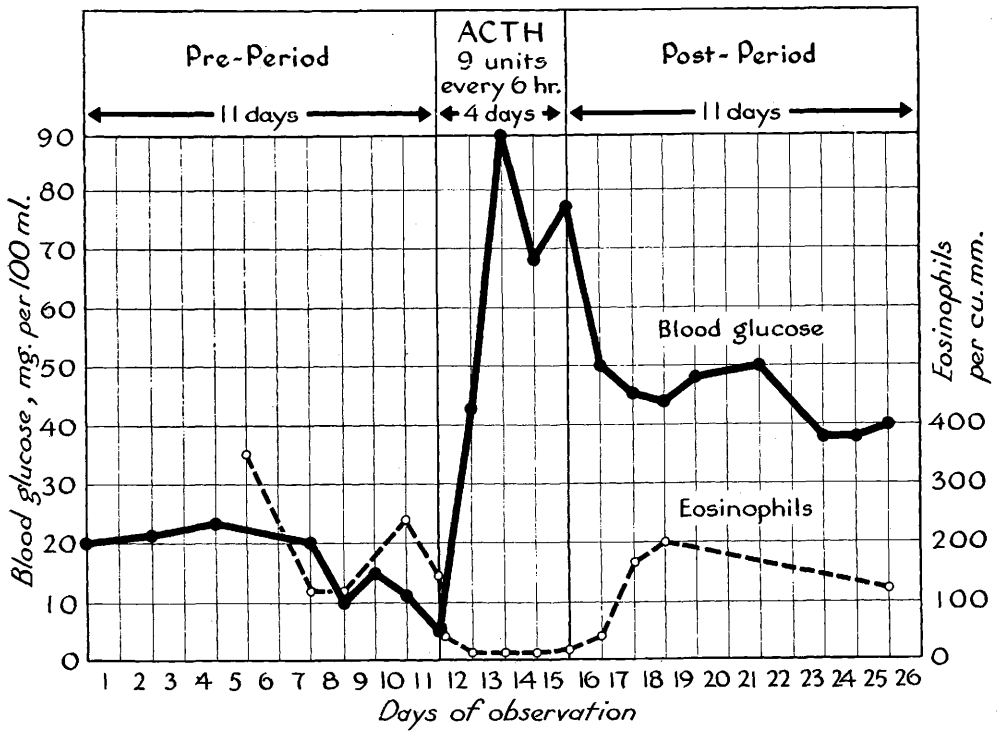

FIG. 1.
Effects of pituitary adrenocorticotrophic hormone (ACTH) on fasting blood glucose and eosinophil count in B.G.

period of similar length. Sugar tolerance tests were performed in the pre-period and again during the latter part of the ACTH period in all cases. In this test blood glucose was determined immediately before and fifteen minutes, 1 hour, 2 hours, 3 hours, 4 hours and 5 hours after termination of the intravenous infusion of a 25% solution of glucose (0.5 g glucose per kg of body wt.). The insulin sensitivity test was carried out by the "insulin-with-glucose" tolerance test proposed by Engel and Scott[8] on the last three patients studied. The ability of the pituitary gland to elaborate or to release ACTH was evaluated in these 3 patients by means of the test proposed by Recant, Forsham and Thorn,[9] according to which a marked decrease in the eosinophil count 4 hours following the intravenous infusion of epinephrine indicates a normal response. The dosage of epinephrine was adjusted to the size of the child. R.R. was given 0.3 mg in 50 ml of saline solution; D.R. 0.4 mg in 60 ml, and Dn. R. 0.5 mg in 70 ml, the infusion running one hour.

Results. The experimental data on the 5 subjects with non-Addisonian hypoglycemia included in this study indicate that their responses to ACTH administration are similar in type to those reported for normal adult subjects.[5,10] Severe hypoglycemia and attendant symptoms were completely abolished during the period of intensive hormone administration and for at least a week after its withdrawal. The sharp fall and the abnormally prolonged low level of blood sugar in the glucose-tolerance curve, which represented the characteristic response of each of the patients during the control period, were

[8] Engel, F. E., and Scott, J. L., *Proc. Amer. Soc. Clin. Invest.*, Atlantic City, N. J., May 2, 1949, p. 17.

[9] Recant, L., Forsham, P. H., and Thorn, G. W., *J. Clin. Endocrin.*, 1948, **8**, 589.

[10] Forsham, P. H., Thorn, G. W., Prunty, F. T. G., and Hills, A. G., *J. Clin. Endocrin.*, 1948, **8**, 15.

found to be absent during the period of ACTH administration, the curve becoming essentially normal. None of the 5 patients showed sugar in the urine at any time during the entire period of study, except for the mild glycosuria which occurred at the height of the glucose tolerance test made in the ACTH period. Insulin hypersensitivity was likewise counteracted to a large extent by the ACTH in the cases tested. The eosinophil cell counts 4 hours after infusion of epinephrine averaged 31/cmm compared to the average initial count of 133. This 79% fall was interpreted as indicating normal capacity on the part of the pituitary to produce ACTH in the 3 cases tested.

The eosinophil cells of the blood fell precipitately from the normal range (between 100 and 350/cmm) to between 0 and 10/cmm within 4 hours after ACTH was first administered and remained at this low level so long as the hormone was given at 6-hour intervals. While the fasting blood sugar during the post-period tended to remain for a number of days at levels intermediate between the hypoglycemic values of the pre-period and the normal values of the ACTH period, the eosinophils returned to their original normal counts fairly promptly, usually within a day. Responses of the blood glucose and eosinophil cell count to ACTH administration are illustrated in Fig. 1 which presents data obtained on B.G., the most severe case in the series. Changes in the other four cases were very similar to these. The only untoward effects of the ACTH were a transient vasopressor reaction after each injection and a moderate tendency to oliguria during the first day or two of intensive administration. These effects appear to be due to contamination with posterior pituitary hormones.

During a follow-up period of 94 days subsequent to the "post-period" shown in Fig. 1, ACTH was administered to B.G. in a dose of 18 mg once every 2 days. The fasting blood sugar was then determined at the end of each 48-hour period, when it would presumably be at its lowest level. The values so obtained were found to range between 40 and 68 mg/100 ml throughout the period, except for a few days when the patient refused part of her diet because of an upper respiratory infection. Two of the morning values at that time were 24 and 16. Her diet, which was unrestricted, was taken avidly throughout the remainder of the period and the gain in weight was satisfactory. She learned to walk alone in the interval. No clinical signs of hypoglycemia or of toxic side effects were observed at any time. On the contrary, the ACTH appeared to be almost as specific for the control of this severe hypoglycemic disorder as insulin is for the control of diabetes mellitus.

The urinary excretion of 11-oxycorticosteroids and 17-ketosteroids was increased as a result of the ACTH injections by percentages ranging between 75 and 400. At the same time, the uric acid excretion increased 50 to 100%. Whereas intensive administration of ACTH has been reported to induce a negative nitrogen balance in most normal adult subjects, a positive balance was maintained in all periods in these very young, growing subjects, as long as the full diet was taken. However, the magnitude of the positive balance was less during the period of intensive ACTH administration than during the pre- and post-periods. In the one patient, B.G., whose stool, as well as urine, analyses have been completed up to date, the phosphorus balance was likewise slightly positive in all periods. The size of the positive balance during the ACTH period, however, was less than that for the fore-period and the after-period. Sodium and chloride showed no significant tendency to increased retention during the ACTH period, but showed a fairly marked increase in excretion in the post-period. The comparatively low NaCl content of the diet may account in part for the low degree of retention. Potassium showed a small negative balance during the ACTH period but positive balances for the pre-period and the post-period.

There were small but consistent changes in the potassium and inorganic phosphorus of the blood serum of all 5 of the subjects as a result of ACTH administration. In every instance both elements fell to lower levels. The fasting K values, which were slightly elevated in 3 and near the upper limit of normal in 2 of

the cases, were decreased from an average of 5.80 meq/1 in the pre-period to 5.06 meq/1 at the end of the ACTH period. At the same time the P was decreased from an average for the group of 3.03 to 2.79 meq/1. These decreases in serum K and P are interpreted as an indication of glycogen deposition during the ACTH period rather than being due to increased excretion. The magnitude of the decrease in retention of these two elements appeared to be far too small to influence blood composition.

The mucoprotein and the non-specific hyaluronidase inhibitor of the serums of the patients were both found by our colleagues, R.A. Good, V. C. Kelley, and D. Glick, to be increased at the end of the ACTH period by nearly 100% of the control levels. These elevations were transient, however, essentially normal values being found 4 days after withdrawal of the hormone.

Summary. The effects of ACTH on the fasting blood sugar level and glucose tolerance test; on the potassium and inorganic phosphorus content of the serum; on the nitrogen, phosphorus, chloride, sodium and potassium balances; on the urinary excretion of uric acid, creatinine and adrenal corticosteroids and on the blood eosinophil counts were determined in 5 young children with non-Addisonian (familial) hypoglycemia. The type of response to ACTH was similar in all respects to that of the normal adult. However, under the conditions of this experiment, instead of producing a transient state of diabetes mellitus, as it does in the normal subject, the ACTH appeared merely to reverse the hypoglycemic tendency, with return of the fasting blood sugar level and the glucose tolerance curve to normal. While the eosinophil count returned to normal promptly upon withdrawal of ACTH, the blood sugar remained above the threshold for hypoglycemic reactions for at least 10 days without ACTH in the most severe case in the series (Fig. 1). Administration of 18 mg of ACTH in one dose every 48 hours thereafter served to maintain this one-year-old patient in an essentially non-hypoglycemic state for more than three additional weeks. Results of the study suggest that ACTH may prove to be as effective in the control of this non-Addisonian hypoglycemic disorder as insulin is in the control of diabetes mellitus.

Received June 10, 1949. P.S.E.B.M., 1949, **71**.

17255. Prevention of Chemotherapeutic Effects of 4-Amino-N^{10}-Methyl-Pteroylglutamic Acid on Mouse Leukemia by Pteroylglutamic Acid.*

J. H. BURCHENAL,† M. N. KUSHIDA, S. F. JOHNSTON AND M. A. CREMER. (Introduced by C. P. Rhoads.)

From the Section on Leukemia, Division of Experimental Chemotherapy, Sloan-Kettering Institute for Cancer Research, New York City.

Certain derivatives of pteroylglutamic acid (PGA) which have in common the substitution of an amino group in the 4 position of the pteridine ring have a demonstrable effect against some strains of transplanted mouse leukemia[1-3] and against solid tumors in mice

* This investigation was supported, in part, by a research grant from The National Cancer Institute of The National Institute of Health, United States Public Health Service, and, in part, by a research grant from The American Cancer Society.

† Fellow of The American Cancer Society, recommended by the Committee on Growth of The National Research Council.

1 Burchenal, J. H., Burchenal, J. R., Kushida, M. N., Johnston, S. F., and Williams, B. S., *Cancer*, 1949, **2**, 113.

2 Law, L. W., *J. Nat. Can. Inst.*, in press.

3 Burchenal, J. H., Johnston, S. F., Burchenal, J. R., Kushida, M. N., Robinson, E., and Stock, C. Chester, PROC. SOC. EXP. BIOL. AND MED., 1949, **71**, 381.