Leucine-sensitive Hypoglycaemia Treated with Diazoxide

B. D. BOWER*, P. H. W. RAYNER, and L. STIMMLER†

*From the Department of Paediatrics and Child Health, University of Birmingham,
and the Children's Hospital, Birmingham*

It has been estimated that about one-third of children with so-called idiopathic hypoglycaemia are leucine sensitive (Mabry, Di George, and Auerbach, 1960). However, the total number of leucine-sensitive cases reported is still relatively small. In 1960, Di George and Auerbach knew of no more than 30 cases and only a few have been reported since then. We therefore describe two further cases, one of which has been followed up for five years. All authors are agreed that until recently treatment has been difficult. However, the advent of diazoxide, a hyperglycaemic benzothiadiazine derivative, may in future make treatment easier. Our two patients were given this drug and we report the clinical results together with observations of its effect upon blood insulin levels.

Clinical Details and Preliminary Investigation

Case 1. He is the second child in a family of three, the other children being normal. He was born at term following an easy forceps delivery and the neonatal period was normal. At the age of 2 months he developed convulsions. Initially these took the form of typical infantile spasms of the flexor type; later minor fits occurred consisting of episodes in which he became quiet and limp. Although he first smiled at 4 weeks, subsequent progress was slow and at the age of 5 months, when first seen at the Birmingham Children's Hospital, head control and visual fixation were poor. A convulsion occurred during his first out-patient attendance during which a blood sugar estimation was 54 mg./100 ml. He was admitted to hospital. Further investigations at that time showed: developmental quotient (DQ), 35; EEG, grossly and diffusely abnormal and epileptic hypsarhythmia; blood calcium 9.9 mg./100 ml., and skull x-ray examination normal.

The significance of the slightly low blood sugar was not appreciated at the time. It was later noticed that he was unrousable for periods after feeds. Blood sugar estimations 30 minutes after feeds gave values of 30 and 31 mg./100 ml. This suggested leucine sensitivity as a

cause for these hypoglycaemic episodes, and the diagnosis was confirmed by an oral leucine sensitivity test (Table). This showed a pronounced fall in the blood sugar to 25 mg./100 ml. at 45 minutes, when a convulsion occurred. Treatment consisted of reducing the leucine content of his diet together with added glucose supplements. He had previously been fed with a proprietary dried cows' milk (Ostermilk No. 2). The leucine content of another proprietary preparation (S.M.A. Wyeth) is only 40% of cow's milk and he was accordingly fed with this preparation, but large quantities of added glucose (120 g./day) were required before the hypoglycaemic episodes were satisfactorily controlled. His protein intake at this stage was only 12 g./day.

TABLE
Oral Leucine Sensitivity Test

Time	Blood Sugar (mg./100 ml.)
30 minutes before leucine	87
0 minute	83
L-leucine (150 mg./kg. orally)	
15 minutes	62
30 minutes	29
45 minutes	25
60 minutes	28
90 minutes	44
120 minutes	74

At the age of 13 months his protein intake was increased to 20 g./day, without any hypoglycaemic episodes occurring. Examination showed developmental retardation but no focal neurological abnormalities. During the following year he remained well but episodes of loss of consciousness occurred intermittently following foods such as eggs or cheese, or after physical exertion. His weight gain and musculature were poor. At the age of 2 years his height was on the 10th centile, having fallen from the 25th centile, and his weight was on the 3rd centile. An attempt was therefore made to increase his protein intake. However, on a diet containing 40 g. protein per day, post-prandial blood sugars fell to 35-40 mg./100 ml. despite the addition of 100 g. glucose per day. A further leucine sensitivity test was performed. From a fasting blood sugar of 55 mg./100 ml. the level fell rapidly, and the test was discontinued at 60 minutes when the blood sugar had reached 27 mg./100 ml. Blood insulin estimations were performed: in the fasting

Received October 24, 1966.

* Present address: Radcliffe Infirmary, Oxford.

† Present address: Guy's Hospital, London.

state the value was $6 \mu\text{U/ml}$. and at 60 minutes $80 \mu\text{U/ml}$. Dietary management was continued, and at the end of a further two years he was able to tolerate a normal diet, but glucose supplements were still required with and between each meal. He became drowsy and occasionally unconscious if more than one egg was eaten each day. At the age of 4 years he was admitted for treatment with diazoxide.

Case 2. This girl was the second in a family of two, with an uneventful birth and perinatal history. When 3 weeks of age she became drowsy and reluctant to feed and episodes of arm twitching developed at intervals of 2-4 hours. Within a few days the episodes increased in frequency and generalized convulsions occurred. Investigation revealed a normal blood sugar of 90 mg./100 ml. , but on lumbar puncture the CSF sugar was only 6 mg./100 ml. Treatment with phenobarbitone, phenytoin, and pyridoxine was ineffective in controlling the convulsions. Within one week, signs of cerebral damage in the form of hypertonia and increased deep tendon reflexes were found. The EEG was grossly and diffusely abnormal and epileptic.

At the age of 7 weeks she was transferred to the Birmingham Children's Hospital for further investigation. Physical examination confirmed the presence of early spastic cerebral palsy. The blood sugar value before a feed of 5% dextrose was 38 mg./100 ml. and 30 minutes after the feed it was 65 mg./100 ml. Blood sugar before a milk feed was 32 mg./100 ml. , but 30 minutes after the feed the value was only 17 mg./100 ml. These results were highly suggestive of leucine-sensitive hypoglycaemia. A leucine sensitivity test was performed as described below (Fig. 1). The result demonstrates a fall in blood sugar level coincident with a dramatic rise in serum insulin levels. During the test no clinical symptoms of hypoglycaemia occurred. Frequent convulsions occurred following cows' milk feeds but satisfactory control of hypoglycaemia was established with feeds of S.M.A. (Wyeth) and glucose supplements. However, 60 g. of additional glucose were required each day to prevent post-prandial hypoglycaemia.

Effects of Diazoxide Therapy

Our previous experience had shown that a leucine sensitivity test performed in the fasting state could produce profound hypoglycaemia with symptoms. To avoid this a modified test was devised. Preliminary experiments showed that a continuous intragastric infusion of $100 \text{ mg. glucose/min.}$ could after an equilibration period of approximately two hours produce a constant blood sugar level. L-leucine $150 \text{ mg./kg. body weight}$ was then given intragastrically, and blood sugar estimations were performed at 15-minute intervals during the first hour and at 30-minute intervals for the following hour. Serum insulin levels were assayed at 15-minute intervals during the first hour. The blood sugar was estimated by a modified Folin and Wu method and the serum insulin by immuno-chemical assay (Morgan and Lazarow, 1963).

Case 1. A leucine sensitivity test was performed

FIG. 1.—Oral leucine sensitivity test (Case 2). Continuous intragastric infusion of glucose 100 mg./min.

before starting treatment at age 4 years. The results are shown in Fig. 2. A definite hypoglycaemic response was detectable and was coincident with a rise in the serum insulin levels. Diazoxide in a dose of $12 \text{ mg./kg. body weight}$ was started and the hyperglycaemic effect was assessed by measuring daily blood sugar levels before and after the midday meal. During the first 3 weeks of therapy a steady rise in post-prandial levels was demonstrated. At the start of this period the level was $65-75 \text{ mg./100 ml.}$, and at the end it was $100-110 \text{ mg./100 ml.}$ At the same time it proved possible to reduce the quantity of glucose added to his diet from 20 g. to 5 g. with each meal. After 3 weeks of therapy with diazoxide a further leucine sensitivity test was performed (Fig. 3). Unfortunately the intragastric tube blocked 13 minutes after the leucine was given and this occurrence contributed to the hypoglycaemia effect observed, the blood sugar falling from 108 mg./100 ml. to 58 mg./100 ml. at 30 minutes. However, the insulin response following leucine was much reduced, a rise of only two units being recorded.

Case 2. At the age of 3 months diazoxide, in a dose of 12 mg./kg. was started and the hyperglycaemic

FIG. 2.—Oral leucine sensitivity test (Case 1). Continuous intragastric infusion of glucose 100 mg./min.

FIG. 3.—Oral leucine sensitivity test (Case 1) after three weeks of diazoxide therapy. Continuous intragastric infusion of glucose 100 mg./min.

FIG. 4.—Post-prandial (30 min.) blood sugar levels before and after diazoxide therapy.

FIG. 5.—Oral leucine sensitivity test (Case 2) after 10 weeks of diazoxide therapy. Continuous intragastric infusion of glucose 100 mg./min.

response was followed by daily blood sugar levels before and after the midday feed. A satisfactory response was soon apparent (Fig. 4), and it subsequently proved possible to reduce the glucose supplements from 60 g. to 15 g./day. After 10 weeks of therapy, during which time no convulsion occurred, a further leucine sensitivity test was performed. The result is shown in Fig. 5. This clearly demonstrates the lack of any hypoglycaemia or rise in blood insulin following leucine administration.

These results show that diazoxide can inhibit leucine-induced hyperinsulinism. In both patients diazoxide administration has been continued. Their dietary management has been much easier than it was formerly and neither patient has had any symptoms of hypoglycaemia during six and seven months of therapy, respectively.

Side-effects of diazoxide therapy. The recognized side-effects of diazoxide include gastric irritation, dependent oedema, postural hypotension, supraventricular tachycardia, hyperuricaemia, and hirsutism. The majority of these have occurred in adult patients who were receiving diazoxide therapy for hypertensive disease. The close similarity of diazoxide to other benzothiadiazine derivatives suggest the possibility of bone-marrow depression.

In our experience the drug has been well tolerated, the only gastro-intestinal upset which occurred was a short period of diarrhoea in Case 2, which may have been due to an intercurrent infection. It subsided without stopping treatment. A transient macular rash occurred in Case 1 shortly after treatment had been started, and it faded with the addition of an antihistamine drug. No haematological abnormalities have occurred and liver function tests and serum uric acid estimations have remained within normal limits.

The most prominent side-effect observed has been hirsutism. Case 1 displayed increasing hirsutism after the first two months of therapy, a thick growth of hair developing over the forehead, eyebrows, extremities, and in particular over the lumbo-dorsal spine (Fig. 6). This reached proportions which the child's parents found distressing and necessitated a reduction in dosage to 5 mg./kg. At this level the hirsutism has not progressed. The hirsutism did not involve the pubic or axillary regions and no features to suggest any acceleration of pubertal development have been noted. Case 2 developed a fine downy growth of hair over the shoulders and spine which has not increased. Wilson, Stone, Okun, and Russell (1964) reported the development of hirsutism in adult women treated with diazoxide: the distribution was similar to that in Case 1. It was not associated with any increase in urinary 17-ketosteroid excretion.

Unfortunately hypoglycaemic brain damage had already occurred in both patients before diazoxide treatment was started. In Case 1 this was less severe than at first feared. At 4 years and 10 months intelligence testing on the Terman Merrill scale showed an intelligence quotient (IQ) of 93. At the age of 7 months Case 2 showed signs of cerebral damage in the form of

generalized hypertonia and hyperreflexia. She was microcephalic (skull circumference 40 cm. ($15\frac{7}{8}$ in.)) with a mental age of 3 months (Griffiths Mental Development Scale).

FIG. 6.—Hirsutism after two months of diazoxide therapy (Case 1).

Discussion

The nature of the metabolic defect in leucine sensitivity is still incompletely understood. An important step in the understanding of the cause of the hypoglycaemia was the finding of increased serum insulin levels in leucine-sensitive patients following the oral administration of leucine (Payne and Woolf, 1959; Yalow and Berson, 1960). From the experiments of Butterfield, Whichelow, Wright, and Woolf (1960), it seems clear that leucine has no direct effect on the glucose uptake of peripheral tissues in patients sensitive to this amino acid. It has been shown that sulphonylureas produce a decrease in the plasma sugar level by direct stimulation of beta-cell insulin production. Pre-treatment with chlorpropamide has induced leucine-sensitive hypoglycaemia in normal subjects as a result of excessive insulin secretion (Fajans, Knopf, Floyd, Power, and Conn, 1963). It seems likely, therefore, that the excessive insulin secretion in leucine-sensitive hypoglycaemia results from the stimulation of the pancreatic beta-cells. Histological reports in

these cases have been few, but the finding of beta-cell degranulation (Cochrane, Payne, Simpkins, and Woolf, 1956) or hyperplasia (Rosenthal, Metz, and Pirani, 1964) suggests a state of prolonged secretory stimulation.

The majority of cases of leucine-sensitive hypoglycaemia recorded to date have shown moderately severe mental retardation, and microcephaly is a common feature. The only recorded case with normal development is a child described by Griese and Wenzel in 1965, who was developmentally normal at 2 years of age. Gentz, Lehmann, and, Zetterström (1962) suggest that brain atrophy may be more common in leucine-sensitive hypoglycaemia than in other varieties. The hypoglycaemia is likely to be most profound in the neonatal period or early infancy at a time when the developing brain is most susceptible to hypoglycaemic damage. Prevention of cerebral damage, therefore, demands prompt recognition of the condition and effective control of further hypoglycaemia. Earlier diagnosis will follow the increasing awareness of this condition which is now apparent. The modified leucine sensitivity test, as described, should prevent significant hypoglycaemia during diagnosis.

The treatment of this condition has previously been based on dietary principles. By feeding a diet low in L-leucine together with frequent carbohydrate supplements, attacks of hypoglycaemia can be reduced. Human breast milk has a leucine content of only 40% of cows' milk. A proprietary preparation, S.M.A. (Wyeth), has a leucine content similar to human breast milk and this preparation provides a suitable milk feed in infancy. Leucine is an essential amino acid. Rose, Wixom, Lockhart, and Lambert (1955) suggest that for a normal healthy adult the safe (twice minimal requirements) intake is approximately 2.2 g./day of leucine and 1.4 g./day of isoleucine. For growing children, however, the requirements may be several times greater. To control hypoglycaemia in many leucine-sensitive children, daily intakes considerably below optimal growth requirements may be needed, leading to suppression of growth (Rosenthal *et al.*, 1964). This difficulty is well illustrated by Case 1 in which a daily protein intake of 20 g. proved inadequate for optimal growth but barely capable of controlling post-prandial hypoglycaemia.

In face of this difficulty several authors have reported attempts to control the hypoglycaemia with other therapeutic measures. Adrenocortical hormones, either as adrenocorticotrophin (ACTH) or synthetic steroid preparations have been commonly employed. At first sight their hyperglycaemic effect would appear to provide a useful approach, but this

results largely from the stimulation of gluconeogenesis, and the subsequent increase of free serum amino acids including leucine may in fact aggravate hypoglycaemia. Frequent hypoglycaemic convulsions occurred in a case described by Griese and Wenzel (1965) despite large doses of ACTH gel. Mabry *et al.* (1960) treated a patient with ACTH and adrenal steroids in a sufficiently large dosage to produce a severe Cushingoid state without control of the hypoglycaemia. In addition, long-term steroid therapy may suppress growth.

Long-acting preparations of glucagon may be of value, but regular injections are necessary and the regulation of dosage is difficult and variable. Griese and Wenzel (1965) report the successful use of a long-acting epinephrine, but 8-hourly subcutaneous administration was required, the dose being regulated by the blood sugar level. Several authors advocate pancreatectomy as a means of controlling the hypoglycaemia if other measures are unsuccessful (Grumbach and Kaplan, 1960; Koop, Hamilton, Baker, and Kaye, 1966). This is an operation with an appreciable mortality and morbidity and was unsuccessful in one of Cochrane's cases (Cochrane *et al.*, 1956) and in the case described by Griese and Wenzel (1965).

Human growth hormone (HGH) has also been used to treat hypoglycaemia of organic origin in adults (Mahon, Mitchell, Steinke, and Raben, 1962) and 'idiopathic hypoglycaemia of infancy' (Soyka, Molliver, and Crawford, 1964). However, the use of HGH is limited by its scarcity and by other hormonal side-effects, so that prolonged treatment is not feasible.

Thiazide derivatives, the first of which, chlorothiazide, was introduced in 1957, have proved satisfactory diuretics and mild hypotensive agents. The search for a more potent hypotensive agent led to the preparation of a benzothiadiazine derivative, diazoxide (3 methyl 7 chloro 1-2-4 benzo thiadiazine 1-1 dioxide), in the laboratories of the Schering Corporation, New Jersey (Rubin, Roth, Winbury, Topliss, Sherlock, Sperber, and Black, 1961). Disturbed carbohydrate tolerance following therapy with thiazide diuretics was described by Wilkins (1959) and Freis (1959) who found that these agents caused hyperglycaemia in some hypertensive patients. Dollery, Pentecost, and Samaan (1962) used diazoxide in combination with an ordinary thiazide diuretic to treat two hypertensive patients. In both patients diabetes mellitus developed acutely within four weeks of the start of therapy. Serum insulin studies in one of the patients after the onset of diabetes showed that the insulin-like activity was low, suggesting that the diabetes was caused by an

inhibitory action on the pancreatic beta-cells. In both patients the level returned to normal within two weeks of discontinuing treatment. Samaan, Dollery, and Fraser (1963) later confirmed a lowering of serum insulin-like activity in four patients with diabetes mellitus precipitated or worsened by benzothiadiazine derivatives. The inhibition of insulin release following treatment with diazoxide and trichlormethiazide was also confirmed by Seltzer and Allen (1965).

A drug capable of inhibiting insulin release, such as diazoxide, has obvious application in leucine-induced and other hypoglycaemic states which are associated with excess insulin production. Ernesti, Mitchell, Raben, and Gilboa (1965) reported the successful use of diazoxide to control hypoglycaemia in a 76-year-old woman with an islet cell tumour of the pancreas. Wolff, Nabwangu, Staquet, Viktora, Yabo, and Zarday (1965) report its use in treating hypoglycaemia occurring in association with adenocarcinoma, islet cell tumour, and of 'functional' origin. Drash and Wolff (1964) used diazoxide in treating a 4-year-old boy with leucine-sensitive hypoglycaemia who had previously failed to respond to ACTH corticosteroids, testosterone, human growth hormone, and partial pancreatectomy. Following therapy with diazoxide (4 mg./kg.) a steady rise in post-prandial blood sugar occurred and the patient was able to tolerate a 26-hour fast.

In our experience diazoxide has proved an advance in treatment of this condition. Before its use in Case 1 dietary management had been difficult and the child's parents had found strict dietary control difficult to achieve. No episodes of hypoglycaemia have occurred in either case during diazoxide therapy.

The occurrence of hirsutism may be alarming to both parents and physician. In Case 1 we felt that its extent justified a reduction in dosage. The effects of hypoglycaemia become less damaging with increasing age, and his progress suggests that therapy may eventually be discontinued. During infancy, however, the risks of hypoglycaemia outweigh the emotional effects of hirsutism.

A drug which is related to compounds known to cause bone-marrow depression must be prescribed with caution and though no haematological effects have been observed in this study a careful watch must obviously be maintained. The precise place of diazoxide in the treatment of this serious condition will only become clear with further experience of patients treated with it.

Summary

Two cases of leucine-sensitive hypoglycaemia are

described. Both cases showed raised serum insulin levels during leucine-induced hypoglycaemia. Treatment with diazoxide, a hyperglycaemic benzothiadiazine derivative, resulted in inhibition of the hyperinsulinism and satisfactory control of the hypoglycaemia.

Diazoxide represents an advance in the treatment of this condition.

We are grateful to the following for their help in the investigation of these patients; Professor P. J. Randle, the late Mr. Harold Salt, Dr. D. N. Raine, Dr. Pat Hughes, and Mr. W. T. Simpson of Allen & Hanburys Ltd., who supplied the diazoxide; and members of the nursing staff, particularly Sister Joan Woodward. We thank Dr. H. J. W. Fisher for allowing us to investigate Case 2.

REFERENCES

- Butterfield, W. J. H., Whichelow, M. J., Wright, P. H., and Woolf, L. I. (1960). Hypoglycaemic response to leucine in a leucine-sensitive man. *Nature (Lond.)*, **188**, 70.
- Cochrane, W. A., Payne, W. W., Simpkins, M. J., and Woolf, L. I. (1956). Familial hypoglycemia precipitated by amino acids. *J. clin. Invest.*, **35**, 411.
- Di George, A. M., and Auerbach, V. H. (1960). Leucine-induced hypoglycemia: a review and speculations. *Amer. J. med. Sci.*, **240**, 792.
- Dollery, C. T., Pentecost, B. L., and Samaan, N. A. (1962). Drug-induced diabetes. *Lancet*, **2**, 735.
- Drash, A., and Wolff, F. (1964). Drug therapy in leucine sensitive-hypoglycemia. *Metabolism*, **13**, 487.
- Ernesti, M., Mitchell, M. L., Raben, M. S., and Gilboa, Y. (1965). Control of hypoglycaemia with diazoxide and human growth hormone. *Lancet*, **1**, 628.
- Fajans, S. S., Knopf, R. F., Floyd, J. C., Jr., Power, L., and Conn, J. W. (1963). The experimental induction in man of sensitivity to leucine hypoglycemia. *J. clin. Invest.*, **42**, 216.
- Freis, E. D. (1959). Clinical pharmacology and use of chlorothiazide in the treatment of hypertension. In *Hypertension: The First Hahnemann Symposium on Hypertensive Disease*, ed. J. H. Moyer, p. 545. Saunders, Philadelphia.
- Gentz, J., Lehmann, O., and Zetterström, R. (1962). Studies on leucin-induced hypoglycemia. *Acta paediat. (Uppsala)*, **51**, 169.
- Griese, G. G., and Wenzel, F. J. (1965). Leucine-sensitive hypoglycemia treated with long-acting epinephrine. *Pediatrics*, **35**, 709.
- Grumbach, M. M., and Kaplan, S. L. (1960). Amino acid and alpha-keto acid-induced hyperinsulinism in the leucine-sensitive type of infantile and childhood hypoglycemia. *J. Pediatr.*, **57**, 346.
- Koop, C. E., Hamilton, J. P., Baker, L., and Kaye, R. (1966). Subtotal pancreatectomy in idiopathic persistent hypoglycaemia. In *British Association of Paediatric Surgeons. Arch. Dis. Childh.*, **41**, 224.
- Mabry, C. C., Di George, A. M., and Auerbach, V. H. (1960). Leucine-induced hypoglycemia. I. Clinical observations and diagnostic considerations. *J. Pediatr.*, **57**, 526.
- Mahon, W. A., Mitchell, M. L., Steinke, J., and Raben, M. S. (1962). Effect of human growth hormone on hypoglycemic states. *New Engl. J. Med.*, **267**, 1179.
- Morgan, C. R., and Lazarow, A. (1963). Immunoassay of insulin: two antibody system. *Diabetes*, **12**, 115.
- Payne, W. W., and Woolf, L. I. (1959). Familial hypoglycaemia precipitated by amino acids. *Mod. Probl. Pædiat.*, **4**, 369.
- Rose, W. C., Wixom, R. L., Lockhart, H. B., and Lambert, G. F. (1955). The amino acid requirements of man. XV. The valine requirement; summary and final observations. *J. biol. Chem.*, **217**, 987.
- Rosenthal, I. M., Metz, R., and Pirani, C. (1964). Congenital leucine-sensitive hypoglycemia. *Amer. J. Dis. Child.*, **107**, 343.
- Rubin, A. A., Roth, F. E., Winbury, M. M., Topliss, J. G., Sherlock, M. H., Sperber, N., and Black, J. (1961). New class of anti-hypertensive agents. *Science*, **133**, 2067.
- Samaan, N., Dollery, C. T., and Fraser, R. (1963). Diabetogenic action of benzothiadiazines. Serum-insulin-like activity in diabetes worsened or precipitated by thiazide diuretics. *Lancet*, **2**, 1244.
- Seltzer, H. S., and Allen, E. W. (1965). Inhibition of insulin secretion in 'diazoxide-diabetes' (Abstr.). *Diabetes*, **14**, 439.
- Soyka, L. F., Molliver, M., and Crawford, J. D. (1964). Idiopathic hypoglycaemia of infancy treated with human growth hormone. *Lancet*, **1**, 1015.
- Wilkins, R. W. (1959). New drugs for the treatment of hypertension. *Ann. intern. Med.*, **50**, 1.
- Wilson, W. R., Stone, D. B., Okun, R., and Russell, R. P. (1964). Metabolic effects of diuretic and non-diuretic benzothiadiazines. (Abstract). *ibid.*, **60**, 317.
- Wolff, F. W., Nabwangu, J., Staquet, M. J., Viktora, J. K., Yabo, R., and Zarday, Z. (1965). Hormonal regulations of hypoglycemia due to diazoxide and its application in hypoglycemic states. *Diabetes*, **14**, 469.
- Yalow, R. S., and Berson, S. A. (1960). Immunoassay of endogenous plasma insulin in man. *J. clin. Invest.*, **39**, 1157.