Serum levels of vancomycin following multiple 1-g. doses in Case 3. The shaded areas represent periods of dialysis.

estimations were made. Some accumulation of the drug in the serum over the six-week treatment period was found in Case 3, but this was not a consistent finding in all the patients.

Discussion

Though vancomycin-resistant staphylococci were reported to be very uncommon when the drug was first introduced, we felt it was important to carry out sensitivity tests on a range of hospital staphylococci to be sure that the situation had not altered. Since the size of the zone of inhibition obtained when using the disc method of sensitivity testing bears no relation, for vancomycin, to the minimum inhibitory concentration of the organism, minimum inhibitory concentrations of vancomycin were measured for a selection of staphylococci. The results are similar to those reported some time ago (Griffith and Peck, 1955-6; McCormick *et al.*, 1955-6; Kirby and Divelbiss, 1956-7), and there seems to have been no change in the sensitivity of hospital staphylococci to the antibiotic; this may be attributable to its rather limited clinical use.

Vancomycin is a difficult antibiotic to assay by the agar plate diffusion technique, and it was essential to do a minimum of two separate estimations on each serum sample. Occasionally serious discrepancies occurred; these results

were discounted and the assays were repeated. Even with prediffusion at 4°C. the zones of inhibition obtained are apt to be small compared, for instance with the penicillins, and the dose-response line is steep.

The assay results obtained in six patients showed that levels of vancomycin above 2.5 µg./ml. persist in the serum for about seven days. Since vancomycin inhibits most staphylococci at a concentration of 2.5 µg./ml. and is only minimally removed during dialysis, shunt site infections can be treated with intermittent infusion of the antibiotic into the shunt. In patients with normal renal function a therapeutic regimen of 1 g. of vancomycin every 12 hours has been recommended (Kirby and Divelbiss, 1956-7). For anuric patients Lindholm and Murray (1966) suggested that 1 g. of vancomycin every 10 to 14 days would produce therapeutic serum levels. On the basis of our results, 1 g. of vancomycin every seven days is necessary to maintain sufficient levels of the drug for effective treatment of staphylococcal infection. If a dose of 0.5 g. were used, more frequent injections would be required. Since vancomycin may accumulate over several weeks' treatment, estimation of the serum level may well be required in each patient.

The treatment schedule of 1 g. of vancomycin every seven days led to pronounced clinical improvement of the shunt site infection within a week in four of the six patients, and within two weeks in one more. It failed in only one case. Since only one infusion of the drug was given each week at the time of a routine visit to the hospital for dialysis, and since oral and intramuscular routes were avoided, the patients were not inconvenienced in any way.

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Requests for reprints should be addressed to Dr. I. Phillips, Department of Clinical Microbiology, St. Thomas's Hospital, London S.E.1.

REFERENCES

- Dutton, A. A. C., and Elmes, P. C. (1959). *British Medical Journal*, **1**, 1144.
- Geraci, J. E., Heilman, F. R., Nichols, D. R., and Wellman, W. E. (1958). *Proceedings of the Staff Meetings of the Mayo Clinic*, **33**, 172.
- Griffith, R. S., and Peck, F. B. (1955-6). *Antibiotics Annual*, p. 619.
- Kirby, W. M. M., and Divelbiss, G. L. (1956-7). *Antibiotics Annual*, p. 107.
- Lindholm, D. D., and Murray, J. S. (1966). *New England Journal of Medicine*, **274**, 1047.
- McCormick, M. H., Stark, W. M., Pittenger, G. E., Pittenger, R. C., and McGuire, J. M. (1955-6). *Antibiotics Annual*, p. 606.

Serum Lipid Levels in Hyperosmolar Non-ketotic Diabetic Coma

P. D. BEWSHER,* M.B., CH.B., M.R.C.P.ED. ; J. C. PETRIE,† M.B., CH.B., M.R.C.P. ; H. G. J. WORTH,‡ PH.D.

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Summary: Levels of serum lipids were measured in two patients with hyperosmolar non-ketotic diabetic coma. Each lipid fraction was raised in a patient with severe hyperglycaemia and the degree of disturbance was less in a patient with moderate hyperglycaemia. The studies suggest that a block in the hepatic uptake of free fatty acids is not the explanation for the lack of ketosis.

Introduction

Though many cases of hyperosmolar non-ketotic diabetic coma have been reported (Sament and Schwartz, 1957; Danowski and Nabarro, 1965) and the association of glucose intolerance with raised blood lipid levels is recognized (Bierman and Porte, 1968) there are no well-documented studies of blood lipid levels in this condition. Two cases of hyperosmolar non-ketotic diabetic coma, one with pronounced hyperglycaemia, have been studied with particular reference to serum lipid levels.

*Senior Lecturer in Therapeutics, University of Aberdeen.

†Lecturer in Therapeutics, University of Aberdeen.

‡Lecturer in Chemical Pathology, University of Aberdeen.

Case 1

A 69-year-old farmer had a long history of exacerbations of airways obstruction precipitated by infection. For six years intermittent antibiotics and corticotrophin had been prescribed. There was no personal or family history of diabetes mellitus. One month before admission symptoms and signs of cardiac failure were noted and he was treated with frusemide and potassium supplements. One week before admission he developed a fungal mouth infection, polydipsia, and polyuria. He became progressively drowsy and nauseated. On admission he was thin and very dehydrated. Physical examination confirmed the signs of diffuse airways obstruction and there was abdominal tenderness. An irregular twitching of head and limbs was a prominent feature.

Investigations.—Urine contained over 2% glucose, but no acetone. Blood sugar 1,150 mg./100 ml., serum sodium greater than 160 mEq/l., bicarbonate 35 mEq/l., urea 95 mg./100 ml., creatinine clearance 62 ml./min. Serum osmolality, measured by freezing-point depression, was 381 mOsm./kg. The serum amylase was normal and the serum was not lactescent. The lipid levels are shown in the Table.

In the first 24 hours he was given 248 units of soluble insulin and 8 litres of 0.45% saline in 2.6% glucose with appropriate potassium supplements. Within four hours the blood sugar fell to normal limits and the serum bicarbonate, urea, and osmolality fell towards normal levels. An oral glucose tolerance test confirmed diabetes mellitus. The patient was maintained on chlorpropamide and a 1,500-calorie diet, and one year after admission the blood sugar, serum bicarbonate, urea, and osmolality were within normal limits.

Case 2

A 75-year-old woman with a 12-year history of maturity onset diabetes mellitus was maintained on chlorpropamide and a 1,500-calorie diet. She had a one-year history of cerebrovascular insufficiency, and over the 12 days before admission she had become increasingly drowsy and uncooperative. On admission she was obese, very dehydrated, and had bilateral cataract.

Investigations.—Urine contained over 2% glucose, but no acetone. Blood sugar 366 mg./100 ml., serum sodium 155 mEq/l., bicarbonate 29 mEq/l., urea 125 mg./100 ml., osmolality 321 mOsm./kg. The serum lipid levels are shown in the Table. She was treated with 6 litres of 0.45% saline in 2.6% glucose and 20 units of soluble insulin. On discharge she was maintained on chlorpropamide and a 1,500-calorie diet. Four months after admission the serum urea, blood sugar, and osmolality remained within normal limits.

Methods and Results

Serum lipids were extracted according to the method of Folch, Lees, and Sloane Stanley (1957) and fractionated on a silicic acid column, monitored with a liquid/liquid chromatograph, and each fraction was assayed colorimetrically (Worth and MacLeod, 1969). Control values were obtained from 12 fasted normal subjects and are expressed as means $\pm$ one standard deviation.

In the severely hyperglycaemic and hyperosmolar patient (Case 1) each serum lipid fraction measured was initially raised, though the increases were less pronounced in the monoglycerides and diglycerides, with the most striking abnormality in the triglyceride and free fatty acid fractions. After four days the triglyceride rise persisted but by day 9 it had reverted to normal levels. The serum lipid levels in the patient with only mild hyperglycaemia and hyperosmolality (Case 2) were only slightly different from the control values, and a small rise in the triglyceride and free fatty acid fractions was noted.

Discussion

Cases of hyperosmolar non-ketotic diabetic coma are being recognized more often than in the past, but the mechanism by which this syndrome develops is not understood. The levels of circulating plasma insulin (Ashkar, 1969) are reported to be normal or even slightly raised. Detailed lipid analyses have not been described, though Seftel, Goldin, and Rubenstein (1967) noted raised serum free fatty acid levels in their patient, suggesting that the absence of ketosis resulted from a block either in the hepatic uptake of free fatty acids or in their oxidation to ketone bodies. We have confirmed the rise in serum free fatty acid levels in this condition, but our finding of raised levels of triglycerides suggests that the hepatic uptake of free fatty acids is not inhibited. Conversion of circulating free fatty acids to triglyceride in the liver is said to be accelerated by high glucose levels (Carruthers 1969), which is in keeping with our findings in these patients.

In rats hypernatraemia causes glucose intolerance (Nitzan and Zelmanovsky, 1968), but the relative importance of hyperosmolality or hyperglycaemia in producing raised blood lipids is not clear. Hyperosmolality alone does not entirely explain the raised serum lipid levels, as our patient with less severe hyperosmolality also had less severe hyperglycaemia. Since the difference between the osmolality in the two cases appears to be due to the high blood sugar level in Case 1, we would suggest that hyperglycaemia rather than hyperosmolality is more closely related to the high serum lipid levels.

The lack of significant ketosis in this condition has not been satisfactorily explained. A critical level of plasma insulin may be important in the development of hyperglycaemia without ketosis (Sølvsteen, Olsen, and Hansen, 1968; Johnson, Conn, Dykman, Pek, and Starr, 1969). If the insulin available is insufficient to increase the uptake of glucose by muscle, yet sufficient to inhibit lipolysis in adipose tissue, a dissociation between hyperglycaemia and ketosis is possible. The raised levels of free fatty acids described by Seftel *et al.* (1967) and ourselves, however, would indicate that lipolysis is not inhibited. Hyperglycaemia inhibits ketogenesis in the liver (Mirsky, Heiman, and Broh-Khan, 1937), but this must only be an incomplete effect or diabetic ketoacidosis would never arise. Ketogenesis and cholesterol synthesis from 2-carbon fragments share a common pathway to the level of β -

Serum Lipid Levels

	Controls	Case 1					Case 2
		On Admission	20 hours	4 days	9 days	15 days	On Admission
Blood sugar (mg./100 ml.)	74 $\pm$ 5	1,150	246	70	90	100	366
Osmolality (mOsm./kg.)	289 $\pm$ 10	381	314	—	—	290	321
Cholesterol esters (μ mole/ml.)	2.76 $\pm$ 0.28	5.42	3.24	1.98	2.46	1.96	2.84
Triglycerides (μ mole/ml.)	1.40 $\pm$ 0.28	10.47	5.23	3.33	1.77	1.00	2.50
Free fatty acids (μ mole/ml.)	0.302 $\pm$ 0.142	1.438	1.112	0.528	0.590	0.568	0.695
Cholesterol (μ mole/ml.)	1.04 $\pm$ 0.10	2.30	1.66	1.12	1.32	0.94	0.95
Diglyceride (μ mole/ml.)	0.208 $\pm$ 0.130	0.387	0.272	0.338	0.217	0.251	0.120
Monoglyceride (μ mole/ml.)	0.300 $\pm$ 0.148	0.520	0.500	0.460	0.478	0.448	0.200
Phospholipids (μ mole/ml.)	3.83 $\pm$ 0.84	7.20	5.20	3.32	3.66	3.64	3.36

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hydroxy- β -methyl glutaryl CoA. If the greatly raised cholesterol levels in hyperosmolar non-ketotic diabetic coma are due to accelerated formation rather than decreased degradation or excretion, then any block in ketone production should occur beyond the initial stage of synthesis.

Further explanations for the lack of ketosis in this condition would be increased urinary excretion of ketones or an increase in their utilization. The lack of significant ketonuria does not support the former suggestion, and there are no reports that the turnover of ketone bodies is increased.

REFERENCES

- Ashkar, F. S. (1969). *Diabetes*, **18**, 344.
Bierman, E. L., and Porte, D., jun. (1968). *Annals of Internal Medicine*, **68**, 926.
Carruthers, M. E. (1969). *Lancet*, **2**, 1170.

- Danowski, T. S., and Nabarro, J. D. N. (1965). *Diabetes*, **14**, 162.
Folch, J., Lees, M., and Stanley, G. H. S. (1957). *Journal of Biological Chemistry*, **226**, 497.
Johnson, R. D., Conn, J. W., Dykman, C. J., Pek, S., and Starr, J. I. (1969). *Diabetes*, **18**, 111.
Mirsky, I. A., Heiman, J. D., and Broh-Kahn, R. H. (1937). *American Journal of Physiology*, **118**, 290.
Nitzan, M., and Zelmanovsky, S. (1968). *Diabetes*, **17**, 579.
Sament, S., and Schwartz, M. B. (1957). *South African Medical Journal*, **31**, 893.
Seftel, H. C., Goldin, A. R., and Rubenstein, A. H. (1967). *Lancet*, **2**, 1042.
Sølvsteen, P., Olsen, V. V., and Hansen, E. L. (1968). *Acta Medica Scandinavica* **184**, 83.
Worth, H. G. J., and MacLeod, M. (1969). *Journal of Chromatography*, **40**, 31.

Preliminary Communications

Azathioprine in Pemphigus Vulgaris

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INTRODUCTION

Pemphigus vulgaris is almost invariably fatal if untreated. Systemic corticosteroids in high doses will usually suppress the disease, but in most cases maintenance therapy must be continued indefinitely (Lever and White, 1963), and many patients develop side-effects from this treatment. The disease may be due to an immunological disorder (Beutner *et al.*, 1968), and immunosuppressive drugs therefore offer a rational alternative to corticosteroid therapy. Though immunosuppressive drugs are now widely used in a variety of conditions, there have been few direct comparisons of their therapeutic efficacy relative to their toxicity in man. Lever and Goldberg (1969) used methotrexate to control pemphigus vulgaris but found fairly high doses were required. In view of the increasing evidence of hepatic damage due to long-term methotrexate therapy (O'Rourke and Eckert, 1964; Coe and Bull, 1968; Muller *et al.*, 1969) we have sought an alternative immunosuppressant. Possibly azathioprine (Imuran) has a high therapeutic index relative to other immunosuppressive drugs (Corley *et al.*, 1966; *New England Journal of Medicine*, 1968; Bach *et al.*, 1969), and we report here our experience with azathioprine in the treatment of four cases of pemphigus vulgaris.

METHODS

In all four cases the diagnosis of pemphigus vulgaris was confirmed histologically by finding intraepidermal bullae with acantholytic cells. The patients had received systemic corticosteroid therapy for four to seven years before azathioprine was started, and regular attempts to reduce their maintenance dose of corticosteroids had enabled a reasonably accurate estimate of their steroid requirements to be made. All had mildly active pemphigus lesions at the start of the trial. Azathioprine was added, in a daily divided dose of 2.5 mg./kg. body weight, to the maintenance dose of corticosteroid, and after four weeks the steroid was gradually reduced. When levels of up to 3 mg. prednisone daily were reached the azathioprine was withdrawn, and was restarted when definite evidence of relapse was obtained. Steroid reduction was then continued to zero.

The patients were assessed at two-weekly intervals and haemoglobin concentration, white cell count, platelet count, liver function tests, and blood urea concentration were determined before starting azathioprine and at each subsequent attendance. Before beginning azathioprine, autoantibodies to intercellular material were estimated by the indirect immunofluorescent technique of Beutner and Jordon (1964).

CASE HISTORIES AND RESULTS

The results of azathioprine therapy are shown diagrammatically in the Chart.

Effect of azathioprine therapy in three cases of pemphigus vulgaris.