lysis, and urease were negative. On tellurite medium the colonies were medium in size, convex, rough, and dark grey. The toxigenicity test using an Elek plate was negative. The organism was identified as a non-toxicogenic *Corynebacterium diphtheriae*, gravis type. Dr. R. E. Weaver, Communicable Disease Centre, Atlanta, U.S.A., confirmed the identification. Sensitivity test results in minimum inhibitory concentrations ($\mu\text{g/ml}$) were as follows: gentamicin 0.01, penicillin 1.0, tetracycline 0.5, cephalothin 0.5, chloramphenicol 1.0, erythromycin 0.01, clindamycin 0.06, and lincomycin 0.25.

Discussion

Corynebacterial endocarditis is rare. In most reports the organisms are described as a 'diphtheroid' without detailed identification (Merzbach *et al.*, 1965; Reid and Greenwood, 1967; Davis *et al.*, 1963; Dismukes *et al.*, 1973; Manhas *et al.*, 1972; Stein, Harken, and Dexter, 1966). In most cases the organisms have been isolated from blood cultures in living patients, but in many of these cases other organisms were isolated in addition to the corynebacteria (Reid and Greenwood, 1967; Stein *et al.*, 1966). 'Diphtheroids' have also been isolated from blood cultures after cardiopulmonary bypass surgery for the repair of cardiac valvular defects (Davis *et al.*, 1963) and from infected prosthetic valves (Dismukes *et al.*, 1973; Manhas *et al.*, 1972; Stein *et al.*, 1966). The latter may present either early or late in the postoperative period.

Pike (1951) reported a case of endocarditis due to toxogenic *C. diphtheriae* and referred to earlier reports of cases in which nontoxogenic organisms were isolated. Fuacial, nasal, or cutaneous diphtheritic lesions may or may not be present in such cases. Corynebacterial species are widely distributed, being found in the soil and atmosphere and as contaminants in blood cultures. Certain strains, including *C. hofmanni*, *C. xerosis*, and nontoxogenic *C. diphtheriae*, are saprophytes in man. They are therefore potential causes of endocarditis.

Bacterial endocarditis in most cases affects valves deformed by acquired or congenital heart disease. Our patient had no congenital heart lesion and the macroscopical and microscopical appearances of the chordae tendinae and myocardium seemed to exclude antecedent rheumatic carditis. Normal valves may occasionally be the seat of endocarditis, particularly when caused by virulent organisms. The implication of our case is that any organism isolated from a patient with a clinical diagnosis of infective endocarditis should be regarded as the possible pathogen and not dismissed as a contaminant. Organisms should be identified fully and

not reported in vague terms such as 'diphtheroid', so that a more accurate idea of the source and nature of the organism may be obtained.

Summary

Fulminant endocarditis affecting the mitral valve in an 11-year-old boy was caused by a nontoxogenic strain of *Corynebacterium diphtheriae*.

We thank Professor John Wainwright for the necropsy findings.

REFERENCES

- Davis, A., Binder, M. J., Burroughs, J. J., Miller, A. B., and Finegold, S. M. (1963). Diphtheroid endocarditis after cardiopulmonary bypass surgery for the repair of cardiac valvular defects. *Antimicrobial Agents and Chemotherapy*, p. 643. Ed. by J. C. Sylvester. American Society for Microbiology, Ann Arbor, Michigan.
- Dismukes, W. E., Karchmer, A. W., Buckley, M. J., Austen, W. G., and Swartz, M. N. (1973). Prosthetic valve endocarditis. Analysis of 38 cases. *Circulation*, **48**, 365.
- Manhas, D. R., Mohri, H., Hessel, E. A., and Merendino, K. N. (1972). Experience with surgical management of primary infective endocarditis. A collected review of 139 patients. *American Heart Journal*, **84**, 738.
- Merzbach, D., Freundlich, E., Metzker, A., and Falk, W. (1965). Bacterial endocarditis due to corynebacterium. Report of 2 cases. *Journal of Pediatrics*, **67**, 792.
- Pike, C. (1951). Corynebacterial endocarditis with report of a case due to toxogenic *Corynebacterium diphtheriae*. *Journal of Pathology and Bacteriology*, **63**, 577.
- Reid, J. D., and Greenwood, L. (1967). Corynebacterial endocarditis. A report of two cases with review. *Archives of Internal Medicine*, **119**, 106.
- Stein, P. D., Harken, D. E., and Dexter, L. (1966). The nature and prevention of prosthetic valve endocarditis. *American Heart Journal*, **71**, 393.

RONALD van der HORST,* DENNIS DYER, and ARTHUR HALLETT

Department of Cardiology, Wentworth Hospital, and the Department of Bacteriology, University of Natal, Medical School, Durban, South Africa.

*Correspondence to Dr. R. van der Horst, 24 Musgrave Centre, Musgrave Rd., Durban, South Africa.

Peritoneal dialysis and exchange transfusion in a neonate with argininosuccinic aciduria

Peritoneal dialysis has been used in conditions such as renal, cardiac, and hepatic failure. Although it has been used in the management of coma in urea cycle deficiency states (Siegel and Brown, 1973), its effectiveness in controlling ammonia intoxication has been questioned (Siegel and Brown, 1973; Saudubray *et al.*, 1973). In a patient with

hyperammonaemia secondary to argininosuccinic aciduria we found peritoneal dialysis of considerable value in reducing blood ammonia levels, in contrast to the limited success of exchange transfusion in the same patient.

Case report

A male infant, weighing 3.35 kg at birth, was admitted at 60 hours of age because of poor sucking, groaning, vomiting, and increasing respiratory distress during the previous few hours. The pregnancy and delivery had been uneventful. The infant, normal at birth, was breast fed at 36 hours. He became drowsy, hypotonic, and vomited the next feed. A sib of the patient had died soon after birth of a similar condition. Though no diagnosis has been made biochemically this led us to suspect an inborn error of metabolism.

On admission the blood pH was 7.41, PCO_2 15 mm, base excess—11 mEq/l, and serum bicarbonate 9.9 mEq/l. The arterial concentration of ammonia was 535 $\mu\text{g}/100$ ml and a large amount of argininosuccinic acid (ASA) was found in the urine and cerebrospinal fluid (CSF) by high voltage electrophoresis screening. A diagnosis of argininosuccinic aciduria was made within a few hours of admission. The patient was fed exclusively by intravenous fluids (dextrose 20%, 1500 ml/24 h per m^2) through a superior vena caval catheter; sodium bicarbonate was added to correct the metabolic acidosis. An exchange transfusion, using citrate as an anticoagulant, was carried out to try to decrease the ammonia levels. However, the ammonia concentration increased to 850 $\mu\text{g}/100$ ml. Another exchange transfusion, with fresh heparinized blood, was no more successful (Fig.). On the fourth day of life peritoneal

Methods and results

Ammonia levels in plasma samples and peritoneal dialysate fluid samples were determined by the Fenton (1962) method. In children and adults the normal values remain below 100 $\mu\text{g}/100$ ml, but may increase up to 150 $\mu\text{g}/100$ ml during the first 72 hours of life. A summary of ammonia concentrations in plasma is shown in the Fig. along with the treatment. Routine screening of the patient's urine and CSF by high voltage electrophoresis (Whatman 3M filter paper 40×20 cm, pyridine buffer pH 5.3, 80 V/cm for 30 min) showed a large amount of the free form as well as the two anhydrides of ASA. Quantitative amino acid analysis, performed by ion exchange chromatography by the method of Cusworth and Westall (1961), confirmed the high urinary excretion of ASA, which was 6.5 g/24 h on the fourth day (undetectable in normal newborns). The urea cycle enzyme activities, argininosuccinic lyase and arginase, in liver obtained half an hour after death were determined on a frozen aliquot within 3 weeks by the method of Brown and Cohen (1959). The argininosuccinic acid cleavage enzyme activity was less than 0.5% of control values but the arginase activity was within the normal range (Table).

TABLE

Activities of urea cycle enzymes in liver ($\mu\text{mol}/\text{min}$ per g wet weight of tissue)

	Patient	Controls
Argininosuccinate lyase	0.03	4.7
Arginase	530	238–800

FIG.—Effect of exchange transfusion and peritoneal dialysis on blood ammonia. *Total excretion per 12 hours.

dialysis was started in a final attempt to control hyperammonaemia. At the end of the same day a cardio-respiratory arrest necessitated intubation and artificial ventilation. The patient's condition deteriorated gradually, despite continuing the dialysis, and he died on the seventh day.

Exchange transfusion. The first exchange was with 250 ml of 24-hour-old citrated blood: 10 ml was exchanged over a period of 8 min. The second exchange was with 300 ml of fresh heparinized blood with the same procedure. Despite these exchange transfusions there was a gradual increase in plasma ammonia concentration up to 850 $\mu\text{g}/100$ ml during the first exchange, which was not significantly reduced by the second exchange (Fig.).

Peritoneal dialysis. Dialysis, through a left flank incision, was with warmed commercial dialysate (Dianeal G 174) to which 5 mEq/l of KCl and 250 mg/l of ampicillin were added. Exchanges of 40 ml/kg were performed in a 60-min cycle; the fluid was infused over a period of 15 min. After an equilibration period of 25 min the dialysate was allowed to drain up to 20 min. Since the child developed renal failure after the cardiac arrest a hypertonic dialysate (Dianeal G 184) was used in one out of every three cycles. During the 20-min period when peritoneal dialysate was being drained 150 ml of 20% lactulose was given rectally in order to trap the gut ammonia. Immediately before dialysis the arterial ammonia concentration was 735 $\mu\text{g}/100$ ml and 15 hours after the procedure it had fallen to 300 $\mu\text{g}/100$

ml; analysis of dialysate confirmed that the fall in ammonia concentration was due to dialysis. A total of 5 mg of ammonia was removed in the dialysate during the first 6-hour period (Fig.).

Discussion

The clinical outcome in hyperammonaemia secondary to a urea cycle enzyme defect seems to be related to the plasma and/or CSF ammonia concentration. Neonatal death tends to occur when this concentration exceeds 300 $\mu\text{g}/100\text{ ml}$. However, patients detected in later childhood seem to have increased ammonia tolerance, perhaps as a function of brain maturation. Peritoneal dialysis has had variable success in reducing ammonia levels in congenital ammonia intoxication. A transient lowering was observed in the case of Saudubray *et al.* (1973) and no significant reduction was achieved in the case reported by Siegel and Brown (1973).

Peritoneal dialysis was performed in our case because of the failure of the two exchange transfusions to decrease the ammonia levels. Neither the use of citrated blood to increase the activity of the tricarboxylic acid cycle nor fresh blood which minimized the transfused blood ammonia content helped the patient. Unfortunately no ammonia determination was performed on the stored blood used for the exchange transfusion to estimate the ammonia load during this procedure. In addition, the secondary effects of the exchange transfusion on the liver blood flow remain obscure. Haemoglobin may be specifically toxic to the liver by producing more ammonia and/or by increasing portosystemic shunting (Bessman, 1967).

Our data confirm that peritoneal dialysis is more efficient in decreasing blood ammonia levels when there is a high concentration gradient but, after an initial fall, it fails to lower the plasma ammonia concentration below 300 $\mu\text{g}/100\text{ ml}$ (Saudubray *et al.*, 1973). Since the amount of ammonia cleared during the dialysis seems to be constant the lack of a further decrease in plasma suggests a persistent high rate of endogenous production of ammonia compared to the clearing rate of the dialysis. A major stress such as an intercurrent infection or surgery is known to provoke metabolic decompensation through an increase in catabolism. The peritoneal dialysis itself may act as a stress and thus help to maintain a metabolic disturbance.

The question of the inexorable course in our patient may now be considered. Despite the dramatic decrease in plasma ammonia concentration to 300 $\mu\text{g}/100\text{ ml}$ this level may still be toxic to the immature brain, which seems to be more vulner-

able than that of older children or adults (Burton, Corsellis, and Russell, 1970). It is also possible that the very high levels of ammonia had produced some irreversible damage in our patient. There may have been other metabolic changes to explain the fatal course of his disease. The very high blood concentration of ammonia with minimal or even absent protein intake suggests a high catabolic rate. On the other hand, the continuous urea production, shown by the constant urea concentration in the dialysate as well as in the plasma, suggests that the urea cycle was still operative during the first 24 hours of dialysis until the major metabolic decompensation produced by the cardiac arrest, which could have been the precipitating factor.

Nicholas and Freeman's (1972) recent studies in premature infants suggest that ammonium ion is primarily incorporated by transamination with α -ketoglutarate to form glutamate and then glutamine and only secondarily metabolized via the urea cycle. In hyperammonaemic conditions α -ketoglutarate, a tricarboxylic acid cycle (TCA) intermediate, may somehow be used excessively in the ammonia detoxification, thus shunting it away from the TCA cycle and leading to an impairment of the energy production. The synthesis of α -ketoglutarate from the TCA cycle intermediates also seems to be inhibited by high levels of ammonia and glutarate (Szutowicz, Stepein, and Angielski, 1974). On the other hand, the early postnatal period is of special importance since at that time the TCA cycle appears to be impaired (G. Verellen and R. De Meyer, in preparation). Therefore even if ammonia levels are reduced by peritoneal dialysis an effectively operated Krebs' cycle appears to be important to stop the vicious cycle of high ammonia production from excess protein catabolism.

Summary

Peritoneal dialysis rapidly reduced blood ammonia concentration in this child with argininosuccinic acid-lyase deficiency, whereas exchange transfusion did not. Yet this reduction in plasma ammonia level did not produce clinical improvement. We speculate that the effects of ammonia intoxication on the highly susceptible neonatal metabolism are due to an accumulation of toxic products and to an altered energy metabolism. Both aspects must be considered in any attempt to treat congenital hyperammonaemia.

We are indebted to Dr. F. Hommes, Department of Paediatrics, Groningen, Netherlands, for carrying out the enzyme assays on the hepatic tissue; to Mr. A. Has-

soun for the ammonia assay; and Dr. J. Dochain for giving us the opportunity to examine this patient.

REFERENCES

- Bessman, S. P. (1967). In discussion on argininosuccinic acidemia. *American Journal of Diseases of Children*, **113**, 164.
- Brown, G. W., and Cohen, P. P. (1959). Comparative biochemistry of urea cycle synthesis. I. Methods for the quantitative assay of urea cycle enzymes in liver. *Journal of Biological Chemistry*, **234**, 1769.
- Bruton, C. J., Corsellis, J. A., and Russel, A. (1970). Hereditary hyperammonaemia. *Brain*, **93**, 423.
- Cusworth, D. C., and Westall, R. G. (1961). Determination of argininosuccinic acid by ion exchange chromatography. *Nature*, **192**, 555.
- Fenton, J. C. (1962). The estimation of plasmic ammonia by ion exchange. *Clinica Chimica Acta*, **7**, 163.
- Nicholson, J. F., and Freeman, J. M. (1972). Metabolism of compounds labeled with ^{15}N by an infant with congenital hyperammonemia. *Pediatric Research*, **6**, 252.
- Saudubray, J. M., Cathelineau, L., Charpentier, C., Boisse, J., Allaneau, C., Le Bont, H., and Lesage, B. (1973). Déficit héréditaire en ornithine-carbamyl-transférase avec anomalie enzymatique qualitative. *Archives Françaises de Pédiatrie*, **30**, 15.
- Siegel, N. J., and Brown, R. S. (1973). Peritoneal clearance of ammonia and creatinine in a neonate. *Journal of Pediatrics*, **82**, 1044.
- Szutowicz, A., Stepien, M., and Angielski, S. (1974). The inhibition of rat brain ATP; citrate oxaloacetate-lyase by $\pm$ -glutamate. *Journal of Neurochemistry*, **22**, 85.

BAUDOUIN FRANCOIS*, GUY CORNU, ROGER DE MEYER†
Department of Paediatrics, Louvain University, Belgium

*Correspondence to Dr. B. François, Department of Paediatrics, University of Louvain, St. Raphael's Clinics, Kapucijnenvoer 35, B3000 Leuven, Belgium.

Decreased antihaemophilic globulin and leucocyte response to epinephrine in preterm infants

The ability of the reticuloendothelial system (RES) and the spleen to respond quickly and efficiently to challenge is thought to be an important factor in the prevention of acute fulminant infections. The increased susceptibility of preterm infants to infections cannot be explained by the deficiencies in their immune system, and the importance of the RES has therefore recently received attention (Gotoff, 1974). Measurement of RES and splenic activity is difficult, especially in preterm neonates, and the idea of splenic hypofunction in preterm infants comes from indirect evidence.

Holroyde, Oski, and Gardner (1969) observed a high percentage of 'pocked' erythrocytes in the blood of preterm infants, similar to those seen in patients after splenectomy, which they thought was due to the immaturity of the RES, mainly of the spleen. Others (Casper, Rodey, and Thatcher, 1974) noted their presence in patients with a nonfunctional spleen. Acevedo and Maurer (1963)

showed that preterm newborns do not remove erythrocytes containing Heinz bodies as efficiently as normal babies, therein resembling splenectomized individuals.

Epinephrine injections (or infusions) have well-known, if not well-understood, effects upon the RES and the spleen, and two of them are easily investigated. They are (1) the rise in the number of formed elements such as leucocytes in the circulating blood (Chatterjea, Dameshek, and Stefanini, 1953), and (2) the rise in the level of plasma antihaemophilic activity (Ingram, 1961). Information about these effects of epinephrine in neonates is lacking, so we investigated them.

Subjects and methods

Two groups of neonates (groups 1 and 2) between the ages of 2 and 4 days and two groups of older children (groups 3 and 4) were studied.

Group 1. Comprised 21 perterm infants (15 males, 6 females) with a gestational age of about 30–38 weeks and a birthweight of 1100–2400 g.

Group 2. Comprised 23 term infants (12 males, 11 females) whose birthweight was between 2900 and 3440 g.

Group 3. Comprised 20 children aged 3 months to 6 years (10 males and 10 females) who served as a control group. All were apparently healthy and had no haematological disorder.

Group 4. Comprised 13 children who had had splenectomy (12 because of trauma, 1 for spherocytosis) and 1 with congenital asplenia syndrome.

With written parental permission, blood was drawn from a vein before and 30 minutes after a subcutaneous injection of epinephrine 0.01 mg/kg. A leucocyte count was made simultaneously, on capillary blood. The antihaemophilic activity of the plasma was estimated by the one-stage PTT test (Rodman, Barrow, and Graham, 1958), using a commercial antihaemophilic globulin-deficient serum (Dade Laboratory).

Results

Tables I and II summarize our findings. Before the injection there was not much difference between the four groups either in antihaemophilic activity or leucocyte count. But the differences in the response to epinephrine were highly significant. While the term infants reacted similarly to the controls the response of the preterm neonates was negligible, as was that of the splenectomized group. No differences were observed between males and females in any of the four groups.

Discussion

Our data suggest that preterm neonates fail to react normally to epinephrine injections, so far as