Parathyroid hormone secretion during exchange transfusion

R. D. G. MILNER and J. S. WOODHEAD

From the Department of Child Health, University of Manchester, and the Department of Medical Biochemistry, Welsh National School of Medicine, Cardiff

Milner, R. D. G. and Woodhead, J. S. (1975). *Archives of Disease in Childhood*, 50, 298. **Parathyroid hormone secretion during exchange transfusion.** Plasma concentrations of calcium, phosphate, citrate, albumin, and parathyroid hormone (PTH) were measured during and after exchange transfusion of infants suffering from haemolytic disease using blood anticoagulated with acid-citrate and dextrose (ACD) or heparin. Pretransfusion plasma PTH and phosphate both correlated positively with postnatal age but not with each other. Transfusion with ACD blood caused a twelvefold rise in plasma citrate levels but no significant change in plasma calcium, phosphate, or PTH of the infant, despite the concentration of these substances being lower in the donor blood. The concentration of calcium, phosphate, and albumin was higher in heparinized than in ACD donor blood, and infants transfused with heparinized blood showed no change in the plasma concentration of any substance measured during transfusion. The addition of 50 µg glucagon to ACD donor blood had no effect on PTH secretion. 3 hours after transfusion there was a rise in the plasma PTH of infants who had received ACD blood but not in those given heparinized blood. Transfusion with ACD blood caused a net loss of calcium, phosphate, and albumin from the infant, whereas transfusion with heparin blood did not. Both types of transfusion caused a net loss of PTH but this was significantly greater in those given ACD blood.

These results show that transfusion with ACD blood results in increased secretion of PTH, probably due to the fall in ionized calcium concentration caused by the citrate load.

The effect of citrate in lowering the ionized serum calcium concentration of blood used for exchange transfusion has been calculated theoretically (Farquhar and Smith, 1958) and directly (Darnlow, 1971). More recently calcium specific electrodes have been used to show that the serum ionized calcium falls by half during exchange transfusion, that therapeutic calcium given during the procedure is ineffective, and that restoration of a normal serum ionized calcium concentration occurs spontaneously and rapidly after transfusion (Perkins *et al.*, 1971; Radde *et al.*, 1972; Gershanik, Levkoff, and Duncan, 1973; Maisels *et al.*, 1974). In the adult cow a fall in serum ionized calcium stimulates parathyroid hormone (PTH) secretion (Sherwood *et al.*, 1966) and for this reason we investigated the PTH response of the human infant during exchange

transfusion. Transfusions using blood preserved with acid-citrate and dextrose (ACD) were compared with transfusions in which heparinized blood was employed, and marked changes in serum ionized calcium were not expected to occur.

Patients and methods

Twenty-seven exchange transfusions were performed for hyperbilirubinaemia in infants suffering from haemolytic disease of the newborn. All transfusions were performed via the umbilical artery or vein as described previously (Milner *et al.*, 1972; Cser and Milner, 1975). 10 transfusions were performed with ACD blood, 9 with ACD blood to which 50 µg glucagon had been added, and 8 with blood anticoagulated with heparin. The 10 infants having ACD transfusions were between 37 and 40 weeks' gestational age and 2.28 and 3.06 kg birthweight. The 7 infants having ACD + glucagon transfusions were 37 or 38 weeks' gesta-

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tional age and between 2.25 and 3.26 kg birthweight. The 7 infants having heparin transfusions were between 35 and 40 weeks' gestational age and 2.12 and 3.66 kg birthweight. 5 infants were studied more than once.

In blood samples collected before, during, and after transfusion as described previously (Milner *et al.*, 1972; Cser and Milner, 1975), estimations were made of the haematocrit, plasma concentration of albumin (Doumas, Watson, and Biggs, 1971), calcium by atomic absorption spectrophotometry, phosphate (Fiske and Subbarow, 1925), and citrate (Zender, 1969). Plasma PTH concentration was measured by an immunoradiometric assay (Addison *et al.*, 1971) using a guinea pig antibody raised to bovine PTH which showed good cross-reactivity with human PTH. Before assay, samples were stored frozen at -20°C . Samples were assayed against a reference preparation of human PTH (kindly provided by Dr. J. L. H. O'Riordan) which was 2.5% pure using an antiserum (code 71/69) generously provided by the Medical Research Council. The lower limit of detection of the assay was 0.1 ng/ml and for the purposes of statistical calculation plasma samples in which PTH could not be detected were assigned a value of 0.1 ng/ml.

Results

Before transfusion. The infants in the 3 groups were comparable in gestational age, birthweight, and pathology. Ages at the start of transfusion ranged from 4 to 137 hours. Plasma PTH concentration before transfusion was positively correlated with postnatal age (Fig.) ($r = 0.408$; $P < 0.05$). Cases 1 and 2 who had high plasma PTH concentrations in the first day of life were rhesus-affected female twins of 37 weeks' gestation and birthweights 2.28 and 2.66 kg. Pretransfusion plasma phosphate also increased with postnatal age ($r = 0.448$; $P < 0.05$), but there was no correlation between pretransfusion plasma PTH and phosphate concentrations, and no significant relation between pretransfusion plasma calcium or albumin and postnatal age.

With the exception of phosphate, donor blood concentrations of all substances were similar in the two types of ACD transfusion and these were pooled for comparison of ACD with heparin donor blood (Table I). Mean plasma concentration of albumin and calcium in ACD donor blood was lower than that in heparin donor blood due to the diluting effect of 120 ml ACD solution in each unit of blood. Plasma phosphate in the ACD donor blood was low for the same reason, and in heparin donor blood the phosphate concentration was raised due to the presence of phosphate buffer in the plastic collecting bags.* PTH was undetectable in 11 of

FIG.—Scatter diagram of pretransfusion serum PTH plotted against postnatal age. Lines join observations made on the same infant. Infants 1 and 2 were twins. ACD transfusions ●; heparin transfusions ○.

the 19 ACD donor blood samples and in 3 of the 8 heparin donor samples.

At the start of transfusion mean plasma concentration of phosphate was higher and calcium significantly lower in infants transfused with heparinized blood than in those receiving ACD blood (Table I). This was probably a small sample phenomenon in that in each case the heparin group differed significantly from only one of the two ACD subgroups which were of similar size.

During transfusion. During transfusion the plasma concentration of all substances in the infants receiving ACD blood did not differ significantly from that of those transfused with ACD blood enriched with glucagon. It was concluded that glucagon was without effect on plasma albumin, calcium, citrate, phosphate, or PTH during exchange transfusion and the results of the two groups were pooled for comparison with the changes taking place during heparin transfusion.

*Fenwal Blood Pack, Travenol Laboratories Ltd.

TABLE
Mean ($\pm$ SE) plasma concentration of albumin, phosphate,

Substance measured	Donor	0 ml
<i>ACD transfusions</i>		
Albumin (g/100 ml)	2.80 $\pm$ 0.13 (9)	3.25 $\pm$ 0.14 (9)
Phosphate (mg/100 ml)	2.98 $\pm$ 0.29 (9)	6.23 $\pm$ 0.44 (9)
Calcium (mg/100 ml)	5.54 $\pm$ 0.31 (9)	6.54 $\pm$ 0.35 (9)
Citrate (g/l)	4.57 $\pm$ 0.18 (9)	0.04 $\pm$ 0.01 (9)
PTH (ng/ml)	0.22 $\pm$ 0.06 (10)	0.70 $\pm$ 0.18 (10)
<i>ACD + glucagon transfusions</i>		
Albumin (g/100 ml)	2.79 $\pm$ 0.10 (9)	3.08 $\pm$ 0.12 (9)
Phosphate (mg/100 ml)	2.22 $\pm$ 0.19 (9)*	5.02 $\pm$ 0.44 (9)
Calcium (mg/100 ml)	5.28 $\pm$ 0.15 (9)	6.79 $\pm$ 0.47 (9)
Citrate (g/l)	4.29 $\pm$ 0.12 (9)	0.03 $\pm$ 0.01 (9)
PTH (ng/ml)	0.11 $\pm$ 0.06 (9)	0.72 $\pm$ 0.19 (9)
<i>All ACD transfusions</i>		
Albumin (g/100 ml)	2.80 $\pm$ 0.08 (18)	3.16 $\pm$ 0.09 (18)
Phosphate (mg/100 ml)	2.85 $\pm$ 0.17 (18)	5.62 $\pm$ 0.31 (18)
Calcium (mg/100 ml)	4.40 $\pm$ 0.16 (18)	6.67 $\pm$ 0.29 (18)
Citrate (g/l)	4.43 $\pm$ 0.11 (18)	0.04 $\pm$ 0.01 (18)
PTH (ng/ml)	0.17 $\pm$ 0.04 (19)	0.71 $\pm$ 0.13 (19)
<i>Heparin transfusions</i>		
Albumin (g/100 ml)	3.98 $\pm$ 0.17 (8)‡	3.25 $\pm$ 0.18 (8)
Phosphate (mg/100 ml)	9.16 $\pm$ 0.63 (8)‡	7.05 $\pm$ 0.39 (8)
Calcium (mg/100 ml)	6.66 $\pm$ 0.11 (8)‡	5.80 $\pm$ 0.27 (8)
PTH (ng/ml)	0.24 $\pm$ 0.04 (8)	0.41 $\pm$ 0.10 (8)

Level of significance : *P < 0.05, †P < 0.01, ‡P < 0.001 for comparison of heparin transfusions with all ACD transfusions, or of ACD + glucagon transfusions with ACD transfusions.
No. of observations shown in parentheses.

During ACD transfusion there was a gradual fall in plasma albumin concentration so that at the end of transfusion the mean concentration was similar to that in the donor blood. In heparin transfusions the mean plasma albumin rose to a level halfway between that in the infant before transfusion and that in the donor blood. Mean plasma albumin concentrations were significantly higher in heparin transfusions from the 200 ml point onwards.

A similar change took place in plasma phosphate concentration during heparin transfusions, but during ACD transfusion there was no fall in plasma phosphate despite the donor plasma phosphate being half that in the infant before transfusion. Likewise, plasma calcium level in ACD transfusions remained steady despite the mean donor plasma calcium being two-thirds that of the infants' before transfusion.

In both types of transfusion the pretransfusion mean plasma PTH was higher than that in the donor blood and was maintained at a relatively constant level throughout. The wide variation in plasma levels between infants, partly a function of postnatal age, meant that though the plasma PTH levels in ACD transfusions were almost twice as high as in heparin transfusions there was no significant difference between the two groups. In 4

infants aged 4, 4, 6, and 15 hours receiving ACD transfusions, no PTH was detectable at any time. Despite the citrate concentration in the donor blood being 100 times higher than that in the infant, the mean plasma citrate level in the infant rose only twelvefold during the procedure.

Analysis of the total amounts of each substance injected and withdrawn from the infants revealed marked differences between ACD and heparin transfusions (Table II). ACD transfusions were characterized by a significant net loss of calcium, phosphate, and albumin, whereas in heparin transfusions no significant loss or accumulation of these substances by the infant occurred. In both types of transfusion there was a net loss of PTH but this was three times greater in ACD than in heparin transfusions. If the ACD transfusions in which PTH was never detected were included in the calculation of net PTH loss, the figure became 54.71 ± 12.01 ng/kg, which was not significantly greater than that occurring in heparin transfusions. The net balances of PTH in the 5 infants who had more than one transfusion are shown in Table III. In 3 PTH loss increased and in the other 2 PTH loss was high in all transfusions.

After transfusion. Blood samples were col-

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calcium, citrate, and PTH during exchange transfusion

100 ml	200 ml	300 ml	400 ml
3.06±0.13 (9)	3.02±0.11 (9)	2.91±0.09 (9)	2.83±0.10 (9)
6.27±0.47 (9)	6.30±0.46 (9)	6.39±0.42 (9)	6.39±0.39 (9)
6.69±0.33 (9)	6.72±0.38 (9)	6.85±0.32 (9)	7.11±0.41 (9)
0.38±0.03 (9)	0.46±0.05 (9)	0.56±0.07 (9)	0.54±0.06 (9)
0.69±0.17 (10)	0.73±0.16 (10)	0.71±0.16 (10)	0.65±0.14 (10)
2.90±0.07 (9)	2.80±0.09 (9)	2.72±0.08 (9)	2.63±0.09 (9)
5.41±0.39 (9)	5.50±0.39 (9)	5.52±0.35 (9)	5.35±0.32 (9)
6.75±0.42 (9)	6.73±0.39 (9)	6.86±0.38 (9)	6.77±0.29 (9)
0.35±0.03 (9)	0.43±0.03 (9)	0.52±0.13 (9)	0.53±0.19 (9)
0.69±0.19 (9)	0.71±0.21 (9)	0.88±0.26 (9)	0.77±0.20 (9)
2.98±0.07 (18)	2.91±0.07 (18)	2.82±0.06 (18)	2.73±0.07 (18)
5.84±0.30 (18)	5.90±0.30 (18)	5.95±0.27 (18)	5.87±0.25 (18)
6.72±0.27 (18)	6.72±0.27 (18)	6.86±0.25 (18)	6.94±0.25 (18)
0.37±0.02 (18)	0.45±0.03 (18)	0.54±0.04 (18)	0.53±0.04 (18)
0.69±0.12 (19)	0.72±0.12 (19)	0.79±0.14 (19)	0.71±0.11 (19)
3.28±0.18 (8)	3.34±0.13 (8)†	3.46±0.16 (8)‡	3.58±0.13 (6)‡
7.52±0.48 (8)†	7.72±0.51 (8)†	7.78±0.39 (8)‡	8.47±0.57 (6)‡
6.46±0.26 (8)	6.44±0.30 (8)	6.45±0.37 (8)	6.92±0.22 (6)
0.45±0.11 (8)	0.44±0.11 (8)	0.43±0.10 (8)*	0.37±0.12 (6)*

lected hourly for 3 hours after ACD transfusion in 8 infants and at 3 hours after transfusion in 6 who had received heparinized blood. In 1 of the 8 PTH was never detected. The mean ($\pm$ SE) plasma PTH concentration at the end of transfusion in 7 infants was 0.29 ± 0.09 ng/ml, in 7 infants at 1 hour 0.26 ± 0.08 ng/ml, in 6 at 2 hours 0.37 ± 0.14

ng/ml, and in 5 at 3 hours 0.44 ± 0.16 ng/ml. Paired analysis showed that a small but significant rise in plasma PTH of 0.13 ± 0.04 ng/ml occurred 3 hours after transfusion. The mean ($\pm$ SE) plasma PTH of the 6 infants receiving heparinized blood was 0.38 ± 0.12 ng/ml at the end of transfusion and 0.37 ± 0.11 ng/ml 3 hours later.

TABLE II

Amounts of albumin, calcium, phosphate, citrate, and PTH infused and removed during exchange transfusion with ACD or heparin blood

Metabolite or hormone	Mean $\pm$ SEM amount infused or removed/kg body weight		
	In	Out	Balance
<i>ACD transfusions</i>			
Calcium (mg)	3.67±0.16	5.73±0.35 (18)	-2.06±0.29†
Phosphate (mg)	1.78±0.12	4.89±0.30 (18)	-3.11±0.24†
Citrate (mg)	311±13	36.1±2.5 (18)	+275±12
Albumin (g)	1.92±0.12	2.44±0.11 (18)	-0.52±0.04†
PTH (ng)	13.52±3.56	83.10±13.60 (15)	-69.58±12.60*
<i>Heparin transfusions</i>			
Calcium (mg)	6.14±0.45	6.43±0.39 (8)	-0.28±0.30
Phosphate (mg)	8.47±0.84	7.77±0.67 (8)	+0.70±0.41
Albumin (g)	3.68±0.32	3.41±0.25 (8)	+0.27±0.14
PTH (ng)	21.13±3.23	44.37±10.72 (8)	-23.24±12.49

Level of significance. *P < 0.05, †P < 0.001 for comparison with heparin transfusions.
No. of observations shown in parentheses.

TABLE III
Net balance of PTH (ng/kg) in infants who had more than one exchange transfusion

Case no.	Exchange transfusion		
	1	2	3
1	- 51.2(8)	- 92.2(17)	—
2	-118.1(5)	-111.6(16)	-129.6(39)
3	- 2.8(4)	- 34.0(21)	- 33.0(46)
4	—	- 52.5(33)	- 94.2(78)
5	-109.7(86)	—	-128.5(132)

Figures in parentheses show age in hours at which transfusion occurred.

Discussion

This study has shown that infants respond to exchange transfusion with ACD blood by increasing PTH secretion, the evidence being the greater net loss of PTH during transfusions with ACD blood than with heparinized blood and the rise in plasma PTH 3 hours post-transfusion. One difficulty in comparing two groups of exchange transfusions stems from the rise of plasma PTH levels with postnatal age. The most striking feature of individual transfusions was that the plasma PTH level remained relatively constant despite the donor plasma PTH being usually lower. These findings are partly in agreement with those of Tsang *et al.* (1973) who observed no rise in plasma PTH in paired samples during the first week of life and who claim to have shown an increase of PTH secretion during ACD exchange transfusion which was dependent on both gestational and postnatal age. The range of gestational age of our infants was too narrow for comment on the first point but the positive correlation of pretransfusion PTH with postnatal age suggests that the resting level of secretion increases after birth as did the secretory response to ACD in 3 of the 5 infants who had more than one transfusion.

The heparin transfusions were studied as a control group for comparison with ACD transfusions. Unfortunately, in addition to the difference in citrate, and hence ionized calcium concentration, the diluting effect of 120 ml ACD solution and the presence of phosphate buffer in the heparin collection bags resulted in significant differences in the total plasma calcium, phosphate, and albumin concentrations between the two types of donor blood. Plasma albumin concentration in both types of transfusion and plasma calcium and phosphate concentrations in the heparin transfusions rose or fell towards the donor level as the transfusion progressed. In contrast, plasma cal-

cium and phosphate concentrations during ACD transfusions did not fall despite appreciably lower donor levels. The mobilization of calcium and phosphate from the extravascular compartment maintained homeostasis of plasma concentrations as shown by the significant negative net balance of each substance occurring in ACD but not in heparin transfusions. This was probably due to increased PTH secretion, though it was not proven.

The addition of 50 µg glucagon to ACD blood results in high plasma glucagon in the infant by the end of transfusion with effects on glucose mobilization and insulin secretion (Milner, Chouksey, and Assan, 1973). Glucagon has been reported to stimulate calcitonin release from perfused thyroid glands (Care, Bates, and Gitelman, 1970) and indirect evidence suggests that chronic hyperglucagonaemia may lead to parathyroid hyperplasia (Paloyan, 1967). It was of interest then to see if raising plasma glucagon levels had any acute effect on calcium balance or PTH release during exchange transfusion. The negative results may have been caused by glucagon having no effect on the PTH secreting cell or because the citrate load was already causing maximum stimulation of PTH secretion.

The finding of an increased PTH secretion after birth fits well with the morphological observations of Kaplan (1942), who noted that parathyroid hypertrophy occurred in 11 of 13 infants dying between the fourth and tenth day of life but in none of 20 dying during the first 3 days.

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Correspondence to Dr. R. D. G. Milner, Room 61, Research Floor, St. Mary's Hospital, Manchester 13 0JH.