Measurement of serum parathyroid hormone, with particular reference to some infants with hypocalcaemia

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Fairney, A., Jackson, D., and Clayton, B. E. (1973). *Archives of Disease in Childhood*, 48, 419. **Measurement of serum parathyroid hormone, with particular reference to some infants with hypocalcaemia.** Parathyroid hormone (PTH) in serum has been determined by a double antibody radioimmunoassay which is suitable for use in infants. The concentration in normal infants rose after birth from a mean level of 97 pg/ml in cord blood to 217 pg/ml at 6 days of age. It remained at this level in the older infants and children. In a group of infants with hypocalcaemia and many complicating factors, the PTH concentration was significantly lower than normal. All but one of the affected infants maintained a normal serum calcium without treatment 1 year after the episode of hypocalcaemia. It is suggested that prolonged immaturity of parathyroid function may contribute significantly to the aetiology of hypocalcaemia and may be related to immaturity of the mechanisms responsible for converting glandular PTH to secreted PTH.

An increase in the number of neonates with hypocalcaemia has been reported (Baum, Cooper, and Davies, 1968; Begum, Yudkin, and Dormandy, 1968; Eades, 1968; Pugh, 1968). The decline of breast feeding and the use of artificial milks with a high content of phosphate appear to be aetiological factors. It is possible that abnormal function of the parathyroids may be important too, but the lack of an easily available sensitive assay for parathyroid hormone (PTH) has made it difficult to study the role of the parathyroid glands in hypocalcaemia of infants.

This paper reports a radioimmunoassay for serum PTH which has been adapted from the standard double antibody technique for growth hormone (Morgan and Lazarow, 1963; Jackson, Grant, and Clayton, 1968), which requires only micro quantities of serum, and which used an antiserum known to cross-react with human PTH. Since the method is capable of measuring normal values in the neonate, it has been used to study hypocalcaemia in a series of complex infants referred to The Hospital for Sick Children.

Patients and methods

Blood samples and subjects. Capillary blood samples were obtained by heel prick from 13 babies with hypocalcaemia. They were mostly unusual compared with infants with classical neonatal hypocalcaemia and had many complicating features. The clinical details are shown in Table I. During the interval between the age at which hypocalcaemia was first diagnosed and the age at which PTH was measured (see Table VI), hypocalcaemia persisted or was intermittently corrected by treatment. In order to establish a normal range, assays were also performed on cord bloods from healthy infants, on capillary blood obtained by heel prick on the 6th day of life at the time of the Guthrie test in healthy babies, on capillary blood obtained from older babies and children thought to have normal parathyroid function when they were having blood collected for other tests, and on samples from healthy adult volunteers. Blood was also obtained from 12 adults with hypoparathyroidism resulting from surgery. Blood was allowed to clot for less than 2 hours at room temperature, the serum being separated by centrifugation and stored at -20°C in acid-washed glass bottles until required for assay. Whenever possible, plasma was collected for estimation of calcium.

Estimation of calcium. Plasma calcium was measured by atomic absorption in all subjects except in

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TABLE I
Clinical details of babies with hypocalcaemia

Case no.	Clinical presentation	Reason for estimating plasma calcium	Age at which hypocalcaemia was first diagnosed (dy)	Associated lesions	Treatment at this hospital for hypocalcaemia	Treatment required 1 year later to maintain normal calcium
<i>Boys</i>						
1	Vomiting from 2 dy of age; convulsions from 6 dy of age	Convulsions	10	Nil	Calcium	Nil
2	Convulsions at 8 dy of age, followed by heart failure	Twitching	18	Atrial septal defect; ventricular septal defect; persistent ductus arteriosus; preductal coarctation; partial anomalous venous drainage	Calcium	Died
3	Acute renal failure after i.v. pyelogram for urinary retention; twitching developed	Routine estimation	5	Congenital hydronephrosis; congenital hypothyroidism; congenital hypoparathyroidism	Calcium and vitamin D	Nil
4	Cyanosed 24 hr after birth	Convulsions	11	Transposition of great arteries; ventricular septal defect; probably pulmonary stenosis	Calcium	Nil
5	Floppy baby with odd facies and heart failure; referred to this hospital 7 wk, hypocalcaemia still present	Routine estimation	2	Pulmonary atresia	Calcium and SMA	Nil
6	Progressive cyanosis after birth	Twitching after surgery for diaphragmatic hernia	1	Right diaphragmatic hernia left cleft lip and palate	Calcium	Nil
7	Tachycardia; heart failure	Convulsions	5	Nil; no cardiac lesion found	Calcium and vitamin D	Nil
8	Snuffles; apnoeic attacks; referred to this hospital at 7 wk for persistent hypocalcaemia; receiving Cow and Gate	Twitching	30	Nil	Changed to SMA	Nil
9	Poor feeder; vomiting from 4th dy; convulsions from 5th dy; transferred to this hospital at 6 wk for persistent hypocalcaemia	Convulsions	5	Bronchopneumonia with severe bronchospasm; repair of left inguinal hernia; hypoparathyroidism	SMA and calcium supplements continued; dose of vitamin D increased	Oral calcium and vitamin D
<i>Girls</i>						
10	Increasing cyanosis from 3rd day	Convulsion after cardiac catheterization at 6 dy of age	6	Transposition of great arteries; ventricular septal defect; mental retardation	Calcium	Nil
11	Feeding difficulties	Respiratory arrest	2	Tracheo-oesophageal fistula; hydrocephalus	Calcium	Nil
12	Feeding difficulties	Convulsions	4	Oesophageal atresia without tracheo-oesophageal fistula; stenosis, lactose intolerance	Calcium	Nil
13	Twitching	Twitching	4	Nil	Calcium and vitamin D	Nil

the normal babies in whom insufficient sample was available. Using an Eppendorf flame photometer, similar normal infants gave values for infants who were breast fed and those who were artificially fed on the

first day of life of 8.1 ± 0.5 and 8.3 ± 0.4 mg/100 ml (mean $\pm$ SD), respectively, rising to 8.7 ± 0.9 and 9.2 ± 0.3 on the 8th day (T. M. Barratt, unpublished observations).

Assay of parathyroid hormone. Materials used included (1) iodination (a) highly purified bovine PTH (b.PTH),* (b) ^{125}I ;† (2) standard (a) b.PTH, purified on Sephadex, 2000 MRC units/mg (b.PTH), (b) serum from a patient with primary hyperparathyroidism (kindly donated by Professor C. E. Dent); (3) antiserum; guinea-pig antiserum to b.PTH;‡ (4) precipitating serum; antiguinea-pig γ -globulin from rabbits; (5) 3 diluents were used in the system: A was hypoparathyroid serum (kindly donated by Dr. W. H. Taylor and Professor C. E. Dent) and was used for the standard curve and any test sera requiring further dilution; B was veronal buffer (0.1 mol/l.) + hypoparathyroid serum (6 : 1) and was used for the antiserum and labelled PTH; C consisted of 2 parts diluent B, 1 part 0.1 mol/l. EDTA and 1 : 1000 normal guinea-pig serum and was used to equalize incubation volumes. Parathyroid hormone was labelled with ^{125}I by a modified Hunter and Greenwood technique (Greenwood, Hunter, and Glover, 1963; Yalow and Berson, 1966; J. S. Woodhead, personal communication, 1971), stored in aliquots at -20°C and diluted for the assay so that $30\mu\text{l}$ labelled hormone gave 100 counts per second. The labelled material produced 75 to 80% counts bound in the presence of excess PTH antibody and less than 10% counts bound in the absence of PTH antibody. The assay procedure is shown in Table II. The control

TABLE II
Assay procedure for PTH

Solution	Volume (μl)
Antiserum 1 : 40,000	100
Standard PTH: 50–3,200 pg/ml b.PTH or dilutions of primary hyperparathyroid serum or patient samples	50
Diluent C	50
Preincubate at 4°C for 24 hr	250
^{125}I PTH	30
Incubate at 4°C for 72 hr	
Precipitating serum	50
Incubate at 4°C for 24 hr	

Centrifuge twice at 1500 g for 15 minutes after washing with veronal buffer; count precipitate for 100 seconds.
b.PTH, bovine parathyroid hormone.

*Wilson Laboratories, Chicago, U.S.A.

†Radio Chemical Centre, Amersham, Bucks, U.K.

‡Gift from Wellcome Research Laboratories, Beckenham, Kent, and Division of Biological Standards, Mill Hill, London.

incubation consisted of 'buffer blanks' composed of $400\mu\text{l}$ diluent C + labelled hormone + precipitating serum. Two sera, one of known high PTH value and one of low, were included in each assay. When sufficient serum was available 'serum blanks' composed of 1 : 2 dilution of serum to be assayed + diluent C + labelled hormone + precipitating serum were included for each patient. The reliability of the assay was assessed by performing recovery experiments (see Table III). The coefficient of variation was 39% using values obtained from samples in duplicate assays, 23% using values obtained from samples assayed at different dilutions, and, using two different sera assayed in 11 consecutive assays, was 29% and 8.8%. The lower limit of sensitivity for the assay was 39 pg b.PTH/ml.

Results

The concentrations of serum PTH are shown in the Fig. Further details concerning the normal infants are given in Tables IV and V. The PTH

TABLE IV
Serum PTH in normal neonates

Feed*	Serum PTH (pg/ml b.PTH)	
	Cord blood	6th day
Boys		
Breast	10	310
Breast	200	410
SMA	35	155
Ostermilk	75	140
SMA	30	125
Cow and Gate	90	225
SMA	390	220
Girls		
Ostermilk	25	215
Ostermilk	180	310
SMA	60	135
SMA	165	220
Breast	60	150
Breast	40	205
SMA	None detected	220

*See Table VII.

concentration rose significantly ($P < 0.01$) from a mean level in cord blood of 97 pg/ml to 217 pg/ml at 6 days of age, all except 1 infant showing the increase. There was no significant further

TABLE III
Recovery of b.PTH

	Amount of b.PTH added (pg)				
	50	150	300	600	1200
Mean amount recovered	56	149.5	355.6	627	1218
Mean % recovered	112	99	118	104	102
No. of experiments	4	4	4	4	4

FIG.—Serum PTH in normals and in hypocalcaemic infants. *Case 4 excluded from statistical analyses (see text).

increase in the older infants or children aged 1 to 6 years. Levels in the adults were higher ($P < 0.001$) compared with those in the infants and children.

Details of the infants with hypocalcaemia are

TABLE V
Serum PTH in older normal babies

Case no.	Sex	Age (wk)	Serum PTH (pg/ml b.PTH)
14	M	4	120
15	M	6	240
16	F	8	215
17	F	12	200
18	M	12	380
19	M	24	180
20	F	32	170
21	F	36	120

shown in Table VI. PTH concentrations were lower in this group ($P < 0.01$) compared with those found between 6 days and 36 weeks of age, the mean value being 130 pg/ml (excluding Case 4 who was probably recovering). The levels were not as low as those found in the adults with hypoparathyroidism. Analyses of the infants feeds are shown in Table VII.

Discussion

PTH has been measured in neonates and adults, and in children up to 6 years of age. Some time during later childhood or adolescence there must be a rise in PTH concentration in the serum, and this will require further study.

Classical neonatal tetany was first described by Kehrer in 1913 and subsequently shown to be associated with low serum calcium levels (Howland and Marriott, 1917). The babies in our study, however, did not form a homogeneous group and the majority were not considered to have the classical picture of neonatal hypocalcaemia. Their

TABLE VI
PTH and calcium concentrations in infants with hypocalcaemia

Case no.	Age at estimation (dy)	Feed*	Plasma Ca (mg/100 ml)	Serum PTH (pg/ml b.PTH)
<i>Boys</i>				
1	10	Cow and Gate	6.9	75
2	21	ND	6.4	65
3	25	SMA	6.2	216
4	13	ND	8.8†	485
5	53	Cow and Gate	5.5	118
6	8	ND	5.8	147
7	40	ND	4.6	107
8	54	SMA	6.0	72
9	48	Ostermilk	4.8	88
<i>Girls</i>				
10	11	ND	5.8	245
11	5	Parenteral	6.8	100
12	8	Cow and Gate	5.8	140
13	6	Ostermilk	5.1	182

*See Table VII

†This infant had a plasma Ca of 6.9 mg/100 ml 3 days before the specimen for PTH was taken.

ND, National Dried Milk.

TABLE VII
Composition of milks

Abbreviation	Milk	Composition of milk (mg/100 ml)		
		Ca	P	Mg
C & G	Cow and Gate (full cream)	97.2	73.9	10.1
ND	National Dried (full cream)	110	90.8	13.4
Ostermilk	Ostermilk I	106	83.6	12.3
SMA	SMA	50	40	5.0
BM	Breast milk	29	14	3.0

presentation was varied (see Table I) and there were many complicating factors.

Three mechanisms for the pathogenesis of neonatal tetany have been suggested (Bakwin, 1937): vitamin D deficiency, parathyroid hypofunction, and reaction to a high phosphate intake, the latter two being the subject of much subsequent debate and research. In 1942, Kaplan reported hypertrophy of the parathyroid glands in 14 out of 53 infants aged between birth and 30 days. In none was there evidence of renal disease or rickets. On reviewing the necropsy records (Gardner *et al.*, 1950), the hypertrophy was found to be associated with an excessive intake of phosphorus from cow's milk feeds and the relation between artificial feeding and hypocalcaemia has been noted subsequently (Baum *et al.*, 1968; Begum *et al.*, 1968; Eades, 1968; Pugh, 1968; Oppé and Redstone, 1968).

Although radioimmunoassays for measuring PTH have undoubted limitations, helpful determinations have been reported from a number of centres (Berson *et al.*, 1963; Reiss and Canterbury, 1968; Arnaud, Tsao, and Littledike, 1971; O'Riordan, Watson, and Woodhead, 1972). Shortage of materials of human origin, immunological differences between species, and the fact that native glandular hormone is immunologically different from secreted hormone (Arnaud, Tsao, and Oldham, 1970; Sherwood, Rodman, and Lundberg, 1970) all add to the problem of measuring PTH in patients.

Our study of hypocalcaemic infants showed that all were fed on artificial milks, that there was a preponderance of boys and a frequent association with congenital abnormalities of the thorax and reduced concentration of serum PTH. In spite of severe hypocalcaemia the parathyroid glands in these babies appeared to be incapable of responding to this stimulus by an increase in secretion of PTH. Unfortunately, blood samples for PTH assays were unavailable during the follow-up, but the maintenance of a normal serum calcium 1 year after

the hypocalcaemic episode suggests that most of them had only a transient disturbance of parathyroid function. Some of our infants had features similar to those with transient congenital idiopathic hypoparathyroidism described by Fanconi and Prader (1967) in whom the aetiology was thought to be a primary congenital hypoplasia or dysplasia of the parathyroids with a subsequent compensatory hyperplasia.

Our results provide further evidence for a functional immaturity of the parathyroid glands which may be more prolonged in infants in whom hypocalcaemia develops. Though our results were obtained in a rather unusual group of infants, it seems likely that they would apply to a series of infants with classical neonatal hypocalcaemia and this requires further study. In view of the differences between glandular and secreted PTH, it is possible that functional immaturity may be related to hypofunction of enzymes concerned with the conversion of glandular PTH to secreted PTH. This cannot be studied until methods for measuring different types of PTH become available.

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