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Normal Ranges and Diagnostic Value of Serum 5'Nucleotidase and Alkaline Phosphatase Activities in Infancy

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Belfield, A., and Goldberg, D. M. (1971). *Archives of Disease in Childhood*, **46**, 842. **Normal ranges and diagnostic value of serum 5'nucleotidase and alkaline phosphatase activities in infancy.** Determinations of serum alkaline phosphatase (APase) and 5'nucleotidase (5Nase) activities have been carried out in 159 children investigated for possible disease of liver or bones, and in 147 children in whom these possibilities never arose. The latter group, which served as a control population, showed 5Nase levels very much lower than those encountered in a normal adult population. APase values in this group rose after birth, fell after the first year, and remained stable until puberty when the range increased slightly.

APase was raised in 73% of children with hepatobiliary disease, 65% of children with bone disease, and 28% of children in whom there was no evidence of disease of either type. The corresponding figures for 5Nase activity were 87%, 6%, and 3%, and the degree of increase encountered in hepatobiliary disease was proportionately higher than the increase of APase seen in the same subjects. It is concluded that 5Nase is superior to APase in the diagnosis of hepatobiliary disease in infancy.

The interpretation of serum alkaline phosphatase (APase EC 3.1.3.1., orthophosphoric monoester phosphohydrolase) activities in children is difficult because of the age dependence of the normal range. In contrast, serum 5'nucleotidase (5Nase, EC 3.1.3.5. 5'-ribonucleotide phosphohydrolase) activity is said not to vary with age, and has been recommended in place of APase in the investigation of hepatobiliary disease in children (Hobbs, Campbell and Scheuer, 1968). Lack of an adequate normal range and the unsatisfactory nature of many existing methods for 5Nase assay have hindered the implementation of this recommendation.

Difficulties in 5Nase assay are caused by the presence in serum of non-specific phosphatase which hydrolyses the substrate used for 5Nase assay, and unless this non-specific phosphatase activity is suppressed, falsely high 5Nase activities are obtained. These difficulties have now been overcome (Belfield and Goldberg, 1968; Belfield and Goldberg, 1970), and a study of 5Nase activity in various diseases in adults using this method has been reported (Belfield and Goldberg, 1969b).

This paper describes a comparative study of 5Nase and APase in various diseases affecting children. The normal range for both these enzymes in children has been determined and the dependence of these ranges on age has been investigated.

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Materials and Methods

Sera were collected from patients attending the Sheffield Children's Hospital or referred for laboratory investigation by general practitioners. Selection of patients for this series depended on a request being made by the physician for serum APase or bilirubin estimations. In a few cases 5Nase was specifically requested together with the investigations mentioned above. Patients, whose ages ranged from birth up to and including 15 years, were divided into the following groups.

Hepatobiliary disease (30 cases). 20 patients had the clinical and biochemical features of infectious hepatitis. There were 2 cases of neonatal hepatitis and 1 case each of chronic hepatitis, homologous serum jaundice, portal vein thrombosis, and Gilbert's disease. 4 cases had extrahepatic biliary obstruction, 2 being due to biliary atresia, 1 to a choledochal cyst, and 1 to a cavernous malformation of the portal vein.

Physiological jaundice and haemolytic disease (14 cases). 11 children included in this group were jaundiced at or shortly after birth, several as a consequence of Rh-haemolytic disease. In addition, 3 cases of haemolytic anaemia, 2 of which were due to sickle-cell disease are included.

Bone disease (17 cases). Cases in this group had no evidence of liver or biliary tract disease. 9 had rickets, and 2 had osteomalacia secondary to renal failure. There were, in addition, 2 sisters with idiopathic hyperphosphatasia associated with bowing of the legs, 2 cases with tumours involving the bones, 1 case of hypoparathyroidism following thyroid operation, and 1 case of hypercalcaemia investigated for metabolic bone disease.

Other metabolic disease (50 cases). This category contained no cases known to have disease of the bones, biliary tract, or liver. There were 4 children in whom no final diagnosis was available; of these 1 case, probably of viral myocarditis, may have had liver involvement; 2, in which liver involvement was definitely excluded, had APase activities well outside normal limits; the fourth had pyrexia of unknown aetiology. The remaining children were distributed as follows: renal disease, including urinary infections (10 cases); gastrointestinal disease, including gastroenteritis and ulcerative colitis (11 cases); acute infections, including tuberculosis (9 cases); pulmonary disease, including acute asthma and mucoviscidosis (7 cases); endocrine disease (3 cases); myopathy (3 cases); anaemia (2 cases); acute poisoning (1 case).

No metabolic disease (48 cases). This group included 10 cases with well-compensated congenital cardiac disease for preoperative assessment; 3 cases of epilepsy and 9 of mental deficiency. 2 hepatitis contacts were investigated and found to be normal, as were 2 children of short stature, one child suspected to have ingested laburnum seeds, and one with a discharging postoperative sinus. One case of chromosome translocation with multiple congenital malformations was included. The remaining 19, of whom 9 had feeding problems, were investigated for miscellaneous complaints and found to be normal.

Normal subjects (147). These were children in hospital who had no obvious bone or hepatobiliary disease. They were divided into the following groups: 0 to 4 weeks; 5 to 11 weeks; 3 to 11 months; 1 to 5 years; 6 to 10 years; 11 to 16 years.

Measurement of enzyme activities. Serum 5Nase activity was measured by a coupled kinetic assay in which adenosine formed by hydrolysis of adenosine-5'-monophosphate in the presence of β-glycerophosphate is converted to inosine by adenosine deaminase, with a consequent decrease in extinction at 265 nm. This technique is described in detail elsewhere (Belfield and Goldberg, 1969b). Results are expressed as International Units per litre at 37 °C (IU/l.).

APase was measured by a modified version of the Kind and King (1954) procedure in which quantities were scaled down to use only 5 μl serum and the extinction measured in a Beckmann-Spinco microcolorimeter. Results are expressed as King-Armstrong Units per 100 ml (KAU/100 ml).

Calculation of normal range. To take into account the effects of age and sex, large samples are needed. In this study the only large body of subjects available were patients in hospital. Though no children having diseases affecting the bones, biliary tract, or liver were knowingly included, some may have accidentally been included. The problem of extracting a normal range from a mixed hospital sample has been discussed by Hoffman (1963), and his method has been used in this study.

Results

Normal ranges. Serum APase activity was highly dependent upon age (Table I). The values rose after birth and remained high for the first year, but then declined to remain stable throughout infancy, the upper limit showing a slight increase once more at puberty and adolescence. 5Nase activity was dependent neither upon age nor upon sex in the range examined, and the upper limit of the normal range calculated by the method of Hoffman (1963) was taken as 8 IU/l. This is lower than that obtained for a normal adult population (Belfield and Goldberg, 1969b). Fig. 1 combines the present data with those of our previous study in adults to demonstrate this difference and to show the rise in the normal mean value with increasing age in adult life.

TABLE I

Serum APase Activity in Children and Adolescents Measured by Method of Kind and King (1954)

Age		Nos.	Mean Activity (KAU/100 ml)	SD*	Upper Limit of 97·5–Centile*
Range	Mean				
0–4 wk	15 dy	24	10·0	3·8	17·6
5–11 wk	7·0 wk	18	19·4	6·5	32·4
3–11 mth	6·3 mth	24	18·5	4·4	27·3
1–5 yr	2·6 yr	43	13·6	3·9	21·4
6–10 yr	7·8 yr	21	12·5	3·2	18·9
11–16 yr	13·4 yr	17	12·7	4·8	22·3

*The normal ranges were extracted from a mixed hospital sample by the method of Hoffmann (1963).

Clinical subjects. These are summarized in Tables II and III and in Fig. 2.

Hepatobiliary disease. Most children in this group had raised serum 5Nase activity, and the

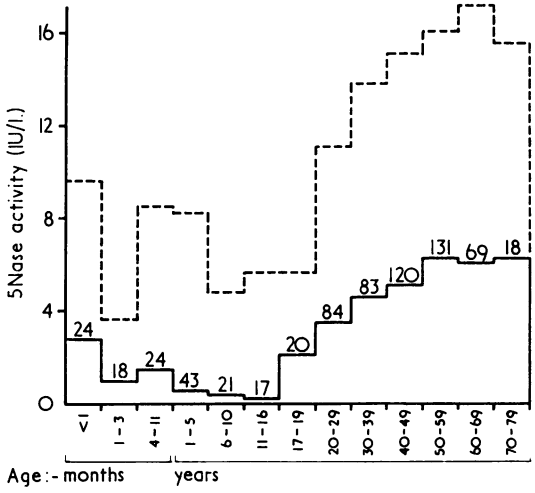

FIG. 1.—*Distribution of serum 5Nase activity with age. Method of Belfield and Goldberg (1969b). Solid line indicates mean and broken line 2 SD above mean. Numbers of subjects in each group indicated above mean.*

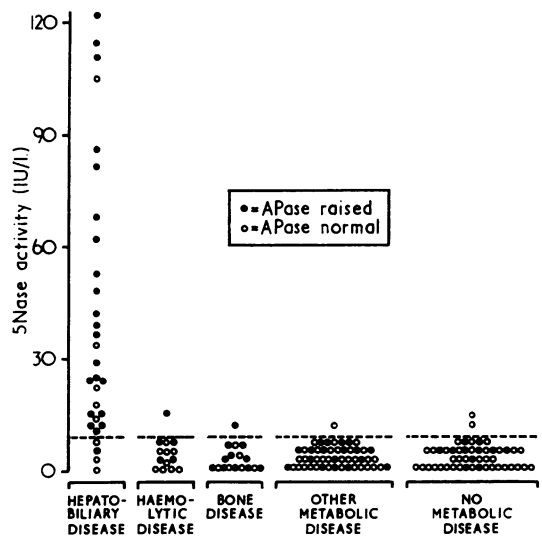

FIG. 2.—*Serum 5Nase activity in children with various diseases and conditions. Open circles indicate a raised APase; solid circle a normal APase for the age of the patient. The dotted line denotes the upper normal limit for 5Nase (8 U/l.) in children.*

TABLE II

Serum Alkaline Phosphatase Activity in Various Diseases of Children

Disease State	% Raised	Range	Median	16–84 Centile
Hepatobiliary disease (30)	73	13–105	31	16–48
Haemolytic disease (14)	36	7–32	18	8–26
Bone disease (17)	65	14–52	28	16–38
Other metabolic disease (50)	36	6–60	18	11–27
No metabolic disease (48)	23	4–30	17	13–22

TABLE III

Serum 5'Nucleotidase Activity in Various Diseases of Children

Disease State	% Raised	Range	Median	16–84 Centile
Hepatobiliary disease (30)	87	0–167	28	8–78
Haemolytic disease (14)	7	0–14	3	0–7
Bone disease (17)	6	0–9	0	0–5
Other metabolic disease (50)	2	0–9	1	0–5
No metabolic disease (48)	4	0–14	2	0–5

extent of increase in relation to the upper normal limit was more than twice that of APase in the same patients. 3 of the 4 cases with normal 5Nase activity also had serum APase activities which fell within normal limits for their ages. Serum APase activity was normal in 5 of 24 cases in which 5Nase was raised. The highest 5Nase and APase activities were found in a case of biliary atresia, but greatly increased 5Nase activities were also found in cases with neonatal hepatitis and portal vein thrombosis. The patient with homologous serum jaundice had normal APase and 5Nase activities, but transaminase activities on the same specimen were about 100 times the normal values.

Physiological jaundice and haemolytic disease. 5 of 14 patients had raised serum APase activities, and in one of these, a case of Rh-haemolytic disease with septicaemia, 5Nase activity was also raised. There were no cases of raised 5Nase activity in patients with normal APase activity.

Bone disease. Only 1 of 17 children, a case of rickets, had a raised serum 5Nase. 11 patients, including the child with raised 5Nase, had raised serum APase activity.

Other metabolic disease. Only 1 child, a case of infectious mononucleosis, had a raised serum 5Nase; this was associated with a normal APase activity. Clinical or subclinical liver disease is extremely common in this disease (Dunnet, 1963). 18 cases had raised serum APase activity but in few were the increases large. There were 2 cases with APase activities of 45 and 60 KAU/100 ml in which no firm diagnosis was made but in whom liver involvement was excluded.

No metabolic disease. 2 patients had raised

serum 5Nase activities; one was the child with multiple congenital abnormalities; the other was under observation after ingestion of laburnum seeds. In both instances APase activity was normal. 11 patients had raised serum APase activities but none exceeded 30 KAU/100 ml.

Discussion

Normal range for serum APase in infancy. Posen and colleagues who commented on the low values in the newborn obtained a mean APase activity of 16·6 KAU/100 ml in the serum of 13 healthy neonates (Kitchener *et al.*, 1965). This value is about 50% higher than that obtained by us, and is in line with the activation of APase induced by magnesium used by these authors in their method. Clark and Beck (1950) used p-nitrophenyl phosphate as substrate and reported serum APase ranges in a large series of seemingly normal children. They described a dramatic fall from high values encountered in the first year of life beyond the age of 2, but did not include observations during the neonatal period. The present study reconciles the above reports in demonstrating that the low values encountered shortly after birth rise rapidly during the first year and decline to a plateau which persists until puberty.

Diagnostic value of 5Nase and APase determinations. The results described show that serum 5Nase is more reliable than APase in the diagnosis of hepatobiliary disease in children, the former being increased more frequently than the latter. Only in 1 patient in this disease category was the 5Nase activity within normal limits when APase was raised, whereas the APase activity was within normal limits on 5 occasions when 5Nase was raised. Patients with physiological jaundice and haemolytic disease frequently had raised serum APase activities, but only once, in a patient with septicaemia, was the 5Nase activity also raised.

Some authors have been impressed by the superiority of 5Nase over APase determinations in the investigation and differential diagnosis of hepatobiliary disease in adults (Hill and Sammons, 1967), especially in relation to the early detection of hepatic malignant metastases (Schwartz and Bodansky, 1965; Smith *et al.*, 1966; Van der Slik *et al.*, 1970). In two fairly large series utilizing different methods of enzyme analysis we did not find 5Nase assays helpful in the differential diagnosis of jaundice, but concluded that they were more reliable than agar-gel electrophoresis in distinguishing bone disease from liver disease as the source of raised serum APase activity (Belfield and Goldberg, 1969a, 1969b). This conclusion is broadly in agreement with the earliest reports describing the clinical significance of serum 5Nase activity (Dixon and Purdom, 1954; Wachstein and Sigismondi, 1958), though a recent report comparing polyacrylamide disc electrophoresis of APase with serum 5Nase assay in this clinical situation favoured the former (Connell and Dinwoodie, 1970).

The proportion of children with raised 5Nase activities found in the groups without hepatobiliary disease (4 out of 124 patients) is that which would be expected from a normal range defined so as to contain 95% of the normal population. Serum APase activity was often raised in the three categories in which neither hepatobiliary nor bone disease was found. We have not seen such a high rate of non-specific APase increase documented previously in childhood illnesses. Whether this reflects a generalized systemic response to disease, or subclinical abnormality of liver and/or bones not detectable in other ways, or even liberation of enzyme from other damaged tissues, must for the present remain an open question; but the problem is an important one that clearly demands an independent investigation.

In view of this lack of both sensitivity and specificity, determination of serum APase activity adds little to the diagnosis of hepatobiliary disease in infancy. Serum 5Nase assay is much to be preferred in this situation. Recent methodological contributions to the problem of measuring the activity of this enzyme have led to a simple and sensitive colorimetric assay suitable for paediatric work and well within the scope of the smallest hospital laboratory (Belfield, Ellis, and Goldberg, 1970) as well as a more sophisticated kinetic procedure at 340 nm (Ellis, Belfield, and Goldberg, 1970) which has been adapted for assay by automatic reaction rate analysis (Goldberg, Ellis, and Wilcock, 1971) and is therefore more attractive to the larger laboratory. The availability of these methods should encourage paediatricians to employ 5Nase determinations on a routine scale.

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