VARIATION IN THE PROTEOLYTIC ACTIVITY OF CHILDREN'S STOOLS

A STUDY OF MECONIUM AND STOOLS FROM NORMAL AND ILL CHILDREN

BY

JOHN L. EMERY

From the Department of Pathology, Children’s Hospital, Sheffield

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In most paediatric clinics the examination of stools for trypsin activity has become a routine screening test as an aid to the diagnosis of fibrocystic disease of the pancreas.

The test is an old one (Hecht, 1908), and although recently re-introduced by Shwachman, Patterson and Laguna (1949) for use in fibrocystic disease, the examination of stools for trypsin has in the past been proposed as an aid to the diagnosis of other diseases such as rickets (Lukacs, 1926).

There is a physiological and a little experimental basis for examining faeces for trypsin as an indication of pancreatic function. It is known that the pancreas is the gland chiefly responsible for the intestinal secretion of trypsin and alkali, and it has been shown in animals that if all the pancreatic ducts are tied trypsin activity disappears from the stools (Carpi, 1923).

The clinical advantage of testing for trypsin activity of stools lies in its simplicity compared with the obviously more valuable examination of aspirated duodenal juice. On occasion there is considerable difficulty in obtaining duodenal samples and the procedure is not suitable as a rapid out-patient routine.

The amount of pancreatic secretion varies greatly, both with the individual and the diet (Thomas, 1950; Kahn and Klein, 1932; Snyder and Liume, 1936; Abramson, 1935), and it is probable that any residue in the faeces would vary to an even greater extent. To assess the clinical value of estimating faecal trypsin it is necessary to know the extent of this variation.

Klumpp and Neal (1930) found a relatively high level of trypsin secretion in infancy compared with that in later childhood, and Maday and Dancis (1947) found no depression of proteolytic enzymes in immature infants. McDougal (1950) found normal enzyme activity in the presence of severe malnutrition; four of 165 children showed a transitory partial suppression of activity in infections. Maddock, Farber and Shwachman (1943) found a smaller rise in trypsin levels after stimulation with secretin in six patients with malnutrition compared with five normal infants.

Veghelyi (1949), studying children over the age of 11 months, found no difference in duodenal enzyme value at different ages, and he was unable to relate the fluctuation in levels that he obtained with diet. His aim, however, was to establish mean levels and he used pooled specimens which would eliminate recording the widest variation in enzyme content.

In the recent clinical use of testing for faecal trypsin it was soon realized that anomalous results occurred; that children with gross fibrocystic disease can on occasion show trypsin activity in the stool (Johnstone, 1950; Johnstone and Neter, 1951), and from our own observations, that no trypsin activity may be found in apparently normal children.

It is also obvious that fibrocystic disease is neither clinically (Gibbs, Bostick and Smith, 1950) nor histologically (Andersen, 1948; Emery, 1951a) an ‘all or none’ disease, and a gross clinical syndrome may only be expected in what are probably the severe and irreversible cases.

It seemed necessary, therefore, to ascertain the degree of variation in faecal trypsin activity of normal children and also the variation in trypsin activity related to other incidents in the child.

Material and Methods

Two separate series of stools were obtained.

For the assessment of the normal trypsin range three groups of 100 normal children were used. Meconium was obtained from 100 normal infants born at a municipal maternity unit. In all cases the first specimen of meconium obtained from the child was examined. The stools from older children were from my own and my colleagues’ children, ‘well baby’ welfare clinics, children in residential hostels for normal children in the Sheffield region, and a few selected normal children admitted to the surgical side of the Children’s Hospital for surgical anatomical conditions such as strabismus.

In the general series, which contained over 1,000 stools, the stools were obtained from children in the
Sheffield Children's Hospital and in long stay and convalescent hospitals in the Sheffield area. No selection was made other than excluding cases of fibrocystic disease and very ill children.

Stools were collected in waxed cartons and examined usually within 24 hours of being passed, resting meanwhile in the refrigerator.

Tryptic activity was estimated using a simplified modification (Emery, 1951b) of that described by Andersen and Early (1946). The gelatine photographic film method was not used as it was no more rapid and thought to introduce too many uncontrollable factors. The recent observations of Gaffney (1951) appear to confirm this view.

Specimens in the normal series were examined to a final negative dilution whereas those in the general hospital series were examined at a dilution of 1 in 5, 10, 50 and 100 only.

Results

Tryptic Activity in Stools from Normal Healthy Children. These values are shown in Fig. 1.

MECONIUM. In the consecutive series from 100 normal newborn infants one specimen had no tryptic activity, two had tryptic activity only at a dilution of 1 in 2, four at a dilution of 1 in 4 and 12 at a dilution of 1 in 8. The distribution curve (Fig. 1) has a mean titre of around 1 in 30.

Breast and Bottle-Fed Infants. The distribution of the titre of the tryptic activity in this group is seen in Fig. 1. No specimen showed absence of activity, or activity only to a dilution of 1 in 2 or 1 in 4. One showed activity at a dilution of 1 in 8 and two at 1 in 16. The mean level of tryptic activity is in the region of 1 in 150. The tryptic activity in this group is higher than that in the first specimen of meconium.

Weaned Children on Mixed Diets up to the Age of 7 Years. The distribution of tryptic activity in this group (Fig. 1) has approximately the same mean as in the breast-bottle fed infants but the standard deviation of the curve is more than twice that of the younger group of children. Four children showed no tryptic activity, one activity at a dilution of 1 in 2, four at 1 in 4, eight at 1 in 8 and six at 1 in 16.

Variation in Tryptic Activity of Stools in Hospital Patients Related to Other Observations. These variations are considered in relation to the consistency and pH of the stool, and to the age, height, weight, nutrition, clinical disease of the child, and to other factors.

Tryptic Activity and Consistency of Stool. Nine hundred and eighty-nine stools were examined; 763 were described as bulky and soft, and 226 as small and hard. When these stools were analysed in degrees of tryptic activity it was found (Table 1) that the general distribution of numbers with different tryptic activity in the two groups appeared similar; 23% of the small, hard stools compared with 18% of the bulky, soft stools had tryptic activity of 1 in 10 or less.

There is no obvious relationship between the size and consistency of the stools and the tryptic activity.

Tryptic Activity and pH of Stool. Of 1,003 stools in which pH and tryptic activity (Table 2) were examined, 122 (12%) had a pH below 6, 649 (65%) a pH between 6 and 8 inclusive and 230 (23%) above 8.

When the percentage of these showing no tryptic activity or tryptic activity at a dilution of 1 in 5.
only are compared, all three groups give almost identical figures.

There appears to be no relationship between the tryptic activity of the stool and its pH.

**Tryptic Activity Related to Age.** In the general survey of stools the numbers in each age group were as follows (Table 3). Birth to 5 months, 498 children appears and tryptic activity normal limits, was 5 over 6 months and the numbers, identical figures.

Table 3

<table>
<thead>
<tr>
<th>Tryptic Activity Related to Age</th>
<th>0-5 months</th>
<th>6 months</th>
<th>1 year</th>
<th>2 years</th>
<th>3 years</th>
<th>4 years</th>
<th>5-13 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>3</td>
<td>1</td>
<td>9</td>
<td>2</td>
<td>22</td>
<td>17</td>
<td>2</td>
</tr>
<tr>
<td>1/5</td>
<td>1</td>
<td>2</td>
<td>4</td>
<td>8</td>
<td>29</td>
<td>22</td>
<td>13</td>
</tr>
<tr>
<td>1/10</td>
<td>2</td>
<td>2</td>
<td>24</td>
<td>14</td>
<td>69</td>
<td>12</td>
<td>12</td>
</tr>
<tr>
<td>1/50</td>
<td>3</td>
<td>8</td>
<td>11</td>
<td>14</td>
<td>46</td>
<td>8</td>
<td>6</td>
</tr>
<tr>
<td>1/100</td>
<td>45</td>
<td>84</td>
<td>160</td>
<td>125</td>
<td>315</td>
<td>25</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>54</td>
<td>97</td>
<td>208</td>
<td>163</td>
<td>481</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

54; 6 to 11 months, 97; 12 months to 2 years 11 months, 208; 3 years to 4 years 11 months, 163; over 5 years, 481.

Stools with no tryptic activity and activity only in a dilution of 1 in 5 showed an incidence of 7-4% in the children under 6 months; 3-1%, 6 to 11 months; 5-3%, 1 to 2 years 11 months; 6-2%, 3 years to 4 years 11 months and 10-6% over 5 years.

With this very artificial grouping the lower incidence of trypsin-free stools over the age of 6 months and the subsequent increase appears to be of more than chance occurrence. None of these were clinically suggestive of the coeliac syndrome.

**Weight.** Of the 943 instances in which the weight was available, 798 were within normal limits, 142 below normal and 12 above normal. Trypsin was absent in 26 (3-3%) instances of the normal group, twice (1-5%) in the below normal group and once in the over-weight group. If the figures for minimal trypsin activity are included the incidence of minimal trypsin activity is 6-8% in the normal weight and 4-6% in the wasted. There appears to be no correlation between the tryptic activity of the stool and wasting in these children.

**Height.** A similar finding to height was found. Of 498 children of normal height, 5% had minimal trypsin activity compared with one (2-5%) of 38 children below normal.

**Nutrition.** Seven hundred and ninety-seven specimens were described as being from children of normal nutrition, 165 from wasted and 38 from oedematous.

The proportions showing no tryptic activity were six (5%) in the wasted, 27 (4-6%) in the normal and one (2-6%) in the oedematous. If those showing activity only in the dilution of 1 in 5 are included the proportions are 12-4%, 10-6% and 10-6%.

The general results of these analyses of weight, height and nutrition are that there appears to be no relation between reduced activity of the stool and general nutrition.

**Relation Between Tryptic Activity and Clinical Disease.** The diseases were grouped as (1) gastro-intestinal, consisting of any child admitted for any dietary disturbance or surgical or functional disorder of the alimentary tract (97 case specimens); (2) respiratory (228 case specimens); (3) cardiovascular, the majority children with rheumatic heart disease (185); (4) nephritis (49); (5) other diseases, consisting of a mixed group of largely surgical conditions, many being from an orthopaedic hospital (417); (6) coeliac, children showing the coeliac syndrome in whom fibrocystic disease had been eliminated by other means (24).

Of the case specimens with intestinal disease 14%, had no tryptic activity and a further 7% at a dilution of 1/5 only. In the respiratory group seven (3-1%) had none and 13 (5-7%) minimal trypsin activity. In the nephritic group one (2%) had no and four (8%) minimal activity. In the miscellaneous group 11 (2-6%) had none and 34 (8-2%) minimal activity.

It will be noted that there was no difference between the nephritic, respiratory, cardiovascular and miscellaneous groups. The intestinal group showed a surprisingly high incidence, approximately one stool in five showing low activity, an incidence significantly higher than in the miscellaneous group. The ages of the children in the intestinal group showed no gross difference from the other groups.

**Relation Between Tryptic Activity and Mouth-Anus Stool Passage Time.** Stool passage time, using markers, was investigated in 167 specimens only. No relationship between the time of the intestinal passage and the degree of tryptic activity was found.
TRPYTIC ACTIVITY RELATED TO PRESENCE OF MUSCLE FIBRES IN STOOLS. Muscle fibres were seen microscopically in 658 (66%) of the 1,003 stools examined; in 59 (6%) they were present in very large numbers. No fibres were seen in 345 (34%) of the stools. Relationship between the presence of muscle fibres and trypsin activity is shown in Table 5.

Table 5

<table>
<thead>
<tr>
<th>Trypsin Activity</th>
<th>Presence of Muscle Fibres</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0</td>
</tr>
<tr>
<td>0</td>
<td>17</td>
</tr>
<tr>
<td>1/5</td>
<td>17</td>
</tr>
<tr>
<td>1/10</td>
<td>37</td>
</tr>
<tr>
<td>1/50</td>
<td>31</td>
</tr>
<tr>
<td>1/100</td>
<td>243</td>
</tr>
<tr>
<td>Total</td>
<td>345</td>
</tr>
</tbody>
</table>

There appears to be no statistically significant relation between the presence of muscle fibres and trypsin activity.

Discussion

The tube method of proteolytic assay used in this study does not give exactly parallel results with the x-ray gelatine film method. But the results we obtained in children of different age groups are roughly parallel to the figures quoted by Shwachman, Patterson and Laguna (1949) in their 'normal' series, i.e. non-fibrocystic. They found that none of 28 premature infants had negative stool trypsin; 4·3% of 233 infants; 12% of 133 children aged 2-5 years, 46% of 74 children aged 5 to 10 years and 75% of 32 over 10 years. The corresponding figures in our series are for children under 6 months, 7·4% negative; 6-11 months, 3·1%; 1-2 years 11 months, 5·3%; 3-4 years 11 months, 6·2% and over 5 years, 10·6%, that is to say, there is a similar increase in the incidence of trypsin-negative stools in later childhood.

The distribution curves (Fig. 1) from healthy children illustrate this same tendency, as the spread of distribution appears much greater in the older than in the bottle-breast fed infants.

A possible conclusion from these findings is that the absence of proteolytic activity in the stools in the first years of life is rarer than in later childhood, and thus its occurrence is probably of greater clinical significance.

In older childhood, on the basis of the gelatine film test used by Shwachman et al. (1949), a negative reaction may be expected in about a half of the non-fibrocystic stools; in our hands the corresponding figure is around a tenth. The implication of this in the use of this test as a screening test for disease of the pancreas will be obvious. Shwachman et al. concluded by stating that 'at least three specimens of stool should be examined and whenever doubt arises duodenal intubation is indicated,' but, on the basis of their own findings in the age groups 5 to 10 years, the chance occurrence of three consecutive negative stools would be expected in about 1 in 10 of hospital patients. In our hands the tube dilution method of stool trypsin assay appears to be more sensitive and the chance of a series of three negative stools more than 1/100, i.e. more than ten times less likely than the film method.

It is obvious that the use of stool trypsin in the diagnosis of fibrocystic disease of the pancreas can only be of value if used in conjunction with other features. Consideration should also be given to the observation of Johnstone and Neter (1951), who found positive gelatine film tests in 62% of patients with cystic fibrosis of the pancreas.

It had been partly expected that, in view of the postulated association of pancreatic change with glandular changes in the bronchi, that low trypsin levels would be more than usually common in children with chest infections. This does not appear to be the case.

Among the disease groups studied the incidence of low trypsin activity appeared to be remarkably consistent in all, except those presenting with disorders of the gastro-intestinal tract. This is particularly interesting if related to the observation of Baggenstoss (1948) that in routine necropsies an unusually high incidence of cystic and plugged acini was found in patients dying of infective and obstructive lesions of the alimentary tract. We do not know if the pancreatic changes in his cases or trypsin changes in the present series are primary or secondary to the alimentary disturbance and the present study takes us no further.

Summary

A survey was made of the variation in trypsin activity in meconium and in the stools from normal and general hospital patients.

The trypsin content of meconium appeared to be less than in later childhood.

Weaned children had the same mean level of trypsin activity as unweaned children but showed a greater variation in titre.

Approximately one in 10 of older children had minimal or no trypsin activity in the stools.

No relationship was found between the trypsin activity of the stools and the consistency, pH, the presence of undigested muscle fibres in the stools, the height, weight or general nutrition of the children.
An increased incidence of trypsin-low stools was found in cases with disorders of the alimentary tract. No increase was seen in children with respiratory infections, cardiovascular disease or nephritis.

The finding of trypsin-free stools appears to have a higher clinical significance in younger than in older children.

It is a pleasure to acknowledge the help of the nursing staff at the Sheffield Children’s Hospital, Nether Edge Hospital, the Jessop Hospital, King Edward Orthopaedic Hospital, Ash House Hospital School, the Moss Residential Nursery, and many other individuals for their assistance in collecting specimens of stool.

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