SERUM ALKALINE PHOSPHATASE AFTER FAT INGESTION: AN IMMUNOLOGICAL STUDY

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SUMMARY

1. Normal volunteers were given standard oral loads of cream, dextrose and skimmed milk. Serum alkaline phosphatase rose by a mean of 26%, 5 hr after fat ingestion. The rise was due, predominantly, to the appearance of intestinal alkaline phosphatase in the circulation.

2. Sera of secretors show greater intestinal alkaline phosphatase activities after fat ingestion than sera of non-secretors.

Serum alkaline phosphatase (SAP) rises after fat ingestion in humans (Langman et al., 1966; Warnock, 1968) and in rats (Jackson, 1952). Recent work in this unit has provided immunological evidence that, in the rat, the fat-induced increase in SAP activity is due predominantly to intestinal alkaline phosphatase (Saini & Posen, 1969). This paper demonstrates, also by an immunological technique, that the much smaller fat-induced increase in SAP in man is similarly due to intestinal alkaline phosphatase.

MATERIALS AND METHODS

Preparation of antigen

A macroscopically normal human small intestine was obtained at autopsy 2 hr after death. The mucosa was scraped off and alkaline phosphatase was prepared from this by the method of Horne, Cornish & Posen (1968). The powder obtained after acetone fractionation was dried in a dessicator at room temperature and stored at −20° until required. It had a specific activity of 2.42 King Armstrong units (Kind & King, 1954) per mg of protein and was used for injection into rabbits without further purification.

Injection protocol

Powdered intestinal alkaline phosphatase was dissolved in 0.9% NaCl containing 0.01% sodium merthiolate so as to give a concentration of 2–3 mg protein/ml. One part of this...
solution was mixed with 2 parts of complete Freund's adjuvant (Difco Laboratories, Detroit) so that a water-in-oil emulsion was formed. 1·0 ml of this emulsion was injected intramuscularly into four rabbits at weekly intervals for 3 weeks. The rabbits were bled 1 week after the last injection and the rabbit serum was heated for 14 hr at 56° in order to destroy alkaline phosphatase activity. The heated antiserum was used without further purification. Similarly heated sera from two uninjected rabbits were used for control experiments. No antibody activity was lost during the heating procedure (Kleerekoper, unpublished).

The effect of different volumes of one of the antisera on the alkaline phosphatase activity of the antigen is shown in Fig. 1. This antiserum had no detectable effect on the alkaline phosphatase activities of extracts of pooled human liver, bile, bone and placenta or on an extract of pooled rat intestine.

**Incubation technique**

The heated antiserum was diluted 1 : 50 with distilled water; 1·0 ml of this diluted material (containing 0·02 ml antiserum) was incubated with 0·05 ml of test serum for 30 min at 37°, and for 96 hr at 4° (Birkett et al., 1966). After centrifugation at 4° for 20 min at 4000 rev/min, alkaline phosphatase activity of the supernatant was compared with that of the same test serum similarly incubated with control rabbit serum.
Alkaline phosphatase assay

Two methods were used to assay alkaline phosphatase activity. The first of these was the automated phenylphosphate method of Marsh, Fingerhut & Kirsch (1959) which was used to determine total SAP activity and to determine SAP activity in the presence of $5 \times 10^{-3}$ M L-phenylalanine (Fishman & Ghosh, 1967). The other method consisted of an automated micro-assay with 4-methylumbelliferyl phosphate as a substrate. Enzyme activity determined by this method is expressed in MUP units, one such unit being the amount of enzyme hydrolysing 1 µmole 4-methylumbelliferyl phosphate/min at 37° under the conditions employed (Cornish, Neale & Posen, 1970). The 4-methylumbelliferyl phosphate method was used to determine SAP activity in the antibody studies.

ABO blood groups and secretor status were determined by the standard procedures (Mollison, 1963). Electrophoresis of SAP on cellulose acetate was carried out as previously described (Posen et al., 1967; Horne et al., 1968).

Fat ingestion

Twenty apparently healthy hospital workers whose clinical details are given in Table 1, were fasted overnight. At approximately 09.00 hours they were given 8-9 g of cream (stated fat content 38-0%) per kg body weight. Blood was taken in the fasting state and at 3 and 5 hr after ingestion of the cream. No food was taken in the interim. In two of the subjects (M.K. and M.H.) the experiment was repeated and continued for 8 hr. The sera were kept at $-20^\circ$ until required. Alkaline phosphatase activity was determined in all serum samples with and without $5 \times 10^{-3}$ M L-phenylalanine in the assay system. Fasting and 5-hr serum samples were incubated with rabbit antiserum and with control rabbit serum as described under ‘incubation technique’.

Carbohydrate and protein ingestion

Ten of the subjects were given 1·5 g skimmed milk powder (50% carbohydrate, 38% protein, less than 1% fat) per kg body weight on another occasion. Blood was again taken in the fasting state and 3 hr later. In nine of the subjects the experiment was repeated a third time with 0·28 g dextrose per kg body weight and blood samples were collected as for the skimmed milk experiment. In all subjects there was an interval of at least 1 week between experiments.

Definitions

‘L-Phenylalanine sensitive SAP’ was defined as the difference between SAP activity with and without L-phenylalanine ($5 \times 10^{-3}$ M) during assay. This definition differs slightly from that of Inglis, Krant & Fishman (1967). ‘Antibody sensitive’ SAP was defined as the difference between the SAP activity of a sample incubated with control rabbit serum and that of the same sample incubated with antiserum.

RESULTS

Table 1 shows that 5 hr after the ingestion of cream there was a significant rise in mean serum alkaline phosphatase ($P<0.001$ for both methods). Table 1 also shows that there was a significant rise in L-phenylalanine-resistant and antibody-resistant material. It may be calculated from Table 1 that there was a significant rise in both L-phenylalanine-sensitive and antibody-
TABLE 1. The rise in SAP in twenty normal subjects after the ingestion of 8-9 g of fat/kg body weight

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age</th>
<th>Sex</th>
<th>Blood group</th>
<th>Secretor status</th>
<th>Total SAP (KA u/100 ml)</th>
<th>Total SAP (MUP units/l)</th>
<th>SAP with $5 \times 10^{-3}$ M L-phenylalanine (KA u/100 ml)</th>
<th>SAP after incubation with antiserum (MUP units/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.W.</td>
<td>26</td>
<td>F</td>
<td>0</td>
<td>se</td>
<td>4.3 5.7</td>
<td>2.06 2.58</td>
<td>3.3 3.9</td>
<td>1.93 2.09</td>
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<td>R.L.</td>
<td>22</td>
<td>M</td>
<td>0</td>
<td>Se</td>
<td>8.2 10.3</td>
<td>4.05 5.25</td>
<td>6.4 7.2</td>
<td>3.76 4.23</td>
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<tr>
<td>P.F.</td>
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<td>M</td>
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<td>Se</td>
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<td>5.06 6.03</td>
<td>7.7 8.5</td>
<td>5.27 4.12</td>
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<td>B.B.</td>
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<td>M</td>
<td>0</td>
<td>se</td>
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<td>4.10 4.88</td>
<td>6.6 7.0</td>
<td>3.91 4.54</td>
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<td>0</td>
<td>Se</td>
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<td>5.72 7.44</td>
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<td>0</td>
<td>se</td>
<td>10.0 10.9</td>
<td>4.43 5.45</td>
<td>8.6 9.2</td>
<td>4.50 4.89</td>
</tr>
<tr>
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<td>M</td>
<td>0</td>
<td>Se</td>
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<td>7.27 8.24</td>
<td>9.9 10.1</td>
<td>6.42 7.64</td>
</tr>
<tr>
<td>D.K.</td>
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<td>M</td>
<td>0</td>
<td>Se</td>
<td>9.4 12.2</td>
<td>5.15 6.05</td>
<td>6.5 7.2</td>
<td>4.91 4.89</td>
</tr>
<tr>
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<td>0</td>
<td>Se</td>
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<td>3.34 4.70</td>
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<td>Se</td>
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<td>2.48 3.55</td>
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<td>Se</td>
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<td>4.99 5.82</td>
<td>7.5 6.1</td>
<td>4.75 4.70</td>
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<tr>
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<td>B</td>
<td>se</td>
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<td>4.80 6.81</td>
<td>5.6 9.2</td>
<td>4.70 5.69</td>
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<td>M</td>
<td>A</td>
<td>se</td>
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<td>8.17 8.48</td>
<td>12.0 13.0</td>
<td>7.83 8.20</td>
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<td>A</td>
<td>Se</td>
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<td>5.8 9.8</td>
<td>3.14 3.39</td>
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<tr>
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<td>M</td>
<td>A</td>
<td>Se</td>
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<td>4.85 5.17</td>
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<tr>
<td>M.K.</td>
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<td>4.00 4.00</td>
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<tr>
<td>A.I.</td>
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<td>M</td>
<td>A</td>
<td>se</td>
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<td>4.6 4.8</td>
<td>3.19 3.29</td>
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<tr>
<td>Mean</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>9.05 11.37</td>
<td>4.53 5.53</td>
<td>6.77 7.68</td>
<td>4.24 4.58</td>
</tr>
<tr>
<td>SD</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>±2.60 ±2.64</td>
<td>±1.42 ±1.51</td>
<td>±2.13 ±2.38</td>
<td>±1.41 ±1.51</td>
</tr>
</tbody>
</table>

$P$ value for correlated means: $<0.001$
sensitive SAP ($P<0.001$ for both calculations) and that the rise in the antibody-sensitive fraction was significantly ($P<0.001$) greater than the rise in the antibody-resistant material.

There was a wide variation between individuals, some subjects showing very little SAP elevation, while in others the SAP activity at 5 hr had increased by more than 40% above the fasting value. The highest absolute rise was 4 KA units/100 ml. The mean SAP at 3 hr was 10.57 KA units/100 ml, an increase of 1.52 KA units over the mean fasting value ($P<0.001$). In the two individuals in whom the experiment was continued for 8 hr, no rise in SAP activity occurred after 5 hr.

The apparently greater rise in total SAP activity amongst secretors than amongst non-secretors could not be validated statistically ($P>0.1$). However, the mean antibody-sensitive activity at 5 hr (calculated by subtracting antibody-resistant from total activity) was significantly greater amongst secretors than amongst non-secretors ($t = 2.12, P<0.05$).

Table 1 also shows that 4-methylumbelliferyl phosphatase activity paralleled phenylphosphatase activity very closely ($r = 0.96$). The rise in ‘antibody-sensitive’ SAP at 5 hr was significantly correlated with the rise in L-phenylalanine-sensitive material in individual subjects ($r = 0.46, P<0.001$).

Fig. 2 shows mean total SAP, mean L-phenylalanine-sensitive SAP and mean antibody-sensitive SAP in the fasting state and 5 hr after the ingestion of fat. It is seen that the increase in SAP activity is due predominantly to material sensitive to L-phenylalanine and to antibody.
Cellulose acetate electrophoresis was carried out on the sera of the first ten subjects listed in Table 1. Only two of these (D.K. and C.C.) showed a slow-moving band in the fasting state while all sera showed such a band following the ingestion of cream.

There was no rise in SAP activity after the ingestion of skimmed milk or dextrose. The mean fasting SAP in the skimmed milk experiment was $9.1 \pm 2.4$ KA units/100 ml, while at 3 hr the mean SAP was $8.8 \pm 2.3$ KA units/100 ml. Similar values were obtained in the dextrose experiment.

**DISCUSSION**

This study confirms earlier work (Langman *et al.*, 1966; Inglis *et al.*, 1967; Warnock, 1968) which showed a rise in SAP following the ingestion of fat and the absence of a similar rise following the ingestion of carbohydrate and protein. It also confirms the work of Warnock (1968) who showed that the appearance of slow-moving SAP after fat ingestion occurred in all subjects regardless of their blood groups or secretor status. This study provides good evidence, by a specific method, that at least some of the increment in circulating alkaline phosphatase after fat ingestion is due to intestinal material.

We have previously shown (Saini & Posen, 1969) that the rise in SAP in rats on a high-fat diet is only partially accounted for by material sensitive to anti-rat intestinal alkaline phosphatase antibody. Fig. 2 shows that the much smaller rise in SAP in humans is also due, in part, to alkaline phosphatase resistant to L-phenylalanine and to anti-intestinal antiserum. The tissue source of this ‘non-intestinal’ alkaline phosphatase which appears in the serum of rats and humans after fat ingestion, remains unknown.

Two other findings are unexplained at this stage. (1) Fasting SAP in the rat rises by a mean of 600% after a fatty diet, whereas the mean rise in humans is approximately 20%. (2) In both species rises in SAP activity occur after fat rather than carbohydrate or protein ingestion. The difference between fat on the one hand and carbohydrate and protein on the other is apparent even when these substances are given in isocaloric quantities (Langman *et al.*, 1966). These and related problems are currently under investigation.

**ACKNOWLEDGMENTS**

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**REFERENCES**


Alkaline phosphatase after fat ingestion


