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# Frequency distribution and 'reference values' of plasma alkaline phosphatase (EC 3.1.3.1) activity in the adult population of a Scottish new town

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SUMMARY As part of a study to establish the prevalence of renal calculus disease, alkaline phosphatase activity was measured by the method recommended by the Scandinavian Society for Clinical Chemistry and Clinical Physiology in the plasma of 3823 adults from the Scottish new town of Cumbernauld. The enzyme activity differs in males and females and increases with age. Reference ranges are presented.

The changes in the activity of alkaline phosphatase with age and sex are well reported (Roberts, 1967; Keating *et al.*, 1969; O'Kell and Elliott, 1970; Wilding *et al.*, 1972; Penttilä *et al.*, 1975), but most authors have measured activities in King-Armstrong units. The method recommended by the Committee on Enzymes of the Scandinavian Society for Clinical Chemistry and Clinical Physiology (1974) is being widely adopted in Europe and has been in use in this hospital since 1973. Reference ranges for this method are not yet well established (a small series is included in Whitaker *et al.*, 1977) and the difference in results makes comparison with 'traditional' King-Armstrong units difficult. Conversion factors between enzyme units measured under different conditions are notoriously unreliable (Rosalki *et al.*, 1975).

This work was undertaken as part of a wider study designed to establish the prevalence of renal calculus disease in the adult population of Cumbernauld (Scott *et al.*, 1977). This new town has largely drawn its population from the west central belt of Scotland in general and the city of Glasgow in particular. The population of the town at the time of the survey was just over 40000.

## Choice of subjects

The Cumbernauld Development Corporation maintains a computerised list of all persons who rent or own a house built by the Corporation (more than 90% of houses in the town). The computer randomly selected 7000 names of householders, alternately male and female names. These randomly selected persons were invited to attend at the local health centre. To account for the small numbers of people living in privately built houses, names were randomly selected by the survey clerk; this allowed all social classes to be included. If a person invited to attend failed to do so, no attempt was made to replace him/her. Owing to publicity in the local press, a number of people volunteered to attend and these have been included with the randomly selected population. The survey took 13 months to complete.

## Blood specimens

These were taken by medical practitioners with the subjects, who were not fasting, in a sitting position. Blood (10 ml) was withdrawn (after swabbing with 70% isopropyl alcohol and allowing the area to dry) from the antecubital fossa using a 20 ml disposable polypropylene syringe and 19 gauge disposable steel needle. A tourniquet was used only if necessary to locate the vein and was released after penetration of the vessel wall. After removal of the needle from the syringe the blood was gently ejected into a polystyrene tube containing solid lithium heparinate and polystyrene beads to aid separation. The blood samples were taken between 1815 and 2100 hours and were centrifuged for five minutes at the end of each session. The separated plasma in polystyrene tubes was stored overnight for analysis on the following day.

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### Measurement of alkaline phosphatase activity

The kinetic method is based on that of Bessey *et al.* (1946) with optimised reaction conditions at 37°C. It uses *p*-nitrophenyl phosphate (10 mmolar) substrate in diethanolamine buffer (1·0 molar) pH 9·8 containing MgCl (0·5 mmolar) and sample size 20 $\mu$l. The reagents were obtained from Boehringer, Mannheim. The reaction rate analyser (LKB 8600) was equipped with sample processor (LKB Ultrolab 2071) and calculator (LKB 8200). The calculator was set to use a $\mu$molar extinction coefficient for *p*-nitrophenol of 16·95 at 410 nm. Printout from the calculator was on an Olivetti Teletype (TE 318).

The samples from Cumbernauld were analysed each day under the same conditions as the hospital patient samples. Quality control sera (Precinorm E and Precipath E from Boehringer, Mannheim) were inserted in every tenth position in the batch, alternating the two sera. The analyses were performed by various laboratory technicians.

### Results

#### QUALITY CONTROL OF THE METHOD

Precinorm E and Precipath E are supplied with 'target values' and ranges assigned by the manufacturers.

Table 1 shows typical results calculated for a three-month period during the survey.

Table 1 *Quality control results*

| | *Assigned, target value (range)* | *Found, mean (2 SD range)* |
|---|---|---|
| Precinorm E | 197 (158-236) | 198 (157-239) |
| Precipath E | 455 (387-523) | 467 (389-545) |

During the 13 months of the study an occasional batch of determinations went 'out of control'. The error was due to a fault in the LKB calculator. Results from these tests were discarded.

#### POPULATION OF SUBJECTS

Of the people selected at random, 3397 aged 19-88 years responded to the request to attend the survey and 622 volunteered. A few refused to have blood taken, and some alkaline phosphatase results were discarded as described above. Results from randomly selected and volunteer subjects were combined. Results from three subjects aged 19 years were discarded. The number remaining was 3823. Results were arranged by sex, and by age groups within each sex. Age was calculated to the nearest 6 months. The age groups chosen were 20-29, 30-39, 40-49, 50-59, 60-69, and > 69 years. Figure 1 shows the survey population (overall) compared to the population of the town and the Scottish population as a whole. Many of the women were taking oral contraceptives which have been recorded as lowering plasma alkaline phosphatase activity (Penttilä *et al.*, 1975). Eight young women were in the early stages of pregnancy (within the first trimester). The results are unlikely to have been influenced by the measurement of detectable amounts of the placental enzyme.

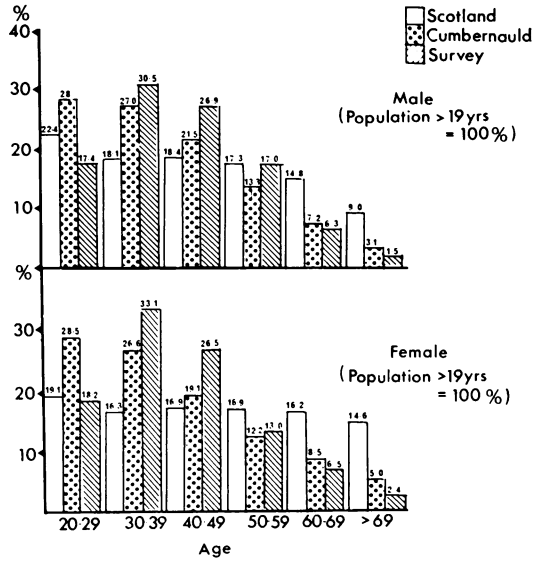


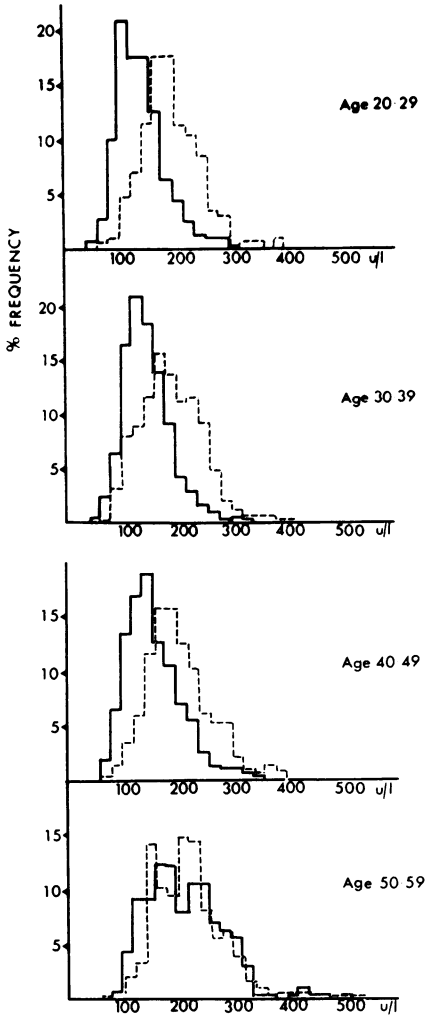

Fig. 1 *Survey population compared to the population of Cumbernauld and of Scotland.*

Histograms of frequency were prepared for each group using a class interval of 10 U/l, and the percent and cumulative percent frequencies were calculated. Obvious pathological results and 'outliers' were excluded from further calculations, but not more than 1% of results were removed from any group. The numbers finally remaining are shown in Table 2.

Table 2 *Distribution of the population by age and sex*

| Age | 20-29 | 30-39 | 40-49 | 50-59 | 60-69 | >69 |
|---|---|---|---|---|---|---|
| Male | 289 | 508 | 448 | 283 | 104 | 31 |
| Female | 389 | 705 | 564 | 278 | 145 | 51 |

As the distribution of the results was obviously not Gaussian, non-parametric methods have been chosen for presentation of results. The histograms in Fig. 2 have been drawn using percent frequency to remove the bias of differing numbers in each group and a class interval of 20 U/l. Figure 3 shows the asymmetry of the distribution, sex difference, and increas-



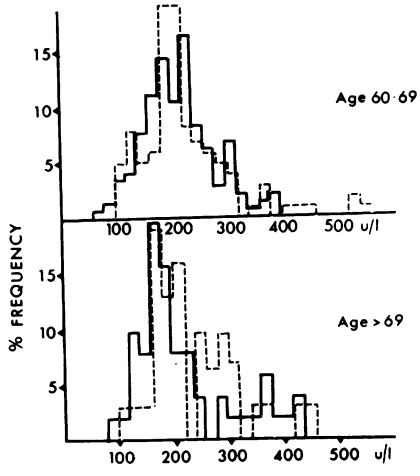




Fig. 2 *Frequency distribution as a function of age and sex: - - - male; —— female.*

ing activity with age, in particular the increase between the fifth and sixth decades in women.

## Discussion

It is recognised that many commonly determined constituents of plasma vary with sex and age but many laboratories continue to use reference values that are not so related. Before this decade many publications described 'normal ranges' derived from the analysis of a small number of samples from healthy young laboratory staff or students and based on calculation of the mean ± 2 standard deviations. With increasing use of multichannel analysers and the advent of population screening, numbers of age/sex related ranges are being published (O'Kell and Elliott' 1970; Wilding *et al.*, 1972), with more attention being paid to their distribution. As it is relatively uncommon for plasma constituents to be distributed normally within a population (Reed *et al.*, 1972) it is obvious that the mean ± 2SD method can be misleading. Much ingenuity has been employed in attempting to find mathematical 'fits' for the distribution found (O'Halloran *et al.*, 1970; Reed *et al.*, 1972; Flynn *et al.*, 1974), and the increasing complexity of these has led to a popular revival of the use of non-parametric methods (Mainland, 1971; Dybkaer and Gräsbeck, 1973; Siest *et al.*, 1975).

The reference ranges here presented (Table 3) are derived from centiles. There seems to be general

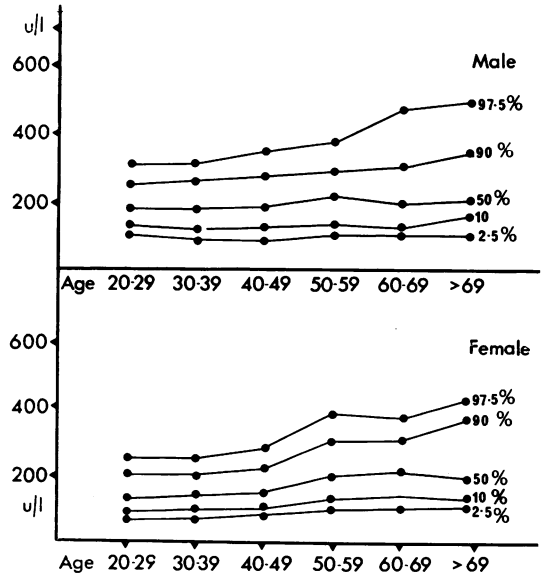



Fig. 3 *Cumulative frequency as a function of age and sex.*

Table 3 *Recommended reference ranges*

| *Age* | *Male* | *Female* |
|---|---|---|
| 20-29 | 100-320 | 70-260 |
| 30-39 | 90-320 | 70-260 |
| 40-49 | 100-360 | 80-290 |
| 50-59 | 110-390 | 110-380 |
| 60-69 | 120-450 | 110-380 |
| >69 | 120-460 | 90-430 |

agreement that such ranges should include 95% of the population studied. The omitted 5% is usually 2·5% from either end of the distribution but where, as with alkaline phosphatase activity, there is a skew in one direction it could be argued that this represents a pathological group within the population and that 5% should be omitted from the top of the range. This problem is discussed by Siest *et al.* (1975). We have presented the 2·5% to 97·5% ranges since the number of pathological values that may have been included is not known. The resulting ranges are somewhat higher than those derived by the mean ± 2 SD method.

The sex difference is most apparent in the younger age groups. In the male the increase with age is almost entirely due to an increasing skew to the right. Figure 3 shows only a small rise in the 2·5% and median values. In female subjects the most marked increase with age occurs between the fifth and sixth decades and is apparent at each centile, as shown in Figure 3. After the menopause the 2·5% and median values are similar to those of the male subjects but the increase in the 90% and 97·5% figures is not so great.

This study has not attempted to define changes attributable to variables other than sex and age. A recent study (Morrison and Shenkin, personal communication) has shown that there is some diurnal variation in the activity of alkaline phosphatase but this is unlikely to cause problems of interpretation. Seasonal variation, if this exists, was not obvious during the 13 months of the study and we have not investigated the effect of posture or drugs.

Because of the large number of subjects studied over more than a year, we are confident that, with the possible exception of our oldest age group, the ranges presented provide good guidelines for the interpretation of alkaline phosphatase activity in the adult.

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