oxidation of *o*-aminophenol is avoided when ascorbic acid is present. Trichloroacetic acid solutions brought to pH 2.25 precipitate proteins if phosphate buffer at the same pH is also present, permitting colour development without further pH adjustment.

2. The colour reaction has been applied to the measurement of glucuronide synthesis by mouse-

liver slices. Rates of synthesis of the order of 0.7 mg. *o*-aminophenol conjugated/g. dry weight of liver/hr. were obtained at the optimum concentration of 0.0025 %.

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The Frequency Distribution of the Zinc Concentrations in the Dental Tissues of the Normal Population

By D. B. CRUICKSHANK, *Sims Woodhead Memorial Laboratory, Papworth, Cambridge*

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In earlier work the author demonstrated the presence of zinc in the dental tissues (Cruickshank, 1936) and studied the increases occurring in persons suffering from active tuberculosis (Cruickshank, 1940). The present paper deals with the way in which zinc concentrations of dental tissues vary throughout the 'normal' population. The experimental results indicate that the population sampled was heterogeneous and divisible into two (or three) groups, each with its characteristic zinc concentrations. The significance of these findings is briefly discussed.

EXPERIMENTAL

Population and material sampled. Over a period of years, the author collected 'sound' teeth extracted, for orthodontic reasons, from secondary school children attending the Public Dental Clinic at Cambridge. All specimens were carefully examined and only fully calcified pre-molars free from visible caries or defects were selected for analysis.

The children, boys and girls, ranged in age from 10 to 17 years, 80% falling between 12 and 15 years. They can be regarded as a random sample of the pre-adolescent secondary school population biased only in the sense that all had a degree of 'overcrowded' dentition calling for orthodontic extraction.

Zinc determinations. These were made by a modification of the dithizone extraction, ferricyanide-iodine titration technique of Sylvester & Hughes (1936) reduced to a micro scale in order to permit quadruplicate or quintuplicate

analyses on the dentine and on the enamel of each single tooth specimen (Cruickshank, 1948). The standard deviation of the 250 standards (20 µg.) run during the tests was $\pm 3.8\%$. Interbatch variations were eliminated by 'batch scattering' the multiple analysis for each specimen. The total number of zinc estimations was about 1250.

Preparation of specimens. Dentine: The 'root' of each pre-molar was cut off just below the crown with a fissure bur, dried to constant weight (105°), ashed in an electric furnace overnight (500°), cooled and pulverized. The four to five analyses were run on samples of this pulverized ash.

Enamel: The 'crown' was first heated in the electric furnace for 0.5 hr. at 270° (pyrometer controlled) to char the dentine. When cool the 'neck' was pressed lightly into a surface of plasticine, surrounded with a 1 in. length of 1 in. diameter rubber tubing, and the hole filled with plaster of Paris. When set, the plaster containing and supporting the 'crown' was removed and trimmed to expose the 'neck'. The charred dentine was then cut out with a rose-head bur. The remaining enamel 'cap' was freed by making a cruciform saw cut into the block and splitting apart; it was washed and ashed overnight (500°), and four to five samples of the pulverized ash were analyzed.

Examination of variables. It was intended to obtain experimental data for 100 persons but exigencies of the war reduced this to 70. The available data were first studied in relation to the known variables of (i) sex, (ii) age of the subject, (iii) position, and (iv) condition of the specimen. As already mentioned all specimens were 'pre-molars' (the zinc contents of incisors, canines, pre-molars and molars differ to some extent). The 'defectives' were sound teeth with no caries but with enamel of slightly less perfect

structure than average. In view of the object of the experiment some knowledge of the effects of these accidental variables on zinc concentrations was imperative.

RESULTS

The results are summarized in Table 1. There is some suggestion of systematic effects; for example in dentine the zinc concentrations in males are 5% greater than in females and in enamel the zinc

by the enamel and dentine frequency curves it is clear that these 'zones' correspond to the *M*, *T* and *C* peaks already noted (Fig. 1). These results are only suggestive; definite proof of the existence of such subgroups must await more extensive and elaborate surveys.

Embryological factors. Dentine and enamel arise from two distinct embryological layers, viz. mesoderm and ectoderm; their calcification is effected by two independent groups of cells, the odonto-

Table 1. Summary of zinc concentrations in the teeth of the normal population classified in relation to certain known variables

Type of tooth	Dentine				Enamel			
	Males		Females		Males		Females	
	Mean Zn conc. (mg./kg.)	No.	Mean Zn conc. (mg./kg.)	No.	Mean Zn conc. (mg./kg.)	No.	Mean Zn conc. (mg./kg.)	No.
All	196	33	205	37	195	53	199	46
Normal	194	26	205	27	195	40	194	36
Defective	201	7	209	9	195	13	206	10
Uppers	198	17	208	20	195	22	188	23
Lowers	194	14	204	16	199	17	208	17
Age of subject (yr.)								
10 and 11	179	3	186	8	177	6	190	12
12 and 13	196	12	213	26	192	24	200	31
14 and 15	193	14	189	2	206	16	191	2
16 and 17	216	4	—	—	204	6	—	—

The S.D.'s of all means lie between ± 20 and 30 mg./kg.

concentrations of lower pre-molars are 15% greater than for upper pre-molars. In both dentine and enamel zinc concentrations appear to increase with age, rising respectively 12 and 15% during a period of 6 to 7 years; but in all these averages there is a wide scatter and a large degree of overlapping. The average zinc concentrations found in these experiments were some 20% higher than those found previously in a mixed adult population (dentine 178, enamel 152 mg./kg.) (Cruickshank, 1936).

Frequency distributions. The observed zinc concentrations were ranged in order of ascending magnitude and the curves (Fig. 1) plotted from a 3-point moving average across 5 mg./kg. class intervals. That for enamel gives a peak at 200 mg./kg. (*M*) with subsidiaries at 170 mg./kg. (*C*) and 220 mg./kg. (*T*). That for dentine is apparently bimodal with a major peak at 190 mg./kg. and another well-developed peak around 220 mg./kg. (*CT*).

A scatter diagram of the independently determined dentine and enamel zinc concentrations was next prepared and the frequency contours plotted (using a moving average across the 5×5 mg./kg. two-dimensional cells). There are three 'zones' of high frequency, two juxtaposed and the third discrete. From the elevation of this 'surface' as given

Fig. 1. Frequency curves of the zinc concentrations in dentine and enamel; and frequency contours of the dentine: enamel correlation surface.

blasts and the ameloblasts. There is therefore no *a priori* reason to anticipate a 'normal correlation' between the respective zinc levels of these two

calcified tissues. Because of this the 'abnormalities' of the frequency surface and curves may have biological significance. A suggested interpretation is that the population sampled was heterogeneous when classified in terms of the zinc concentrations of the two dental tissues examined. These irregularities could not be accounted for in terms of the known variables described above.

DISCUSSION

The main interest of the investigation lies in the demonstration of the possible existence of these population subgroups; other related work has

persons suffering from tuberculosis, in which condition there is evidence of increases in tooth zinc concentrations several years before any clinical manifestations of the disease appear (Cruikshank, 1940). Group *T* may therefore represent pre-tuberculosis subjects or alternatively persons prone to that disease.

If, as seems possible, zinc plays some essential role in calcification processes, its variations in tuberculous and pre-tuberculous(?) persons would be understandable, for 'calcification' of the lesion is one form of resistance against the disease. The therapeutic effects of zinc in tuberculosis are discussed by Ballard (1943).

It is of interest to compare the results of the present experiments with those of an independent investigation into serum alkaline phosphatase levels in normal and tuberculous persons (Murnaghan, 1946). Utilizing Murnaghan's data, Fig. 2 was prepared to show the striking resemblance which exists between the tooth zinc and serum phosphatase distributions (note particularly the bimodal frequency curves). There is a further resemblance in that the zinc and phosphatase increases (in tuberculosis) are unaccompanied by any increase in serum calcium and phosphorus (Table 2). Such close parallelism can scarcely be accidental.

Subgroup *C* is not discussed in this paper as there is as yet no experimental evidence of its nature or origin.

SUMMARY

1. The zinc concentrations in the dentine and enamel of pre-molars of a group of seventy secondary school children were determined by a modified dithizone ferricyanide-iodine titration method.
2. After eliminating secondary variables there remained an apparent irregularity in the frequency distribution indicating possible division of the subjects into one major (*M*) and two minor (*T* and *C*) groups each in respect of their zinc concentrations. One group (*T*) showed characteristics identical with those found in tuberculous subjects, characteristics which are known to appear years before clinical manifestation of the disease; it is therefore

Fig. 2. Resemblance between the distributions of the tooth zinc (0.5 x (dentine + enamel values)) and serum alkaline phosphatase levels in the normal population and in persons suffering from active tuberculosis (see also Table 2).

already indicated this possibility (Cherry, 1924, 1925; Cruickshank, 1939). If group *M* be taken as the mean type then group *T* differs from it in having a higher zinc concentration in both enamel and dentine. This is exactly the difference found in

Table 2. *The relative specificity of the dentine zinc and serum alkaline phosphatase increases in tuberculosis*

	Normal		Tuberculous		Significant increase
Serum calcium (mg./100 ml.)	11.03 ± 0.547	(130)*	10.36 ± 0.5	(55)*	No
Serum inorganic phosphorus (mg./100 ml.)	3.38 ± 0.509	(130)*	3.38 ± 0.49	(55)*	No
Alkaline phosphatase (units/100 ml.)	6.49 ± 2.60	(130)*	9.05 ± 4.4	(55)*	Yes (<i>P</i> = < 0.00006)
Dentine ash (g./100 g.)	73.85 ± 1.41	(41)	73.81 ± 2.11	(74)	No
Dentine zinc (mg./kg.)	178 ± 21	(55)	192 ± 26	(75)	Yes (<i>P</i> = 0.0007)

All the results are given as mean ± s.d. and (in brackets) no. of specimens analyzed.
* After Murnaghan (1946).

suggested that group *T* may represent pre-tuberculous or tuberculosis-prone persons.

3. The close correspondence which exists between the tooth zinc and the serum alkaline phosphatase levels, both in health and in tuberculosis is discussed, and it is suggested that the role of zinc

in tuberculosis may be connected with calcification of the lesions.

I wish to express my great indebtedness to Mr W. Baird Grandison and his staff at Cambridge for very full and cordial co-operation, and also to Mr Sampson for his technical skill in preparing the enamel specimens.

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A Biochemical Study of *Pseudomonas prunicola* Wormald

1. PECTIN ESTERASE

By G. BARBARA MILLS, *The Biochemical Laboratory, University of Cambridge*

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Certain species of *Pseudomonas* are responsible for the production of canker in stone fruit trees (Wormald, 1930). Erikson (1945), working with a strain of *Ps. mors-prunorum* Wormald, showed that the pathogen penetrated the tissues of the host intercellularly, plasmolysis of the cell contents and disintegration of the cell walls being followed by gradual invasion by the bacteria. This suggests that destruction of the pectic substances of the middle lamella of the host cells by extracellular pectic enzymes produced by the bacteria may play a primary part in facilitating the invasion of the tissues. Consequently, an investigation of the pectic enzymes of organisms involved in this invasion has been undertaken. The present paper describes investigations of an extracellular pectin esterase secreted by *Ps. prunicola* Wormald, an organism causing a similar disease in cherry and pear trees.

EXPERIMENTAL

Organism. For this work a strain of *Ps. prunicola*, isolated from an infected pear tree at East Malling Research Station, was used. This is a Gram-negative, non-sporing rod, falling into Dowson's Group II (Dowson 1939), the type species of which is *Ps. fluorescens* Migula.

Cultivation. The organism was grown in Roux bottles on a medium consisting of a tryptic digest of casein containing 0.4% citrus pectin and 0.01% marmite. Growth took place at 25° and lasted for 7–10 days.

Preparation of enzyme. The cells were centrifuged off, and the enzyme in the supernatant fluid concentrated as follows. Solid $(\text{NH}_4)_2\text{SO}_4$ was added to a final concentration of 40% saturation, and the resulting precipitate discarded. Further

$(\text{NH}_4)_2\text{SO}_4$ was then added to give a final concentration of 90% saturation. The precipitate, containing the enzyme, was allowed to rise to the surface of the liquid, and the fluid beneath siphoned off and discarded. The precipitate was collected and dialyzed for 24 hr. in collodion sacs against running tap water. The enzyme, now in solution within the dialyzing sac, was precipitated by the addition of 3 vols. of methanol at room temperature, spread on a dish, and allowed to dry at 37°. The dry precipitate was powdered in an agate mortar; such preparations of the pectin esterase can be kept indefinitely. For use, the powder was rubbed up in water (30 mg./ml.), and the solution brought to pH 7.5 with *N*-NaOH.

Substrate. Throughout the work the pectin used was a commercial citrus pectin, which was kindly provided by Chivers and Sons, Ltd., Histon, Cambs. This contained at least 30% invert sugar; the methoxyl content of the powder was estimated by the gravimetric micro-Zeisel apparatus and found to be 6.2%. For the enzyme studies a 3% solution, brought to pH 7.8 with *N*-NaOH, was used.

Estimation of activity. A manometric technique was used to estimate pectin esterase activity. Since the action of the enzyme liberates acidic groups on de-esterification, the activity can be followed by the evolution of CO_2 from a suitable bicarbonate buffer. The manometer cups contained 0.5 ml. of 0.3 *M*- NaHCO_3 solution, 0.5 ml. enzyme solution, and 1.0 ml. pectin solution, and were filled with a N_2/CO_2 gas mixture containing 5% (v/v) CO_2 ; at the experimental temperature (37°) this gave a pH of 7.8.

RESULTS

Confirmation that the manometric reaction studied is a de-esterification. In order to ascertain that the reaction studied is in fact the hydrolysis of an ester linkage, the relationship between the liberation of