gland. Rapid excretion of locally-introduced dye by the eccrine sweat glands, as described earlier(4), is consonant with the physiologic activity of these glands in the formation, over long periods, of large quantities of a fluid composed principally of water. Prompt excretion of locally introduced dye by the apocrine sweat glands suggests that these glands also have the capacity to form sweat, in part at least, immediately, *i.e.*, at time of apocrine sweating. However, the apocrine sweat glands produce a turbid or milky secretion containing significant concentrations of protein, lipid and carbohydrate in addition to ammonia and electrolytes. It seems likely that these substances are formed or secreted slowly by the secretory cells with a fluid or merocrine component added, perhaps by simple diffusion, at the time of apocrine sweating as indicated by the dye excretion studies. Variations in turbidity, color and natural fluorescence of apocrine sweat in the same individual could readily be explained on the basis of this theory of apocrine secretion.

In view of these observations, it would be well carefully to reexamine the apocrine sweat gland microscopically in an effort to delineate the cytologic mechanisms responsible for the proposed phases of apocrine sweat secretion. Histologic study of dye-treated skin is in progress but has not yet revealed the presence of selective channels, canaliculi, secretory vacuoles or other structures which could readily explain the merocrine component of apocrine sweat secretion.

*Summary.* The prompt excretion of locally-introduced fluorescein sodium in axillary apocrine sweat has been described. This observation suggests that the secretion of apocrine sweat includes a fluid or merocrine component formed at the time of apocrine sweating.

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## The Citrate Content of Teeth. (28432)

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Fluoride has been shown to increase the "crystallinity" of bone apatite of the rat (1-3) and of the human(4,5) with a concomitant decrease in the citrate content of the bone(6-7). The increase in "crystallinity," *i.e.*, a decrease in specific surface and/or reduction in strain would produce a less reactive and a more stable crystal structure. If a similar situation obtains in dental enamel (8), another parameter would be introduced to help elucidate the efficacy of fluoride in reducing caries. Preliminary information, however, is desirable on the citrate content of dental tissues of various species before the relation of "crystallinity" to fluoride may be studied. Data on citrate content of human teeth appear to be limited to the work of Free(9), Zipkin *et al.*(10), Stack(11) and Hartles *et al.*(12) indicating that dentin and enamel contain about 0.9% and 0.1% citrate, respectively. In addition, Thunberg(13) has reported about 0.5% citrate in the teeth of cats, cattle, and swine, and Free(9) found 0.54% and 0.11% citrate in dog's dentin and enamel, respectively. Leaver *et al.*(14) recently reported that rat incisor dentin and enamel contained 0.046% and 0.032% citric acid, respectively, while the molar dentin con-

TABLE I. Citrate Content* of Teeth of Various Species (Dry, Fat-Free Basis, %).

| Species | Dentin | Enamel | 100 E/D |
|---|---|---|---|
| Man† | .880 (194) | .100 (122) | 11.3 |
| Monkey | .510 ( 20) | .053 ( 16) | 10.4 |
| Dog | .437 ( 24) | .065 ( 24) | 14.9 |
| Cow | .425 ( 6) | .022 ( 6) | 5.2 |
| Sheep | .334 ( 3) | .033 ( 3) | 9.9 |
| Puma | .269 ( 4) | .040 ( 4) | 14.9 |
| Fox | .252 ( 8) | .054 ( 6) | 21.4 |
| Pig | .140 ( 5) | .010 ( 5) | 7.1 |
| Mean | | | 12.5 |

* Mean coefficient of variation is 12.5. Number of samples is given in parentheses. Mesh size <60, >100.

† Zipkin and Piez(10).

tained 0.405% citrate acid. Irie(15) has also presented data on the citrate content of developing tooth germs of dogs.

In this study, therefore, data are presented on citrate concentration of the dentin and enamel of various species. Citrate was determined on teeth of the cow, monkey, dog, sheep, puma, fox, pig, as well as on the white rat, white mouse, cotton rat, deer mouse or peromyscus and the hamster. Studies on the relation of the fluoride concentration of dental tissues to citrate and X-ray diffraction patterns will be presented separately.

*Methods.* The incisors, canines, first premolars, second premolars, first molars, second molars and third molars of man(10) and monkey were each pooled separately by tooth type for citrate analysis. For dog, puma, fox and pig, the incisors, canines, premolars and molars were pooled separately; for cow, sheep and rodents, the incisors and molars were separately pooled. In all cases, only adults of sufficient age to have a complete permanent dentition were studied. The crowns were separated from the roots, comminuted, extracted with alcohol for 8 hours and ether for 4 hours. The fraction ground to pass a 60-mesh, but not a 100-mesh sieve, was separated into dentin and enamel according to Manly and Hodge(16). Preliminary experiments demonstrated that a liquid of Sp.Gr. 2.65 used for separation of rat, hamster and human dental powders applied as well for separation of powdered cow, sheep and pig crowns. That is, no change was observed in the percent of powder that was floated until a Sp.Gr. of 2.9 was reached. Dry, fat-free samples of the dental tissues of the human, dog, cow, pig and rat were ashed at 550° for 3 hours.

Citrate was determined by a combination of the Taylor(17) and the Ettinger *et al.*(18) procedures as previously employed in this laboratory(6). Data are also presented on the dentin and enamel of the teeth of some pre-historic animals and fish ranging in age from a few thousand to 175 million years.

*Results.* The highest concentration of citrate in the teeth of the "large" mammals was found in the dentin (0.880%) and enamel (0.110%) of the human, whereas the same tissues of the pig contained 0.140% and 0.010% citrate, respectively (Table I). The enamel to dentin citrate (100 E/D) varied from 5.2% in the cow to 21.4% in the fox, with a mean ratio of 12.5%.

The mean concentration of citrate in the molar enamel of the rodent was also about 12% of that of the molar dentin (Table II). It should be noted, however, that the enamel of the continuously erupting rodent incisor contains approximately 30% as much citrate as the dentin. It is also apparent that the dentin and enamel of the incisors contain a lower concentration of citrate than the corresponding dental tissues of the molars,

TABLE II. Citrate Content* of Teeth of Various Rodents (Dry, Fat-Free Basis, %).

| | Molars | | | Incisors | | |
|---|---|---|---|---|---|---|
| Species | Dentin | Enamel | 100 E/D | Dentin | Enamel | 100 E/D |
| W. rat | .470 (18) | .054 (11) | 11.5 | .110 (15) | .024 (14) | 21.1 |
| Mouse | .332 (1) | .054 (1) | 16.2 | .048 (1) | .016 (1) | 33.3 |
| C. rat | .287 (1) | .013 (1) | 4.5 | .036 (1) | .016 (1) | 44.3 |
| Peromyscus | .137 (1) | .025 (1) | 18.3 | .038 (1) | .011 (1) | 28.9 |
| Hamster | .125 (3) | — | — | .021 (3) | — | — |
| Mean | | | 12.6 | | | 31.9 |

* Mean coefficient of variation is 12.5. Number of samples analyzed is given in parentheses. Mesh size <60, >100.

TABLE III. Citrate Concentration of Teeth of Some Prehistoric Species (Dry, Fat-Free Basis, %).

| Species | Age in million years | Dentin | Enamel |
|---|---|---|---|
| Marine shark | 60 | .0 | .0084 |
| Mammoth | * | .0054 | .0074 |
| Rhinoceros | 12 | .0052 | .0149 |
| Titanthere | 50 | .0397 | .0 |
| Shark | 20 | .0353 | .0047 |
| Phytosaur | 175 | .0237 | — |
| Crocodile | 60 | .0316 | .0015 |

* Age—a "few thousand years".

with the decrease in citrate concentration being proportionally greater in the dentin than in the enamel.

The ash content of the enamel of the teeth of the human, cow, dog, pig and for the incisors and molars of the rat was similar; namely, 95.0%, 93.4%, 96.0% 94.5%, 95.2% and 94.7%, respectively. The ash content of the dentin of the teeth of these species was also similar; namely, 76.8%, 75.5%, 73.2%, 74.2%, 75.5% and 75.3%, respectively.

The findings on citrate content of fossil teeth are quite striking. The age of the specimens ranged from a few thousand to 175 million years of age. In one case, no citrate was detectable in the dentin, and in another, none in the enamel. The citrate values were unexpectedly disproportionate between dentin and enamel, being higher in the latter in half of the specimens. The values seem extremely low, although no data appear to be available on citrate content of the teeth of the modern shark, rhinoceros, or crocodile.

*Discussion.* Analysis of the teeth of 13 species of animals, representing a number of mammalian orders revealed no consistent pattern. For example, the teeth of man and monkey, both primates, contained significantly different amounts of citrate. The dentin of these 2 species contained 0.880% and 0.510% citrate, respectively, while the enamel contained 0.100% and 0.053%, respectively. The dental tissues of the dog and fox, both canines, also contained different amounts of citrate. The dentin showed 0.437% and 0.252% citrate, while the enamels had 0.065% and 0.054% citrate, respectively. It is noteworthy that the dental tissues of the pig contained considerably less citrate than corresponding tissues of the human although reports indicate that both species show similar embryonic stages of development and similar skeletal growth patterns(19). The citrate content of the dental tissues of the dog and cow presented here are consonant with those of Free(9) and Thunberg(13), respectively, Our data on the pig are considerably lower than those presented by Thunberg(13).

It is clear also, that within the rodents, there was considerable variation in citrate content of the dentin and enamel. It is interesting to note that there was about 12% as much citrate in the enamel as in the dentin in the teeth of "large" mammals (Table I) and in the molars of the rodents (Table II). However, there was about 32% as much citrate in the enamel of the growing incisors of the rodents as in the dentin. This increase in the enamel to dentin citrate ratio was largely at the expense of dentin citrate, since the decrease in dentin citrate was proportionally larger than the decrease for the enamel citrate. Our data on concentration of citric acid in rat molar dentin (0.470%) approximates the value of 0.405% presented by Leaver *et al.*(14). However, our value of 0.110% for citric acid content of rat incisor dentin is considerably higher than that given by Leaver *et al.*, of 0.046%(14). The concentration of citric acid in the incisor enamel was comparable from both laboratories, 0.024% and 0.032%(14), respectively. It is possible that differences in diet may explain the differences to some extent, since Carlsson and Hollunger(20) have reported that the level of Vit. D intake may affect the citric acid content of rat incisors.

The citrate content of enamel may have important relevance to caries susceptibility. Previous studies from this laboratory have shown that fluoride may increase the "crystallinity" of bone apatite(1-5). If a similar effect of fluoride on enamel can be demonstrated, despite the larger crystal dimensions for enamel apatite(21), another parameter may be introduced to help explain the inhibitory effect of fluoride on caries. Hartles (22) has suggested that enamel citrate may act as a substrate for certain microorganisms,

and that fluoride may prevent its utilization. It has also been reported that addition of citrate to an *in vitro* calcifying system reduces the size of "calcium phosphate crystals"(23). The concentration of citrate in the dental tissues assumes importance, therefore, since it may affect the "crystallinity" of the enamel apatite *in vivo*, irrespective of the fluoride exposure. It should be noted that man has the highest enamel citrate content of the species studied and coincidentally is the most caries susceptible. It is striking that no other constituent of mineralized tissues reported to date varies as much as citrate among various species.

Of interest is the finding that fossil teeth contain only small concentrations of citrate, perhaps due to bacterial degradation or replacement by silicate or fluoride. Thunberg (24) reported that the citrate content of bone decreased with age. The bone-find of maximum age was taken from a Stone Age dwelling of approximately 8000 years ago and contained 0.02% citrate(24). The citrate content of the fossil teeth in the present study bore no relationship to their age, although a number of the samples had considerably less citrate than 0.02% and were 50-60 million years old. Two of the dental tissue had no demonstrable citrate. However, the dentin of the titanthere (50 million years old) contained about 0.04% citrate. The prediction of age of prehistoric fossils from the citrate of the dental tissues, therefore, would seem to be unlikely.

*Summary.* Dentin and enamel from 13 species comprising the primate, canine, bovine, feline, porcine and the rodent were analyzed for citrate. A wide variation in citrate concentration of the dental tissues was found. The dentin and enamel of the teeth of man contained 0.880% and 0.100% citrate, respectively. Similar dental tissues of the monkey, another primate, contained 0.510% and 0.053% citrate, respectively. The dentin and enamel of the teeth of the pig contained 1/6 and 1/10 as much citrate, respectively, as the same dental tissues in the human. The dentin and enamel of the molars of the rodent contained more citrate than the corresponding tissues of the incisors. A 4- to 5-fold variation was seen in the citrate content of the dentin of molars and incisors among the 5 rodents. The white rat had the highest concentration of citrate in the dental tissues, namely, 0.470% and 0.054% in molar dentin and enamel, respectively, and 0.110% and 0.024% in incisor dentin and enamel, respectively. The dentin and enamel of the rapidly growing incisors of the rodent contained less citrate than the same dental tissues of the molars. The possible relationship of citrate content of the dental tissues to caries and to crystallinity of dentin and enamel apatite is discussed.

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# Comparison of the Physico-Chemical and Physiological Behavior of Natural Inulin with Its Radioactive Derivatives.* (28433)

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The commercial availability of $C^{14}$ and $H^3$ labelled inulin derivatives† has prompted use of these substances as radioactive inulin tracers in the measurement of glomerular filtration rates and extracellular spaces. Although evidence has been presented(1,2,3) indicating the validity of the labelled derivatives as physiological inulin tracers, it will be shown that subjected to certain *in vitro* conditions in which molecular size, diffusibility, or charge were important variables, the labelled derivatives utilized in this study *separated* from the non-labelled compound.

While measurements of urine specific activity at various times after a single intravenous injection of inulin-$C^{14}$OOH and inulin to a dog revealed no glomerular differentiation (constituting evidence that both substances filtered at equivalent rates in this physiological process) the possibility of a more rapid rate of extrarenal removal of radioactive inulin from the circulation was suggested by data presented below. These findings emphasize that careful attention must be given to establishing the validity of the labelled derivatives as inulin tracers in any particular situation before employing them as analytical tools.

*Materials and methods.* The radioactivity labelled inulin derivatives were purchased from New England Nuclear Corp., Boston, Mass. Several lots of inulin-$C^{14}$OOH were used, No. 35-211-5 (S.A. 3.0 $\mu$c/mg), and No. 35-92-21 (S.A. 1.36 $\mu$c/mg), and lot No. 46-114-5 (S.A. 27 $\mu$c/mg) of inulin-$OCH^3$. Non-radioactive inulin was Pfanstiel (4045), Difco (446208) or Warner-Chilcott 10% solution (Control No. 205191).

Tritium and carbon$^{14}$ were counted in the Technical Measurement Corp. LP-1 liquid scintillation counter. Ordinarily 0.1 ml of aqueous inulin-containing solution was mixed with 3.0 ml absolute ethanol and 10 ml toluene, containing 0.3% diphenyloxazole (DPO) and 0.01% 1,4-di-2-(5 phenyloxazolyl) benzene (POPOP). Urines containing much inulin precipitated under these conditions and 1 ml of 1 M Hydroxide of Hyamine 10-X was added to each counting vial to keep the inulin in solution. Internal standards of labelled toluene were added and counted to verify a consistent counting efficiency.

Paper electrophoresis was performed in 0.075 $\mu$ Veronal buffer at pH 8.6 with the Spinco Model R apparatus. Carbon$^{14}$ activity of the papers was counted on the Forro paper chromatogram scanner coupled with Nuclear-Chicago Model 1615-B ratemeter and Texas Instruments Rectiriter recorder.

* This paper is based on work performed under contract with U. S. Atomic Energy Commission at University of Rochester Atomic Energy Project, Rochester, N. Y.

† Tritium label is introduced by reacting inulin with dimethylsulfate, only a small amount being used so as to methylate a very low proportion of the hydroxyl groups. In synthesizing the carbon$^{14}$-labelled inulin derivative a cyanohydrin is formed with $KC^{14}N$ and subsequently hydrolyzed to give inulin-$C^{14}$OOH. This additional carboxyl group undoubtedly was responsible for the anionic behavior of the $C^{14}$ labelled derivative. Both labelled derivatives are re-crystallized from ethanol and stated by the supplier to possess molecular weights between 3000-4000.