SUMMARY

1. Two hitherto undescribed colouring matters, *nalgiovensin* and *nalgiolaxin*, have been isolated from the mycelium of laboratory cultures of *Penicillium nalgiovensis* Laxa.

2. Nalgiovensin, $C_{18}H_{16}O_8$, crystallizes in orange needles or plates, m.p. 199–200°. It has been shown to be *dextro*-4:5-dihydroxy-7-methoxy-2-hydroxypropylanthraquinone in which the nature of the hydroxypropyl group, whether $—CHOH.CH_2.CH_3$, $—CH_2.CHOH.CH_3$ or $—CH(CH_3).CH_2OH$, is at present undetermined.

3. Nalgiolaxin, $C_{18}H_{15}O_8Cl$, crystallizes in yellow needles or plates, m.p. 248–248.5°. It has been shown to be a monochloroderivative of nalgiovensin and is believed to be *dextro*-1(or 8)-chloro-4:5-dihydroxy-7-methoxy-2-hydroxypropylanthraquinone.

4. Nalgiolaxin is believed to be the first example of a derivative of anthraquinone containing chlorine which has been recorded from any natural source.

5. Nalgiovensin and nalgiolaxin are both dextro-rotatory in chloroform solution and are of unusual interest since optically active, naturally occurring, derivatives of anthraquinone are rare except those which occur as glycosides.

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The Influence of a High-Sucrose Diet on the Calcium and Phosphorus Percentage of the Rat Femur, and a Comparison with its Effect on the Enamel and Dentine of the Rat Incisor Teeth

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The enamel and dentine of the incisors of rats maintained on a diet containing 67% sucrose contained significantly higher percentages of calcium and phosphorus than the same tissues of animals maintained on a stock diet of Lever cubes (Hartles, 1951). The calcium/phosphorus ratios on the high-sugar diet were, however, significantly lower than those on the stock diet showing that there was a relatively greater increase in phosphorus than in calcium.

The left femur of each of these animals was removed at the same time as the teeth and kept for subsequent analysis. The work reported here is concerned with the effect of the high-sugar diet on the percentage of calcium and phosphorus in these bones, and includes a comparison with the effect on the incisor teeth. The results show that in the femur there is no significant difference in the calcium and

phosphorus percentage or the calcium/phosphorus ratios on the two diets. The high-sugar diet, therefore, affected the incisor teeth but not the bone.

EXPERIMENTAL

Animals and diet. The animals and diets were those used in the tooth study (Hartles, 1951): group I, twenty-two animals maintained on a diet of Lever rat cubes; group II, twenty-seven animals weaned at 21 days on to the diet containing 67% sucrose.

The animals were killed with ether, the left femora removed at the same time as the teeth and placed in absolute ethanol for 48 hr. The atomic Ca/P ratio of the stock diet was 1.36 and of the high-sugar diet 1.35.

Treatment of bones. Each femur was stripped of adhering tissue, broken into small fragments, wrapped in a small filter-paper parcel and placed in absolute ethanol overnight.

The fragmented femora were then extracted with ether for 6 hr. and dried at 110° for 3 hr. The dry, fat-free bones were pulverized in a percussion mortar so as to pass through a 60-mesh wire sieve. Tissue debris and non-mineralized tissue were removed from the powdered bone by flotation in a bromoform-acetone mixture of sp.gr. 1.5. This is admittedly a somewhat arbitrary treatment, but it was found that material separated up to sp.gr. 1.5 and not in any quantity above that value. The procedure has the merit of producing a standard material for analysis. In this report the analytical figures for bone refer to that portion of the dry, fat-free femur of density greater than 1.5.

Analytical procedure. Samples of bone (50–100 mg.) were added to 10 ml. of 5N-HCl and allowed to stand for 1 hr. The mixture was made alkaline with ammonia, re-acidified to pH 5.2 with acetic acid and made up to 50 ml. The acid-insoluble residue was removed by filtration, and calcium and phosphorus determinations were carried out on the filtrates as previously described (Hartles, 1951).

RESULTS

The results for the calcium and phosphorus determinations and the calcium/phosphorus ratios are given in Table 1, together with those for dentine and enamel for comparison. It is interesting to note that the calcium/phosphorus ratio for the rat femur approximates to that of the incisor enamel rather than the dentine.

slight changes in the teeth of dogs (Smith & Light, 1945). Clarke & Smith (1935) showed that rats maintained on diets almost completely lacking in minerals still continued to deposit small amounts of minerals in the incisor teeth, although the bones were greatly altered. Any conditions, therefore, which appear to affect the developing tooth rather than the bone are of interest. Gaunt & Irving (1940) showed that large variations in the calcium/phosphorus ratio of the diet affected the incisor teeth and bone of rats in a different way. When the calcium/phosphorus ratio was 4.0 much better teeth were formed than at a ratio of 0.5. The effect on the bone was the exact converse; at a calcium/phosphorus ratio of 4.0 the bones were severely rachitic, whilst at the lower ratio of 0.5 bone of more normal structure was formed. The present work shows that the high-sugar diet which affects the composition of the enamel and dentine does not change that of the bone.

In view of this finding it is of interest to study the degree of correlation between the amounts of calcium and phosphorus in the three tissues.

Teeth. On the stock diet there is a high degree of correlation between the calcium in enamel and dentine and the phosphorus in enamel and dentine (the probability that the correlation is due to chance

Table 1. *Percentages of calcium and phosphorus in the dry fat-free femur and incisor teeth of rats on a stock diet and a high sucrose (67%) diet*

(Tooth results reproduced from Hartles, 1951.)

	Ca (%) Mean $\pm$ S.E.M.	P (%) Mean $\pm$ S.E.M.	Ca/P atomic ratio Mean $\pm$ S.E.M.
Stock diet:			
Femur	25.41 $\pm$ 0.18	12.25 $\pm$ 0.13	1.608 $\pm$ 0.016
Dentine	26.00 $\pm$ 0.29	13.54 $\pm$ 0.16	1.486 $\pm$ 0.012
Enamel	33.17 $\pm$ 0.37	15.99 $\pm$ 0.15	1.607 $\pm$ 0.014
High-sugar diet:			
Femur	25.57 $\pm$ 0.22	12.21 $\pm$ 0.07	1.623 $\pm$ 0.014
Dentine	26.75 $\pm$ 0.23	14.57 $\pm$ 0.12	1.425 $\pm$ 0.014
Enamel	35.06 $\pm$ 0.31	17.27 $\pm$ 0.11	1.569 $\pm$ 0.012

There was no significant difference between the percentages of calcium and phosphorus in the dry, fat-free femora of rats maintained on the stock diet and of those maintained on the high-sucrose diet. Neither was there any significant difference in their calcium/phosphorus ratios.

This finding is in contrast to the results for the enamel and dentine of the incisor teeth of the same animals (Hartles, 1951).

DISCUSSION

In most experiments conducted on bone and teeth it has been found relatively easier to influence the composition of bone than that of the developing tooth. For example, rachitogenic diets produce only

being 1 in 50 and < 1 in 100, respectively), i.e. if the calcium and phosphorus contents are high in the enamel there is a strong probability that they will be high in the dentine and vice versa.

On the sugar diet, however, the probability of high dentine calcium being associated with high enamel calcium is very small, and there is no correlation between dentine and enamel phosphorus. Values for the partial correlation coefficients are given in Table 2.

Teeth and bone. On the stock diet values calculated for the correlation coefficient show no correlation between calcium or phosphorus in enamel and bone, and for dentine and bone there is correlation only for phosphorus (the probability of its being due to chance is < 1 in 100).

Table 2. *Values of partial correlation coefficients for calcium in enamel, dentine and bone, and phosphorus in enamel, dentine and bone*

	Calcium		Phosphorus	
	Dentine	Enamel	Dentine	Enamel
Stock diet				
Bone	0.176	-0.072	0.458*	-0.198
Dentine	—	0.488*	—	0.650*
$n - 3 = 19$				
Sugar diet				
Bone	-0.269	-0.278	-0.297	0.388*
Dentine	—	0.269	—	0.116
$n - 3 = 24$				

* Significant correlation.

Table 3. *Values of correlation coefficients between calcium and phosphorus in enamel, dentine and bone*

	Stock diet Calcium			Sugar diet Calcium		
	Enamel	Dentine	Bone	Enamel	Dentine	Bone
Phosphorus:						
Enamel	0.628	—	—	0.482	—	—
Dentine	—	0.629	—	—	0.276	—
Bone	—	—	0.435	—	—	0.282
$n - 2 = 20$				$n - 2 = 25$		

On the sugar diet for enamel and bone there is a doubtfully significant negative correlation for calcium and a significant positive correlation for phosphorus (the probability of its being due to chance is < 1 in 20). For dentine and bone there are doubtfully significant negative correlations for both calcium and phosphorus. Values for the partial correlation coefficients are given in Table 2.

The calcium/phosphorus ratio. This ratio is widely used in investigations on teeth and bone and it is important to know, therefore, the degree of correlation between calcium and phosphorus in any of the tissues.

On the stock diet there are high degrees of correlation between calcium and phosphorus content in enamel, dentine and bone, especially in the two former tissues (the probability of their being due to chance are respectively < 1 in 100, < 1 in 100 and < 1 in 20). The effect of the high-sugar diet is to reduce the significance of the correlation in enamel and to render the figures for dentine and bone doubtfully significant. The values for the correlation coefficients are given in Table 3.

It can be concluded from the above data that on a nutritionally sound laboratory stock diet there is a high degree of correlation between enamel and dentine calcium and phosphorus content in the rat incisor, even though the calcium/phosphorus ratios of the two tissues may differ (Sobel & Hanok, 1949; Hartles, 1951). The effect of the high-sugar diet is to lessen markedly this correlation, particularly in the case of phosphorus.

Enamel is ectodermal and dentine mesodermal in

origin; even so, if the processes leading to the deposition of minerals were identical in enamel and dentine it would be expected that alteration of the dietary regimen should affect both tissues equally.

Murray (1936) has pointed out that magnesium can replace calcium in a mineralized tissue to a certain extent. This may be a reason why in the cases of enamel and bone, and dentine and bone, on the stock diet the only significant correlation is between dentine phosphorus and bone phosphorus. In the bone, itself, the sucrose diet has not significantly altered the mean values for calcium, phosphorus or the calcium/phosphorus ratio, but the correlation between calcium and phosphorus is reduced to doubtful significance.

Murray (1936) found wide differences in the composition of the mineral constituents of whole teeth in different animals, in different types of teeth in the same animal, and in bones and teeth of the same animal and in different tissues of the same tooth. She suggests that mineralization is an active and specific cell process and not merely a precipitation reaction. Experiments with radioactive isotopes (^{32}P) have confirmed the dynamic nature of bone (Chievitz & Hevesy, 1935, 1937); similar experiments with teeth have shown that ^{32}P is taken up but to a lesser degree (Manley & Bale, 1939). The continually growing rat incisor shows greatest uptake of the isotope at the root, and as the tooth grows the amount in the outer portions increases. The isotope also enters the molar teeth, but the uptake is only one-third of that in the incisor.

There is therefore enough evidence to show that the minerals of the teeth are not static. The apparent stability of the minerals of the teeth may be due to a dynamic state in which the equilibrium is much more resistant to change than is the case in bone, although it must be remembered that bone has a much greater blood supply than teeth.

The enzyme now known as alkaline bone phosphatase was first observed by Robison & Soames (1924); it is present in both teeth and bone. In enamel, the enzyme does not actually occur within the ameloblast but outside in the stratum intermedium (Bevelander & Johnson, 1945; Morse & Greep, 1947*a, b*). On the other hand, phosphatase occurs actually within the odontoblast and the adjacent pulp during mineralization of the dentine (Bevelander & Johnson, 1946). Histochemical methods have revealed that the ameloblasts contain more phosphorus than do the odontoblasts (Hintzsche & Baumann, 1933). By the use of microincineration techniques Hampf (1940) showed that the mineral content of the ameloblasts differed considerably from that of the odontoblast. There is therefore some evidence to suggest that the ameloblasts and odontoblasts differ chemically and biochemically.

The influence of the high-sugar diet on the enamel, dentine and bone of the rat lends support to the view that the processes of mineralization in the three tissues are significantly different.

SUMMARY

1. The calcium and phosphorus content and the calcium/phosphorus ratios in the rat femur were the same whether the animals were raised on a stock diet of Lever cubes or a diet containing 67 % sucrose.

2. This is in contrast to the effect on the enamel and dentine of the incisor teeth of the same rats (Hartles, 1951), and makes it clear that the 'sugar' disturbance is confined to the teeth and does not include bone.

3. The correlation between the amounts of calcium and phosphorus in enamel, dentine and bone has been evaluated.

4. The findings support the view that the processes of mineralization in the three tissues are significantly different.

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Studies on the Metabolism of the Protozoa

1. METABOLISM OF THE PARASITIC FLAGELLATE, *TRYPANOSOMA LEWISI*

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Trypanosoma lewisi has its natural environment in the blood stream of the rat. In this medium, oxygen, glucose and carbon dioxide are readily available to meet the energy requirements of the flagellate. Biot, Biot & Richard (1911) first indicated the important part played in the life of *T. lewisi* by glucose, which according to Reiner, Smythe & Pedlow (1936) is metabolized with the production of acid. Searle & Reiner (1941) showed that under aerobic conditions, almost 50 % of this acid was acetic acid, and stated

that carbon dioxide had a pronounced stimulatory effect on the respiration. Under anaerobic conditions, they found that very little glucose was used, unless pyruvate or carbon dioxide was also present. In this latter case, they demonstrated the fixation of carbon dioxide, with the production of succinic, lactic, pyruvic and acetic acids. Moulder (1948*a, b*) showed that, in the adult phase, *T. lewisi* was able to oxidize glucose more efficiently than in the reproductive phase, and that the trypanosome was also