some way with density relationships. Eklund's statement that there is no physical reason why the temperature profile of a stable column of water cannot cross the line of maximum density surely needs challenging. If two points on a profile that is crossed by the line of maximum density are considered, one point on each side of the line, then the water at the point to the right will be too warm for maximum density and that on the left of the line will be too cold. As the points approach the line the system will gain entropy by mixing in such quantities as are necessary to achieve the temperature of maximum density. If no external forces are present the "reshuffling" process will proceed until stability is attained along the temperature curve for maximum density. The example provided by Eklund of a lake isothermal at 3.9°C having a temperature profile crossing the line of maximum density is by no means convincing unless it can be shown that this condition does in fact occur under calm conditions. Lussana, whose work is quoted by Dorsey (4) and by Eklund in support of his calculations, deduced a figure of 197 atm as the pressure at which the temperature of maximum density is 0°C. This work is not referred to by Strøm, who considered that Pushin and Grebenshchikov had fixed this point with great certainty as 600 atm. It is unfortunate that the discrepancy between physical determinations should be so great.

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Cariostatic Effect of Phosphates

Abstract. *The caries-preventive effect of phosphate additives in cariogenic diets fed to white rats has been further demonstrated. Diammonium phosphate, β-glycerol phosphate, and sodium phytate again were shown to be caries-inhibiting, as was a 1,6-fructose diphosphate. The caries-inhibiting action of organic phosphates may coincide with a caries-protective factor presumed lost in the refining of sugar and in the processing of certain cereal foods.*

Previous publications have presented numerous results and reviewed data demonstrating unusual cariostatic effects of phosphates in experimental rats and hamsters (*1*). This report presents data on an anticaries property of a fructose phosphate, and adds to previous evidence of cariostatic effects of sodium phytate, β-glycerol phosphate, and diammonium phosphate (*1*). The data prompted speculation regarding the identity of caries-preventive organic phosphates with a so-called "caries-protective factor," presumed lost during the refining of sugar and in the processing of certain cereal foods (*2, 3*).

As discussed by Shaw (*4*), a low incidence of caries among certain populations often occurs where the oral environmental effect of the food is regarded as a dominant caries-preventing factor. Osborn, Noriskin, and Staz postulated in 1937 that crude cereals and sugars contain substances which inhibit dental caries but are removed during the refining process (see *2*). Osborn (*3*) reported a reduction in decalcification of teeth in vitro by cooked brown flour. The inhibitory effect of unrefined sugars, various hexose-phosphates, calcium phytate, and calcium glycerol phosphate was investigated (*2, 3*). Jenkins *et al.* (see *2*), in extensive experiments on white and brown flour, confirmed the evidence of Osborn *et al.* "that cooked brown flour contains a substance which reduced the solubility of teeth *in vitro*," and concluded "that certain organic phosphates, including phytate, reduce the solubility of calcium phosphate and teeth." These may be active substances in brown flour.

It is of interest that oat hulls (*5, 6*), as well as rice, pecan, peanut, and cotton seed hulls (*7*), have a cariostatic property. While efforts have been made to isolate an anticaries factor from such seed hulls, so far as is known such a factor has not been identified as an organic phosphate, for example, phytin. The major effort has been to identify an ether, alcohol, or acid water extract as a caries-preventive, brought about by an effect on the oral flora (*5*).

The experimental regimen of this type of study has been described (*1*). Dry ground bread, prepared by a local bakery, constituted 67 to 73 percent of the diet, together with 18 percent glucose (commercial cerelose) and 4 to 8 percent corn starch. Essential vitamins (Nutritional Biochemical Company vitamin mix), 1.09 percent L-lysine·hydrochloride, 0.5 to 2.0 percent NaCl, and 1.37 percent $CaCO_3$ were added. Phosphorus supplements replaced an equivalent quantity of corn starch. The diets contained 10 to 12 percent protein, and cariogenicity was not dependent on an excessive quantity of sugar. The animals were killed after a 60- to 90-day experimental period, and the lower molar teeth were examined for caries, essentially all the smooth surface type (*1*).

In the first series of experiments (1a, 1b, 1c, 1d in Table 1) the cariostatic effects of 0.55 percent, 1.11 percent, and 3.33 percent of $(NH_4)_2HPO_4$ were compared. In the second series (2a, 2b, 2c) the primary objective was to study a hitherto uninvestigated sugar phosphate, namely, the trisodium salt of fructose 1,6-diphosphate. In a third series the cariostatic effects of $(NH_4)_2HPO_4$ and sodium phytate were compared. Both the phytate and phosphate were evaluated in previous studies (*1*). Anticaries effects of the various additives are measured by comparison of control and corresponding test groups, in terms of percent of carious rats, carious molar teeth, and caries severity scores. That all control groups do not develop identical caries experiences is not unexpected in experimental caries research. Results can be evaluated, therefore, only by comparison of a test group with its specific control group. Rats of all comparable experimental groups are littermates.

As shown in Table 1, the cariostatic effect of $(NH_4)_2HPO_4$ was again demonstrated. Three different concentrations of $(NH_4)_2HPO_4$ reduced the incidence of caries from 91.4 percent to 20.5, 34.3, and 20.3 percent, respectively. A severity score of 13.2 was reduced to 1.0, 1.8, and 0.8. Under these conditions as little as 0.55 percent $(NH_4)_2HPO_4$ was very cariostatic, concomitant with a low concentration of 0.24 percent phosphorus in the diet. This effect of $(NH_4)_2HPO_4$ stands in striking contrast to results of previous

Table 1. The cariostatic effect of various phosphates in the white rat.

Expt. No.	Phosphate additive and percentage in diet: Additive	Percentage	Dietary Ca (%)	Dietary P (%)	No. of rats in group	Carious rats (%)	Carious lower molar teeth (per rat)	Caries severity score (per rat)
1a	Control	0.00	0.59	0.10	35	91.43	3.83	13.2
1b	$(NH_4)_2HPO_4$	0.55	0.60	0.24	39	20.51	0.56	1.0
1c	$(NH_4)_2HPO_4$	1.11	0.59	0.36	35	34.28	1.17	1.8
1d	$(NH_4)_2HPO_4$	3.33	0.58	0.87	34	20.29	1.77	0.8
2a	Control	0.00	0.59	0.14	40	100.00	5.23	26.6
2b	β-glycerol phosphate*	4.54	0.61	0.60	40	30.00	0.78	1.3
2c	Fructose 1,6 diphosphate†	4.20	0.61	0.63	33	15.21	0.33	0.5
3a	Control	0.00	0.62	0.11	27	96.30	3.41	10.6
3b	$(NH_4)_2HPO_4$	0.64	0.64	0.25	28	23.10	0.38	0.6
3c	Sodium phytate	0.71	0.63	0.23	26	42.32	1.31	2.6

* The sodium salt. † The trisodium salt.

experiments in which $Ca_3(PO_4)_2$ and $CaHPO_4$ had no cariostatic action (*1*) and indeed as much as 0.64 and 0.63 percent phosphorus was present in the diets (*1*).

The mechanism of the anticaries effect of inorganic phosphates remains unresolved (*1, 8*). There is strong evidence that this action lies within the oral environment and it appears not to become effective by way of a systemic metabolic pathway. Thus the striking difference in the cariostatic effects of $(NH_4)_2HPO_4$ compared with $Ca_3(PO_4)_2$ and $CaHPO_4$ is probably due to a difference in solubility which determined availability within the oral environment. Previously $CaHPO_4$ became cariostatic only when sodium chloride was present in the diet, perhaps through its solubilizing effect (*1*). Intubation of Na_2HPO_4 likewise has had limited cariostatic action (*1, 8*).

The highly significant cariostatic action of fructose 1,6-diphosphate is of particular interest. This caries inhibition is comparable to that resulting from β-glycerol phosphate, phytin, sodium phytate, and calcium phytate (*1*). All the rats in the control group (experiment 2a) had caries, and the severity score reached an unusually high average value of 26.6. Caries incidence was reduced from 100 percent to 30.0 percent and 15.2 percent, and average severity scores to 1.3 and 0.5. The diets which produced very little caries in experiments 3b and 3c contained but 0.25 and 0.23 percent of phosphorus, comparable to 0.24 percent phosphorus in the similar diet of experiment 1b.

The mechanism of the anticaries effect of organic phosphates as studied in this and other experiments poses a problem no less complicated than that of the inorganic phosphates. Nonetheless, their anticaries activity is suspected of being localized within the oral cavity. Perhaps through salivary or bacterial hydrolysis, inorganic phosphorus could become available in the oral milieu, but it seems likely that these compounds could be active as intact molecular entities. As noted above, the results of Osborn *et al.* (*2*) and particularly those of Jenkins *et al.* (*2*) suggest that their effect may be due to a stabilization of the oral tooth surfaces, that is, the teeth become more resistant to acid. These investigators note further that "this effect is observed in experiments with buffers (that is, in the absence of saliva) and is, therefore, evidence of a property of organic phosphates as such, and is quite independent of the capacity of some of these substances to act as substrates for phosphatases during incubation with saliva and thus be a source of inorganic phosphorus" (*2*).

Pertinent to this problem also is the following, quoted from a U.S. dentifrice patent of 1960: "The present invention is predicated upon the discovery that a dentifrice containing as an essential ingredient certain organic phosphates and their water soluble salts, will reduce the dissolving action of acids on dental enamel" (*9*). Furthermore, according to Manly and Manly (*10*), the solution rate of enamel in acid was reduced by a solution of cephalin, or one of its component phosphatides, when brushed onto human enamel. These investigators postulated that a cephalin film, formed on a tooth surface, becomes impermeable to other ions (*10*). Indeed, there is need to differentiate between the possible oral environmental action and effect of phosphate in contrast to its role as an essential systemic nutrient.

A clinical trial conducted in Sweden is reported to have demonstrated an anticaries effect of an inorganic phosphate, $CaHPO_4$, when used as a dietary additive in the bread, sugar, and small pastries at a school lunch menu (*11*). A 3-year study has been completed in the United States in which $CaHPO_4$ was an additive to flour used for baking purposes. The baking products were a part of the diet of groups of children residing in boarding schools (*12*). However, the results of this study were negative. Another similar study conducted in this country by Bibby (*13*) was also negative. Nonetheless, further evaluation of the cariostatic potential of phosphates, organic and inorganic, for the control of human caries warrants serious consideration, with emphasis on appraisal of the "protective factor" presumably lost during the refining of sugar and in the processing of certain factors.

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