though where it has been used it has given satisfactory results.

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HEAVY GLASSWARE IN THE LABORATORY

THIS note was suggested by that of Dr. J. Howard Brown who recently described in these columns the use of milk bottles with a crown seal for bacteriological laboratory purposes. In the laboratory with which the writers are connected the replacement of the fragile and expensive laboratory glassware by heavy or commercial products has been constantly increasing. The type commonly known as a prescription bottle, in the 6 oz. and 12 oz. sizes, has been found particularly desirable. The modern heavy glass bottle is so well annealed that it will stand repeated heatings in the autoclave with no clouding of the glass and with a negligible amount of breakage.

Instead of the crown seal, we have used the screw cap seal with excellent results. The screw caps can be obtained with various types of liners, of which the cardboard-waxed paper combination has proved the best. Such caps can be placed on loosely in situations where ventilation is desired. For many purposes an unlined cap is best. It permits of practically no evaporation on long storage and the closure is sufficient to prevent all contamination. The easy removal of the screw cap and its re-use an indefinite number of times is an advantage over the crown seal.

A prescription bottle, which has one side nearly flat, is used in this laboratory for a variety of purposes. It serves admirably for the storage of bacteriological media, for the water used as dilution blanks in quantitative bacteriological work, and in many instances in place of the familiar petri dish. About 15–20 cc of agar is sterilized in the bottle, which is placed with the flat side down for the agar to harden, when the bottle is used in place of a petri dish. When used in the student laboratories in place of the petri dish for isolation purposes or for quantitative work, there is less difficulty from contamination and much less desiccation of the agar than with the petri dish.

The screw cap when placed loosely on the bottle permits of sufficient diffusion of oxygen to support the growth of the obligate aerobic organisms and serves to keep out contamination as well if not better than a cotton plug. In moist situations there is no danger of molds growing through the seal as often occurs with cotton plugs.

These bottles are very satisfactory for the growth of mass cultures of bacteria for the preparation of vaccines and antigens. During the past three years many thousand cultures of the legume-nodule bacteria have been grown and distributed to the farmers of the state in such bottles. Before the adoption of the screw cap, bottles with cork finish were used. The cork-finish bottles were plugged with cotton during the incubation period and this cotton plug was replaced with a paraffined cork for shipment. With the screw-cap bottle, the cap is placed on loosely during the incubation period and then tightened for shipment. The use of the screw cap has materially lessened the labor of preparation and has actually resulted in a lower percentage of contamination. The inoculation of the agar for these cultures is done by a spray process similar to that employed in applying lacquer or paint. An ordinary atomizer with supply pipe dipping into a suspension of the inoculant is supplied with compressed air which passes through a sterile three-foot length of 2 inch iron pipe which is packed with cotton. Two men can easily inoculate 800 bottles an hour with this apparatus.

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SPECIAL ARTICLES

A PRELIMINARY NOTE ON THE SIGNIFICANCE OF THE PHOSPHORUS INTAKE IN THE DIET AND BLOOD PHOSPHORUS CONCENTRATION, IN THE EXPERIMENTAL PRODUCTION OF CARIES-IMMUNITY AND CARIES-SUSCEPTIBILITY IN THE RAT[1]

INTRODUCTION

RECENTLY Hoppert, Webber and Canniff[2] have reported that rats fed stock diets develop caries[3] of the molars at three months of age. The composition of the stock diet as published consists of whole ground corn 60 parts, whole milk powder 30 parts, linseed oil 6 parts, alfalfa 3 parts and sodium chloride 1 part.

When the 60 parts of corn were omitted from the diet and replaced by either 60 parts of oatmeal or 60

[1] The Biochemical Laboratory, School of Hygiene and Public Health, The Johns Hopkins University, Baltimore, Maryland.

[2] C. A. Hoppert, P. A. Webber and T. L. Canniff, SCIENCE, 74: 77, July 17, 1931.

[3] Dental caries in the rat means a breaking down of tooth structure in the molars of rats resulting in a cavity formation such as has been described in a previous communication (H. Klein, *J. Dental Research*, 11: 151, February, 1931). The use of the term "dental caries in the rat" means exactly what the term implies and does not carry with it any implication or suggestion that such caries in the rat is the same as that known as human dental caries.

parts of that portion of whole ground corn which passes through a sixty-mesh sieve, no caries were observed in the experimental rats. These workers conclude or suggest that the higher incidence of caries on the diet containing 60 parts whole ground corn is due to the impaction in the molars of large particles of corn, resulting in fermentative processes, so giving rise to dissolution of tooth substance. This deleterious effect on the teeth, they report, is prevented by substituting in the diet 60 parts of that portion of whole ground corn which passes through a sixty-mesh screen.

This speculation, namely, that the caries in the rats arises because of particle size, is open to criticism on a number of points. That portion of corn which passes through a sixty-mesh screen has properties which are different from whole ground corn aside from the differences in physical size, and if size of particle was the controlling factor, then oatmeal, which contains large particles, should also cause caries in the diets which they fed. However, oatmeal, as reported by those workers, does not produce caries.

It is the purpose of the present communication to direct attention to the rôle which phosphorus plays in the occurrence of such caries as the above-mentioned writers report.

DISCUSSION

As indicated in the preceding paragraphs, reports on the relation of diet to the incidence of dental caries in rats would indicate that high caries incidence may result from the rats receiving a diet containing large particles of whole ground corn.

If the calcium and phosphorus content of the diets reported by these workers is determined, interesting correlations are established between the occurrence of caries in rats and the level of phosphorus fed, as is indicated in Table I. It will be noted from the table that as the amount of phosphorus is increased in the diet the incidence of caries decreases, the ratio of calcium to phosphorus remaining practically constant.

A study of these phosphorus values seems to indicate that size of particle is not the factor which controls the incidence of caries in the rat, since oatmeal does not produce caries in the proportions as fed by Hoppert, Webber and Canniff. The phosphorus figures, however, indicate that dental caries in the rat appears where phosphorus intake falls to 0.4802 grams per 100 grams of diet where Ca intake is .3424 per cent. Where oatmeal is substituted for the corn, the phosphorus content of the diet is increased to 0.5666 grams per 100 grams of diet, and when sifted corn, which is higher in phosphorus than whole ground corn (see Table II), is substituted for whole ground corn the phosphorus content of the diet is increased to 0.5282 grams per 100 grams of diet. In both of the latter instances the caries incidence is zero, where Ca intake is .4012 and .3490 per cent., respectively.

TABLE I

CALCULATIONS* ON THE DIETS REPORTED BY HOPPERT, WEBBER AND CANNIFF

Diet	Parts	Phosphorus	Calcium	Ca/P ration	Caries	Nature of diet
Whole corn	60	.1740	.0084			
Milk powder	30	.2520	.2760	0.7/1	Per cent.‡ 90–100	Coarse
Alfalfa	3	.0071	.0339			
Linseed meal	6	.0471	.0241			
NaCl	1					
	100	.4802	.3424			
Oatmeal	60	.2604	.0672			
Milk powder	30	.2520	.2760	0.7/1	Per cent.‡ zero	Coarse
Alfalfa	3	.0071	.0339			
Linseed meal	6	.0471	.0241			
NaCl	1					
	100	.5666	.4012			
Corn sifting	60	.2220	.0150†			
Milk powder	30	.2520	.2760	.66/1	Per cent.‡ zero	Fine
Alfalfa	3	.0071	.0339			
Linseed meal	6	.0471	.0241			
NaCl	1					
	100	.5282	.3490			

* Calculations based upon figures given by Forbes.[4]

† This figure is for bolted cornmeal.

‡ This information on percentage incidence supplied through the courtesy of Dr. Hoppert by personal communication.

For the past two years results in the McCollum rat colony have indicated that the level of phosphorus

TABLE II

DETERMINATION OF PHOSPHORUS IN WHOLE CORN AND CORN SIFTINGS WHICH PASS THROUGH NO. 60 SIEVE*

Substance	Sample weight	Treatment	Phosphorus in percentage per 100 grams of substance dried at room temperature
Whole cornmeal*	2.0003	ash	0.295
Hoppert sample	2.0513	ash	0.295
No. 60 sifted corn*	2.0012	ash	0.370
Hoppert sample	2.0014	ash	0.3685

* These samples were kindly supplied by Dr. Hoppert.

[4] E. B. Forbes and M. H. Keith. Technical series, Bull. No. 5, Agricultural Experiment Station, 1914.

in the diet is important in the production and prevention of experimental dental caries in rats. Eighty-eight per cent. of rats fed a diet containing 0.23 grams of phosphorus per 100 grams of diet show dental caries at the end of 140 days of feeding. One hundred and forty rats fed diets containing 0.410 grams of phosphorus per 100 grams of diet show a percentage incidence of caries of 5 per cent.

The studies of McCollum, Simmonds, Park and Shipley, on bone growth and calcification, demonstrated the great importance, for the deposition of calcium salts in the osseous tissues, of certain ratios between these elements. The studies of Howland and Kramer, and others, have demonstrated the dependence of calcification on the inorganic phosphorus content of the blood, and especially on the product of the concentration of calcium and phosphate ions in the blood. We have found that a similar relation exists between blood composition and the incidence of dental caries. Using available data[5] on the composition of rat blood on diets unbalanced in calcium-phosphorus, we have been able to calculate that a critical level of blood phosphorus exists ($10.5 \pm .5$ mg) under which rats develop caries and above which rats are immune from dental caries.

Blood phosphorus concentration is determined in great measure by total calcium, total phosphorus and total vitamin D intake. Therefore the maintenance of a level of blood phosphorus above the caries-immunity level ($10.5 \pm .5$) is dependent upon these three factors. The level of phosphorus intake which will maintain the caries-immune level of blood phosphorus increases with increasing calcium intake and decreases with increasing vitamin D intake.

SUMMARY

(1) Estimations of the phosphorus content of diets reported to produce caries in rats indicate that rations containing 0.4802 grams of phosphorus per 100 grams of diet, or less than 0.4802 grams of phosphorus per 100 grams of diet, tend to induce dental caries in rats if Ca intake is 0.3424 per cent.

(2) Estimations of the phosphorus content of diets reported to produce rats immune to dental caries indicate that such diets contain 0.5282 or more grams of phosphorus per 100 grams of diet and 0.4012 or less grams of calcium per 100 grams of diet.

(3) Experimental evidence is presented which indicates that the level of phosphorus in the diet is an important factor in producing caries-susceptibility and caries-immunity in rats.

(4) We have also found that a relation exists between blood composition (phosphorus) and the incidence of dental caries in rats. Caries arises in rats whose blood phosphorus falls below a critical level (about $10.5 \pm .5$ mg[6] of phosphorus per 100 grams of serum), while those rats whose blood phosphorus concentration is $10.5 \pm .5$ or above are immune from dental caries.

(5) It is indicated that this blood figure is dependent upon the level of phosphorus, calcium and vitamin ingested in the diet.

In a forthcoming paper we shall present the results of an extensive review of the recorded observations of our own and other laboratories on the relation between diet and susceptibility to dental caries in the rat and our conclusions from examining these data in the light of the working hypothesis (blood phosphorus critical level) here presented.

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[5] Benjamin Kramer and J. Howland. In press.

ON THE ULTRA-VIOLET PHOTOMICROGRAPHY OF LIVING CELLS

AN important advantage of the ultra-violet microscope in biology is its ability to photograph directly many cell structures which ordinarily are seen only in fixed and stained preparations. In this way one can study these structures without fear that they may be coagulation artifacts. To do this with complete assurance, however, it is often necessary to work with living and not merely with fresh material. All the ultra-violet photographs thus far published have been made with the original microscope of Köhler (Zeiss)[1] or the modification due to Barnard (as built by Beck)[2]. These instruments, though well suited to the photography of fixed preparations, require exposures which are too long for living cells both because of the highly injurious action of the ultra-violet light and because of the loss of fine detail through Brownian movement and protoplasmic streaming.

It has been found that only simple modifications are required to give photographs with the immersion

[6] This figure is obtained by relating the calcium, phosphorus and vitamin D intake and percentage incidence of caries of the Hoppert, Webber and Canniff rats, with the blood calcium and phosphorus values of Kramer and Howland.

[7] Research fellow supported by grants from the American Dental Association and the Dental Staff of the Johns Hopkins Hospital.

[1] A. Köhler, *Zeit. f. wiss. Mik.*, 21: 129, 273 (1904); *ibid.*, 24: 360 (1907); A. Köhler and F. Togby, *Arch. f. Augenheilk.*, 99: 263 (1928); E. Grawitz and Grüneberg (photography by A. Köhler), "Die Zellendes menschlichen Blutes im ultraviolettem Licht" (Leipzig, 1906); H. v. Schrötter, *Virch. Arch. f. Path. Anat. u. s. w.*, 183: 343; F. F. Lucas, *Proc. Nat. Acad. Sci.*, 16: 599 (1930); *J. Morph.*, 52: 91 (1931).

[2] J. E. Barnard, *Lancet*, 2: 117 (1925); *J. Roy. Mic. Soc.*, 47: 253 (1926); R. W. G. Wyckoff and A. L. Ter Louw, *J. Exp. Med.*, 54: 449 (1931).