heated serums agglutinated the organisms slightly but such agglutination did not interfere with motility of the parasite. The preparation of parasites diluted with thioglycolate medium gave results similar to preparations of heated serums and parasites except that agglutination did not occur.

Discussion. Nuttall(3) and Buchner(4) were the first to demonstrate that normal blood and serum were bactericidal for certain bacteria. Nuttall(3) observed that the bactericidal property of normal serum was destroyed by heating at 56°C for 30 minutes. For many years thereafter "complement," a term used by Ehrlich(5) for the heat labile substance together with natural antibody, was considered the only bactericidal substance in normal serum. Recently, however, Pillemer and his associates(6) have demonstrated in normal human serum and other serums a substance which they named properdin. In the presence of complement and Mg^{++}, properdin participates in bactericidal, virus-neutralization and hemolytic activities. Properdin, like the factor in serum which destroys *T. vaginalis,* is also thermolabile. Feldman (7) has reported a toxoplasmal antibody activator in the serum-properdin system. Recently Borsos and Warren (Am. Soc. Tropical Med. 1957) described 2 factors in serum of adult chickens; the first was thermolabile and lysed *Trypanosoma cruzi,* the second was thermostabile and agglutinated this parasite.

This paper is limited to the demonstration that normal serum from human beings and various animals kills and disintegrates *T. vaginalis,* and that this ability is lost after heating at 56°C for 30 minutes. In current studies attempts are being made to determine the nature of the active substance and its effect on other parasites.

Summary. Human serums and serums from several species of animals have the capacity to kill and disintegrate *T. vaginalis* with great rapidity. This activity of serum is destroyed by exposure of the serum to 56°C for 30 minutes.

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Received April 21, 1958. P.S.E.B.M., 1958, v98.

Influence of Inorganic Ions on Fluoride Retention in the Rat.* (24087)

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Previous work has indicated that rats retain less fluoride in their carcass from a fluoride drinking water than from a chemically pure sodium fluoride solution when equal quantities of fluoride are given(1). These differences were thought to be due to mineral content of natural water, indicating that inorganic ions interfere with either fluoride absorption or skeletal retention. There is evidence for this assumption by Weddle and Muhler(2), and the theoretical studies of Feldman, Morken, and Hodge(3). The present study investigated several different inorganic ions, constituents of natural water supplies, for their influence on fluoride retention. Calcium, magnesium, iron, and phosphorus were chosen for investigation. These were evaluated for their ability to affect fluoride retention by comparing retention in the carcass of rats receiving fluoride from an aqueous solution containing the different inorganic ions with retention from a mineral-free water containing only fluoride.

* This work was supported in part by grant from Indiana State Board of Health.

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TABLE I. Results of Carcass Analyses of Rats Receiving Either a Pure Sodium Fluoride Solution (A) or One Supplemented with Calcium, Magnesium, Iron, and Phosphate (B).

Group	No. of animals	Avg ash wt (g)	μg F given		F found μg		F found ppm		Net % retained	
A	9	5.258	731	2*	602	15*	116	3*	63.9	5.7*
B	9	5.466	732	1	503	14	92	2	49.1	5.7
Control	9	5.066			143	9	29	2		

* Stand. error.

Methods. The animals were weanling Sprague-Dawley strain rats. Littermates were used in all instances. The fluoride content of animals from each litter was determined at beginning of experiment so that fluoride retention during the experiment could be calculated. All fluoride solutions were made in advance for the entire study period, and stored in paraffined glass carboys. This insured that all groups received water of identical fluoride concentration. Carefully weighed amounts of the respective inorganic compounds were then added to the fluoride solutions. Concentrations of inorganic ions were decided upon by taking values of Indiana State Board of Health for representative cities in Indiana. The levels used were thus consistent with those found in natural communal sources. Calcium was studied at levels from 0 to 200 parts per million; magnesium from 0 to 160 parts per million; iron from 0 to 20 parts per million; and phosphorus from 0 to 80 parts per million. Fluoride concentrations were either 1 or 2 parts per million. All solutions were made using redistilled, mineral-free water. Calcium and magnesium were added as the chlorides, iron as ferrous sulfate, phosphorus as sodium dihydrogen phosphate, and fluoride as sodium fluoride. All chemicals were reagent grade. Animals were housed individually in raised screen cages in a temperature-controlled room. Water consumption was measured daily by recording the amount dispensed and spilled during drinking, using technics already described(1). Pair-drinking procedures assured uniform ingestion of fluoride water by all the animals. In all studies, a low-fluoride stock corn diet ($F = 0.5$ μg/g) was available *ad libitum*. At termination of an experimental period of either 4 or 6 weeks the animals were sacrificed and the whole carcasses analysed for fluoride by methods previously described (4).

Results. Table I summarizes the influence of a combination of Ca^{++}, Mg^{++}, Fe^{++}, and PO_4^{---} ions on the whole carcass retention of fluoride. Group A received redistilled mineral-free water containing 1 μg F/ml. Group B received the same amount of fluoride as Group A, but the water contained in addition 200 parts per million calcium, 160 parts per million magnesium, 20 parts per million iron, and 80 parts per million phosphate. These data show an appreciable reduction in total amount of fluoride retained by the Group B animals when compared to those of Group A, showing that the combination of ions resulted in significant ($p = 0.02$) inhibition of fluoride retention.

The results of the studies in which only calcium was aded to the fluoride drinking water are summarized in Table II. No significant reduction in retention occurred as increasing

TABLE II. Results of Whole Carcass Analyses of Rats Receiving Fluoride and Varying Levels of Calcium in Drinking Water.

Group	No. of animals	ppm Ca	μg F given		F found μg		F found ppm		Net % retained	
A	10	0	584	10*	516	29*	76	5*	62.0	4.2*
B	4	8	559	9	524	72	75	5	60.6	6.9
C	4	16	567	10	496	16	80	0	55.4	4.1
D	16	22	568	9	496	21	71	4	62.4	4.4
E	11	107	568	6	481	13	64	3	64.3	2.1
F	12	197	596	7	454	14	60	3	55.6	3.9

* Stand. error.

TABLE III. Results of Whole Carcass Analyses of Rats Receiving Fluoride and Varying Levels of Magnesium in Drinking Water.

Group	No. of animals	ppm Mg	μg F given		F found μg		F found ppm		Net % retained	
A	15	0	545	5*	505	14*	73	2*	71.4	3.1*
B	2	10	524	7	483	16	61	11	58.6	2.2
C	2	20	529	3	508	7	75	5	62.8	1.7
D	6	40	531	5	487	20	70	20	74.1	5.2
E	5	80	544	8	474	25	73	4	71.6	6.6
F	2	160	560	2	524	50	70	1	85.2	8.7

* Stand. error.

TABLE IV. Results of Whole Carcass Analyses of Rats Receiving Fluoride and Varying Levels of Iron in Drinking Water.

Group	No. of animals	ppm Fe	μg F given		F found μg		F found ppm		Net % retained	
A	8	0	1103	7*	640	40*	96	5*	54.9	3.8*
B	8	5	1104	7	673	50	95	6	57.5	4.6
C	7	10	1101	7	709	25	98	5	60.7	3.2
D	7	20	1105	8	633	41	96	8	54.0	3.3

* Stand. error.

levels of calcium were added to the drinking water.

The influence of magnesium ions on availability of fluoride for whole carcass retention is found in Table III. There were no significant differences in any of the groups in amount or fluoride given, total amount recovered in the carcass, or in concentration of fluoride in the carcass.

The results of the effect of iron appear in Table IV. Iron at levels of 5 to 20 ppm did not influence availability of fluoride for whole carcass retention.

The effect of phosphate is reported in Table V. These data suggest that phosphate, like magnesium, appeared to facilitate somewhat the storage of fluoride although not in proportion to phosphate concentration studied. Certainly no reduction in storage occurred with addition of phosphate to the water.

Discussion. Considerable attention has been given in the past to factors affecting fluoride metabolism and, especially, fluoride retention in the body when fluoride is used in communal water supplies as a means of partially reducing incidence of dental caries in humans. Present evidence suggests that when fluorides are added to fluoride-deficient water supplies at the level of 1 μg/ml there are no adverse systemic effects occurring from cumulative fluoride retention.

The degree of dental caries reduction afforded by artificial addition of fluoride to communal water supplies containing different interfering inorganic ions is of particular interest, however. The results of such interference may be an inactivation of the metabolically available fluoride to such an extent that degree of protection against dental caries is significantly affected. Because of this possibility, the inorganic composition of the communal water supplies which are being fluoridated may need more attention than given heretofore in order to insure availability of sufficient fluoride to obtain maximum dental caries reduction. It may be that amount of

TABLE V. Results of Carcass Analyses of Rats Receiving Fluoride and Varying Levels of Phosphate in Drinking Water.

Group	No. of animals	ppm PO_4	μg F given		F found μg		F found ppm		Net % retained	
A	9	0	1149	6*	791	22*	106	3*	43.3	2.1*
B	9	20	1129	9	841	28	107	5	48.7	2.8
C	8	40	1151	4	799	21	100	3	44.0	1.8
D	9	80	1141	5	827	25	105	4	46.7	2.1

* Stand. error.

fluoride added to communal water supplies will need to be increased in communities served by water having a high inorganic ion content in order to maintain significant anticariogenic benefits. The data in this study apply to this problem, since they strongly suggest that when fluoride is added to a water supply containing Ca^{++}, Mg^{++}, Fe^{++}, and PO_4^{---} together, significantly less fluoride is retained in the whole rat carcass than when similar amounts of fluoride are added to a mineral-deficient water. The contribution of individual inorganic ions in producing this collective effect is not clearly defined, since when Ca^{++}, Mg^{++}, Fe^{++}, or PO_4^{---} ions are tested separately by measuring fluoride retention in the whole carcass, no significant changes are noted. As a result, additional study is needed of combinations of ions common to communal water supplies to determine the unique properties of the ion combination which make it capable of significantly affecting fluoride absorption or retention.

Summary. 1) The whole carcass retention of fluoride by rats receiving a fluoride drinking water containing a combination of Ca^{++}, Mg^{++}, Fe^{++}, and PO_4^{---} ions was significantly less than in rats drinking a chemically pure fluoride water containing no additional inorganic ions. 2) In contrast, studies of the individual ions at various concentrations show only small fluctuations in the proportion retained. No significant influence on retention was noted when any of the ions were studied singly. 3) The decrease in retention seen in rats ingesting the water containing a combination of ions suggests that the influence of the ions may be additive or synergistic. This may be important since most natural waters contain a group of several inorganic constituents. More attention should be placed on inorganic composition of the communal water supplies before fortifying them with fluoride. It is possible that if the combination of major inorganic constituents of natural waters exceeds a yet-to-be determined concentration, optimal fluoride concentration would need to differ from the now accepted level of 1 ppm in order to produce maximum anticariogenic benefits.

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Received April 21, 1958. P.S.E.B.M., 1958, v98.

Effect of Banana Ingestion on Urinary Excretion of 5-Hydroxyindole Compounds in Normal Adults. (24088)

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Anderson and coworkers(1) have reported that addition of bananas to the diet increases the urinary excretion of 5-hydroxyindole acetic acid (5-HIAA). Their study was done on 4 Rhesus monkeys and 2 sick children. 5-hydroxyindole (5-OH indole) compounds are excreted in larger than normal amounts in the clinical syndrome produced by malignant carcinoid with metastases, and both the roughly quantitative "screening test"(2,3), and the precise quantitative determination(4) are useful laboratory procedures in establishing the diagnosis. Anderson's cited report suggests that high levels of urinary 5-HIAA may be produced in normal patients by banana ingestion, thus leading to an erroneous diagnosis of malignant carcinoid.

The present investigation was undertaken to determine whether urinary excretion of 5-OH indole compounds in normal adults can be affected significantly by addition of bananas to the diet.