groups until about the 20th day, when an abrupt disappearance of pigment was observed at the gingival margin. Thereafter, depigmented enamel progressively replaced pigmented enamel as the teeth erupted. All animals in group 3 had bluish-white incisor teeth after 45 days (indicated as 0 in Fig. 1), although a few retained small yellow areas on a depigmented background. Mandibular incisors of this group presented no significant deviation of pigmentation as compared with those of other groups. However, rats fed a similar diet for a greater length of time exhibited an irregular depigmentation of these teeth.[5] Fig. 1 shows changes of pigment observed in the five groups.

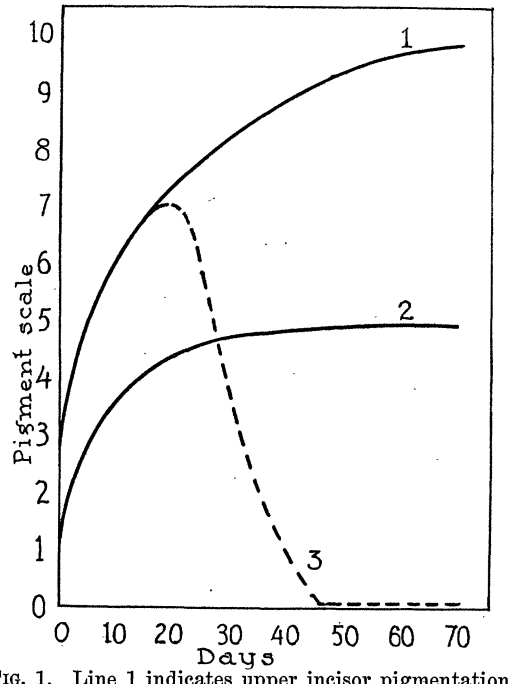

FIG. 1. Line 1 indicates upper incisor pigmentation in groups 1, 2, 4 and 5. Line 2 represents lower incisor pigmentation of all 5 groups. Line 3 shows upper incisor pigmentation of group 3.

No particular differences in eruption and attrition of incisor teeth were found in animals of the first four groups, but in group 5 these processes took place at a somewhat higher rate. Depigmentation was not correlated with any change in the rate of eruption and attrition. The average weight at the end of the experiment was slightly higher in groups 4 (194 g) and 5 (205 g) than in preceding groups (142, 153 and 160 g). Scaly tail developed in all animals on fat-free diets (1, 2). Autopsy revealed brown coloration of adipose tissue only in group 3, confirming previous observations.

[5] H. Granados, K. E. Mason and H. Dam, *Jour. Dental Research* (Proc. 23rd General Meeting International Association for Dental Research), 1945.

These results indicate that depigmentation of incisors in vitamin E-deficient rats requires the presence of fat, presumably unsaturated fatty acids, in the diet, a finding which suggests that the phenomenon is related to some abnormal deposition or reaction of fat in the ameloblasts. A previous observation[6] of persistence of dental pigment in rats reared on an E-deficient diet for 167 days can not be compared with our findings, due to the fact that the diet used was not reported. It is possible that this contradictory finding could be explained by differences in dietary fat content.

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URINARY EXCRETION OF PENICILLIN IN MAN AFTER ORAL ADMINISTRATION WITH GASTRIC ANTACIDS[1]

WHEN given by mouth, penicillin is reported to be destroyed by stomach acid.[2, 3] In subjects suffering from achlorhydria, penicillin is absorbed into the blood after oral administration.[3] Penicillin has been detected in the blood when given by mouth together with sodium bicarbonate.[3] Urine recoveries of intravenously and intramuscularly injected penicillin are reported to average approximately 60 per cent.[4]

These reports suggested to one of us (H.E.A.) that neutralization of gastric acidity might permit the absorption of a significant quantity of penicillin when the drug was given by mouth.

In our first series of experiments, approximately 20,000 units of penicillin in 200 ml of solution containing the antacid to be tested were given by mouth two hours after the morning meal. The percentages of the dose excreted in the urine during each two-hour period thereafter were estimated turbidimetrically using *Staphylococcus aureus* 6538 as the test organism. The unknown samples were compared with standard penicillin curves which were prepared in duplicate each day.

It was found that when penicillin was administered by mouth alone under these conditions, no more than 3 per cent. of the dose was recovered.

When 5 gm trisodium citrate ($Na_3C_6H_5O_7 \cdot 5\frac{1}{2}\ H_2O$)

[6] J. T. Irving, *Nature*, 150: 122, 1942.

[1] This manuscript was submitted for publication in SCIENCE under date of December 9, 1944.

We wish to thank Mr. S. M. Mann, head of the Microbiological Department of the Wyeth Institute of Applied Biochemistry, for making possible the penicillin assays, and Miss Anne Ospeck for carrying out the assays.

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[3] C. H. Rammelkamp and J. D. Helm, Jr., *Proc. Soc. Exp. Biol. and Med.*, 54: 324, 1943.

[4] C. H. Rammelkamp and S. E. Bradley, *Proc. Soc. Exp. Biol. and Med.*, 53: 30, 1943.

TABLE 1

URINARY EXCRETION OF PENICILLIN AFTER ORAL ADMINISTRATION

Subject	Date	Conditions of penicillin administration	Percentage of dose excreted Hours after oral administration					Total recovery
			2	4	6	8	10	
								Per cent.
F.B.E.	4/20/44	32,000 units in 100 ml distilled water	2.2	0	0	0	0	2.2
J.C.	5/16/44	20,000 units plus 5 gm trisodium citrate in 200 ml distilled water.	11.4	6.1	1.8	0	0	19.3
F.W.B.	8/8/44	111,000 units plus 5 gm trisodium citrate in 200 ml distilled water.	13.6	6.7	0.6	0	0	20.9
F.B.E.	3/16/44	22,000 units plus 2.5 gm disodium phosphate in 200 ml distilled water.	12.7	4.9	1.6	0.7	0	19.9
R.T.	7/24/44	24,000 units plus 3 gm sodium bicarbonate in 200 ml distilled water.	5.3	1.5	0	0	0	6.8
H.E.A.	2/1/44	20,000 units plus 20 ml Amphojel (hydrous alumina) in 200 ml distilled water.	11.5	3.1	0.9	0	0	15.5
J.C.	8/8/44	28,000 units in 200 ml milk plus 5 gm calcium carbonate.	6.2	1.2	0	0	0	7.4
J.C.	8/17/44	20,000 units plus 1.0 gm magnesium oxide plus 20 ml Amphojel in 200 ml distilled water.	0	5.0	0	0	0	5.0

were given simultaneously with penicillin, an average of 18 per cent. of the dose was recovered in the urine in twenty-one trials. Occasional recoveries of over 25 per cent. were obtained. Of the amount recovered, an average of 70 per cent. was excreted in the first two hours.

Disodium phosphate ($Na_2HPO_4 \cdot 12\ H_2O$), (2 to 10 gm) was also tested with comparable results. The average recovery of penicillin in five trials was 18 per per cent. In our tests with sodium bicarbonate recoveries ranged from 3 per cent. to 10 per cent. Solid or colloidal antacids such a hydrous alumina, and milk with calcium carbonate were also used.

The results of representative experiments are tabulated in Table 1.

Following these preliminary results, a study was made of some factors affecting urinary excretion of penicillin. In order to obtain data on a greater number of subjects, we determined only total excretion during a period of at least 6 and not more than 8 hours after administration. As shown by Table 1 detectable quantities of penicillin are rarely found in the urine after this length of time. Urine was voided every two hours into a bottle kept stoppered at 0°–3° C. which contained 25 ml of diethyl ether and 100 ml of 0.5 M phosphate buffer at pH 6.4. Control assays showed that the ether and buffer were without effect on the penicillin assays. Urine samples of 10 subjects collected under these conditions, without penicillin having been administered, had no antibiotic activity.

In all cases, the subjects took no food or liquid for at least two and one-half hours after the dose was administered. All subjects ate four hours after the dose was administered.

The results obtained are shown in Table 2.

The data in Table 2 indicate that under the conditions described:

TABLE 2

No. of subjects	Total No. determinations	Conditions of Penicillin administration (Approximately 25,000 units)	Percentage of dose excreted in 6–8 hours	
			Average	Range
13	25	400 cc water, after overnight fast.	12.8	4.2–23.3
5	5	400 cc water plus 0.5 gm disodium phosphate, after overnight fast.	11.8	7.1–16.6
5	5	400 cc water plus 2.5 gm disodium phosphate, after overnight fast.	19.4	13.6–27.2
5	5	400 cc water plus 5.0 gm disodium phosphate, after overnight fast.	10.1	4.9–14.4
6	6	400 cc water plus 0.3 gm trisodium citrate, after overnight fast.	11.9	8.0–21.0
6	6	400 cc water plus 1.4 gm trisodium citrate, after overnight fast.	15.6	6.6–32.5
6	6	400 cc water plus 2.8 gm trisodium citrate, after overnight fast.	16.8	8.4–26.4
6	6	400 cc water plus 7.0 gm trisodium citrate, after overnight fast.	13.9	9.2–18.5
9	15	400 cc water, two hours after breakfast.	5.0	0.0–10.5
6	6	400 cc water plus 0.3 gm trisodium citrate, two hours after breakfast.	6.9	0.0–20.5
6	6	400 cc water plus 1.4 gm trisodium citrate, two hours after breakfast.	10.6	1.7–16.9
6	6	400 cc water plus 2.8 gm trisodium citrate, two hours after breakfast.	10.6	5.6–16.2
6	6	400 cc water plus 7.0 gm trisodium citrate, two hours after breakfast.	11.2	8.2–14.4

(1) Less penicillin is excreted when administered in water two hours after breakfast than when administered in water after an overnight fast. This is probably due to decreased gastric acidity in fasting subjects.

(2) Administration of 1.4 to 7.0 gm of trisodium citrate or 2.5 gm disodium phosphate with penicillin after an overnight fast slightly increases the urinary excretion of penicillin over values obtained after administration of penicillin in water alone under the same conditions.

(3) Administration of 1.4 to 7.0 gm of trisodium citrate with penicillin two hours after breakfast, results in approximately a 100 per cent. increase in excretion of urinary penicillin as compared to administration in water alone under the same conditions.

(4) After oral administration, large individual differences in urinary penicillin excretion occur.

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AEROSOL APPLICATION OF GROWTH REGULATORS TO RETARD ABCISSION OF APPLE FRUITS[1]

GROWTH-REGULATING substances have been used effectively both in water sprays and in dust mixtures to retard the abscission of apple fruits before harvest.[2,3] One gallon of spray with a concentration of 10 ppm of growth substance is used for each bushel of fruit on the tree.

More recently the aerosol method[4] has been reported as a convenient and efficient means of dispensing growth-regulating substances.[5] It has been used for the setting of tomato fruits,[5,6] the development of parthenocarpic tomato fruits,[5,6] the development of blackberry fruits[7] and the destruction of weeds.[8] It is more like a fumigation than a spray and avoids the use of water or dust carrier. The growth substance and a carrier solvent are held under pressure dissolved in a liquefied gas. The mixture is released as a very fine mist. The liquefied gas escapes and carries with it the growth-regulating substance and the solvent. The vapor pressure of the gas is sufficient to disperse the solution into very small particles.

Because of the large size of commercial apple trees and the rapid diffusion and drifting of an unconfined gas, the use of the aerosol method with present equipment in large commercial orchard operations has been considered impracticable. However, its use with small trees suggests itself as a possibility. This paper reports results with growth-regulating substances applied in aerosol form to dwarf and semi-dwarf apple trees for the prevention of pre-harvest drop of fruit.

An aerosol was prepared consisting of .25 per cent. naphthalene acetic acid, .5 per cent. lanolin and 94.75 per cent. dimethyl ether.[5] In addition, for comparison, two water sprays were prepared with naphthalene actic acid at 10 ppm, using .5 per cent. ethyl alcohol as the carrier for the growth-regulating substance for the one spray and .5 per cent. Carbowax 1500 for the other.[9]

Eight- and nine-year-old trees of the McIntosh, Macoun and Kendall varieties were selected for treatment—12 trees of the first and 6 trees of each of the other two. They are varieties which do not retain the fruit well and also which are not overly responsive to the pre-harvest spray. They were on dwarf (Malling IX) and semi-dwarf (Malling I, V and VII) rootstocks. The dwarf trees were 5½ feet in height, 6 feet in spread and averaged about 1 bushel of fruit per tree. The semi-dwarf trees were approximately 12½ feet in height, 10 feet in spread and averaged about 2.5 bushels of fruit per tree.

Initial applications were made on September 18 at the time considered most likely to be effective, and repeated at 7-day intervals on September 25, October 3 and October 10. The aerosol was applied by means of a "Sure Shot Pressure Sprayer" weighing about 2 pounds, and the water spray from a 3-gallon knapsack sprayer. Applications were made during the more quiet part of the day, as early forenoon or late afternoon. Because of the small size of the trees and the accessibility of the fruit, it was possible to apply both the aerosol and the water sprays directly at the fruit from a distance of only a few feet. Average mean temperatures on the days of application were 69° F. September 18, 54° F. September 25, 58° F. October 2 and 55° F. October 10.

Approximately 1/3 (.34) of a gallon of water spray was used for each bushel of fruit, and ½ ounce (.034 of a pound) of aerosol for each bushel of fruit. In terms of growth-regulating substance, 13.8 mgs of naphthalene acetic acid were used per bushel of fruit in the water spray and 34 mgs in the aerosol. This compares with the recommended commercial prac-

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