larger numbers than in untreated preparations stained by the Giemsa method.

Summary. A method for staining *R. orientalis* in yolk-sac smears is described which yields uniformly satisfactory results. The efficacy of the method apparently depends upon the preliminary treatment of smears of infected tissue with lipid solvents.

14992

Dental Caries in the Cotton Rat. IV. Inhibitory Effect of Fluorine Additions to the Ration.*

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Miller[1] and several other workers have observed the inhibitory effect of fluorine upon the type of dental caries induced in the white rat by a coarse particle diet. Studies on the role of fluorine in the prevention of tooth decay have received increasing attention in the past few years. The cotton rat is very susceptible to the development of carious lesions when maintained on a ration containing sucrose or other soluble sugars.[2,3,4] Therefore it is fundamentally important to know the relation of fluorine in the ration to the production of this type of carious lesion.

Experimental. Fluorine as sodium fluoride (reagent grade) was added to the basal ration, 802 + 4% 1:20 liver extract, at levels ranging from 22.5 to 450 p.p.m. In these studies, the identical experimental procedure described by Schweigert *et al.*[4] was followed, with special attention to the division of litters between experimental and control groups. At the end of the 14-week experimental period after weaning, the cotton rats were sacrificed and the heads prepared for the evaluation of the incidence and extent of carious lesions. In addition to the caries evaluation, the teeth were examined for evidence of any toxic effect of the dietary fluorine.

When dextrin is substituted for all the sucrose in the basal ration, the incidence of carious lesions is greatly reduced.[2,3,4] Therefore, analyses of the dextrin and sucrose were made to determine their fluorine content. In this way it was possible to decide whether the inhibitory effect of dextrin upon the formation of carious lesions might be due to its fluorine content. The ashing of the samples was carried out in a manner similar to that described by Crutchfield.[5] The distillation was performed according to the method of Willard and Winter.[6] Determination of the amount of fluorine in the distillate was made by a colorimetric method based upon the bleaching of the alizarin-thorium lake by fluorine in a monochloracetic acid buffer.[7] Readings were made in an Evelyn colorimeter with a 520 filter.

Results. The average weekly rates of growth for the first 6 weeks and for the entire 14-week period are presented in Table I. The rate of growth is expressed as growth of males as outlined by Schweigert *et al.*[4] In most cases where 90 p.p.m. or more fluorine were

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TABLE I.
Effect of Fluorine Additions to the Ration upon Growth and upon Incidence and Extent of Carious Lesions in the Cotton Rat.

	No. of cotton rats	Avg growth/ week for 6 wks, g	Avg growth/ week for 14 wks, g	Avg No. of carious lesions	Avg extent of carious lesions	Avg No. of carious lesions in third molars	Avg extent of carious lesions in third molars
802 + 4% 1:20 L.E.	3	8.5	6.9	19.0	71+	4.3	12+
802 + 4% 1:20 L.E. + 22.5 p.p.m. F	3	8.5	6.6	16.3	57+	3.3	11+
802 + 4% 1:20 L.E.	5	10.8	7.0	27.2	85+	6.4	24+
802 + 4% 1:20 L.E. + 45 p.p.m. F	7	10.3	6.9	20.4	56+	4.7	13+
802 + 4% 1:20 L.E.	4	10.5	6.6	29.9	103+	7.0	27+
802 + 4% 1:20 L.E. + 90 p.p.m. F	7	8.9	5.5	10.3	21+	2.4	6+
802 + 4% 1:20 L.E.	3	9.0	5.9	33.7	102+	8.6	27+
802 + 4% 1:20 L.E. + 135 p.p.m. F	3	6.5	4.4	20.0	51+	3.0	6+
802	3	7.7	4.0	30.0	86+	7.6	24+
802 + 135 p.p.m. F	3	6.6	4.4	2.0	4+	0	0
802 + 4% 1:20 L.E.	3	10.0	6.6	28.0	97+	6.6	27+
802 + 4% 1:20 L.E. + 225 p.p.m. F	5	9.8	6.2	2.6	7+	1.2	4+
802 + 4% 1:20 L.E.	2	10.9	6.8	28.0	94+	6.0	25+
302 + 4% 1:20 L.E. + 450 p.p.m. F	3	9.0	5.0	2.7	7+	2.0	5+

added to the basal ration (802 + 4% 1:20 liver extract), some inhibition in the rate of growth resulted, although in one series the growth rates for the 135 p.p.m. group and the 225 p.p.m. group were approximately the same as their respective controls. The rates of growth and caries incidence and extent in various control groups cannot be directly compared since different litters are represented in each group.

The inhibitory effect of fluorine, as sodium fluoride in the ration, upon dental caries formation is presented in Table I. It can be seen that the ingestion of 22.5 p.p.m. of fluorine did not result in a decrease in the incidence or extent of the carious lesions. However, 45, 90, and 135 p.p.m. of fluorine produced a progressive decrease in both the incidence and extent. At these levels the carious lesions observed were darker in color and not as soft as those observed in the animals on the basal ration without added fluorine. The ingestion of 225 or 450 p.p.m. of fluorine produced the maximum inhibitory effects.

The crowns of the third molars are not completely formed when the cotton rat is weaned. Therefore the third molars of the cotton rats which received fluorine in these experiments were partially formed in the presence of fluorine. No toxic effects were observed in the third molars of those animals which received less than 135 p.p.m. of fluorine in the ration. When 135 p.p.m. were fed, the enamel of the third molars was slightly mottled. At 225 p.p.m. there was a severe mottling while at 450 p.p.m. the mottling was most extensive and some enamel hypoplasia was observed.

In order to determine whether fluorine had a greater inhibitory effect on the occurrence of carious lesions in the third molars, the incidence and extent of carious lesions in these molars were calculated since they were formed during fluorine ingestion. When 45, 90, or 135 p.p.m. of fluorine were fed, the results indicated that the reduction in the incidence and extent of caries in the third molars closely paralleled the reduction in the first and second molars. When 225 or 450 p.p.m. of fluorine were fed, the incidence and extent of carious lesions in the third molars were higher than in the first and second molars.

A slight inhibitory effect of fluorine on the formation of the pigment in the enamel of the incisors was observed when 45 p.p.m. of fluorine were fed. At this level the lower incisors developed a few, irregular white or pale areas. When 90 p.p.m. of fluorine were

fed, the enamel of the lower incisors was almost completely white but that of the upper incisors was still practically normal in color. When 135 or 225 p.p.m. of fluorine were fed, the enamel of the incisors was a uniformly, pearly white, whereas the enamel of the incisors became very chalky with a complete loss of the normal opalescence when 450 p.p.m. of fluorine were ingested.

Analyses of the dextrin and sucrose used in previous carbohydrate experiments indicated fluorine contents of 3 to 4 p.p.m. and 1 to 2 p.p.m., respectively. Therefore, the inhibitory effect of dextrin upon the formation and development of carious lesions was not due to the fluorine content.

Discussion. Miller[1] observed a 90% inhibition of experimental dental caries in the white rat when the rats were given the caries-producing diet plus 250 mg of sodium fluoride per kilogram and drinking water containing 4.2 mg of sodium fluoride per liter. McClure and Arnold[8] reported that the production of dental caries in the white rat was significantly reduced by 125 p.p.m. of fluorine, as sodium fluoride, in the food. Hodge and Finn[9] and Cheyne[10] have reported that the ingestion of 3 mg of fluorine as potassium fluoride decreased the incidence of caries production by 80-85%. This level is approximately twice that shown to be effective by Miller[1] and McClure and Arnold.[8] The levels of fluorine fed by the latter authors[1,8] are approximately comparable to 135 p.p.m. of fluorine in the ration. When 135 p.p.m. of fluorine were fed to cotton rats, as an addition to the basal ration, 802, an inhibition of over 90% was produced. When 135 p.p.m. of fluorine were added to the basal ration which contained liver powder, 802 + 4% 1:20 liver extract, only about 40% inhibition was produced. However, this was possibly due to an increased susceptibility of this particular group to tooth decay since the inhibition observed was not as great as that produced by 90 p.p.m. of fluorine. Previously we have shown that the addition of 4% 1:20 liver extract had no effect upon the production of carious lesions.[2,3] This is also shown when the two groups of controls for the 135 p.p.m. of fluorine studies are compared. The levels of sodium fluoride fed by Hodge and Finn[9] and Cheyne[10] are comparable to about 225 p.p.m. of fluorine at which level 90% inhibition was observed in the cotton rat. Dixon and Cox[11] have reported that 250 p.p.m. of sodium fluoride in the ration produced mottled enamel in the third molars of weanling rats. Cheyne[12] has shown that as low as 150 p.p.m. of fluorine fed as potassium fluoride to day-old rats was sufficient to produce mottling in all molars. Cox *et al.*[13] observed mottling of the enamel of the first and second molars of white rats fed 128 and 256 γ of fluorine per day for the first 21 days of life. Mottled enamel of the third molars was produced in the cotton rat by as low as 135 p.p.m. of fluorine in the ration.

The level of fluorine in the ration of the cotton rat necessary to inhibit the production of carious lesions appears to be very similar to that in the white rat even though the method of producing the carious lesions was very different. The levels of fluorine at which fluorosis of the teeth occurs are also similar in the two species.

It was observed that the reduction of the incidence and extent of carious lesions in the third molars appeared to be the same as the reduction observed in the first and second molars. This is in contrast to previous reports that those teeth formed during fluorine ingestion in man[14,15,16] and in the white rat[17] were

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more resistant to tooth decay than those subjected to fluorine only after tooth development was complete. The reason for this difference is not known.

Summary. Some inhibition of caries resulted when 45, 90, or 135 p.p.m. of fluorine were ingested as supplements to a sucrose basal ration. Depigmentation of the incisors appeared when 45 or more p.p.m. of fluorine were incorporated in the diet while mottling of the third molars occurred when 225 or more p.p.m. were fed. The ingestion of 225 or 450 p.p.m. of fluorine caused a 90% decrease in caries incidence. Thus the amount of fluorine necessary to inhibit caries to this degree was sufficiently high to cause mottling of the developing teeth.

The inhibitory effect of dextrin upon caries development could not be explained on the basis of its fluorine content.

14993

The A-V Conduction System in the Heart of the Guinea Pig.

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The importance of the conducting system in the heart is attested by increasing numbers of references to its structure and function.[1,2,3] Methods of study have involved gross dissection, injection with opaque substances, and histologic studies of serial sections of the heart.

The present work consists of a study of serial sections of young guinea pig hearts stained with Goldner's modification of the Masson trichrome technic.[4] The conducting tissue stains a purplish gray, the myocardium is brick red, and connective tissue is a bright green. In the guinea pig heart, there is not the slightest difficulty, even under low power of the microscope, in distinguishing A-V conducting tissue from ordinary myocardial tissue. Fig. 1 shows the clear-cut distinction between bundle tissue (B) and myocardial tissue (SE) of the interventricular septum. Under high power, the nodal and bundle cells are of the Purkinje type. The A-V node lies in the posterior part of the interatrial septum, as it does in other mammals. The main bundle and its right and left interventricular septal branches can easily be followed through the serial sections to their peripheral connections. The distribution of the right and left septal branches is similar to that described for other hearts. However, in this heart there are additional interventricular septal branches, one of which is shown in Fig. 1 (S). These additional branches descend directly from the main bundle. There are also connections to the septal muscle from proximal as well as distal parts of the right and left bundle branches.

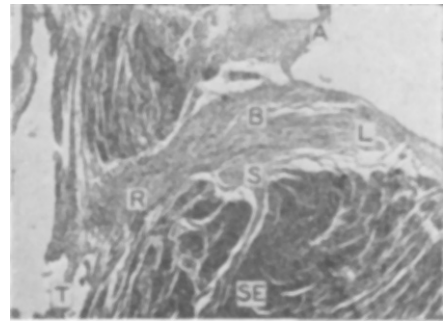

FIG. 1.
Photomicrograph of the main bundle (B) of the A-V conducting system, the septal branch (S), and the beginning of the right (R) and left (L) bundle branches in the interventricular septum (SE) of the heart of a young guinea pig. Semilunar valve of aorta (A), tricuspid valve (T). Frontal section, 10 μ thickness, Masson's trichrome stain.

These muscular connections of conducting tissue may have considerable significance with regard to analysis of function of heart muscle.

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