

material by the methods of detection used. In the cotton rats fed ration 130 much less of the molar crowns had been destroyed by the carious processes; thus the size of the smooth surfaces had not been reduced as much but were as heavily covered with food debris. Again no carious lesions were observed on the smooth surfaces. Thus the relative distribution of carious lesions on the various tooth surfaces in the desalivated cotton rat appeared to be identical with that in the normal cotton rat.

The rate of growth was approximately the same for normal cotton rats receiving rations 100 and 130. The rate of growth for the desalivated cotton rats was appreciably less

than for their intact littermates. This reduction in rate of growth was greatest in the animals which received ration 100 throughout the experimental period.

Summary. The removal of the principal salivary glands of cotton rats prior to maintenance on purified, caries-producing rations resulted in a significantly greater average number and average extent of carious lesions than was observed in their intact littermates maintained under otherwise identical experimental circumstances. No alteration in the relative distribution of the lesions with respect to the various tooth surfaces resulted from the removal of the major salivary glands.

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Inhibition of the Appearance of Phenol Red in Frog Kidney Tubules *in vitro*.

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4'-Carboxyphenylmethanesulfonanilide (caronamide) inhibits concentration of phenol red in the lumina of the tubules of frog kidney slices.[†] This observation suggested that it might be of interest to study the activity of various other substances as inhibitory agents. A number of compounds have been studied. These have been mainly carboxylic acids; however, the present communication is concerned only with derivatives of cinchoninic acid. A recent study in this laboratory has revealed that certain derivatives of cinchoninic acid inhibit a water diuresis in the dog.¹ Some of these compounds have been found to increase uric acid excretion in man.²⁻⁴ The

experiments described in this report show that the appearance of phenol red color in the lumina of the tubules in frog kidney slices is inhibited by certain of these substances.[‡]

Procedure. A modification of the method of Forster has been used.⁵ Both kidneys of a frog (*Rana pipiens*) were removed and sliced transversely into sections approximately 200 microns in thickness. The slices were mixed and distributed to 12 small Petri dishes each containing 5 ml of the solution described by Forster.⁵ The concentration of phenol red used was 0.03 millimoles per liter. The substances being tested were dissolved in this

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† Dr. E. K. Marshall, Jr., in conjunction with Dr. Roy P. Forster, made this observation on the fish kidney (flounder) in Maine in the summer of 1947.

¹ Marshall, E. K., Jr., and Blanchard, K. C., *J. Pharm. and Exp. Therap.*, in press.

² Nicolaier, A., and Dohrn, M., *Deutsches Arch. f. klin. Med.*, 1908, **93**, 331.

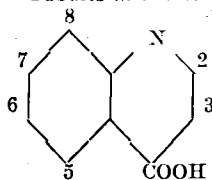
³ Berlingozzi, S., and Donatelli, G., *Boll. Chim. Form.*, 1936, **75**, 387.

⁴ Impens, E., *Arch. de Pharmacodynamie*, 1911-12, **21-22**, 379.

[‡] We wish to thank Dr. K. C. Blanchard for supplying the cinchoninic acid derivatives.

⁵ Forster, Roy P., *Science*, 1948, **108**, 65.

TABLE I.
Effect of Cinchoninic Acid Derivatives on the Concentration of Phenol Red by Frog Kidney Tubules *in Vitro*.



Substituent	Min. inhibitory conc.		
	Cinchoninic derivative mM/1	Caronamide mM/1	Ratio*
None	2	.5	.25
2-Hydroxy	>2	.25	< .12
2-Hydroxy-3-methyl	>2	.3	< .15
2-Hydroxy-3-phenyl	.4	.25	.06
2-Methyl	1	.125	.12
3-Hydroxy-2-methyl	0.1	.3	3
3-Carboxy-2-methyl	>4	.125	< .03
2-Phenyl	.125	.125	1
3-Hydroxy-2-phenyl	.03	.125	4
6-Methoxy	.08	.15	2

* The ratio of the minimal inhibitory concentration of caronamide to the minimal inhibitory concentration of the unknown.

solution before the kidney slices were placed therein. Oxygen was continuously bubbled through the solution in each dish by a small jet made from a 27 gauge hypodermic needle. These 12 jets were fed from a manifold.

Caronamide was used as a standard of comparison. Each experiment included three concentrations of caronamide and two controls containing no drug. In all cases the concentrations of the substances being tested were varied by a factor of two. To avoid subjective errors the minimum concentration of drug which completely prevented the appearance of phenol red color in the tubule lumina was used to evaluate effectiveness. Decrements in drug concentration below the minimal inhibitory level resulted in graded increments in the amount of color seen in the tubule lumina. The minimal inhibitory concentration of caronamide varied somewhat from one experiment to another.

Results and discussion. In Table I the

inhibitory concentrations of various substances are compared with the concentration of caronamide necessary to produce the same effect in the same experiment. Only the 3-hydroxy-2-methyl, the 3-hydroxy-2-phenyl and the 6-methoxycinchoninic acids were more active than caronamide, while the 2-phenyl derivative (cinchophen) was equally active.

The desirability of comparing these results with results obtained *in vivo* in mammals is obvious. We have therefore begun such a study. In preliminary experiments, it appears that some members of this group depress the clearance of phenol red in the dog.

Summary. Cinchoninic acid and nine of its derivatives have been studied with respect to the inhibition of the appearance of phenol red color in the lumina of the tubules of frog kidney slices *in vitro*. Three of these have been found more active in this respect than is caronamide and one equally active.