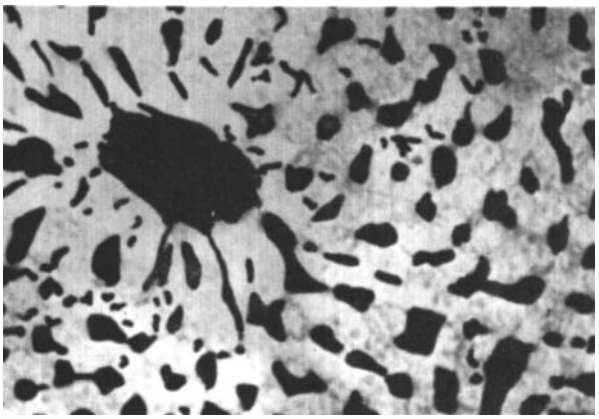

FIG. 1.
Low-power photomicrograph of rabbit liver (300 ×, enlarged 2 ×), fixed in formalin and examined unstained by ultraviolet light. The print has been retouched to emphasize the contrast between non-fluorescent sinusoids and fluorescent hepatic trabeculae, and to remove artifacts which displayed light yellow or green fluorescence.

Summary. Intravenously-administered Rose Bengal can be visualized by fluorescence microscopy in the livers of rabbits and humans. The fluorescence is seen in hepatic polygonal cells, but not in Kupffer cells. It is suggested that the polygonal cells of the normal liver are responsible for the storage of Rose Bengal, and, by analogy, Bromsulfalein.

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Effect of *in vivo* Addition of Selected Nitrofurans on Acid Production in Human Saliva.*

SAMUEL DREIZEN, HENRY I. GREENE, AND TOM D. SPIES.
From the Department of Nutrition and Metabolism, Northwestern University.

Investigation of the relation between nutritional status and dental caries has been a major study at the Nutrition Clinic of the Hillman Hospital, Birmingham, Alabama, since 1941. During the past year an extensive study was made of the effect of the *in vitro* addition of selected nitrofuran compounds on acid production in human saliva, and on the growth and acid production of a pure culture of a strain of the Lactobacillus acidophilus recovered from a carious lesion.[1] The antibacterial action of the nitrofurans has been reported by a number of investigators.[2-4] Our findings showed that 5-nitro-

* Northwestern University Studies in Nutrition at the Hillman Hospital, Birmingham, Ala.

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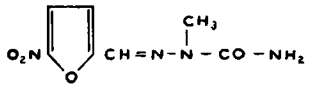
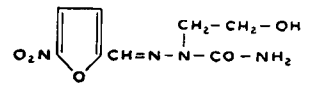


5-nitro-2-furaldehyde-2-β-methyl semicarbazone



5-nitro-2-furaldehyde-2-β-hydroxyethyl semicarbazone

FIG. 1.
Structural formulae of 5-nitro-2-furaldehyde-2-β-hydroxethyl semicarbazone and 5-nitro-2-furaldehyde-2-β-methyl semicarbazone.

2-furaldehyde-2-β-hydroxyethyl semicarbazone and 5-nitro-2-furaldehyde-2-β-methyl-semicarbazone (Fig. 1), in 500 μg quantities, completely inhibited acid production in 10-cc aliquots of paraffin stimulated saliva in 90.5 and 85% of the samples respectively. A significant but incomplete inhibitory effect was observed in all other instances. Both compounds proved to be bactericidal against an oral strain of the Lactobacillus acidophilus, when added in amounts of 3.5 mg and above to yeast extract dextrose broth inoculated with the test organism.

To investigate the possibility that conditions in the mouth not reproducible *in vitro*, such as the absorption potential of the oral mucous membranes, tissue enzymes and the migration of phagocytes to the oral cavity, may interfere with the antibacterial action of 5-nitro-2-furaldehyde-2-β-hydroxyethyl semicarbazone and 5-nitro-2-furaldehyde-2-β-methyl semicarbazone on the organisms associated with dental caries, it was decided to determine whether these compounds retained their effectiveness as inhibitors of acid production when added *in vivo* to the saliva of persons highly susceptible to dental decay.

Materials and Methods. Sixty-nine patients, each of whom had clinical and laboratory evidence of active dental caries, were selected for this study. The clinical examination was made with a mouth mirror and exploring tine and was supplemented in most instances by bite wing or full mouth roentgenograms. Laboratory evidence of prevailing dental caries activity was established by analyzing samples of saliva by the methods of Hadley,[5] Fosdick, Hansen, and Epple,[6] and Dreizen, Mann, Cline and Spies.[7]

Method of Study. Two samples of stimulated saliva comprising 20 cc and 15 to 20 cc respectively were collected in sterile flasks from each patient. An untreated paraffin block served as the salivary stimulant for the first sample and a paraffin block containing 750 μg or 1,000 μg of the test compounds served as the secretory stimulant for the second. The collection time for each sample ranged from 10 to 20 minutes with a 15 minute interval between samples. Paraffin blocks containing 750 μg of 5-nitro-2-furaldehyde-2-β-hydroxyethyl semicarbazone, 1,000 μg of 5-nitro-2-furaldehyde-2-β-hydroxyethyl semicarbazone, 750 μg of 5-nitro-2-furaldehyde-2-β-methyl semicarbazone, and 1,000 μg of 5-nitro-2-furaldehyde-2-β-methyl semicarbazone were used for 20, 23, 7 and 19 patients respectively.

A 10-cc aliquot of the saliva sample obtained with the untreated paraffin block was tested immediately for soluble calcium by the method of Fosdick, Hansen, and Epple.[6] Ten-cc aliquots of the samples obtained with the nitrofuran-treated and untreated paraffin blocks were added to sterile brown vials containing dextrose and tricalcium phosphate. The vials were sealed, shaken, incubated and agitated in a water bath maintained at 37°C. Following a 4-hour period of incubation and agitation, the amount of soluble calcium contained in each of these aliquots was determined. The difference in calcium values between the incubated and unincubated aliquots of saliva for each patient was regarded as a measure of the degree of acid production.

Observations. The effect of the *in vivo* addition of 5-nitro-2-furaldehyde-2-β-hydroxyethyl semicarbazone on acid formation in the

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[5] Hadley, F. P., *J. D. Res.*, 1933, **13**, 415.

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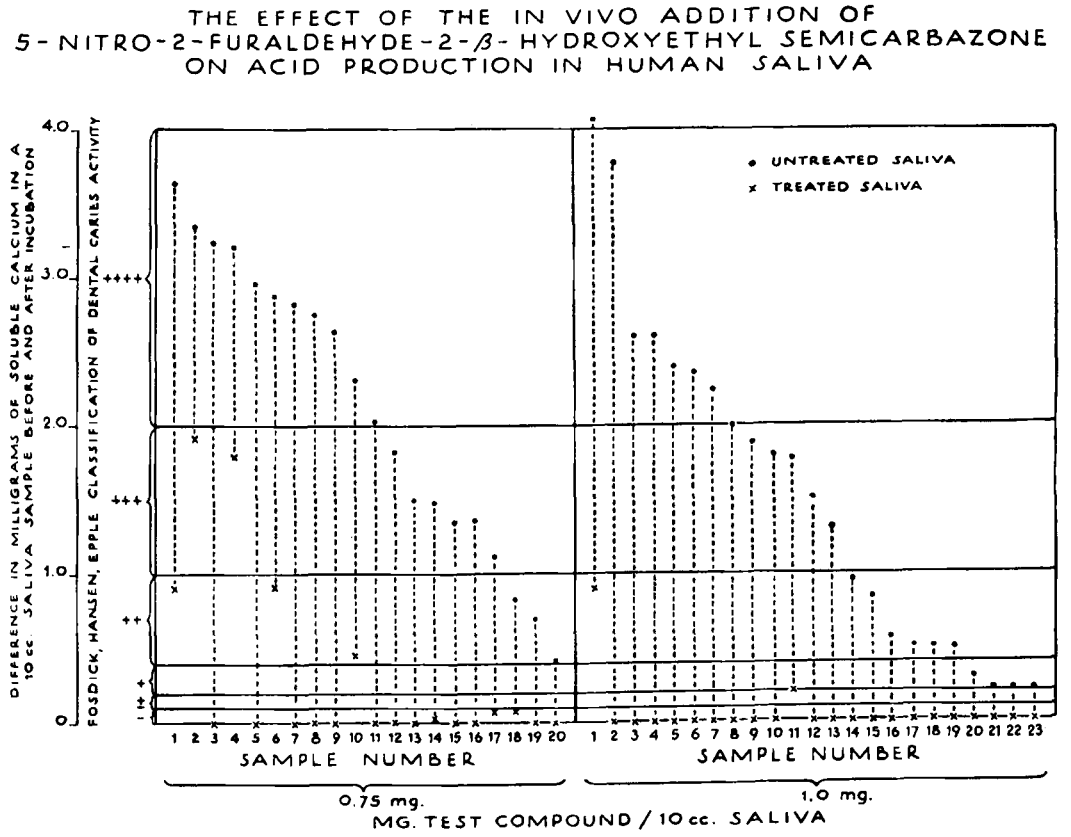

FIG. 2.

The sample numbers are arranged in the order of decreasing dental caries activity in the control aliquot and not in the order in which the samples were collected.

saliva of caries active individuals is shown in Fig. 2. When the compound was added in 750 μg amounts, acid production was completely inhibited in 15 (75%) of the 20 samples tested. A significant partial reduction in acid formation was observed in each of the remaining 5 samples. The *in vivo* addition of 1,000 μg amounts of this compound resulted in a complete inhibition of acid formation in 21 (91%) of the 23 samples tested. Acid production was reduced to a significant extent in the remaining 2 samples.

Fig. 3 demonstrates the results of the addition *in vivo* of 5-nitro-2-furaldehyde-2-β-methyl semicarbazone on acid production in the saliva of caries susceptible individuals. In the 7 instances in which 750 μg quantities of this compound were added, acid formation was completely prevented in only 1 (14%) of the samples, the amount of acid formed in the untreated aliquot of this sample being negligible. Acid production was deterred, however, to a significant degree in each of the remaining samples. When the amount of 5-nitro-2-furaldehyde-2-β-methyl semicarbazone incorporated in the paraffin blocks was increased to 1,000 μg, acid production was completely prevented in 17 (89%) of the 19 samples of saliva in which this concentration was used. A significant partial reduction in the amount of acid formed was noted in the remaining 2 samples.

Discussion. The evidence presented by this study indicates that the prevention of acid formation in saliva by 5-nitro-2-furaldehyde-2-β-hydroxyethyl semicarbazone and 5-nitro-2-furaldehyde-2-β-methyl semicarbazone is not adversely affected in the presence of the tissues and fluids of the oral cavity. In each instance a slightly higher concentration of these compounds was required when added *in vivo* than *in vitro* in order to inhibit acid pro-

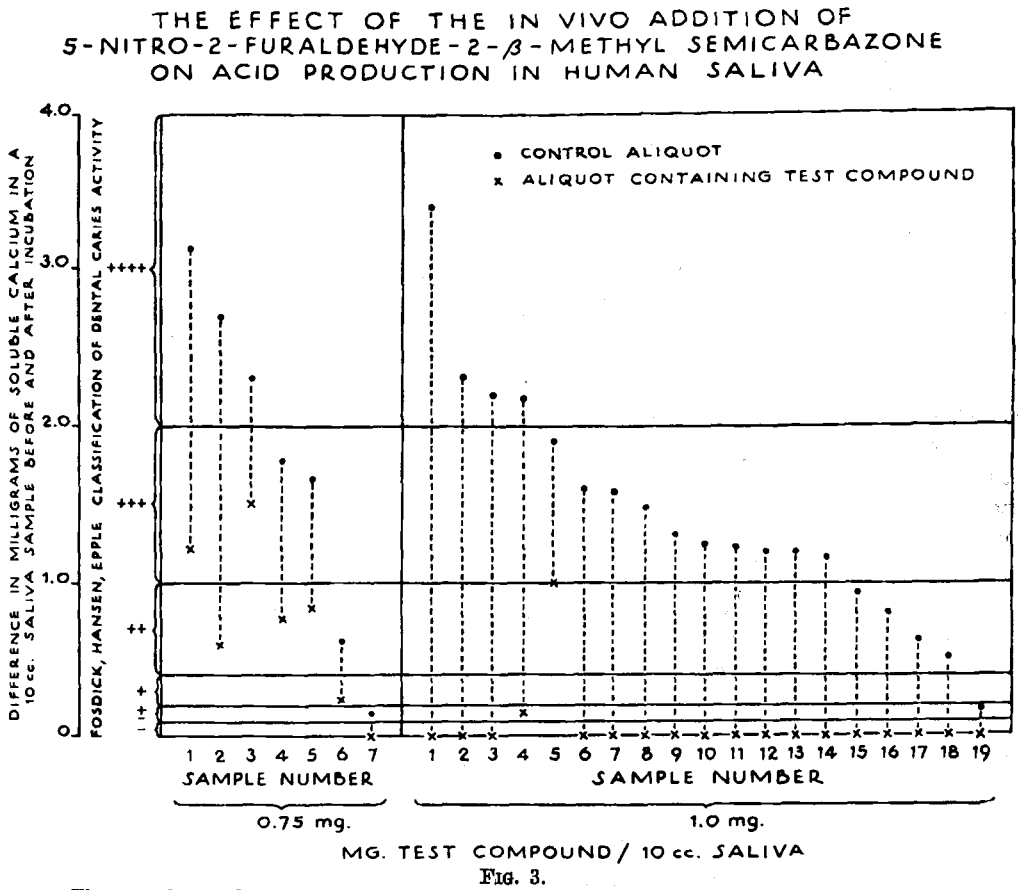

FIG. 3.

The sample numbers are arranged in the order of decreasing dental caries activity in the control aliquot and not in the order in which the samples were collected.

duction in the saliva of caries active patients. The difference may be attributed in part to the unavoidable loss of some of the test material by admixture with the paraffin used as the secretory stimulant, by swallowing, and by retention in the sheltered areas and crevices of the oral cavity.

Each of the test compounds is but slightly soluble in water, the solubility being 1:900 for 5-nitro-2-furaldehyde-2-β-hydroxyethyl semicarbazone, and 1:3000 for 5-nitro-2-furaldehyde-2-β-methyl semicarbazone. It appears, therefore, that the actual inhibitory activity of these compounds may be attributed to a fraction of the concentrations employed in this and in the previous study. The difference in the extent of the solubility of the test compounds may also account for the greater effectiveness of 5-nitro-2-furaldehyde-2-β-hydroxyethyl semicarbazone as an inhibitor of acid production in the saliva when added in 750 μg quantities *in vivo*, as compared with a similar amount of the less soluble 5-nitro-2-furaldehyde-2-β-methyl semicarbazone.

Summary and Conclusions. The incorporation of 1,000 μg quantities of 5-nitro-2-furaldehyde-2-β-hydroxyethyl semicarbazone and 5-nitro-2-furaldehyde-2-β-methyl semicarbazone in paraffin blocks used as salivary stimulants completely prevented acid production in 38 of the 42 saliva samples obtained from caries active individuals. A significant partial reduction in acid formation occurred in the remaining 4 cases. The findings indicate that 5-nitro-2-furaldehyde-2-β-hydroxyethyl semicarbazone and 5-nitro-2-furaldehyde-2-β-methyl semicarbazone are not rapidly destroyed or absorbed by the tissues and fluids of the oral cavity.