Author: Eldon D. Nielson
Biological Research Division

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To: Dr. Murray Senkus
Director of Research

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A. BIOLOGICAL TESTING

I. Smoke Irritancy Studies

Work is continuing on obtaining the standard dose-response curves in the smoke irritancy test for standard cigarettes. All RJR brands, so far tested for a dose-response curve, have produced a fairly straight-line response over a limited range in the test. The cigarettes fall into three general categories by mildness measurement. The increasing order of mildness is unfiltered, Estron filtered, and charcoal-Estron filtered cigarettes.

In addition to testing the mildness of various brands of cigarettes, studies are also under way to determine the relative mildness of the smoke from the component tobaccos and blends thereof. Preliminary data indicate that components and blends may also fall into three groups. The mildest tobaccos in this test were the burley blend, Turkish blend, and flue-cured blend; intermediate in mildness were burley to which 20% carbohydrate had been added, CAMEL blend with no casing, and regular G-7. The regular cased CAMEL blend was least mild of all. These data are still subject to some revision, especially when a complete statistical study of the results is completed.

Further studies have been run to help establish the validity of the test. In one study, various concentrations of croton oil were applied to filter pieces and implanted in experimental animals. The results indicated that differences in the strength of the irritant in a constant weight of nonirritating oil can be distinguished in this test.

II. Pharmacology of Mariolide and Other Smoke Constituents

Work has begun on the study of the pharmacologic properties of mariolide and other constituents found in cigarette smoke. Attention was drawn to mariolide because of its relative high acute toxicity, approaching the LD_{50} range of nicotine. In acute toxicity studies, it had been observed that lethal and sublethal doses of mariolide caused convulsions. Preliminary results indicate that mariolide will lengthen the sleep time, caused by a standard dose of phenobarbital, and appeared to hasten the loss of righting reflex under phenobarbital anesthesia. Preliminary studies on other physiologic parameters, such as respiration, blood pressure, electrocardiogram, heart rate, and body temperature, demonstrated no clear-cut changes from normal.

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III. Thiocyanate Excretion by Rats

Thiocyanate is excreted by animals as a metabolic product of cyanide. Since cyanide will ordinarily come from a few limited sources, such as smoking, attempts have been made to use thiocyanate excretion as a measurement of smoking

Rats injected with 1 mg. of potassium cyanide excreted 2-1/2 times as much thiocyanate in urine samples, collected immediately after injection, as control rats. After receiving cyanide injections for three days in a row, the thiocyanate level in the urine of the injected rats increased to 20 times that of control rats. This reflects a build-up of thiocyanate in the treated animals.

In studying the excretion of thiocyanate, it is important to eliminate the contributions of other potentially interfering materials. Since vitamin B-12 contains the cyanide ion in its structure, it was thought that it might conceivably either react with the thiocyanate reagents or contribute to the observed thiocyanate level in the urine. In tests conducted, it was found that vitamin B-12 does not give red color with the thiocyanate reagents nor does the injection of rats with vitamin B-12 increase the level of thiocyanate in the urine over that of control rats.

IV. Detection of Nicotine in Urine

A method which permits the detection of nicotine in the urine of cigarette smokers has been developed. The method employs the extraction of urine and fractionation of the extracts followed by a gas chromatographic analysis. Using this procedure, it was possible to detect a nicotine peak in the extracts of urine from six different smokers. No peaks in the area of the nicotine peak were found in a similar extract of the urine of two nonsmokers. Nicotine has also been detected in the urine of rats which were given a subcutaneous injection of nicotine.

V. Plant Hormone Activity

Since new supplies of abscisin II (SM24A), a naturally occurring plant hormone, and its isomer (SM25A) have become available, further studies of their biological activity have been undertaken. The effects are being studied on the dropping of flowers (a predominant effect of this hormone) and on rooting of plants. It has been found that the naturally occurring compound, SM24A, stimulates the dropping of flowers whereas the isomeric compound, SM25A, shows no activity. In preliminary tests, both SM25A and SM24A severely inhibited rooting.

B. STARCH BIOCHEMISTRY

I. Production of Amyloglucosidase

Although reproducibility still remains a problem in the production of amyloglucosidase by Aspergillus awamori, the yield was increased from 150

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to 192 units per milliliter by use of a selected strain. In other experiments, it was found that the addition of 1/2 to 1% fumaric acid to the medium raised the yield from 150 to 190 units per milliliter.

II. Evaluation of Enzymes

The amyloglucosidase prepared here was successfully employed in producing a syrup of 55 DE in a period of 96 hours at 60°C. The optimal pH was 5.0 although the reaction proceeded satisfactorily between pH 5 and 6. The glucose-maltose ratio was influenced somewhat by the pH, being 27.8/24.6 at pH 5 and 27.7/26.0 at pH 6.

III. Glucose Isomerase

An additional 36 organisms have been isolated. Glucose isomerase activity could be demonstrated in many of the organisms, but five of them were considerably more active than the others. So far, activity has been observed only among bacteria, all gram negative rods resembling the Pseudomonas.

Organisms capable of converting glucose to fructose in initial screening tests were studied further for their ability to isomerize glucose to fructose. Lyophilized cell preparations were employed successfully. These studies indicate the process to be feasible and will be continued in an effort to develop a commercial process.

IV. Conversion of Starch to Fructose

Experiments will be conducted to determine the feasibility of converting starch to fructose or to a syrup containing significant percentages of fructose. An attempt will be made to isolate phosphorylase from potato for use in the conversion of starch to glucose 1-phosphate. Glycolytic enzymes from yeast will be employed to convert this to fructose 6-phosphate. Some form of phosphatase will then be applied to obtain fructose. If this step-wise pathway can be accomplished in vitro, consideration will be given to the development of a practical process.

C. MISCELLANEOUS

I. Microbial Analysis of Food Products

Examination of dehydrated apples initially and after storage for periods of 7 and 28 days indicated that the sparse microbial population remained unchanged. Chocolate mousse, having a population of about 8,000 organisms per gram initially, showed a decrease to about 4,000 after seven days storage and 28 days storage. This count was not considered excessively high.

Microbial analyses of My-T-Fine egg custard mixes have been carried out as a service to the Food Development program in connection with initial production. Hourly random samples and composites of the complete run of each flavor of custard obtained from both the Hoboken and Pinkneyville plants were examined. Tests for coliform and Salmonella were negative for all samples.

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II. Cigarette Beetle Control

Samples of tobacco were taken at various stages in the stemming process, preceding the redry process. After storage for 29 days, no cigarette beetles or larvae were detected in these samples. They will be held an additional two weeks to permit any larvae or pupae to develop into the easily detected adult beetles.

In another experiment, adult beetles and larvae were placed in small cloth bags along with tobacco and permitted to go through the redrying process. None of the adult beetles or larvae survived this treatment. Beetle eggs were not available at the time these experiments were carried out. These data indicate that tobacco is probably free of beetle contamination at the time it is packed in the hogsheads.

Core samples were taken from the hogshead of tobacco, and the distribution of beetles within the core determined. These results indicated that beetles are present primarily in the outer six inches of tobacco within the hogshead. These results are not in agreement with the observations of leaf people who have observed pockets of beetles at the center of hogsheads in the tobacco purchased from the government.

Eldon D. Nielson

Eldon D. Nielson

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M. S.

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