prehension is that administrative questions arising in a research program may be solved apart from scientific matters or vice versa. In point of fact, the two are apt to be inextricably mingled. In the negotiation and administration of supported research there are and should be four groups involved: the research group at the institution, the scientific staff of the agency, and the administrative offices of both the institution and the agency. It is also desirable that the policy of the supporting agency be flexible enough to adjust to the policy of the institution, within limits. Above all, I wish to emphasize the great importance of a cooperative interest on the part of the research investigators in the program supported by the government agency. This cooperation should be kept extremely close, in order that the agency may meet or even anticipate the needs of the research group, and in order that it may plan effectively. A successfully organized program should have the weight of approval of all its constituents. If this is achieved, government support of research will be abundantly justified.

Technical Papers

Fluorometric Determination of Serum Aureomycin Levels

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In view of the difficulties associated with the bacteriologic assay of serum aureomycin concentrations, a method was developed of determining these concentrations by adsorbing the aureomycin on small columns of silica gel and observing the yellow fluorescence at the top of the column. A fluorometric method of measuring high concentrations of aureomycin has been reported by Kelsey and Goldman (*1*), but their procedure is not applicable to determining the low concentrations found in clinical material.

It was observed that aureomycin had an intense yellow fluorescence in an acid or neutral medium and that after several minutes in an alkaline medium it began to be altered to a compound with a blue fluorescence. Inasmuch as yellow fluorescing compounds are a great deal more uncommon in body fluids and among medications than blue fluorescing compounds, it was felt that a test depending on yellow fluorescence would be less subject to interference than one depending on blue fluorescence. Hence, only neutral solutions were used.

Two-hundred-mesh activated silica gel (Davison) was backwashed with distilled water at 100 ml/min on a column 50 mm × 1 m for 3 hr. This removed the very small particles and gave a suspension of particles of fairly uniform size. To a 20-cm length of 6-mm glass tubing constricted at one end and packed with glass wool, there was added, by means of a capillary pipette, enough of a slurry of the prepared silica gel to form a packed column 3 cm long. Packing was achieved by repeated tapping of the column until no further settling occurred. One ml of serum containing aureomycin was allowed to filter through the silica gel column without vacuum or pressure being applied. The serum was followed by 1 ml of isotonic saline and 1 ml of 95 or 100% ethyl alcohol. The saline served to wash out the serum, and the alcohol intensified the fluorescence. At no time was the surface of the silica gel column allowed to dry out or be mechanically disturbed by the addition of fluids. Standards were prepared, in the same manner, with sera to which 20, 10, 5, 3, 2, 1, 0.5, 0.2, and 0.1 μg of aureomycin hydrochloride[2] had been added. The aureomycin was diluted to the appropriate concentration in distilled water and 0.1 ml of the dilution added to 0.9 ml of serum. The unknowns were visually compared to the standards, after dark adaptation, in a darkened room with the focused light from an 85-watt argon-mercury lamp filtered to remove all wavelengths above 400 mμ. The fluorescence appeared at the top of the column as a yellow band, which varied in width and intensity according to the concentration of aureomycin. Standards must be prepared every day or two, since the fluorescent color tends to fade. The success of this procedure depends in large measure on obtaining uniform silica gel particles and in uniformly packing these particles into a column free of bubbles.

The procedure may be modified to determine aureomycin in urine, spinal fluid, and other media. These modifications and the preparation of permanent standards will be reported elsewhere.

The fluorometric test was checked against bacteriologically determined levels on two healthy subjects who took, respectively, 9.3 and 10.4 mg/kg of aureomycin orally, and on patients who had received a single intravenous dose of aureomycin. The results are recorded in Table 1.

A test for interfering substances was made by adding aureomycin to the sera[3] of patients receiving other medi-

[1] The authors wish to thank Dr. Eleanor Bliss, of The Johns Hopkins Medical School, for her stimulating suggestions and encouragement.

[2] The aureomycin hydrochloride used in these experiments was supplied by the Lederle Laboratories Division of American Cyanamid Company, through the Antibiotics Study Section of the National Institutes of Health.

[3] These sera were obtained through the kind cooperation of the members of the House Staff of The Johns Hopkins Hospital.

cations. Patients with jaundice, cirrhosis of the liver, leukemia, arteriosclerosis, rheumatic fever, acute pneumonia, and other conditions were included in this test.

TABLE 1

A COMPARISON OF FLUOROMETRIC AND BACTERIOLOGIC METHODS OF DETERMINING SERUM AUREOMYCIN CONCENTRATIONS

Patient	Aureomycin dosage	Time after administering dosage	Fluorometric levels μg/ml	Bacteriologic levels μg/ml
C. W.	9.3 mg/kg orally	Control*	0	0
		1 hr	1	0
		2 hr	4	2.5
		4 hr	5	2.5
		6 hr	4	1.25
		8 hr	3	1.25
		12 hr	1	0.3
		24 hr	0.2	0
J. W.	10.4 mg/kg orally	Control*	0	0
		1 hr	1.3	0
		2 hr	1.7	0
		4 hr	3	2.5
		6 hr	2	1.25
		8 hr	3	1.25
		24 hr	0.5	0
W.	200 mg I. V.	Control*	0	0†
		75 min	3	2†
R.	200 mg I. V.	Control	0	0†
		30 min	2	0.5†
B. W.	? I. V.	75 min	1	1†
B. R.	?	30 min	1	0.5†
M. W.	200 mg I. V.	Control*	0	0†
		5 min	15	10†
		15 min	3	1.25†
		30 min	3	1†
		60 min	2.5	1†

* Control tests were performed on patient's sera before aureomycin dosage.

† These bacteriological levels were performed by Dr. Caroline A. Chandler of The Johns Hopkins Medical School.

They had received a variety of medications including sulfa drugs, penicillin, dicumarol, salicylates, and vitamins. In addition, streptomycin and chloramphenicol were each added to a serum containing aureomycin. In no case was anything found which interfered with the fluorometric test.

In the few determinations reported, there is a general correlation between the fluorometric and bacteriological assays. The fluorometric test appears to give slightly higher readings than the bacteriologic test. This suggests that the fluorometric test is measuring not only aureomycin but, in addition, something which is closely allied to and associated with aureomycin and which is bacteriologically inactive. Efforts are being made to define further the accuracy of the fluorometric test.

Reference

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Preparation of C^{14} Uniformly Labeled Fructose by Means of Photosynthesis and Paper Chromatography[1]

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The common carbohydrates, glucose, fructose, sucrose, and starch have been labeled with C^{14} by allowing leaves to photosynthesize in the presence of $C^{14}O_2$. However, only glucose, sucrose, and starch have been prepared with sufficient purity and activity to permit their use in tracer experiments. Fructose has not been available for tracer work because of the difficulty in separating it from glucose following sucrose hydrolysis. Putman *et al.* (*5*) used the calcium-fructose complex methods as described by Bates and associates (*1*). This method has two disadvantages: large amounts of carrier fructose are added, yielding a product of very low specific activity; and the fructose still contains appreciable amounts of glucose.

Recently Partridge (*4*) applied paper chromatography to the qualitative analysis of reducing sugars. The separation of glucose from fructose on a one-dimensional chromatogram using phenol as the solvent is very good (R_f glucose -0.39, R_f fructose -0.51). A 1-in. strip of paper can be used to separate as much as 0.3 mg of fructose from a similar amount of glucose. By using several strips of paper, we have prepared in very pure form milligram amounts of highly active fructose.

After four trifoliate bean leaves had been allowed to photosynthesize in the presence of 2.8 mc of $C^{14}O_2$ (*3*), they were killed in hot 80% alcohol. This alcohol extract contains sucrose, glucose, and fructose as well as other alcohol-soluble substances. After ether extraction and passage through ion exchange columns (Amberlite 100-H and Duolite A-4) to remove polar compounds, H_2SO_4 was added to make the solution 1 *N* and it was kept at 80° C for 10 min. After hydrolysis, the sample was again passed through the ion exchange columns to remove the acid. Most of the glucose was crystallized from the mixture by the addition of carrier glucose followed by the addition of alcohol.

To separate the fructose from the glucose still remaining in the mother liquor, 75 chromatograms (14×1.5-in. strips of Whatman No. 1 filter paper) were set up with 0.1–0.3 mg of sugar per strip. After development with phenol, the strips were air-dried and placed on x-ray plates (Eastman Type K). It is evident from Fig. 1 that a radioautograph of each strip is necessary, since the bands are not always in the same position. In about 2 hr the radioautographs were dark enough to permit tracing of the outlines of the bands onto the chromatograms. The outlined fructose bands were cut out and pooled, giving a total of about 2 g of filter paper. About

[1] Research carried out at Brookhaven National Laboratory under the auspices of the Atomic Energy Commission.