PROBLEMS IN DETECTION AND ISOLATION OF ANTITUMOR ANTIBIOTICS

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In any program for new antibiotics, one is faced with problems at every step: in screening, fermentation, fractionation and isolation, evaluation, process improvement and, ultimately, production. Not only in screening, but at every step from first culture filtrate to final product, the crucial problem is assay, the prompt and precise estimation of how much of the desired substance is present. In no sector of antibiotic research is this problem more vexing than in the quest for an antibiotic effective against neoplasms. For here we lack not only efficient test methods but even one wholly effective drug to serve as a standard of comparison for evaluating leads at an early stage.

The most widely used tests measure inhibition of growth of transplantable neoplasms in rodents. Dr. Stanley will discuss the sequential analysis scheme set up by the Cancer Chemotherapy National Service Center of the National Institutes of Health in an attempt to verify the activity of a primary screening lead. It is obvious that if the test results from the screening system are so suspect that replicate testing is necessary to ensure activity even qualitatively, then this test system cannot be expected to serve well for assay purposes where precise quantitative data are essential. Obviously, there are inherent problems associated with any animal test. In addition to frequently imprecise results, a relatively long test period is required. During this period frozen aliquots of the beers on test must be held in storage, subject to deterioration. And at least equally troublesome precautions have to be taken for maintaining large numbers of microbial cultures in order to ensure the viability of the few promisingly active candidates during the long fermentation, testing, and reporting period.

Rodent tests require a relatively large test sample. This requirement poses problems to the chemist who is striving to isolate the active substance. The preparation of 50 to 100 ml of each test sample is a wasteful and arduous, if not impossible, task for the chemist. And the preparation of large numbers of primary screening beers in sufficient volume for three or more tests in rodents presents problems in logistics that assume staggering proportions.

Testing in rodents involves the problem of toxicity. Not only may the antitumor substance be toxic to the host, but frequently crude beers contain one or more additional toxic components. In the isolation process, these materials may accompany the antitumor activity for a time, with the result that a given sample may contain the antibiotic, but its activity be masked by the larger dilution required because of the extraneous toxic materials.

Another problem that confronts us is tumor variability—variation not only in size and rate of growth but also in sensitivity to inhibition. An example of vari-
ation in sensitivity can be found in some of our experiences at Parke, Davis & Company in the early days of Chloromycetin. Early beers and concentrates and even crystalline Chloromycetin showed minimal but reproducible inhibition of sarcoma 180 in mice. Our early elation subsided and finally vanished when subsequent tests proved totally negative. Eventually, it was concluded that the tumor had probably changed sufficiently to become insensitive to Chloromycetin.

Our experience with azaserine provides another example. Early beers not merely inhibited tumor growth but actually caused regression of the tumor implants. Later, when crystalline azaserine became available, such regression could not be demonstrated with the pure compound or with numerous refermments, although beers and compound remain potent inhibitors of tumor growth. Did this experience reflect loss of a potentiating component present in early beers, or reduced susceptibility of later tumor implants to regression by azaserine?

It is common knowledge that tumors can vary in unpredictable ways. One can select for large tumors or small tumors, for fast-growing tumors or slow-growing tumors—all from the same parent tumor line. When one is screening hundreds of beers a week in thousands of mice, more frequently than not, one observes considerable variation in the size of tumors in the control group. What does this mean? More important, if the range of tumor weight in control groups is from 400 to 2000 mg, how confident can one be that an average tumor weight of 400 mg in a group of treated mice really indicates suppression of tumor growth?

There are other obvious problems associated with animal testing. I suspect that more than one pharmaceutical company has suffered through periods of enforced idleness waiting for problems of chronic disease in mice or shortages of hybrid mice to be cleared up.

All of these problems compound to make it extremely difficult to screen large numbers of culture beers effectively and to select the leads confidently. Probably the most serious aspect of the whole situation is that it is even more difficult, and frequently impossible, to move the leads along rapidly and efficiently.

For an adequate screening program, thousands of cultures must be maintained in the laboratory while awaiting test results. Hundreds of flasks containing thousands of liters of media must be inoculated, incubated, harvested, clarified, perhaps filtered, bottled, frozen, labeled, and transported to the testing laboratory. Transportation, for companies that do not do their own testing, is, in itself, a major operation involving dry-ice packaging and split-second timing to get the packaged materials to the airport in time to be loaded on the proper plane which may then be grounded for eight or more hours because of a fog!

Obviously, we would welcome a substitute for the mouse tests. The cost, not only in dollars, but in thoroughness of work is unsatisfactory, to say the least. Unfortunately, the question of what to substitute for the rodent tests is almost unanswerable in the light, or should I say darkness, of our present knowledge of cancer chemotherapy. The rodent tests with all their failings have, at any rate, been of sufficient value to permit the detection and isolation of antibiotics such as some of the actinomycins, azaserine, mitomycin, and other substances which do

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