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THE DEVELOPMENT OF TISSUE CULTURE SYSTEMS FOR THE EVALUATION OF CANCER CHEMOTHERAPEUTIC AGENTS

The use of tissue culture (TC), especially in tandem with *in vivo* systems, has certain potential advantages in the evaluation of cancer chemotherapeutic agents (CCA). First, much of the data produced by *in vivo* systems can be derived from TC. Second, TC is in the long run potentially less expensive than *in vivo* systems. Third, certain data derived from TC systems appear to point the way to more correct dosage regimens for 5-fluorouracil, which has apparently been used incorrectly for years (Calabro-Jones et al., Cancer Res. 42:4413-4420, 1982). Fourth, the potential exists that cultures of human tumors may in the future be useful in the determination of the sensitivity of these tumors to certain CCA or combinations thereof. Finally, TC techniques interphase with certain important current techniques; i.e., monoclonal antibody production, targeted cancer chemotherapy and genetic engineering. The material presented here is a preliminary study of the effects of Cisplatin and one of its isomers on the cell line 253-J, a human multiple transitional cell carcinoma derived from the urinary tract by Elliot (Elliot et al., J. Natl. Cancer Inst., 53:1341-1349, 1974). Cells were obtained from Dr. Ralph Clayman, University of Minnesota; Department of Urologic Surgery, Minneapolis, Minnesota.

Stock cell cultures were grown at 37 °C as monolayers in 75cm² tissue culture flasks containing RPMI 1640 medium (Grand Island Biological Co., Grand Island, New York), supplemented with 15% newborn calf serum, 10% tryptose phosphate broth, 0.3 units/ml of bovine insulin, 5 μM glutamine, and 100 units/ml each of penicillin and streptomycin. For experiments, 2.0-5.0 x 10^4 cells/25 sq cm flask were seeded into the completed growth medium and incubated at 37 °C for 16-24 hrs before the start of an experiment.

In drug experiments, stock solutions (500 *m*M) of the platinum compounds obtained from the National Cancer Institute, were prepared in complete growth medium by stirring the mixture at 37 °C for 30 min. The culture medium was removed from the flasks before treatment and the cells were treated at 37 °C for 2 hrs by adding the appropriate concentration of drug in 5.0 ml of complete growth medium. The drug treatment was terminated by the removal of the drug containing medium. The cells were washed once with Puck's Saline A followed by the addition of fresh medium to the cultures.

Growth studies were carried out in the following manner: Asynchronous cells were treated with the platinum compounds during the exponential phase of growth and were allowed to proliferate for at least 3 population doublings after drug treatment. Cells were harvested by trypsinization with a solution of 0.25% trypsin and 0.02% sodium EDTA in calcium and magnesium free Puck's Saline A, and counted in a Model ZBI Coulter Counter (Coulter Electronics, Hialeah, Florida). The inhibition of growth was measured by calculating the ratio between the number of cells in treated cultures and those in the untreated cultures run in parallel.

The cell doubling time was determined beginning 16-24 hrs after plating. An initial count was taken to determine the zero time point. Thereafter, cell counts were taken at 24 hr intervals over a period of 5-6 days.

The growth kinetics of 253-J cells are presented in Figure 1. The effect of the addition of increasing concentrations of Cisplatin isomers is presented in Figure 2. The effects of the Cisplatin isomers are similar to those obtained by other researchers with different cell lines (Drewinko, et al., Cancer Res., 33:3091, 1973; Zwelling, et al., Cancer Res., 39:365-369, 1979). These curves appear to be simple exponential types which

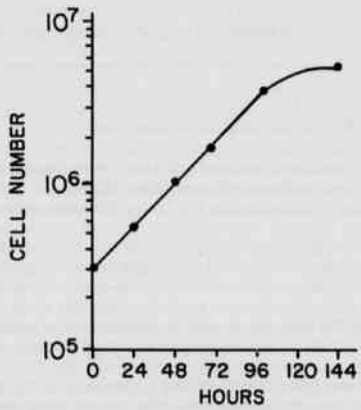

Figure 1. Growth of 253-J cells. Cells seeded into complete growth medium 16-24 hrs before the initiation of experiment. Counts were determined at 24-hr intervals over a period of six days. Each measurement was in duplicate. This figure is the result of three experiments.

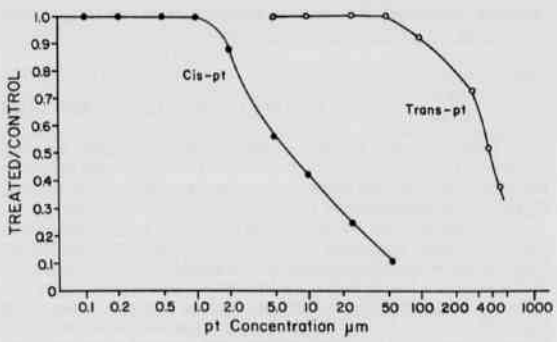

Figure 2. The effect of Cis- and Trans-Dichloroammineplatinum II upon the proliferation of 253-J cells. Asynchronous cells in the exponential growth phase were treated for 2 hrs at 37 °C with increasing drug concentrations. The rest of the protocol is found under methods. Each measurement was done in triplicate and the results are the mean of three experiments.

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are generally seen with alkylating agents or agents which intercalate with DNA (Drewinko, et al., Cancer Res. 33:3091, 1973). This particular line of cells appears somewhat less sensitive to Cisplatin than the lymphoma cells used by Drewinko, Brown and Gottleib (Cancer Res., 33:3091, 1973), however, variations in sensitivity among different cell lines are common occurrences.

The cis-isomer of Cisplatin was about 50-60 times more toxic than the trans-isomer, a phenomenon which has been noted by other workers (Zwelling, et al., Cancer Res., 39:365-369, 1979). This phenomenon is only partially understood. Both agents cause extensive DNA-protein cross linkage and intra- and interstrand DNA cross linkage, however, the degree of interstrand cross linkage more closely correlated with cytotoxicity. The trans-isomer is actually more mutagenic. The mechanism of the specificity for certain tumors which alkylating agents such as Cisplatin display is not understood in view of the wide spectrum of reactions in which these compounds take part. It would appear that the platinum coordination compounds as alkylating agents, must react with very specific sites to produce their characteristic effects.

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FIRST HOST PLANT RECORDS FOR *Chelysomidea guttata* (HERRICH-SCHAEFFER) (HEMIPTERA:SCUTELLERIDAE), WITH NOTES ON THE BIOLOGY AND DISTRIBUTION

Chelysomidea is primarily a tropical-subtropical genus distributed from the southern United States through Mexico, Central America, and into South America. As with most genera of Scutelleridae, little work has been done with *Chelysomidea* aside from species descriptions. The purpose of this paper is to report host plant data and new information concerning the biology of *C. guttata*.

C. guttata has been collected from the southern parts of Louisiana, Mississippi, Alabama, and Georgia; throughout Florida; and from eastern North and South Carolina. One other species in this genus, *C. stictica* (Dallas), is known to occur in the United States from a small area in the vicinity of Brownsville, Texas, which is thought to be the northern limits of its range (Lattin, The Scutellerinae of America north of Mexico (Hemiptera:Heteroptera:Pentatomidae), Unpubl. Ph.D. dissertation, p. 350, 1964).

Blatchley (Heteroptera or true bugs of eastern North America with especial reference to faunas of Indiana and Florida, p. 1116, 1926) reported *C. guttata* being collected from *Ipomoea pes-caprae* (Roth) and scrub oak in Florida. Lattin (1964) reported collecting the species from *Kosteletzkya virginica* (L.) in South Carolina and from *Althaea rosea* (Cav.) in Florida. No feeding activity for *C. guttata* has previously been reported.

Adults and late nymphal instars were observed feeding on *Croton capitatus* (Michx.) and *C. glandulosus* (L.) in September of 1982 in Choctaw County, Alabama and Covington County, Mississippi. All five nymphal instars as well as adults were found on all parts of *Croton* but mainly in groups on the flowering portion of the plants. Gregarious adults and late nymphal instars were also observed.

Several live adults and nymphs were collected from *C. capitatus* and *C. glandulosus* and brought back to the laboratory for rearing. Different stages of nymphal instars were separated and put in pint mason jars with screen tops. Field collected adults were sexed and placed in mason jars, two pairs per jar. Nymphal instars and adults were first fed fresh green beans and raw peanuts. McPherson (The Pentatomoidea [Hemiptera] of northeastern North America with emphasis on the fauna of Illinois, p. 240, 1982) reviewed the literature concerning lab rearing practices for the Pentatomoidea. A high mortality rate occurred within the first month of rearing. In an effort to reduce high mortality, *C. capitatus*, which is abundant in northeastern Arkansas, was collected and placed in the mason jars in lieu of green beans and peanuts. The insects fed on flowering portions of *C. capitatus* which were clipped and placed in small test tubes filled with water. Cotton was used to plug the openings of the test tubes. Food along with paper towelling was replaced three times a week or as necessary. All jars were washed once a week. Specimens were incubated at 25 ± 1°C, 12:12 LD photoperiod and ambient humidity.

The purpose of rearing efforts was to determine the length of the development of the insect, from egg to adult stage, and to determine the length of each individual instar. This part of our study was not completed due to the high mortality rate which occurred within the first month of rearing.

A pair of insects was observed mating on January 3, 1983, but as yet no eggs have been deposited. Further collections of *C. guttata* will be made in order to continue the study into the life cycle of this insect.

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FIRST REPORT OF BRAZILIAN FREE-TAILED BAT MATERNITY COLONIES IN ARKANSAS

Three maternity colonies of the Brazilian free-tailed bat (Molossidae: *Tadarida brasiliensis cynocephala*) have been found in central Arkansas. Previously reported records of *Tadarida* in Arkansas are of individual specimens collected from Ashley, Hempstead and Pulaski counties, either roosting singly or in maternity colonies of the evening bat, *Nycticeius humeralis* (Sealander, A guide to Arkansas mammals, pp. 99-102, 1979; Sealander and Price, J. Mamm., 45:152, 1964).

On July 28, 1982 we investigated a reported bat infestation in the attic of an old two story apartment building in downtown Hot Springs, Garland County. The colony was estimated to have contained 100 individuals. Forty-two bats were captured, examined and released. Six of the bats captured were volant juvenile *Tadarida* and three were volant juvenile *Nycticeius*. Juvenile status was determined by non-closure of the epiphyses of the third and fourth digits. All bats were roosting on the west wall of the attic at the ceiling joist/rafter junction, rendering them extremely difficult to capture. When a light was shone in this area, many of the bats moved outside the attic proper and roosted behind an exterior facer board. It was from behind this facer board that most of the bats launched themselves into flight when initiating their nightly foraging activities. During January, 1983, a check of this roost revealed a portion of the colony used the attic as overwintering quarters.

The second maternity site was found in the attic of an old dormitory building on the campus of Central Baptist College in Conway, Faulkner County, during October, 1982 and represents the northern most distribution of *Tadarida* reported in Arkansas. The colony numbered several hundred individuals and used a 30 centimeter wide air space between a double brick wall and the ceiling joist/rafter junction at the edge of the attic for roosting. Similar roosting sites were selected by *Tadarida* in Louisiana (LaVal, Am. Midl. Nat., 89:112-120, 1973). Both of these roosting sites were located on the west side of the building and warmed considerably during afternoon hours. Judging from the guano that has accumulated to a depth of over 30 centimeters in places, the colony had probably inhabited the attic for a number of years. Verification of this roost as a