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HEALTH EFFECTS OF CHEMICALS:

V. COMPUTER-ASSISTED GENETIC TOXICOLOGY TESTING

The advent of the microchip has accelerated the incorporation of dedicated or time-shared computers, often on line, as integral elements of the modern toxicology laboratory. These most versatile electronic tools have made it possible to undertake massive experiments that formerly would have been considered unmanageable.

Applications differ, and computer assistance is more useful in some areas of specialized research than in others. Such is the case for genetic toxicology, where the diversity of the tests employed and the multiplicity and sheer volume of the data generated make an efficient method of data management and analysis imperative.

Leon S. Otis (Ed.)

Genetic toxicology covers a very broad field, but generally refers to the study of chemicals that adversely affect the genetic material, i.e., the DNA that makes up the genes and chromosomes. Many different endpoints can be used to study these adverse effects, including damage to chromosomes, mutational events, reproductive effects, transformation of cells to a malignant form, and direct damage to the DNA.

All of the various test systems used in this field generate enormous amounts of data that require considerable amounts of time for subsequent compilation and analysis. Until fairly recently, all of these data had to be processed manually, which required countless hours and increased the possibility of human error. The labor-intensiveness of these earlier systems also increased costs to clients. With the advent of relatively inexpensive data management systems, however, it has become possible to reduce these time and cost constraints substantially.

The Cellular and Genetic Toxicology Department at SRI is involved in a wide variety of short term genetic toxicology tests that measure potentially harmful effects to the DNA in both tissue culture and whole-animal assays. To help plan, organize, and complete this complex and interactive testing program, and to analyze the voluminous data produced, the department has developed a computer-assisted data management system using a Digital Equipment Corporation VAX 11/782 computer. Incorporation of this system into our data management procedures has significantly improved efficiency and greatly reduced the possibility of human error. In addition, compliance with Good Laboratory Practice regulations has been greatly simplified by eliminating the need for auditing human calculations and transcriptions as well as establishing "data trails" for use by auditors. The computer is involved in every step of the experimental process — from designing experiments to direct data collection, summary of data, and statistical analysis and production of high-quality tables or graphs for reports. In particular, two test systems that have benefited immeasurably from this technology are *in vivo* DNA repair assays and the L5178Y mouse lymphoma mutagenesis assay.

IN VIVO DNA REPAIR ASSAYS INDICATE POTENTIAL CHEMICAL CARCINOGENICITY OR MUTAGENICITY

Studies have shown that if a chemical interacts with the DNA in any way, there is a good chance that the chemical is a potential carcinogen or mutagen. This DNA interaction can be studied by measuring chemically induced DNA repair. Fortunately, most cells are able to repair damage to the DNA by removing the DNA adduct and resynthesizing the bases to fill in the gap in the molecule. This excision repair synthesis is often referred to as "unscheduled DNA synthesis" or "UDS" because it is DNA synthesis that occurs outside of the regular "scheduled" DNA replication periods. By measuring UDS one can easily obtain some indication as to how much DNA damage has occurred and therefore what the chances are that a chemical may be a carcinogen or mutagen.

The DNA Damage and Repair Program at SRI uses UDS assays both in tissue culture (*in vitro*) systems, and in some relatively new whole-animal (*in vivo*) assays. In the *in vitro* work, cells in culture are exposed to a wide variety of potential carcinogens and examined to determine whether or not these chemicals induce UDS. In the *in vivo* assays, the whole animal is treated, rather than cells in culture, and the responses can be measured in individual target tissues such as the liver, pancreas, or kidney.

An *in vitro* assay is relatively fast and inexpensive, but is somewhat limited in the information it provides. In an *in vivo* system one can study the influence of such complex factors as sex and species differences, diet, chronic exposure, age, synergistic effects, environmental factors, and a myriad of other parameters that may contribute to carcinogenicity. In addition, by examining the response in an

individual target organ, it may be possible to determine not only whether or not a chemical may be a carcinogen, but also in what tissue it will produce tumors.

Both *in vivo* and *in vitro* UDS assays measure DNA repair as incorporation of tritiated thymidine (^{3}H-TdR), a radioactive DNA precursor, using autoradiography. Cells undergoing DNA repair will take up large amounts of ^{3}H-TdR into their DNA, and when a photographic emulsion is placed over the cells, the silver grains in the emulsion will be exposed wherever the DNA contains a radioactive segment. Counting these exposed silver grains gives an indication of whether a chemical has induced UDS.

Until recently manual grain counting took hours for each slide and made routine testing with this procedure impractical. With the new automated system, grains are counted using a "colony counter," which can detect the exposed silver grains using a contrast-discriminator (Fig. 1). Grain counting, therefore, can be accomplished in the time it takes to focus the microscope on a cell, set an electronic aperture over the desired counting region (which is displayed on a TV monitor), and push a count button on the colony counter. The data are fed directly into a file in the computer, eliminating the need to record raw data manually. This system has reduced the time required to score 50 cells per slide from a few hours to an average of less than 10 minutes. In addition, all subsequent processing of data is accomplished by computer programs with no additional entry of data required.

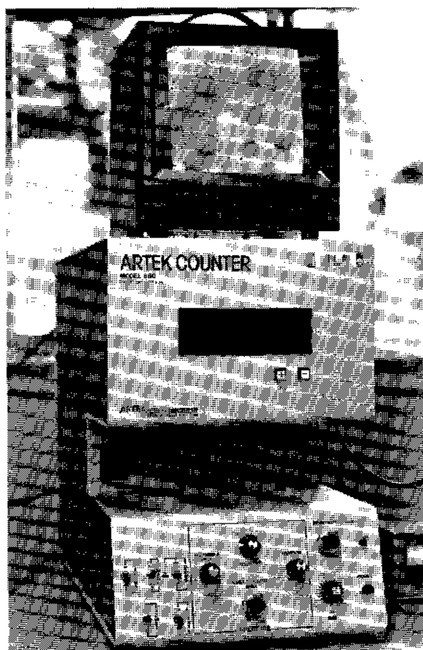

FIGURE 1
The number of silver grains in autoradiographic preparations is rapidly counted by an automated colony counter, and the data are fed directly into a computer for eventual analysis.

Tables 1-4 show some of the sequentially programmed steps of how the data are generated, collected, pooled and summarized in a simple experiment from the planning stages to the final report . . . with all phases coordinated by the computer. The animal treatment schedules, test chemicals, doses, etc. are input by the project leader and appropriate forms are generated by the computer for the coordination of different phases of the experiments (Table 1). After completion of the lab work, data from slide scoring are fed directly into a data file (Table 2). The data from several slides can be pooled to give the totals for individual animals and the data from several animals at a given dose can then be pooled (Table 3). The nuclear grain count (NUC), the cytoplasmic background (BKG), the net grains/nucleus (N.G.), and the percent of cells undergoing DNA repair (% I.R.) are presented for each slide as well as a complete analysis of variance for these parameters.

In the final step, the data for various dose and control groups can be collected by the computer to prepare a summary table for a final report (Table 4). This table shows the three dose groups tested for a coded chemical at the indicated times. At no point is data inputted manually by a human operator. This system has saved tremendous amounts of time and money, and accuracy has increased significantly.

THE MOUSE LYMPHOMA MUTAGENESIS ASSAY DETECTS THE POTENTIAL GENOTOXICITY OF CHEMICALS *IN VITRO*

A second assay that uses computer-assisted data acquisition is the L5178Y mouse lymphoma mutagenesis assay. This assay measures mutations in the thymidine kinase gene that are induced by test chemicals, and serves as a useful indicator of the carcinogenic or genotoxic potential of chemicals. Colonies of mutant cells grown in soft agar are measured using a colony counter very similar to that used in UDS studies. As is done for the UDS assays, all experimental data such as cell counts, colony counts, colony sizes, and other data can be fed directly into the computer.

One example of the usefulness of this computerized system is in the collection, analysis and tabular or graphic display of colony size data. Treating cells with different chemicals may produce mutant colonies of varying sizes, depending on the mode of action of the test compound. Measurement of size differences can provide useful information about the possible genotoxic action of a chemical, and research on this phenomenon is an important part of SRI's ongoing research program. Using an automated colony counter, we can determine the distribution of colony sizes by making colony counts at various size increments. All the colonies are sized, and gradually the smallest colonies are eliminated from the count, leaving only the largest mutant colonies. These data are rapidly collected, a distribution table is constructed (Table 5), and graphs of the data are plotted (Fig. 2).

Using these various techniques for computer-assisted data management, a highly efficient and error-free testing and research program has been developed at SRI. Work is continuing in our laboratory to establish new systems for handling data from other genetic toxicology test systems, such as cytogenetics and other mutagenesis assay systems. While this program is still in its infancy, it has already yielded significant benefits in rapidly, reliably, and economically indentifying potential carcinogens. The assays that we have developed, in conjunction with new genotoxicity assays being developed at SRI and elsewhere, promise effective future management of the vast number of suspect chemicals that require toxicologic evaluation for potential carcinogenicity.

TABLE 1

Following the input of information on the chemicals to be tested in the *in vivo* UDS assay, the computer generates a series of forms to be used by the different laboratory groups involved in the actual experiments. The first three of seven such forms are shown here. Pg. 1 is a summary of the various treatment groups; pg. 2 indicates the appropriate amounts of test chemicals to be weighed out; pg. 3 indicates the treatment schedule with spaces for animal weights, comments, and other parameters. Other pages (not shown) contain perfusion schedules, tissue culture data sheets, and slide coding information.

Experiment # 99 pg. 1 Perfusion Date 6/29/83 Notebook # ______ Page ______
Species:B6C3F1 Mouse Sex:Female

Animal #	Chemical	mg/kg	Route	Time (hr.)	Vehicle	cc/100g body wt.
99T1	CONTROL/water	0	oral	12	water	0.50
99T2	Dimethylnitrosamine	10	oral	12	water	0.50
99T3	Azaserine	20	oral	2	water	0.50
99T4	Azaserine	100	oral	2	water	0.50

Notebook page generated by JON ID# 18730 29-JUN-83 Checked by ______ Date ______

Experiment # 99 pg. 2 Perfusion Date 6/29/83 Notebook # ______ Page ______

Animal #	Chemical	Chemical (mg)	Solvent (ml)	actual wt.	add (ml)	solvent
99T1	CONTROL/water	0.0	1.00	______	______	water
99T2	Dimethylnitrosamine	2.0	1.00	______	______	water
99T3	Azaserine	4.0	1.00	______	______	water
99T4	Azaserine	20.0	0.98	______	______	water

Notebook page generated by JON ID# 18730 29-JUN-83 Checked by ______ Date ______

Experiment # 99 pg. 3 Perfusion Date 6/29/83 Notebook # ______ Page ______

Animal #	Mouse #	Weight (g)	approx. gavage	time gavaged	administered dose	Comments
99T1	______	______	8.30pm	______	______cc	______
99T2	______	______	8.30pm	______	______cc	______
99T3	______	______	7.20am	______	______cc	______
99T4	______	______	7.20am	______	______cc	______

Calibration wt.=______ Measured wt.=______ Balance Ser. No.=______ Chamber Temp.=______
Notebook page generated by JON ID# 18730 29-JUN-83 Checked by ______ Date ______

TABLE 2

Data from UDS slide scoring are collected directly by the computer. The operator inputs essential information (including the slide code, name, and starting slide coordinates) and data from counting are fed directly from the colony counter into the computer file. After the last cell, the operator can input ending slide coordinates and general comments on the slide. The computer automatically indicates the date and time.

```
99G              ST:27.7,110    END:27.9,106.0
880  C= 1.33    scored by JAMES, 15211
0052 0013 0013
0036 0015 0013
0035 0008 0007
0043 0014 0012
0025 0013 0005
0024 0006 0004
0044 0014 0007
0029 0006 0004
0021 0001 0000
0031 0003 0005
0019 0004 0001
0035 0003 0004
0033 0008 0007
0028 0003 0002
0028 0005 0004
0041 0004 0006
0018 0001 0002
0032 0003 0003
0021 0009 0008
0024 0007 0008
0027 0005 0005
0019 0002 0001
0011 0002 0002
0034 0004 0003
0024 0009 0010
0044 0006 0003
0032 0010 0008
0023 0009 0010
0043 0002 0003
0024 0003 0003
0028 0011 0007
0019 0006 0004
0020 0007 0004
0030 0005 0002
0039 0009 0008
0042 0010 0008
0042 0010 0006
0037 0004 0007
0033 0010 0003
0028 0003 0004
0018 0003 0001
0022 0002 0002
0028 0006 0003
0026 0002 0003
0034 0006 0009
0014 0001 0001
0021 0005 0008
0020 0010 0005
0044 0007 0002
0035 0009 0006

      3-JAN-83    13:25:49
 Comments:  GOOD SLIDE
```

TABLE 4

Following completion of all dose groups, a final program collects the data from each group and summarizes the key results in a table that can be printed and included in final reports to clients.

ASSAY FOR INDUCTION OF UNSCHEDULED DNA SYNTHESIS BY 123456
IN THE IN VIVO-IN VITRO HEPATOCYTE DNA REPAIR ASSAY IN RATS

Treatment	Dose (mg/kg)	Time (hr)	n	N.G. mean	N.G. S.E.	% in repair mean	% in repair S.E.
CONTROL/Water	--	2	3	-4.1	2.0	3	1
Dimethylnitrosamine	10	2	3	64.7	11.0	98	5
123456	50	2	3	-1.5	1.5	5	1
		12	3	-0.7	0.6	9	4
	200	2	4	2.5	1.2	16	6
		12	3	14.9	2.1	45	7
	1000	2	3	15.4	1.9	51	9
		12	3	38.8	6.0	84	10

Standard errors (S.E.) represent animal-to-animal variation; n= number of animals treated.
N.G. = net grains/nucleus

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