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An Automated Monitoring System for Fish Physiology and Toxicology

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16. ABSTRACT

This report describes a data acquisition and control (DAC) system that was constructed to manage selected physiological measurements and sample control for aquatic physiology and toxicology. Automated DAC was accomplished with a microcomputer running menu-driven software developed with an extended BASIC. An interface module was built that connected standard sensors and controls to the computer. Digital I/O signals for sample device control and analog signals from sensors were multiplexed through the interface module. Time intervals for automated DAC were user defined, and test data were displayed on a monitor, printed, stored on disk, and transferred to a minicomputer for analysis. Automated measurements were made of temperature, ventilation volume, oxygen content of exposure (inspired) and expired water, and pH of both waters from four in vivo rainbow trout (Salmo gairdneri) preparations. Oxygen uptake efficiency and oxygen consumption were calculated. Urine and expired water samples were also collected from all fish.

Non-automated sampling included ventilation frequency, cough frequency, the electrocardiogram, and aortic blood from an implanted canula. Sampled blood was analyzed for oxygen, carbon dioxide, pH, hematocrit, and hemoglobin. The respiratory-cardiovascular data gathered with this system were used to define fish acute toxicity syndromes (FATS) specific to known modes of toxic action.

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ABSTRACT

A data acquisition and control (DAC) system was constructed that managed selected physiological measurements and sample control for aquatic physiology and toxicology. Automated DAC was accomplished with a microcomputer running menu-driven software developed with an extended BASIC. An interface module was built that connected standard sensors and controls to the computer. Digital I/O signals for sample device control and analog signals from sensors were multiplexed through the interface module. Time intervals for automated DAC were user defined, and test data were displayed on a monitor, printed, stored on disk, and transferred to a minicomputer for analysis. Automated measurements were made of temperature, ventilation volume, oxygen content of exposure (inspired) and expired water, and pH of both waters from four in vivo rainbow trout (Salmo gairdneri) preparations. Oxygen uptake efficiency and oxygen consumption were calculated. Urine and expired water samples were also collected from all fish.

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Key words: respirometer, rainbow trout, fish acute toxicity syndromes, automated system, electrode chamber, ventilation, computer monitoring, cardiovascular, respiratory

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ACKNOWLEDGMENTS

We thank Dan Burrows for his initial design effort on the electronics for this system. Mike Ostraski developed much of the software and constructed a working model of the interface module; his contributions were considerable. We also thank James McKim for his assistance and numerous discussions during the planning stage, and Shelley Heintz for typing the manuscript.

SECTION 1

INTRODUCTION

Quantitative structure activity relationship (QSAR) models make predictions about toxicity of chemicals based upon their physicochemical properties, and it is assumed that all chemicals that kill by the same mode of toxic action can be modeled by a single QSAR (Veith et al., 1983; 1987). Because alternative QSARs are required for different and specific modes of action, optimizing the QSAR approach to predictive toxicology requires an additional systematic effort to define and predict mode of action so that the appropriate QSAR is invoked to make the toxicity prediction for a particular chemical. Detailed mechanistic studies to understand mode of action at the molecular level are simply not possible for all chemicals, but one approach to understanding causal relationships between chemicals and their effects was developed recently and is termed fish acute toxicity syndromes (FATS). These are collections of direct and indirect measures of effect, or clinical signs, manifested in the animal upon exposure to chemicals that are unique and specific to a common mode of action (McKim et al., 1987a). Based on a group of measurable toxic signs involving the respiratory-cardiovascular system in rainbow trout (Salmo gairdneri), FATS have been defined for narcotics, oxidative phosphorylation uncouplers, acetylcholinesterase (AChE) inhibitors, respiratory membrane irritants, and the pyrethroid insecticide fenvalerate (McKim et al., 1987b,c; Bradbury et al., 1987).

FATS testing required data acquisition on 11 respiratory-cardiovascular variables and the capability to monitor more if necessary. Monitoring was performed manually during all previous FATS tests and consumed the full attention of at least three people along with the part-time help of several others during both a seven hour control period and for up to 48-h during the

acutely lethal exposure period. Only two fish could be prepared and tested at one time, and two tests were therefore required to gather sufficient information on four fish to reliably define a FATS. Although measurements were made often enough so that statistical relevance was established, a higher sampling frequency was desirable for greater confidence. Clearly, a more efficient system had to be developed before extensive FATS testing could be accomplished.

The objective here was to develop an automated system to efficiently quantify some of the physiological functions of a whole fish preparation exposed to acutely lethal chemical concentrations. It was desired that the system provide for data gathering on at least four fish, and to do so at predetermined intervals throughout the test including periods of unattended operation. Another requirement was that it remain flexible enough so that sampling and measurement regimes could be changed, singly or collectively, during a test. With these in mind, a system was designed that could be constructed from commercially available sensors, control valves, a personal computer, a specially constructed interface, and menu-driven software to coordinate system activities. Although this report provides some details on how the system was constructed, it is not intended to serve as a construction manual. It should, however, provide enough guidance so that those with access to some technical resources could design and build their own custom system.

SECTION 2

CONCLUSIONS

1. Automated monitoring of respiratory-cardiovascular variables from fish resulted in a considerable savings of time and effort when compared to manual data gathering methods.
2. Automated monitoring provided continual data collection during periods of unattended operation, thus ensuring that data were collected during times when critical changes may have occurred.
3. The real-time sampling and calculation of vital signs permitted judgments on the course of an experiment.
4. Automated monitoring allowed rapid data collection at shorter time intervals than manually possible. A greater number of samples provided for greater statistical reliability.
5. Data were easily manipulated and transferred between computers because they were immediately stored in computer files.
6. Less manual sampling reduced human exposure to potentially hazardous chemicals.

SECTION 3

RECOMMENDATIONS

1. The automated system as described here functioned well and proved suitable for fish acute toxicity syndrome (FATS) testing. Other applications such as pharmacokinetics would also benefit from automated monitoring.
2. More physiological variables should be automated, including ventilation frequency, cough frequency, and heart rate. This would result in more detailed information about the physiological state of an animal in real time.
3. Commercially available components should be used wherever possible when constructing an automated system. This reduces the in-house research and development effort necessary to get such a system into operation.
4. Construction costs for this automated system are estimated at $50,000.

SECTION 4

METHODS AND MATERIALS

This system was designed to perform physiological monitoring on rainbow trout (Salmo gairdneri) that weighed between 0.6 and 1.0 kg and were exposed to a lethal concentration of an organic chemical. This required the integration of several subsystems that included: (1) exposure apparatus that provided water and toxicant delivery; (2) automated sampling and measurement circuits and devices that provided automatic data collection and sampling of physiological functions; (3) non-automated circuits and devices that provided monitoring of those physiological functions that defied automation at this time; (4) a microcomputer system that controlled all aspects of automated monitoring; and (5) an interface that provided all necessary interconnections and switching between the computer and external devices.

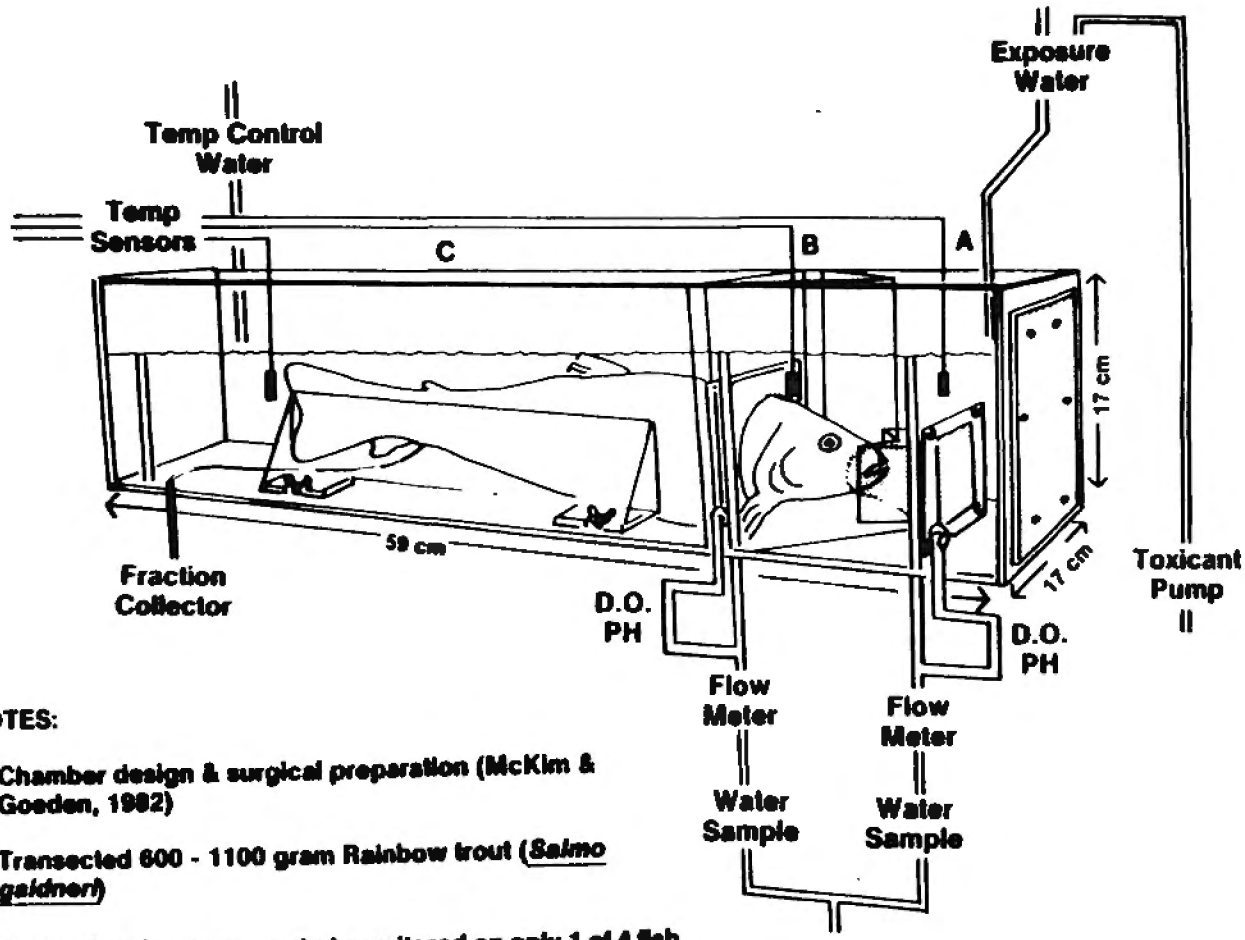
4.1 EXPOSURE SYSTEM

The exposure system consisted of two stainless steel headboxes that fed Lake Superior water at 11 ± 1° into a flow-splitting mix cell, four respirometer-metabolism chambers to hold the fish, and metering pumps that delivered a concentrated aqueous solution of chemical to the mix cell. The mix cell was a 18 cm x 24 cm x 27 cm high glass container that had four 16 mm diameter bored holes in the bottom. Each hole held a neoprene stopper that contained a 10 cm section cut from the tip of a 2 ml disposable pipette. By grinding back the tip so that the bore was about 2 mm in diameter, a 600 ml/min water flow was obtained when the water column height within the mix cell was adjusted to 20 cm. Constant head height was maintained by a float valve that was supported by a glass cover placed over the mix cell. Exposure water was delivered at equal and constant rates through teflon-lined tubes to the A compartment of each fish chamber. Chemical solution was delivered to

the mix cell by 12 volt DC Model RP-BG75 metering pumps (Fluid Metering, Inc., Oyster Bay, NY).

Except for modifications noted below, design and construction of the fish chambers used in this system was that of McKim and Goeden, 1982 (Figure 1). Standpipe position was changed to a bottom rather than side exit in each of the three compartments of the plexiglass fish chamber. Water overflow from the A compartment of fish chamber one and from all B compartment standpipes was directed into the flow measuring devices; other A compartment and all C compartment overflow went directly to drain. From ports located on the sides of the chambers, water was directed to the different sensing electrodes without aeration. Other ports were installed for manual water sampling, and one port was used as the exit point in the C compartment for the urine catheter. Quick-disconnect couplings threaded into the chambers provided convenient connection of sampling tubes.

The exposure apparatus was contained in a specially constructed vented enclosure (Figure 2) to minimize human exposure to potentially hazardous chemicals and to shield the fish from human activity. The framework of the 183 cm long x 91 cm wide x 238 cm high cabinet was constructed of 1.9 cm thick plywood coated with chemically-resistant epoxy paint. Laboratory exhaust connected to the top of the enclosure provided a negative air pressure relative to room air. Sliding glass doors allowed observation and access to the apparatus, yet maintained sufficient negative pressure within the enclosure.



NOTES:

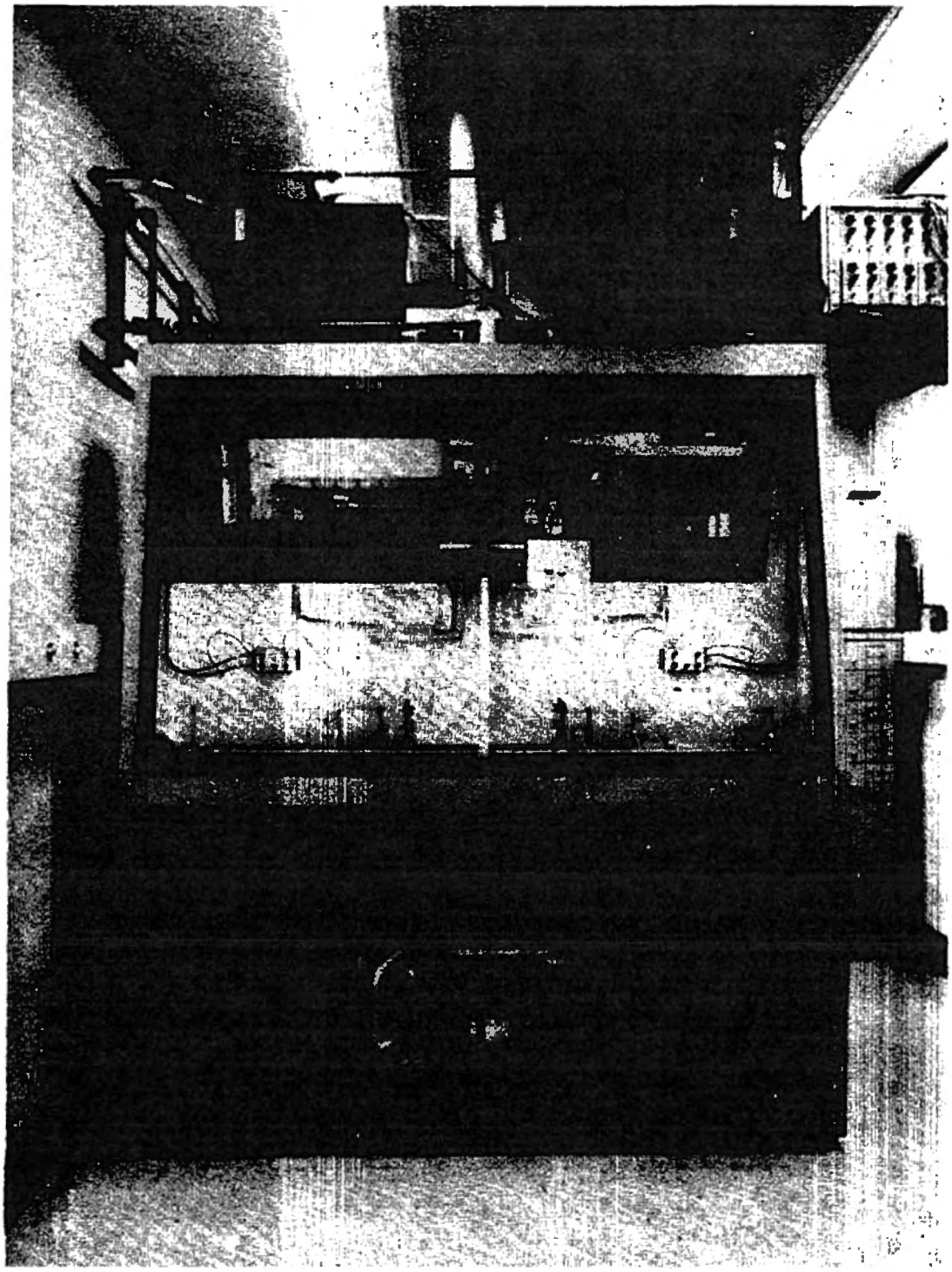
1. **Chamber design & surgical preparation (McKim & Goeden, 1982)**

2. **Transected 600 - 1100 gram Rainbow trout (*Salmo gairdneri*)**

3. **A Chamber (exposure water) monitored on only 1 of 4 fish**
   B Chamber (expired water) (Va) ventilation volume
   C Chamber (fresh water) input for maintaining temp

Fig. 1

Fig. 2



4.2 AUTOMATED DATA ACQUISITION AND CONTROL

Figure 3 shows the overall layout of the data acquisition and control (DAC) system that performed sampling, measurement, and calculation of selected physiological functions. Individual components and their operation are described below. The DAC system was designed to monitor pH, dissolved oxygen (DO), temperature, and flow rate of both the incoming and expired water in which the fish resided during a test. This was accomplished by monitoring the expired water (B compartment) of up to four fish chambers and the incoming water (A compartment) of fish chamber one (Figure 1). The A compartment was designated chamber five, both in illustrations and within the computer program that controlled system operation, but was referred to as chamber 1A on the monitor display. Additionally, samples of both waters and urine fractions from each fish were collected automatically and held for chemical analysis. A single water sample was taken from the A compartment whenever any or all of the B compartments were so scheduled. Also, whenever any fish chamber was monitored for pH, DO or temperature, the A compartment was sampled immediately afterward so that the samples of inspired and expired water were as close in time as possible. This was necessary because the calculations for oxygen uptake efficiency (U_E) and oxygen consumption (VO_2) involved the difference in DO content of both waters at that moment.

4.2.1 Computer System

The computer was an IBM PC/XT version specially built for Analog devices, running at 4.77 MHz, and containing an INTEL 8088 microprocessor, 8087 math coprocessor, 256 KB RAM, and four expansion slots. One of the slots held an additional 384 KB RAM, boosting total memory to 640 KB. The basic system

SYSTEM CONFIGURATION

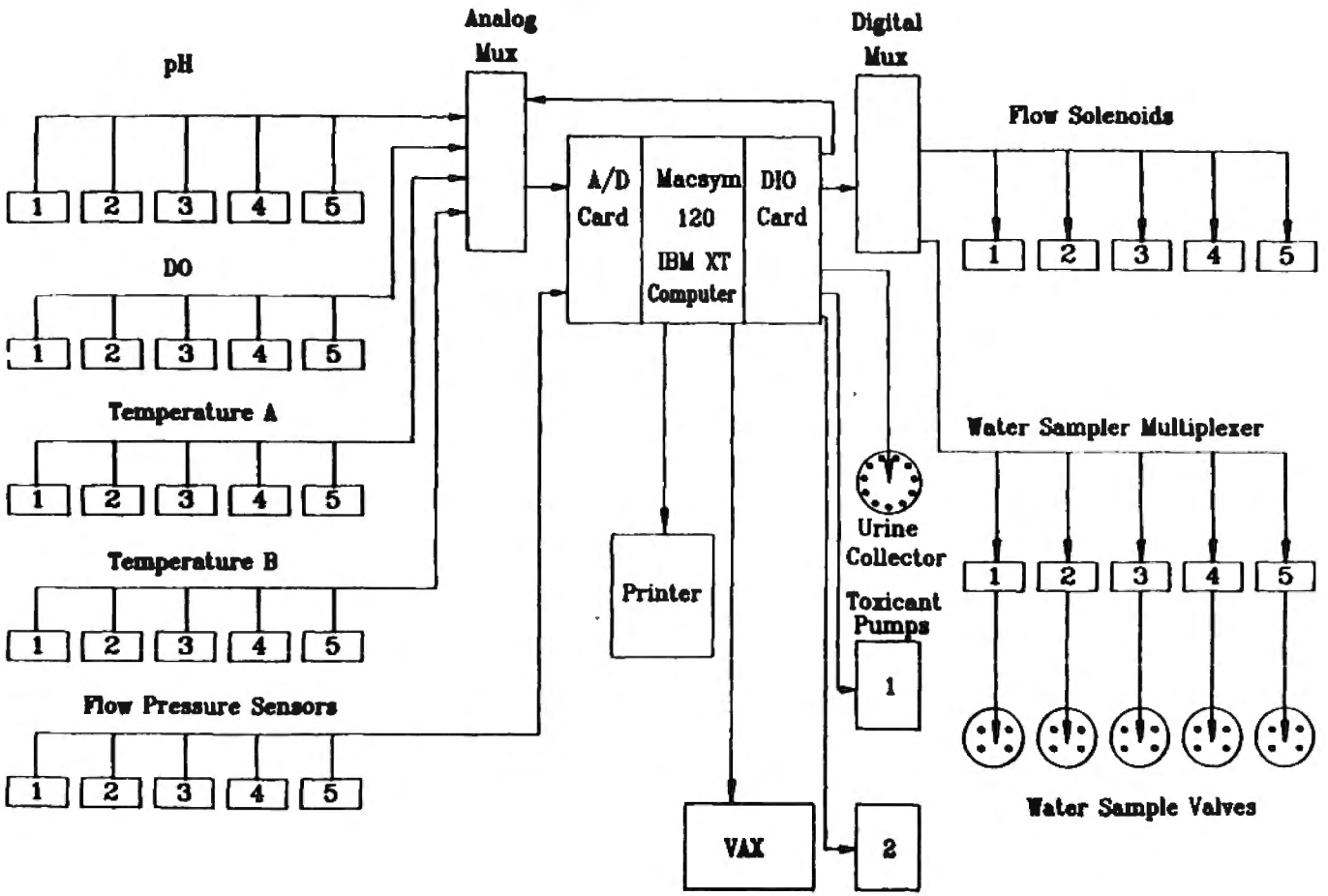


Fig. 3

came supplied with an 83 key sealed membrane keyboard, 130 W power supply, one 5 1/4" floppy diskette drive, a 10 MB fixed disk drive, color/graphics display adapter, and a combination adapter card. The latter provided a serial asynchronous communications port, a parallel printer port, battery backed-up realtime clock, and a thermal warning system to monitor internal system temperature. A 33 cm diagonal, 16 color RGB industrial display with a protective screen served as the system monitor. The entire computer was manufactured to withstand the higher temperatures, vibration, and particulate contaminants found in the industrial environment.

Automated DAC was provided by an MIO120 multifunction board and a DIO120 digital input/output (I/O) board (Analog Devices). The MIO120 contained an input analog section consisting of an analog multiplexer with capabilities for 32 channels of single or pseudo-differential inputs, or 16 channels of full differential input. The automated system described here used ten channels configured as single ended, bipolar analog inputs in the $\pm$ 10 volt range. Six of these channels were reserved for individual analog-to-digital (A/D) conversion on voltage signals coming from the flow sensors, and one channel each for A/D on pH, DO, compartment A temperature, and compartment C temperature signal inputs (Table 1). Analog signals from the DO, pH, and temperature meters were in the 0.6 to 1.2 volt range, and in the 1 to 8 volt range from the pressure transducers used in flow measurement. These signals were fed into the A/D converter which had an A/D conversion resolution of 12 bits (4096 counts). A/D on input signals was accomplished with the MACBASIC command (AIN) used to poll any channel for its current readings; a high speed burst mode was also available for sampling but was not used.

Table 1. Analog and digital I/O channels used for physiological monitoring and controlling system functions in the automated test system.

Computer Card	Channel	Type	Function
MIO120	0	Analog Input	A Temperature
	1		C Temperature
	2		Dissolved oxygen
	3		pH
	4		Chamber 1 pressure transducer
	5		Chamber 2 pressure transducer
	6		Chamber 3 pressure transducer
	7		Chamber 4 pressure transducer
	8		Chamber 5 pressure transducer
MIO120	0	Digital Input	Fraction collector microswitch
MIO120	0	Digital Output	Master A channel select line
	1		Master B channel select line
	2		Master C channel select line
	3		Water sampler - A set
	4		Water sampler - A reset
	5		Water sampler - B set
	6		Water sampler - B reset
	7		Water sampler 3-way valve
DIO120	8	Digital Output	Flow rate 2-way valve
	14		Start toxicant pump 1
	15		Start toxicant pump 2
	21		Advance fraction collector

The MIO120 also contained TTL compatible eight bit digital input and output ports. Each bit represented one channel of digital I/O; hence, eight channels of digital input and eight for output were available. Digital output simply means that, on command, the computer will place close to zero volts (low) or approximately + 5 volts (high) on a digital output channel. These voltages were used to control system functions, such as operating solenoids or motors, by activating or deactivating transistorized circuits that controlled those devices. Digital input means that the computer senses whether a particular input channel has no voltage or about + 5 volts present. This information may come from switches or other devices and was used to determine what course of action was required to perform some system function. All eight digital output channels and one input were used on the MIO120 (Table 1).

The DIO120 was a TTL compatible 24 channel (24 bit) digital I/O card. Although the channels were divided into three eight bit groups (ports) that could be configured for either digital input (readback) or output, each channel (bit) was independently addressable through software control. The system only required four channels for digital output (Table 1).

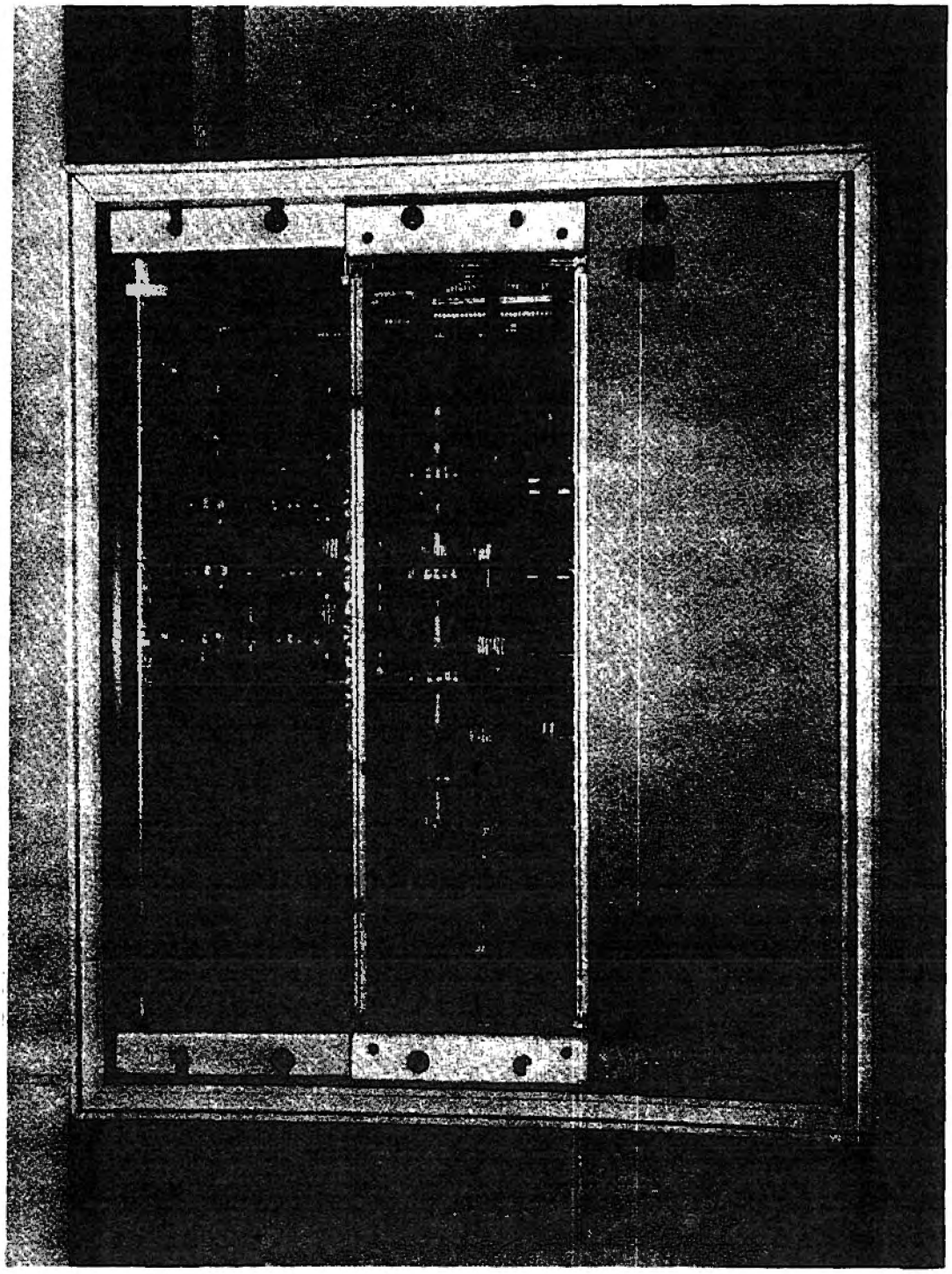
4.2.2 Interface

The interface fulfilled three important needs. First, information in the form of analog signals, or varying voltages, from the meters measuring dissolved oxygen concentration, pH, temperature, and from the pressure transducers for water flow rate must be read into the computer. The interface provided the switching and interconnections between those devices and the correct connection on the A/D card in the computer where the analog

signals were converted, or digitized, into the sort of numerical information that the computer could manipulate to calculate values for those measurements. Secondly, the computer was required to control the operation of solenoids and motors. The interface supplied electronic circuitry and connections so that the solenoids in the two way valves used for flow rate measurements were activated and deactivated at the proper times, as were the solenoids for actuating the three-way valves used in taking water samples. The five-way valve used in taking water samples was also rotated to the correct port at the right time, water sampling initiated, and sampling halted when the bottles filled. Additionally, the toxicant pumps were controlled and the urine fraction collector was advanced through the interface interconnections. Lastly, the interface provided power to operate or control the operation of the different sensors and devices attached to it. An internal power supply provided + 12, + 15, and -15 volts DC to the system, while connections to power supplies external to the interface supplied + 5, -5, and + 24 volts DC.

The interface was constructed within a standard instrument rack that measured 53 cm wide by 39 cm deep x 45 cm high (Figure 4). Two rack mounting frame kits (Vector CA52-HP119) for holding plug in boards, or cards, were installed in the box and 72 pin plugboard receptacles (Vector R636-2) were mounted behind frame kits. Most of the circuitry was built on plug in boards with 72 contacts (Vector 3719-4) that mated with the receptacles on the card frame, and circuit construction techniques used were wire wrap and solder tack. IC wire wrap sockets were mounted to the cards with hot melt glue and wrap ID were used to aid construction. Where necessary, test points were made accessible to the front of the cards.

Fig. 4



The upper card frame held ten receptacles and the lower card frame eight; not all receptacles held cards but were installed to allow future expansion. Cards in the upper frame included an analog multiplexing board and six boards that contained two temperature meter circuits each. The temperature meters monitored the A compartment of chamber one, C compartment waters of each fish chamber, the incoming lake water headbox, and a chilled water bath for the fraction collector, or seven temperatures altogether. One board was a spare. The lower frame held a digital multiplexer, a valve driver board that contained the circuitry used to operate the solenoids in the two-way flow measurement valves and to start the toxicant pumps, and six boards that contained the circuits used to control water sampling from each of the B compartments and the one A compartment; a spare board contained the same circuitry. Three ribbon cables, two 50 conductor and one 34 conductor, connected the interface to the computer. Two of these connected the digital multiplexing board, one going to the DIO120 computer card and the other terminating on the digital I/O section of the MIO120 card. The third cable connected the analog multiplexer to the A/D section of the MIO120.

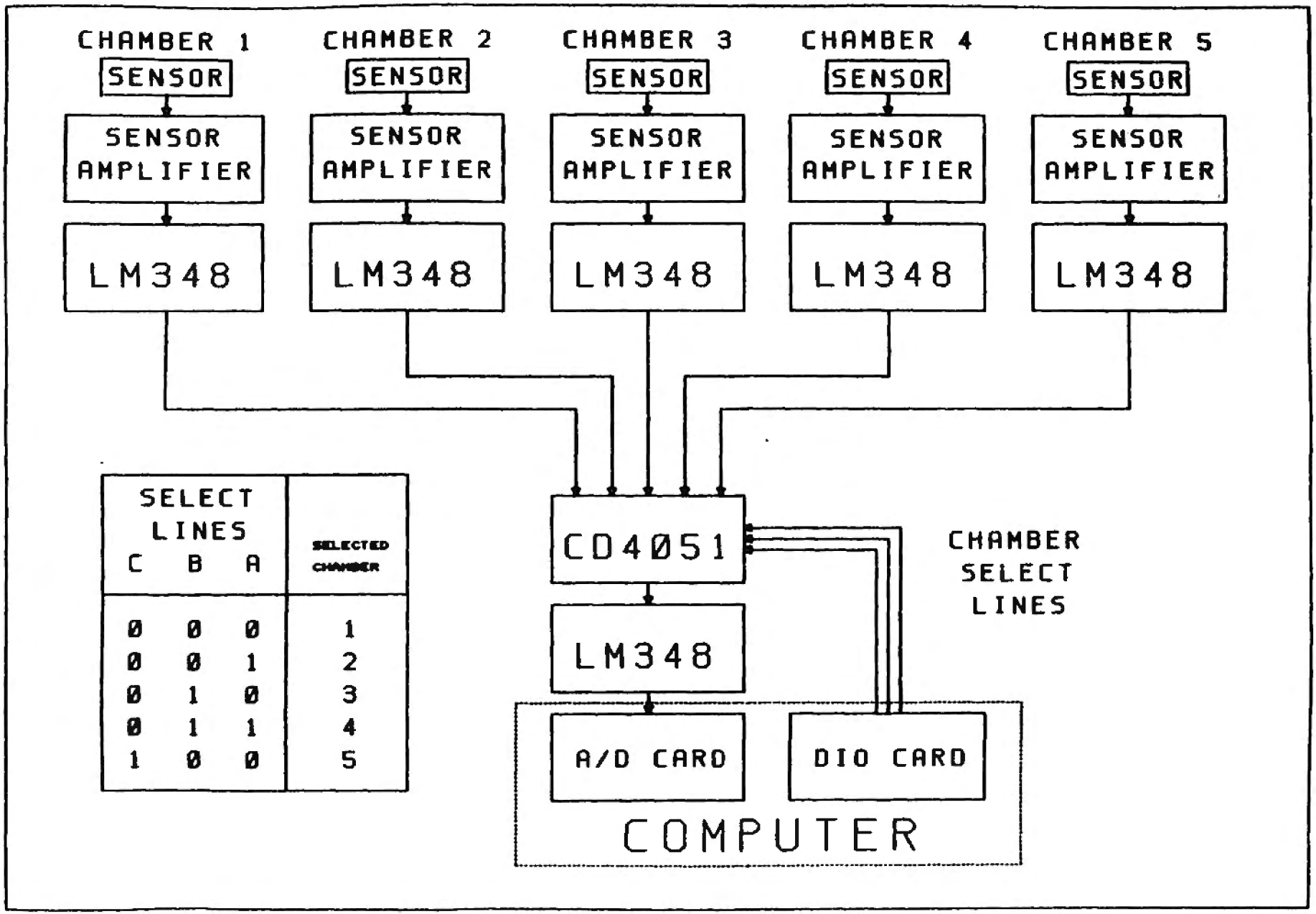
In effect, the analog multiplexer served as a four pole, five position switch. The computer was required to read the pH, DO, and two temperatures (four poles) from five different locations (five positions).

The usual sequence was to begin with chamber one and read DO and pH from the expired water B compartment, the headbox and fraction collector temperatures, and pH, DO, and temperature from the A compartment on chamber one. The latter was referred to as either chamber five or chamber 1A. This sequence was repeated for the remaining chambers two through four, skipping any deactivated chambers.

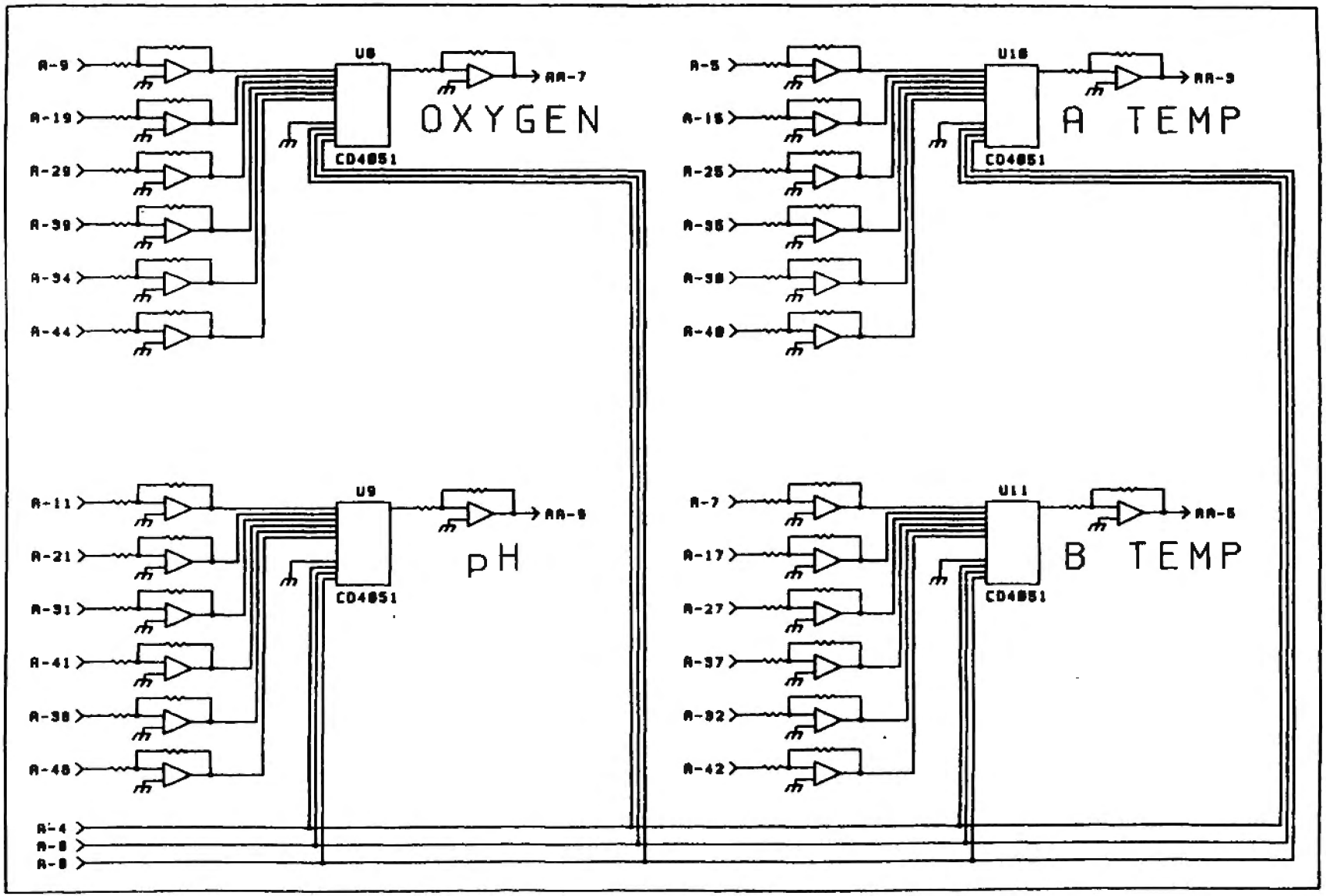
Each of these analog signals could have been fed directly to individual channels on the A/D card but that would have tied up 17 of the 32 available channels; seven for temperatures and five each for DO and pH. Combined with the five A/D channels reserved for reading the pressure transducers used in flow measurement, few channels remained for future expansion. Hence, it was decided to multiplex the above signals into four A/D channels (Table 1).

Figure 5 shows the multiplexing scheme for one of the four required measurements. The CD4051, an eight channel analog multiplexer integrated circuit, was the workhorse of this circuit, and there was one CD4051 for DO, one for pH, and one for each of the two temperature circuits (Figure 6). Although eight positions were available on each CD4051, this application required only six; one for each chamber and a spare. LM348 quad operational amplifiers were used to buffer, or isolate, the CD4051 inputs and outputs from other circuit components. The CD4051 switched from one chamber to another whenever the computer program transmitted the binary code representing the new chamber over the chamber select lines (Figure 5). These lines were designated Master A, B, and C and originated from the digital I/O section of the MIO120 computer card (Table 1), but also passed through inverters on the digital multiplexing board. Because the chamber select lines were common to all the CD4051's, all of the sensors from one chamber became available simultaneously to the computer whenever the CD4051's switched. The multiplexer circuit for one CD4051 and associated LM348's is detailed in Figure 7. The inputs to the circuit were for connecting the meter output of the sensor for each chamber and a spare.

The digital multiplexing board performed several critical functions including:



SELECT LINES C	B	A	SELECTED CHAMBER
0	0	0	1
0	0	1	2
0	1	0	3
0	1	1	4
1	0	0	5


U8
A-9
A-19
A-29
A-39
A-34
A-44
AA-7
OXYGEN
CD4051
U16
A-5
A-15
A-25
A-35
A-30
A-40
AA-9
A TEMP
CD4051
U9
A-11
A-21
A-31
A-41
A-36
A-46
AA-8
pH
CD4051
U11
A-7
A-17
A-27
A-37
A-32
A-42
AA-6
B TEMP
CD4051
A-4
A-8
A-0

19

Fig. 6

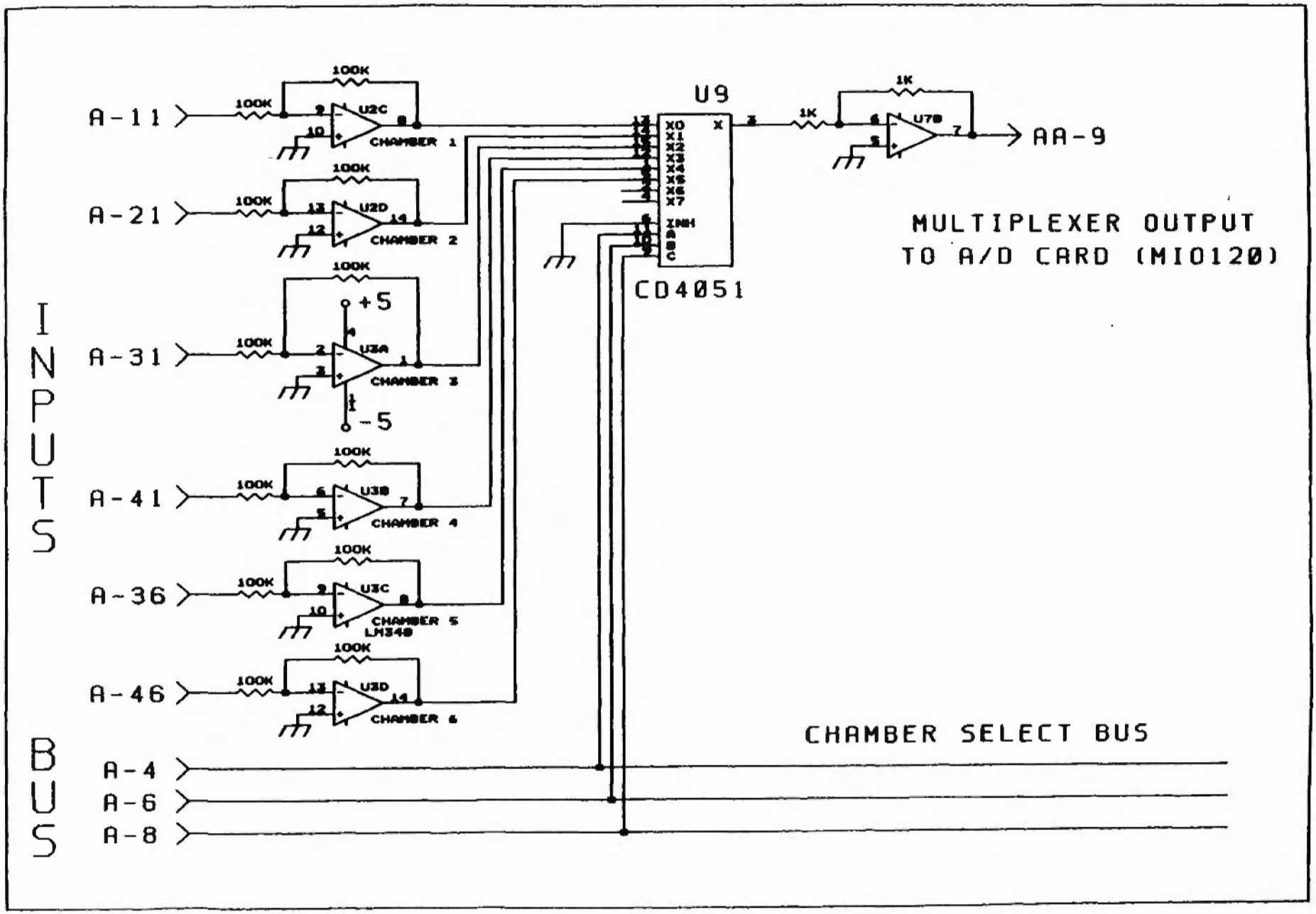

INPUTS
A-11
A-21
A-31
A-41
A-36
A-46
BUS
A-4
A-6
A-8
100K
U2C
U2D
U3A
U3B
U3C
CHAMBER 1
CHAMBER 2
CHAMBER 3
CHAMBER 4
CHAMBER 5
CHAMBER 6
LM348
+5
-5
U9
CD4051
X0
X1
X2
X3
X4
X5
X6
X7
INH
A
B
C
1K
U7B
AA-9
MULTIPLEXER OUTPUT
TO A/D CARD (MIO120)
CHAMBER SELECT BUS

Fig 7

1. Switched to and connected the correct water sampler control board for a chamber into the circuit when the computer called for water sampling from that chamber.
2. Supplied the binary code to the water sampler board to select and fill correct sample bottle (1-4).
3. Supplied the start pulse to begin water sampling.
4. Switched to and activated the proper solenoid valve used for making a flow measurement.
5. Operated one or both toxicant pumps when required.
6. Advanced the fraction collector when required.

The latter two were not multiplexing operations, but required digital output to activate the motor control circuits for those devices.

Whenever the computer program called for a water sample, the inverted binary code representing the desired chamber was passed to the digital multiplexer over the chamber select, or Master A, B, and C, lines. These entered the multiplexer through CD4049 chips (U1) used to invert the signals and isolate the circuit (Figure 8). The control lines were common to three CD4097 differential, eight channel multiplexer/demultiplexer integrated circuit chips designated U5, U6, and U7; the lines were also common to the analog multiplexer. U5, U6, and U7 all had grounded inhibit lines so they were always enabled. The master control lines were connected to the A, B, and C inputs of each CD4097, and the correct binary code switched them to the desired channel, or chamber. The first two CD4097's, U5 and U6, had flip-flops built from CD4001 quad, two-input NOR gates connected to the output of each channel, and each combination of one CD4097 and five flip-flops were designated Series A and Series B. Once the chamber was selected on the

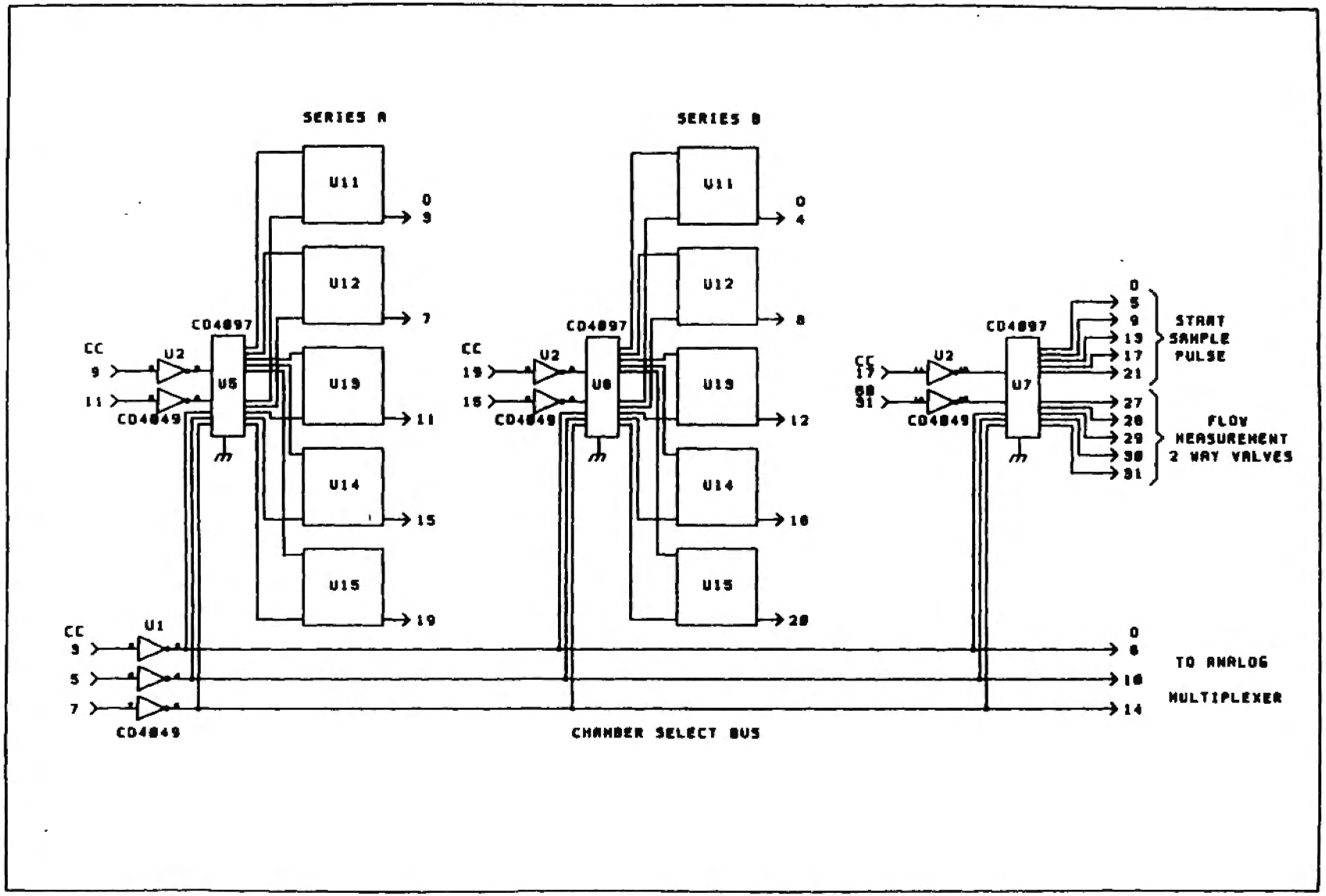

SERIES A
SERIES B
U11
U12
U19
U14
U15
U5
U6
U7
U1
U2
CD4097
CD4049
CC
9
11
19
15
17
68
91
5
7
0
9
7
11
15
19
4
8
12
16
20
5
9
13
17
21
27
28
29
30
31
6
10
14
START SAMPLE PULSE
FLOW MEASUREMENT 2 WAY VALVES
TO ANALOG MULTIPLEXER
CHAMBER SELECT BUS

Fig 8

CD4097's, an inverted set or reset signal was placed on pin 1 or pin 17, respectively (Figure 9). This set or reset information was fed through U5 and U6 and controlled the state of the flip-flops connected to the selected channel outputs. The combination of the A output and B output from the selected channel, paired flip-flops formed the binary code (00-11, decimal 0-3) required to select the correct sample bottle (1-4) for filling. This information was sent to the correct water sampler control board.

For instance, if the computer called for a water sample to be deposited into bottle two on chamber one, the program would first set the chamber select lines so that binary 000 was received by U5, U6, and U7, switching them to channel one. A reset signal was then applied to pin 17 of the Series A CD4097 (U5) resulting in an output of logical zero on pin four (connector three) of the Series A flip-flop U11. Simultaneously, a set signal applied to pin one of U6 resulted in an output of logical one on pin 11 (connector four) of the Series B flip-flop U11. This combination of outputs, AB = binary 01, represented sample bottle number two, and was sent to the water sampler board for chamber one.

The A and B Series of flip-flops and independent sets of AB lines from them to each water sampler control board were required to latch the code for bottle selection. Once latched, the bottle filling procedure could begin and continue uninterrupted while the computer went on to different functions, which may have included activating another water sampler. Once the chamber was selected and the bottle selection latched for water sampling, an inverted start sample pulse was applied to pin one of the third CD4097 (U7) at the moment sampling was to start (Figure 10). Because U7 was also switched to

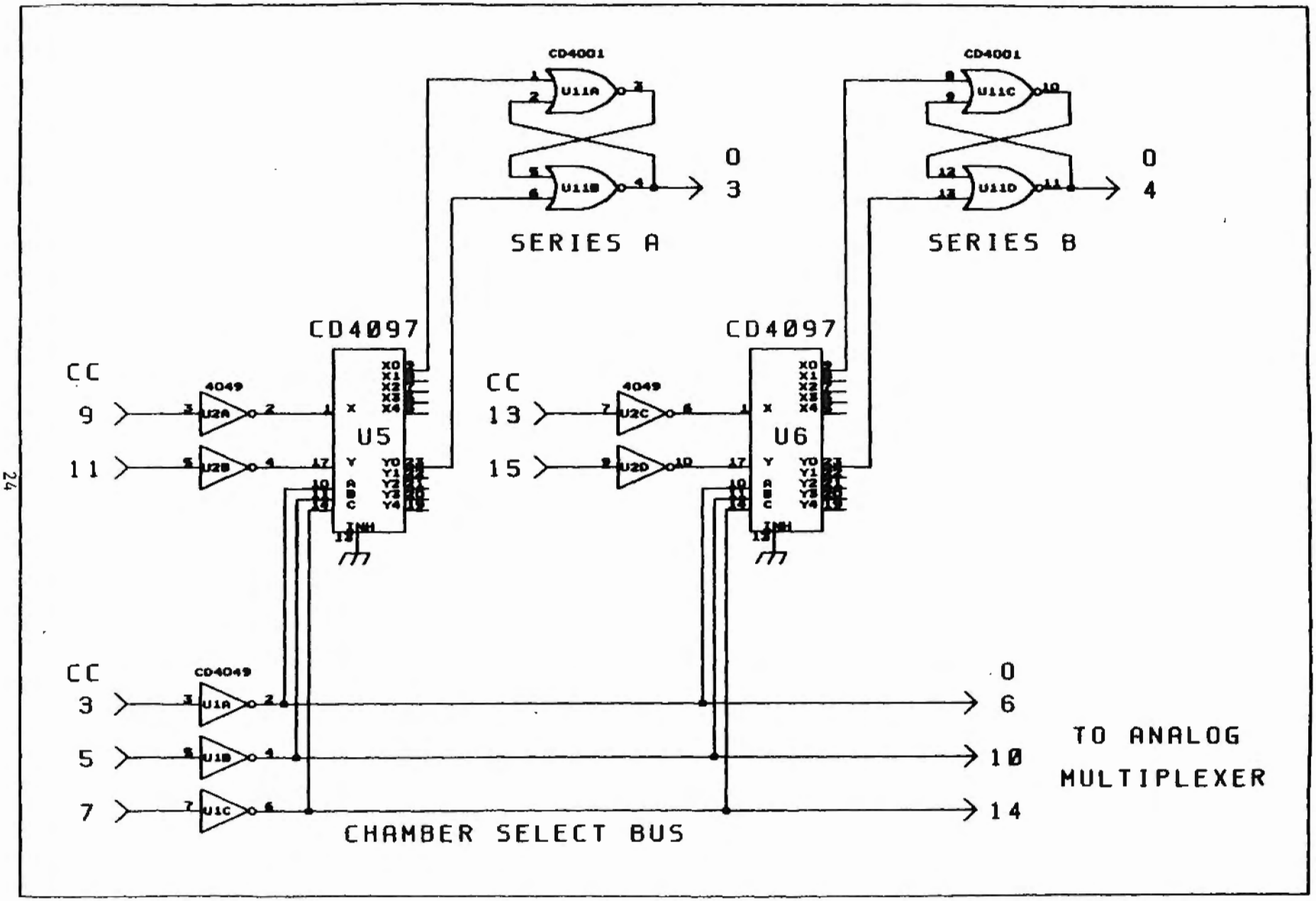

CD4001
U11A
U11B
0
3
SERIES A
CD4001
U11C
U11D
0
4
SERIES B
CD4097
U5
CD4097
U6
CC
9
11
13
15
4049
U2A
U2B
U2C
U2D
CC
3
5
7
CD4049
U1A
U1B
U1C
0
6
10
14
CHAMBER SELECT BUS
TO ANALOG
MULTIPLEXER

Fig 9-1

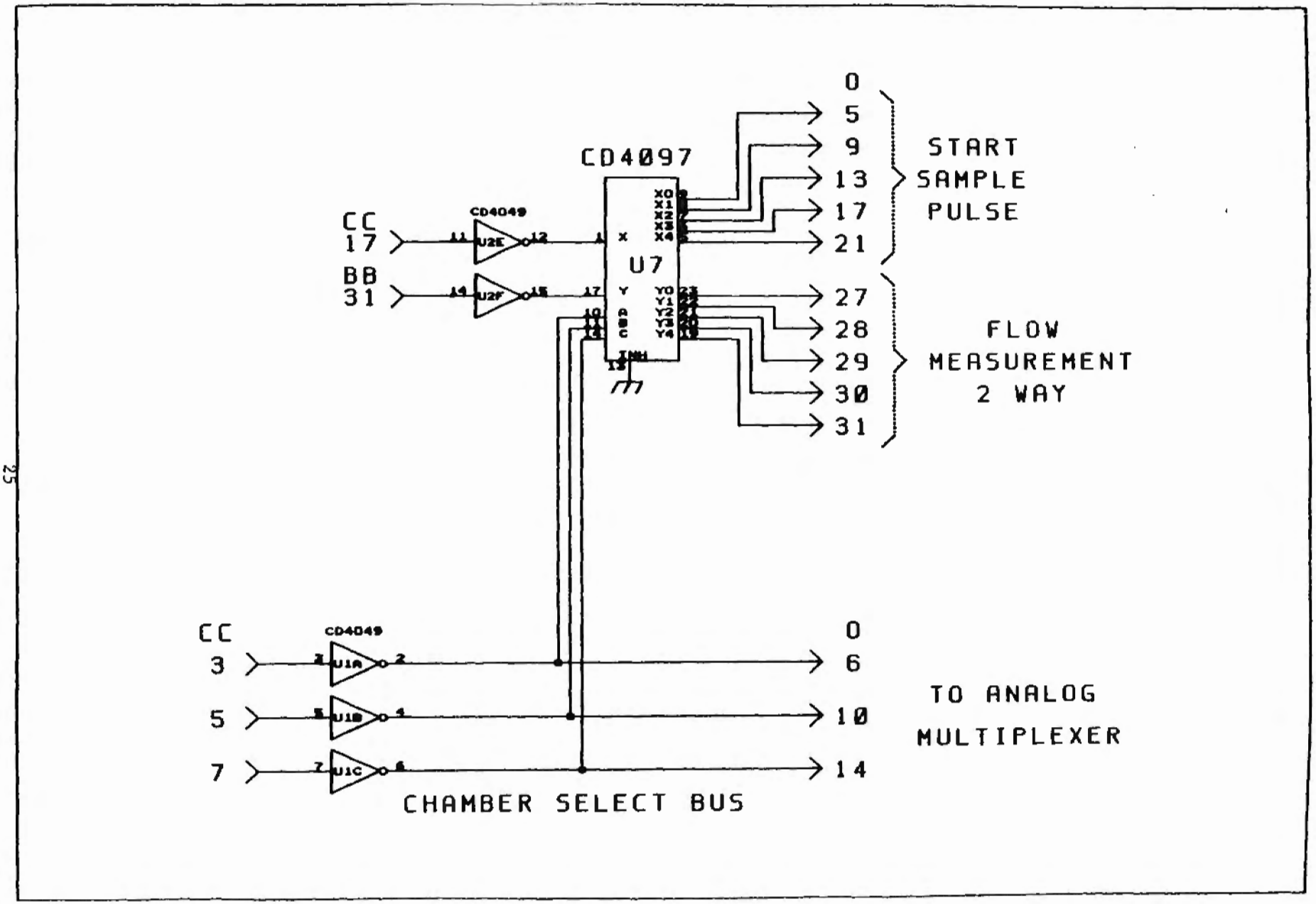

CD4097
U7
CD4049
CC
17
BB
31
U2E
U2F
0
5
9
13
17
21
START
SAMPLE
PULSE
27
28
29
30
31
FLOW
MEASUREMENT
2 WAY
CC
3
5
7
U1A
U1B
U1C
0
6
10
14
TO ANALOG
MULTIPLEXER
CHAMBER SELECT BUS

Fig 10

the same channel as U5 and U6, the start sample pulse was fed to the corresponding water sampler board where it set a latch to begin the bottle filling procedure.

The third CD4097 (U7) also provided the switching necessary to select the proper solenoid valve used in flow measurement. After channel selection by the chamber select lines, an inverted signal placed on pin one was fed through U7 to the corresponding chamber circuit on the valve driver board (Figure 8). Here the + 5 volt signal was passed to the NPN transistors 2N4123 and TIP29 connected as a Darlington pair (Figure 11). This caused the TIP29 to conduct and closed the normally open solenoid valve. This circuit had to remain active throughout a flow measurement.

The valve driver board also contained the circuits necessary to control toxicant pump operation and the fraction collector. Control lines for these circuits originated on the DIO120 card (Table 1). An on/off switch located on the front panel of the interface (Figure 4) provided manual controls for each toxicant pump or placed them under computer control. In either case they could be operated singly, concurrently, or alternately. A request from the computer program to activate either pump brought the input line BB19 or BB17 to a logic zero (Figure 12). This signal was inverted by U3, a 4049 chip, and passed to a Darlington pair where the high output from U3 allowed the TIP29 transistor to conduct and the toxicant pump to run.

Although part of the interface, circuit descriptions for the temperature meters, urine fraction collector, and water sampler control boards are deferred to the sampling and measurement section where they are explained along with their associated hardware.

Fig. 11

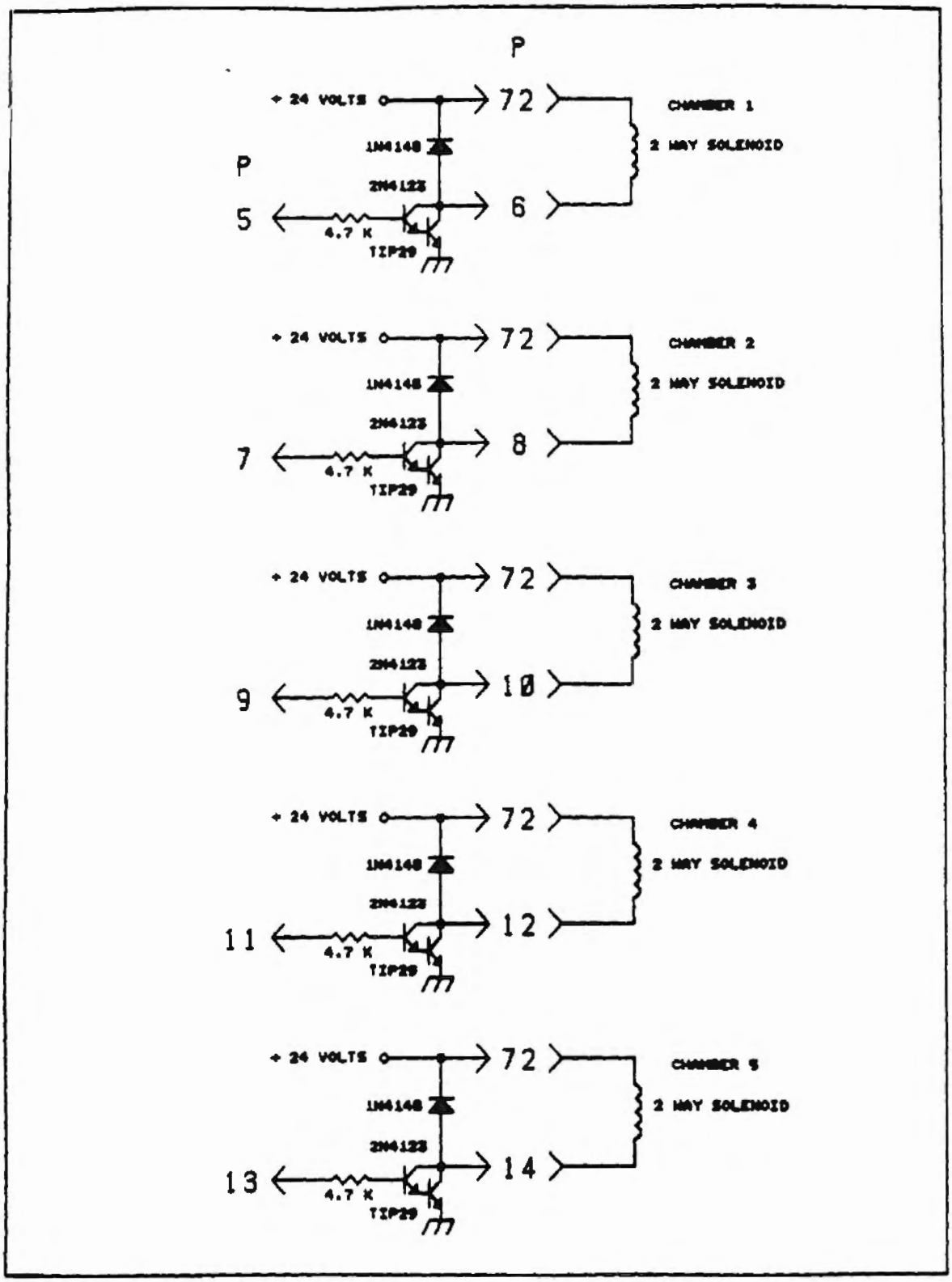
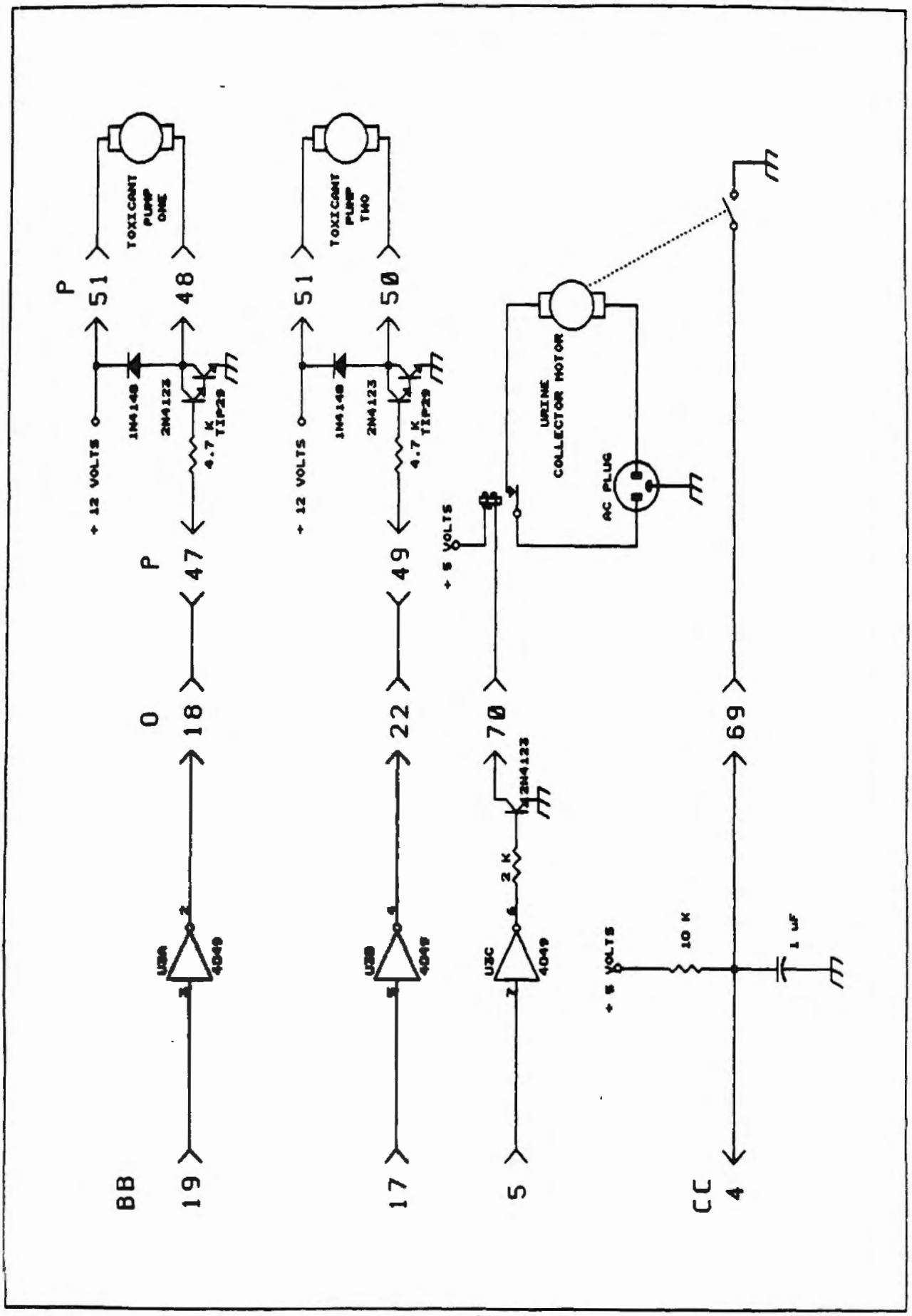

P
+ 24 VOLTS
72
CHAMBER 1
2 WAY SOLENOID
1N4148
2N6122
P
5
4.7 K
TIP29
6
+ 24 VOLTS
72
CHAMBER 2
2 WAY SOLENOID
1N4148
2N6122
7
4.7 K
TIP29
8
+ 24 VOLTS
72
CHAMBER 3
2 WAY SOLENOID
1N4148
2N6122
9
4.7 K
TIP29
10
+ 24 VOLTS
72
CHAMBER 4
2 WAY SOLENOID
1N4148
2N6122
11
4.7 K
TIP29
12
+ 24 VOLTS
72
CHAMBER 5
2 WAY SOLENOID
1N4148
2N6122
13
4.7 K
TIP29
14

Fig. 12


P
51
48
TOXICANT PUMP ONE
+ 12 VOLTS
1N4148
2N4123
4.7 K
TIP29
P
47
0
18
BB
19
U3A
4049
51
50
TOXICANT PUMP TWO
49
22
17
U3B
4049
+ 5 VOLTS
URINE COLLECTOR MOTOR
AC PLUG
70
2N4123
2 K
U3C
4049
5
69
CC
4
10 K
1 uF

4.2.3 Software

The MACSYM 120 included Concurrent CP/M-86 as its operating system, a multi-tasking system that managed the I/O of all devices attached to the computer, provided file management, and loaded and ran the operational program for DAC. This program was coded in measurement and control BASIC (MACBASIC), an expanded version of BASIC that had been optimized for realtime measurement and control. MACBASIC features included special key words to designate common measurement and control functions, a line-by-line compiler that directly translated program statements into code as they were entered, and multi-tasking. The latter permitted several program operations, or "tasks," to be performed concurrently and independently of each other.

The operational DAC program was written in-house and named "TEST." TEST contained 1083 lines of source code and required 55 KB of memory for the undocumented source code or 48 KB for compiled object code. However, TEST was not optimized to save space or to run faster; a more judicious use of code would shorten the program considerably. TEST consisted of a short main program to begin and direct program execution, two tasks running concurrently, and 25 subroutines that performed all the functions required by the main and task portions of the program. The flowchart (Figure 13) shows the main program and task interrelationship, but the reader is referred to the subroutines listed below for a detailed explanation of their operation as they are called by the main and tasks.

4.2.3.1 Main Program and Tasks

After the fish had been prepared and placed in their chambers, the source code for TEST was entered and updated with the current coefficients used in the pH, DO, temperature, and flow algorithms; more will be said about these

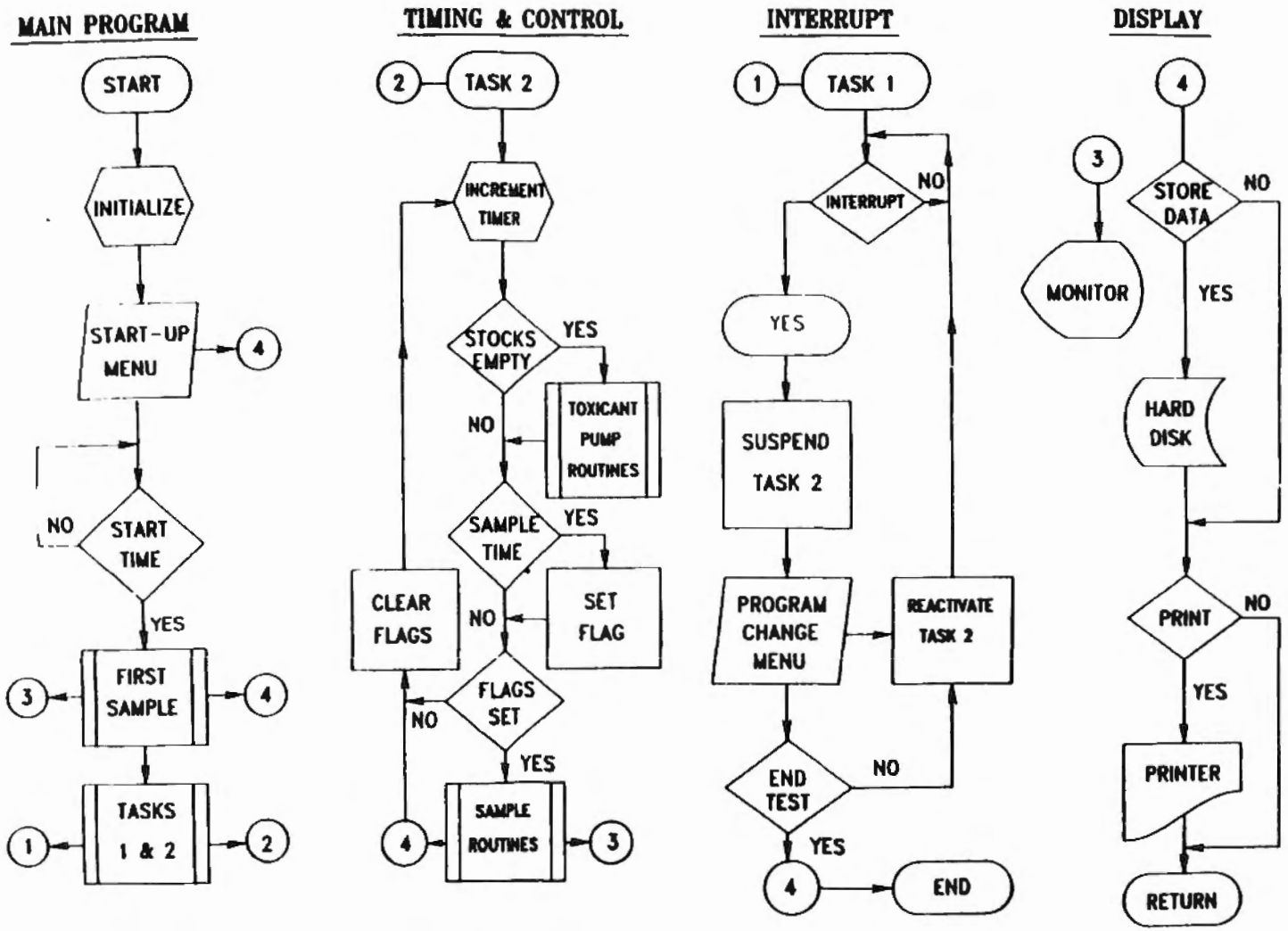

MAIN PROGRAM
START
INITIALIZE
START-UP MENU
4
NO
START TIME
YES
3
FIRST SAMPLE
4
1
TASKS 1 & 2
2
TIMING & CONTROL
2
TASK 2
INCREMENT TIMER
STOCKS EMPTY
YES
TOXICANT PUMP ROUTINES
NO
SAMPLE TIME
YES
CLEAR FLAGS
NO
SET FLAG
FLAGS SET
NO
YES
4
SAMPLE ROUTINES
3
INTERRUPT
1
TASK 1
INTERRUPT
NO
YES
SUSPEND TASK 2
PROGRAM CHANGE MENU
REACTIVATE TASK 2
END TEST
NO
YES
4
END
DISPLAY
4
3
MONITOR
STORE DATA
NO
YES
HARD DISK
PRINT
NO
YES
PRINTER
RETURN

Fig. 13

later. Once TEST was started, the main program made immediate calls to STARTUP and SETSTIM, then waited until the start of monitoring; normally, this was 0500 h the next morning. When monitoring began, calls were made to the routines PHDOTMP and FLOW to perform the first sampling and to PRNTSTOR to save the results. The display monitor was set up to show sampling times, intervals, and results by a call to MONITOR, after which tasks one and two were activated and the main program was exited.

Task two is described first since it assumed control of program execution once it became active. It contained the timing sequence that kept track of what and when to sample and whether or not stock bottles became empty when the toxicant pumps were running. The timing sequence was simply a loop in which, for fish chambers one through four, a flag was set whenever it was time to sample pH, DO, or temperature, make a flow measurement, advance the fraction collector, or take a water sample. The sequence within the loop was as follows: First, a timer variable was updated to the current total time in seconds that had elapsed since the start of the main program. A toxicant pump flag was checked to see if pumps were running; if so, the total liters remaining in the stock bottle(s) were determined by calculating how much had been used based on pumping rate and elapsed time of pump operation. If total liters remaining was < one, a call was made to TOXPMP to either switch pumps along with stock bottles or to stop the pump.

Upon return to the timing loop the interval variables for the fraction collector, pH, DO, temperature, flow, and water sampling were checked to see if it was time to sample. This was done by subtracting the last sampling time for each variable from the current total time. If the difference

was $\geq$ to the initialized sampling interval, a flag was set and the timing loop was completed for that particular fish chamber and then the loop was checked again for all fish chambers to determine whether or not any other sampling flags should be set for that time period. Also, when it was determined that a flag should be set to signal sampling, the current elapsed total time became the new last sampling time for that particular variable. Because the program was occasionally interrupted for some reason and sampling was not executed at a specified time, a factor was calculated and applied to the last sample time update so that upon return to program execution, sampling would still occur at specified times. For example, we preferred to sample DO, pH, and temperature every 15 min on the quarter hour, advance the fraction collector every one or two hours on the hour, and take water samples every four hours on the hour. If the program was interrupted at, for instance, 10 min before the hour and restarted 20 min after the hour pH, DO, and temperature sampling would occur immediately upon restart because the elapsed time since last sampling exceeded the initialized 15 min interval and, without applying the factor, sampling would reoccur at 15 min intervals from that point. However, the readjusted last sample time using the calculated factor for missed sample times would insure that sampling would occur on the quarter hour as desired.

The timing loop for chambers one through four was executed continuously until a flag was set to signal sampling. Once all flags for a particular sampling time was set, a jump was made from the timing loop into a sampling loop. Here, for fish chambers one through four, calls were made to the proper subroutines to perform sampling, print, store, and display the results. Separate loops for timing and sampling were necessary if it was desired to do

these operations in sequential order. If calls were made directly from the timing loop to sampling subroutines when it was time to sample a particular chamber, a larger number of temporary variables and increased code would be necessary to rearrange the data into order since it could not be predicted which fish chamber would be sampled first. Because time was tracked in fractions of a second, it sometimes happened that the timing loop would sense a sampling time when the loop was somewhere other than on chamber one, and, without separate loops, results were often printed and stored in some order other than 1-4.

When all operations were completed for that sampling period, the necessary variables for data collection and handling were re-initialized to their before sampling states, and a jump was made out of the sampling loop and back into the timing loop. Task two continued program execution in this manner until interrupted and TEST was stopped.

Task one was a program interrupt that waited until it sensed a lower case "i" input at the keyboard. At that time it suspended execution of task two and displayed the interrupt menu below:

1. Set or change sample times
2. Advance fraction collector
3. End of file entry
4. Deactivate chamber
5. Start or stop toxicant pump
6. Continue test
7. End test
8. Activate printer
9. Toxicant pump operation

The task would wait until a selection was made from the menu, performed that choice by a jump to the proper subroutine, and redisplayed the menu until continue test or end test was selected. On continue test, MONITOR was called to update the display and program control was relinquished by reactivating task two. End test halted TEST and exited the program.

4.3.1.2 Subroutines

Listed alphabetically and not in the order in which they were called or their order of occurrence within program TEST.

1. ACPRNT - set a flag that signaled the printer was on-line.
2. ADVANCFC - advanced fraction collector one sampling position. ADVANCFC called STEPFC three times to accomplish this.
3. DEACT - queried user for fish chamber number and deactivated all sampling for that chamber by resetting the sample time intervals for pH, DO, temperature, flow, and water samples to 600,000 s. This time period was longer than any foreseeable test duration.
4. DIGIOUT - set digital output lines to select the proper fish chamber for sampling. Chamber number was passed to this formal subroutine as a parameter; each chamber number was specified by binary code and the code was sent out through the Master A, B, and C chamber select lines to the digital and analog multiplexers.
5. DSKERR - MACBASIC had error trapping routines that diverted program execution to another part of the program or a subroutine when certain run time errors were detected. One of these was "channel timeout." Program execution would hang for a specified time when an attempt to perform some operation over an internal

I/O channel could not be completed because of a hardware or software problem, and the program crashed when the default amount of time (2 m) had passed. DSKERR was a subroutine that was called when an attempt to write to disk failed for any reason, and would display a message on the monitor, reset a flag to signal the disk was off-line, and returned control to the next line in the program.

6. ENDTST - was called only from menus. Queried user whether or not test was to be ended, or returned control to program or task that was running. If TEST was to end, ENDTST called EXIT.
7. EOF - some information about the fish and test was entered for printout and storage at the end of the test file. Fish number, sex, stage of maturity, and termination times were entered after prompts. Dose, depuration, redose, and total exposure times were calculated by calls to TIMCALC, and the times where 25 and 75 percent exposure occurred were calculated for each fish. All information was stored on disk and a formatted output was printed for each fish. This subroutine was normally called from the interrupt task menu and only when a test was terminated.
8. EXIT - closed all files and I/O channels. Ended program and exited to MACBASIC.
9. FILEHDR - called from menu displayed by subroutine STARTUP. Information entered for the file header included test number, chemical used in test, toxicant code, date (month, day) that all fish placed in their chambers, fish number, the time (hour, minute) that each fish was placed in chamber, and fish weight.

Information was stored and printed. Also printed was a header containing column labels.

10. FLOW - this subroutine, as did all other sampling subroutines, first called DIGIOUT to set the digital I/O (Master A, B, and C) lines for the chamber being sampled, and noted the time from the system clock (hour, minute) for use in monitor display update after sampling was completed. FLOW closed the solenoid on the flow measuring device and waited one second to allow head pressure to begin building on pressure transducer. Before the flow rate was calculated, A/D conversion of the voltage signal on the pressure transducer output was required. This voltage increased as the water level within the flow device increased. A/D was performed in a loop that cycled from one to up to 60 times. During each passage through the loop, A/D was done on 50 samples from the pressure transducer in less than one second; these were averaged and converted to a digital whole number. When this number exceeded 8.0 volts the water level in the flow device had reached its maximum allowable head height and a jump was made out of the A/D sampling loop. This occurred whenever the flow rate was greater than approximately 200 ml/min. Otherwise, FLOW waited until one second had elapsed, including A/D sampling, and then recycled. If the entire loop was cycled 60 times, then the flow rate was less than 200 ml/min and A/D sampling had lasted 60 seconds. This ensured that sampling was not prolonged during periods of low flow rate. Once A/D conversion was completed, the flow solenoid was opened and flow diverted to drain. FLOW then calculated the volts/min that

the pressure transducer output changed, inserted this value into the appropriate linear equation for the chamber being sampled, and calculated flow rate. A print flag was set and the subroutine exited.

11. HBFCTMP - called from PHDOTMP every time that subroutine was called. Temperature of the incoming lake water within the main headbox and of the cooling water flowing through the fraction collector bath were each obtained by performing 50 A/D conversions on their meter outputs, averaging the mv outputs, and using these values in linear equations.
12. INITFC - called from the STARTUP menu to move the fraction collector into its starting position. Advances were made one step at a time by calling STEPFC. Also used before and after a test to load and unload the fraction collector.
13. INTVLCALC - called from MONITOR to calculate when the next sample times would occur based on last sampling time and present time interval for any particular variable being sampled. Set up temporary variables for times and called INTVLTIM to make the calculations.
14. INTVLTIM - a formal subroutine that had the hour and minute of the last sample along with the sampling interval (in minutes) passed to it, calculated the hour and minute for the next sample, and passed that information back to INTVLCALC.
15. MONITOR - the monitor display was updated after every sampling period to show the results of that sampling, time of sampling, the sample interval, and the time of next sampling for pH, DO, temperature, flow, water sampling on all four B compartments and the A compartment of chamber one. Also, fraction collector advance times were displayed. MONITOR first called

INTVLCALC to calculate next sample times. Formatting of the display was done and all information was sent to the monitor.

16. PHDOTMP - called DIGIOUT to set digital I/O lines for chamber being monitored. Obtained current time (hour, minute) from system clock. Performed A/D on signals from the pH, DO, and temperature meter outputs by taking 1000 samples in rapid succession for each measurement. The average voltage value was inserted into the appropriate linear equation and pH, DO, or temperature calculated. Except when monitoring chamber 1A, PHDOTMP also called HBFCTMP to determine temperatures in the main headbox and fraction collector bath. Before returning, PHDOTMP set the print flag.
17. PRNTMAL - this subroutine used the same error trapping routine as DSKERR to display an error message on the monitor, set a flag to signal that the printer was off line, and continued program execution in the event that the printer malfunctioned and caused an I/O channel timeout.
18. PRNTSTOR - obtained system date and time. Called TIMCALC to calculate the elapsed time between start of program and the last sample time. Kept track of the total number of data records stored on the hard disk. Data were written to disk if the disk write flag was set and printed if the printer flag had been set. PRNTSTOR calculated VO_2 and U_E before printing since only raw data values were stored on disk.
19. SAMINTVL - provided a monitor display of the current settings for pH, DO, temperature, flow, fraction collector, and water sampling time intervals. A change menu was also displayed and any or all time intervals for sampling on any fish chamber or compartment A

could be changed. Although all times were displayed or changed as minutes, the program converted all sample intervals to seconds for timing considerations. Called from either STARTUP or interrupt menus.

20. SETSTIM - set the starting time for program execution. If start hours = 0, returned to main program immediately. Otherwise, the routine sat in a loop checking the clock until starting time arrived. Simultaneously, an interrupt task was activated that served as an interrupt to the waiting period. Upon sensing an interrupt by any input on keyboard, a new start time was entered and the waiting task reactivated. Returned to main program as soon as starting time arrived.
21. STARTUP - this was the main subroutine for starting program execution and was the first call made from the main program. It initialized all variables including dimension statements for all arrays, flags, default sample times, logic levels on the digital I/O lines, and format statements for monitor display of sampled data. After initialization, user was queried whether output should go to printer and hard disk, file names to be used, and whether or not any chambers should be deactivated. Lastly, a menu was displayed that contained options for other pre-test operations and included:
    1. Enter file header information
    2. Enter EOF
    3. Initialize fraction collector
    4. Set sample intervals
    5. Set toxicant pumps
    6. Start test
    7. Exit program

After choices were performed by calls to the proper subroutines, STARTUP was exited by choosing to start test.

22. STEPFC - when called, advanced fraction collector one step at a time. This subroutine was used to position the fraction collector to its starting place or for loading and unloading centrifuge tubes that collected the urine samples. Advancing the fraction collector required monitoring microswitch action to determine whether or not the advance was completed. A safety feature was included so that if the fraction collector continued to advance for more than one second, longer than required to move one step, an error message was printed and displayed, a fail flag set, and the subroutine reset. As soon as the fraction collector was successfully advanced, or after error handling, a return was made to origin of call.
23. TIMCALC - formal subroutine that accepted two different times (month, day, hour, minute) and calculated the difference in hours.
24. TOXPMP - consisted of five distinct sections. The first part, called from STARTUP, initialized the pumps by prompting the user for pump flow rate (ml/min) and stock bottle capacity (liters) and calculated the time required to empty the stock. Also, options included operating two toxicant pumps, either simultaneously or alternating; default condition was one pump operating. Second, a monitor display of current pump settings and operational mode was called from task one, the interrupt task. If changes were required these could be specified. Third, when a call to start the toxicant pump(s) was made from the

interrupt task menu, the timer clock was polled for elapsed time and this became the starting point for calculating the time required to empty the stock bottle(s). The system clock provided the dose start time (month, day, hour, minute). Fourth, when a call was made from the interrupt task menu to stop the toxicant pump(s), the dose stop time was obtained from the system clock (month, day, hour, minute). This was used later along with dose start time to calculate total dose times. Fifth, a call from the timing loop in task two occurred when it was time to switch from an empty stock bottle to a full one. The program continued to switch from one stock bottle to an alternate when empty time arrived so long as the toxicant pumps were initialized to the alternating mode. In this way stock solutions of toxicant were continuously available to the exposure system.

25. WATSAMP - after calling DIGIOUT to select the chamber and obtaining sample time, WATSAMP set the I/O lines on the digital multiplexer to move the five-way valve to correct port, closed the three-way solenoid valve on the drain line so that chamber water was diverted into the sample bottle, and updated the variable keeping track of sample port. Water sampling was done in sequence 1-4 and a notation was made on the printer output when a sample had been taken on a particular port so that sample times were accurately tracked. Once a water sample was started, the program could go on to other sampling without having to wait until the sample bottle had filled; an independent circuit was responsible for unlatching the water sample circuit. The printer flag was set before returning to call origin.

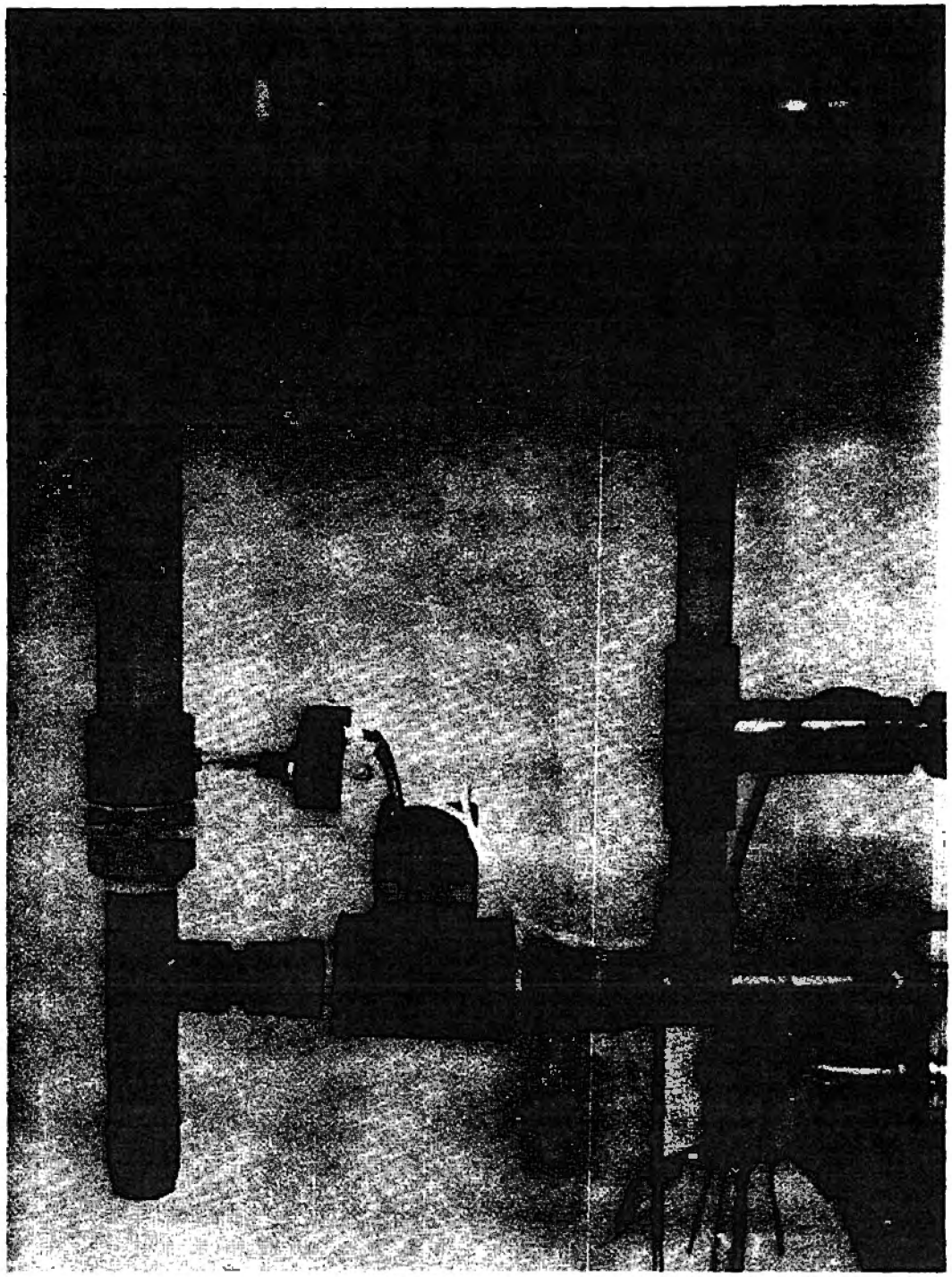
4.2.4 Sampling and Measurement

4.2.4.1 Water Flow

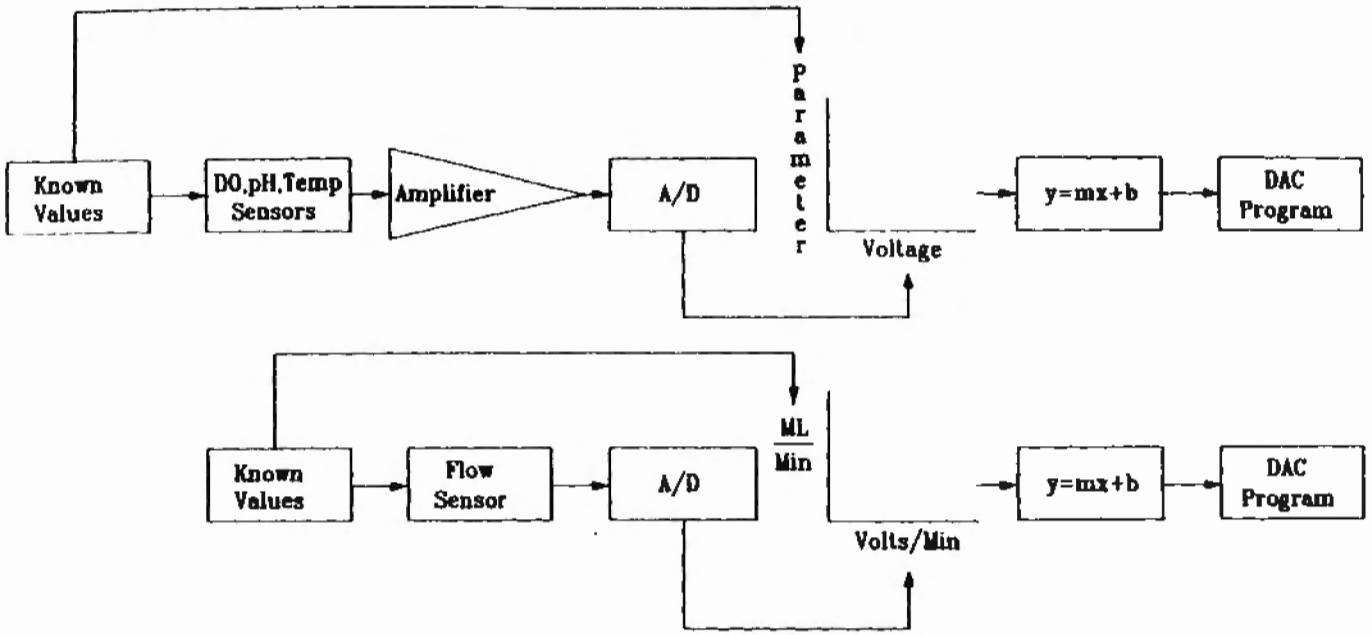
Ventilation volume (V_G) was the amount of water a fish pumped over its gills in a unit of time and was measured directly from the expired water B compartment overflow drain. The expired water drained into a flow measurement device constructed from clear, rigid, series R-4000 PVC pipe (Excelon), a model 160PC pressure transducer (Micro Switch 0-10" H_2O), and a two-way, normally open solenoid (Valcor 16P8408-5). The device was configured similar to a trap found in any drain system (Figure 14). Expired water flowed down a 2.54 cm diameter pipe that was reduced to 1.27 cm diameter at the top of the trap. Tygon tubing connected the pressure transducer to a nipple mounted in the pipe and water left the other side of the trap at a point just above the level of the transducer orifice thereby maintaining about two cm head pressure on the sensor.

During a flow measurement the computer selected and closed the normally open solenoid valve and the pressure exerted on the transducer rose along with the water level as the pipe filled. The computer digitized the increasing voltage output from the transducer as explained in the FLOW subroutine and calculated flow rate based on a volts/min change in output. The regression equations used in the FLOW subroutine were established for each chamber before each test by regressing four or five measured water flows against the rate of change in transducer output voltage (Figure 15). The resulting coefficients for slope (m) and intercept (b) were inserted into the FLOW algorithm before a test, and ventilation volume was calculated as V_G (ml/min) = mx + b where x = volt/min. A flow device was also placed on chamber one, compartment A overflow; this monitored only the difference in flow rate between incoming water and expired water for chamber one.

Fig 14



SENSOR CALIBRATION



Notes:

1. A separate software program is used for calibration.
2. Linear response is assumed.
3. Known values include: a. 0 & saturated for D.O.
   b. pH 7.0 & 10.0 buffers for pH
   c. measured flows between 0–600 for flow

4.2.4.2 Dissolved Oxygen

Determination of oxygen uptake efficiency (U_E) and oxygen consumption (VO_2) by the fish required the measurement of the DO content of both the incoming water and the expired water. Sample ports in the A and B compartments of each fish chamber were connected to needle valves (Clippard, Model MFL-2) that regulated water flow into flow-through electrode cells to approximately 10 ml/min. Sampled water was reconnected to the flow measurement device to maintain accurate V_G readings. The electrode cells (Beckman 572-934) held standard polarographic oxygen sensors (Model 39557, Beckman Instruments, Inc., Fullerton, CA). The electrodes were connected to Beckman Model 0260 oxygen analyzers.

During sampling, the computer selected the chamber through the analog multiplexer and digitized the oxygen analyzer output. The subroutine PHDOTMP calculated DO content by using the correct regression equation. Linear equations were established for each DO sensor and meter by regressing corresponding voltages against two known values, zero and saturated. The zero value for DO was obtained by shorting out the electrode output, and the saturated value of incoming water was determined by the modified Winkler titration method. The slope (m) and intercept (b) of this line were used as coefficients in the PHDOTMP subroutine algorithm and DO was calculated as DO = mx + b where x = volts output by the DO meter. The coefficients were determined and inserted into the subroutine before each test.

The difference in DO content between incoming and expired water was the U_E for that fish at the time of sampling and was expressed as a percentage of the incoming DO. The PHDOTMP subroutine calculated oxygen consumption (VO_2) as:

$$VO_2 = \frac{DO \times U_E \times V_G}{Wt}$$

Where:

VO_2 = mg/g/min

DO = oxygen content of incoming water (mg/L)

U_E = uptake efficiency (percentage)

V_G = ventilation volume (ml/min)

Wt = fish weight (grams)

Values for VO_2 were converted and expressed as mg/kg/h when used in data analysis.

4.2.4.3 pH

These measurements were made similarly to those for DO. Incoming and expired waters were directed through needle valves to combination pH electrodes (Sensorex 450C) mounted in flow-through cells (Sensorex G12255). Water passing through the cells was redirected to the flow measurement device. The pH electrodes were connected to Beckman 3550 pH meters. During sampling, the computer selected the chamber through the analog multiplexer and digitized the pH meter output. The subroutine PHDOTMP calculated pH by using the correct linear equation for that sensor and meter. The coefficients for these equations were established by regressing corresponding voltages against known pH values. These values were obtained by using buffer solutions of pH 7.0 and 10.0 as recommended by the manufacturer to bracket predicted values for the test water; pH of Lake Superior water was close to 8.0. The slope and intercept from this regression equation were used as coefficients in the

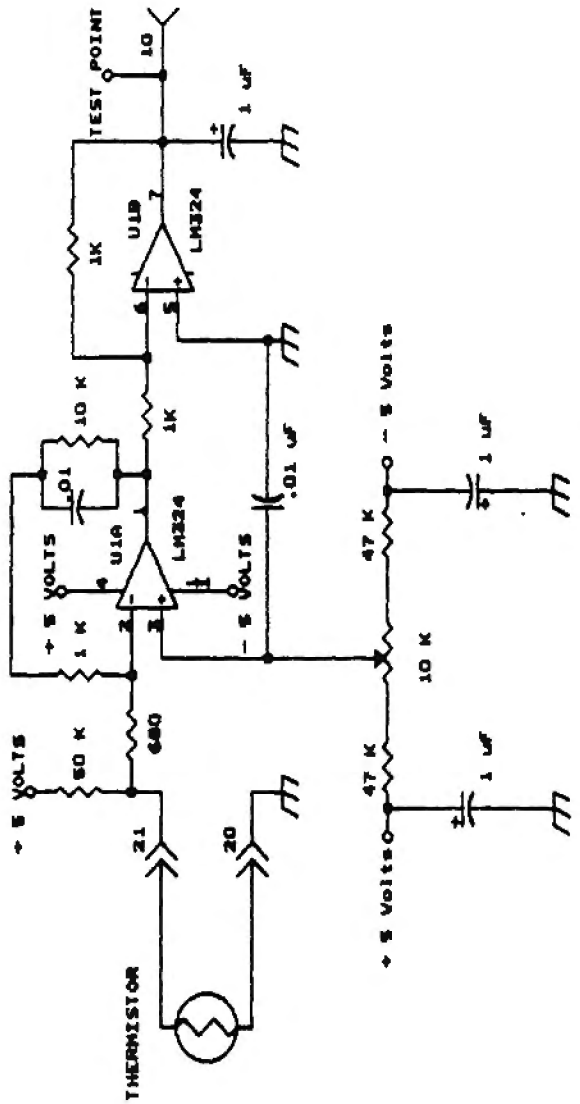
PHDOTMP subroutine and were inserted just before a test. pH was calculated as pH = mx + b where x = volts output by the pH meter.

4.2.4.4 Temperature

Seven system temperatures were monitored; all C compartments, the A compartment of chamber one, the incoming lake water headbox, and the fraction collector cooling bath. YSI thermistor, type 403, stainless steel temperature sensors (Yellow Springs Instrument Co., Inc., Yellow Springs, Ohio) were inserted directly into the compartments being monitored, and custom built circuits amplified the signals from each sensor. There were two temperature circuits on each of six boards in the interface, and each board contained one LM324 quad operational amplifier to support both circuits.

Each circuit used a 50 k ohm resistor in series with the YSI sensor to form a simple voltage divider (Figure 16). Because the YSI thermistor had a negative temperature coefficient, resistance decreased as temperature increased. With the YSI sensor connected to the bottom of the voltage divider, the op amp input sensed a voltage decrease whenever temperature increased. The first stage of the op amp provided an approximate gain of 16 while the second stage was set for unity gain and acted as a buffer stage. All of the capacitors were added for filtering. The 10 k ohm potentiometer set the offset voltage during calibration, and the 47 k ohm resistors connected to the potentiometer provided a more precise adjustment. A test point was supplied to measure the temperature circuit output during calibration. Card level calibration was accomplished by inserting a 7500 ohm resistor in place of the YSI sensor and adjusting circuit output to an arbitrary + 500 mv.

Fig. 16


TEST POINT
10
1 uF
1K
U1B
LM324
10 K
1K
.01
.01 uF
U1A
LM324
+ 5 VOLTS
1 K
- 5 VOLTS
47 K
- 5 Volts
1 uF
10 K
+ 5 VOLTS
50 K
680
47 K
1 uF
21
20
+ 5 Volts
THERMISTOR

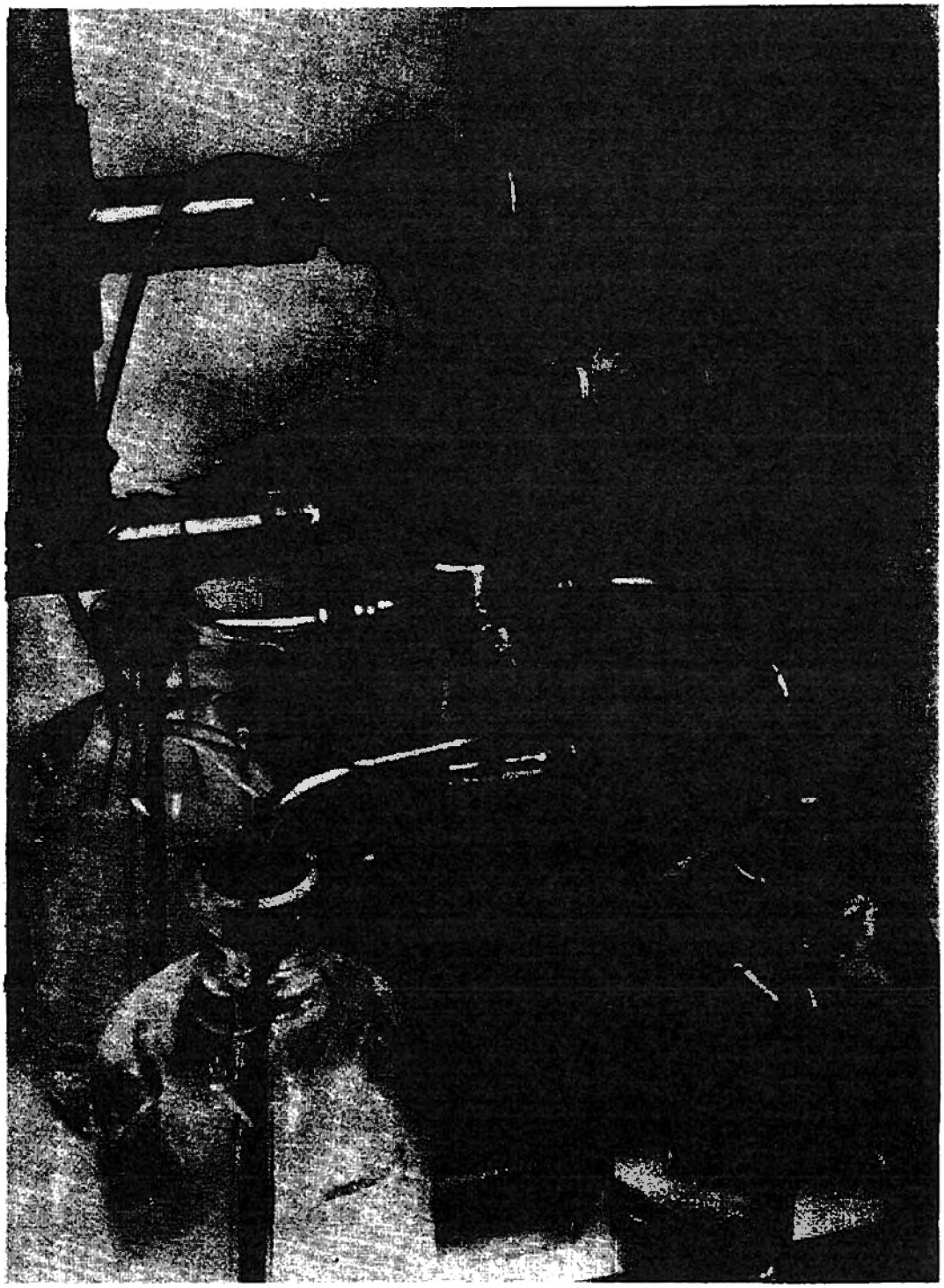
Temperature sampling and calculation were the same as for DO and pH. Pre-test coefficients were obtained by regressing voltage outputs against four or five known temperatures proximal to the expected test temperature. Coefficients were determined for each temperature meter and inserted into the PHDOTMP subroutine. Temperature was then calculated as TMP = mx + b where x = voltage output of the temperature meter.

4.2.4.5 Water

Chemical analysis of incoming exposure water was required for most testing done with this system. Normally, water sampling was done manually from chamber sampling ports. However, an automated sampling device was necessary if samples were needed during periods of unattended operation, and water samplers were built and attached to the drain line coming from each flow measurement device to meet this contingency. A 24 volt DC normally open, three-way valve (Peter Paul Electronics, Model 76 Z 00310GM) was installed in the line so that, ordinarily, water flowed to drain but when switched by the computer drain water was diverted into a sample bottle.

The water sampler was constructed with a five-way valve (Hoke Inc., Creskill, NJ, Model 7841GGY) attached to a 24 volt DC actuator (Hoke, Model 0172L2P) and four one-liter glass bottles (Figure 17). Each bottle was fitted with a neoprene stopper that had two stainless steel wire electrodes inserted through it, a vent hole, and a stainless steel fill tube. The electrodes extended down into the bottle and were part of a water level detect circuit that stopped water flow into the bottle when the desired amount was collected.

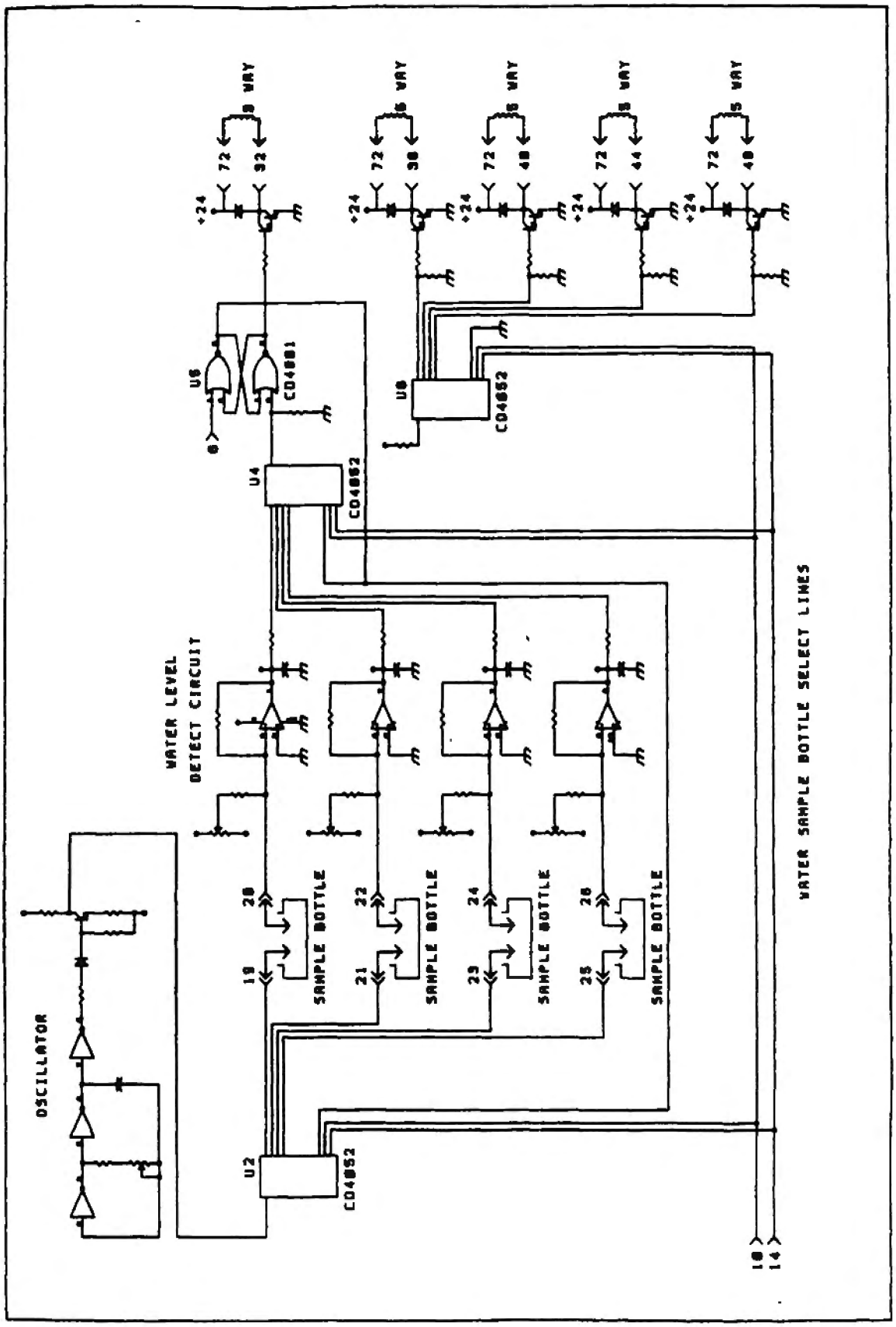
Fig. 17



The interface contained a separate water sampler control board for each sampler unit that controlled sample bottle selection, start of sampling, and sample halt when the bottle filled. Each board was connected to the digital multiplexer by a separate set of control lines that were, by convention, labeled A, B and SET or connectors 10, 14, and 6, respectively (Figure 18). Independent control lines were necessary so that the computer would not have to wait until one sample was complete before starting another. When the computer program selected a particular water sampler to activate, the digital multiplexer placed the binary code for bottle selection on the AB lines as explained previously. These lines were connected to three CD4052 analog multiplexer/demultiplexer integrated circuits labeled U2, U4, and U6, and the AB binary code switched them to the correct channel for that sample bottle (Figure 18). The output of U6 was fed into a Darlington pair causing the TIP29 transistor to conduct and drive the corresponding coil in the five-way valve actuator (Figure 19). This rotated the five-way valve to the correct port and connected the drain line to the sample bottle.

Once the five-way valve was in position, the water sampler control board received a start sample pulse from the digital multiplexer over the set line (connector six). This pulse was accepted by U5, a bistable multivibrator constructed from CD4001 quad, two-input NOR gates and designed to serve as both a water level detect flip-flop and a latch to start and hold the bottle filling procedure (Figure 20). The start sample pulse set the flip-flop and the output from pin four on U5 was applied to another Darlington pair, causing the three-way drain valve to switch from drain to the collect position. This diverted drain water into the sample bottle. The other half of the flip-flop was connected to the inhibit pins of U2 and U4 and with the flip-flop set, a logic low was placed on the inhibit pins, thus enabling both

Fig. 18


5 WAY
72
32
38
48
44
+24
U5
CD4081
U6
CD4052
U4
CD4052
WATER LEVEL
DETECT CIRCUIT
20
19
22
21
24
23
26
25
SAMPLE BOTTLE
OSCILLATOR
U2
CD4052
WATER SAMPLE BOTTLE SELECT LINES
10
14

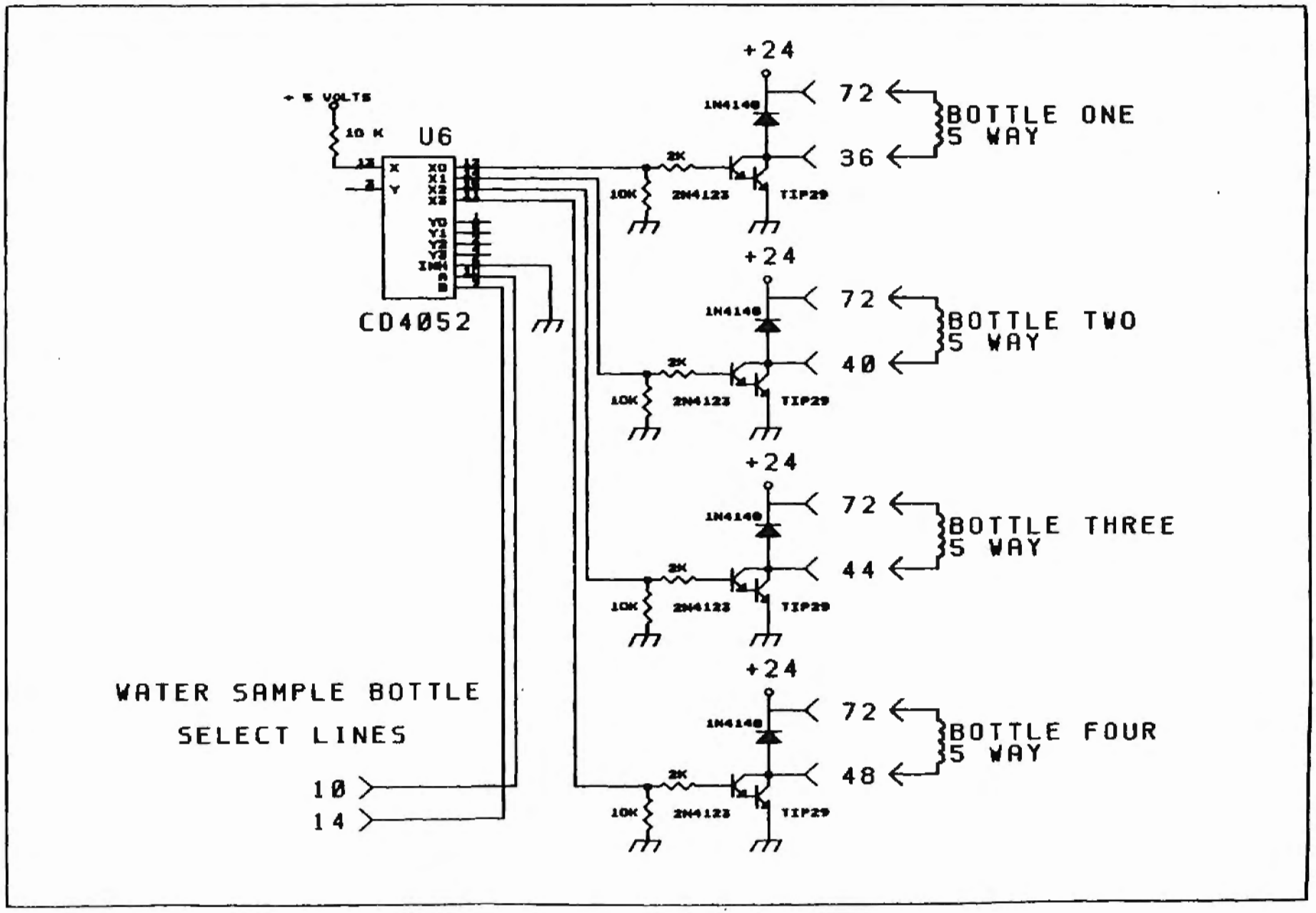

+ 5 VOLTS
10 K
U6
CD4052
+24
1N4148
2K
10K
2N4123
TIP29
72
36
BOTTLE ONE
5 WAY
40
BOTTLE TWO
44
BOTTLE THREE
48
BOTTLE FOUR
WATER SAMPLE BOTTLE
SELECT LINES
10
14

Fig. 19

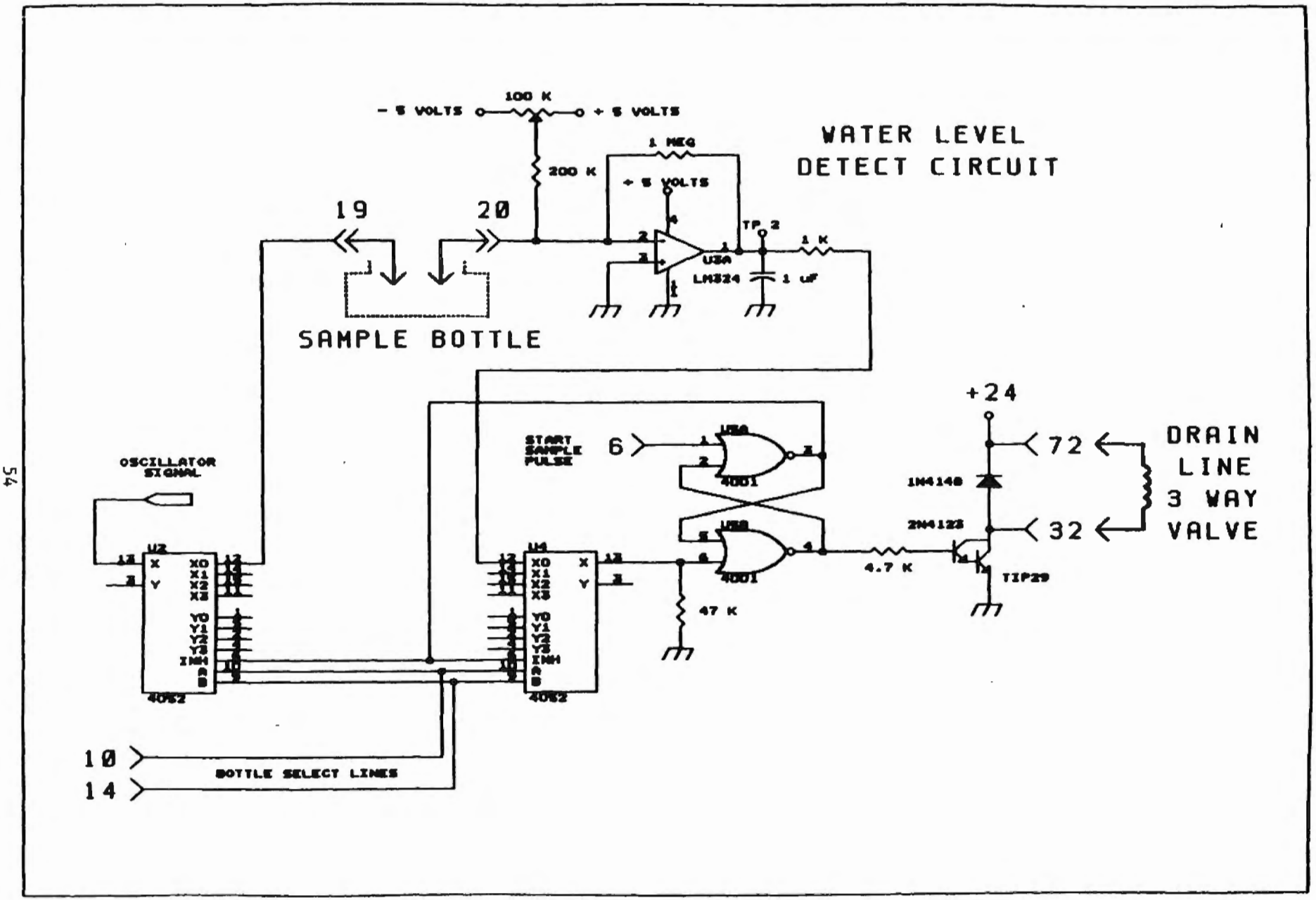

WATER LEVEL
DETECT CIRCUIT
100 K
- 5 VOLTS
+ 5 VOLTS
200 K
1 MEG
+ 5 VOLTS
19
20
SAMPLE BOTTLE
TP 2
1 K
U3A
LM324
1 uF
+24
START
SAMPLE
PULSE
6
U5A
4001
U5B
4001
OSCILLATOR
SIGNAL
U2
4052
U4
4052
1N4148
2N4123
72
32
DRAIN
LINE
3 WAY
VALVE
4.7 K
TIP29
47 K
10
14
BOTTLE SELECT LINES

Fig. 20

U2 and U4. At this point the flip-flop was latched, the water sample started, and the computer free to go on to other functions.

When the AB lines switched U2 and U4, the water level detect lines coming from the electrodes for the correct sample bottle were connected into the circuit (Figure 18). When U2 and U4 were enabled by the start sample pulse, a free running oscillator (Figure 21) fed its signal through U2 and, so long as the bottle was not filled with water, an AC signal was applied to only one electrode. When the water level reached both electrodes the low level AC signal was passed to the detector circuit where the signal was amplified by U3, an LM324 op amp. The amplified signal passed through U4 to pin six of U5 and cleared the water level detect flip-flop allowing the inhibit lines to become active. This inhibited the operation of U2 and U4, shutting down the AC signal going to the selected bottle electrodes, and removed the detected signal from the flip-flop. It was necessary to stop the signal to the flip-flop so that the water sample control circuit could become enabled on the next request for a sample.

4.2.4.6 Urine

The fish urinary catheter was connected to a hypodermic needle which in turn was connected to a port in the C compartment. On the outside another hypodermic needle connected the port to capillary tubing which drained the urine into centrifuge tubes placed in a fabricated plexiglass carrousel mounted on a modified fraction collector (Instrumentation Specialties Co., Lincoln, NE). The fraction collector (Figure 22) was originally designed to operate with 45 positions for small vials, but only 15 of the larger centrifuge tubes per fish could be placed in the custom holder. This meant that the fraction collector had to be advanced three times for each sample

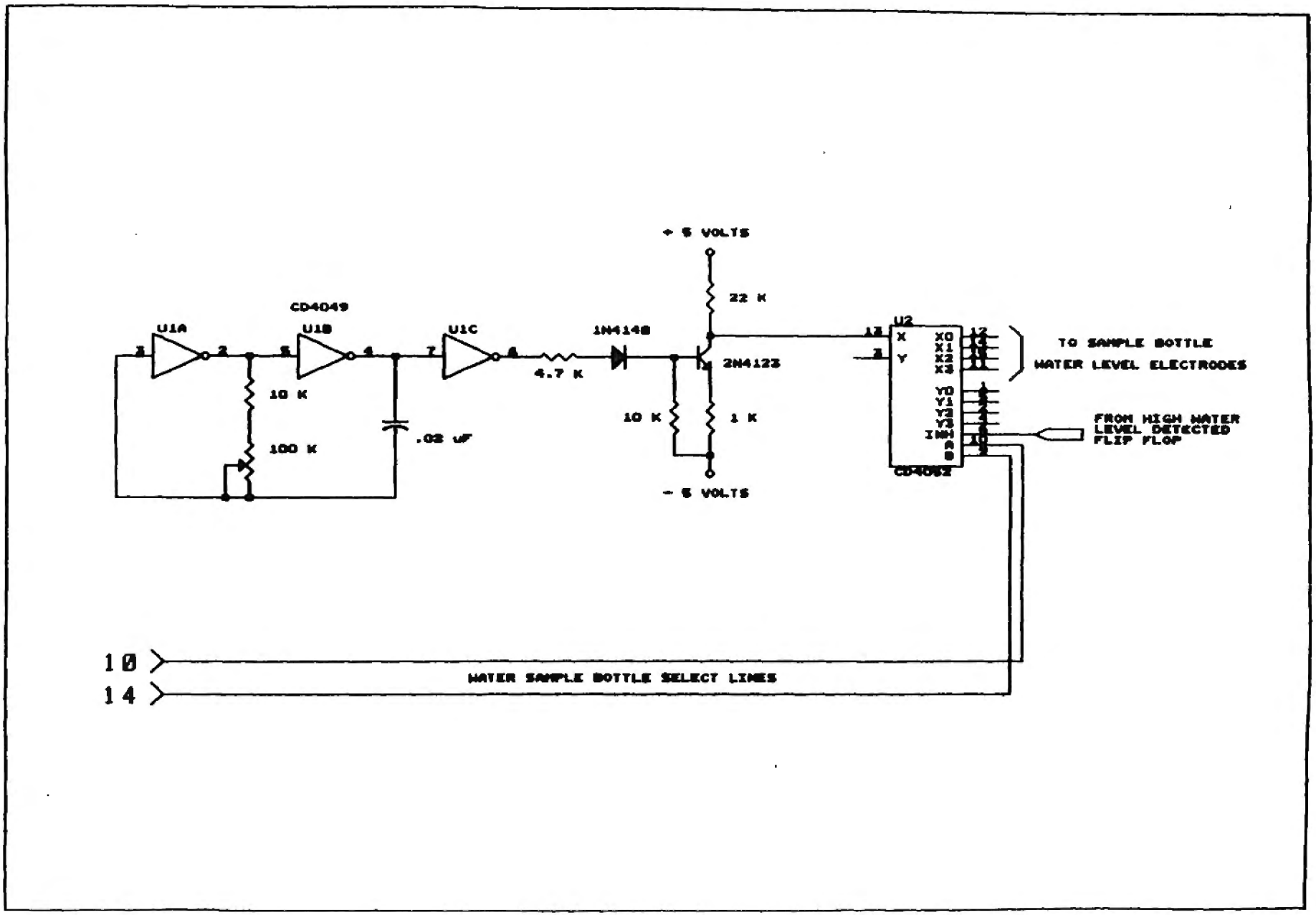
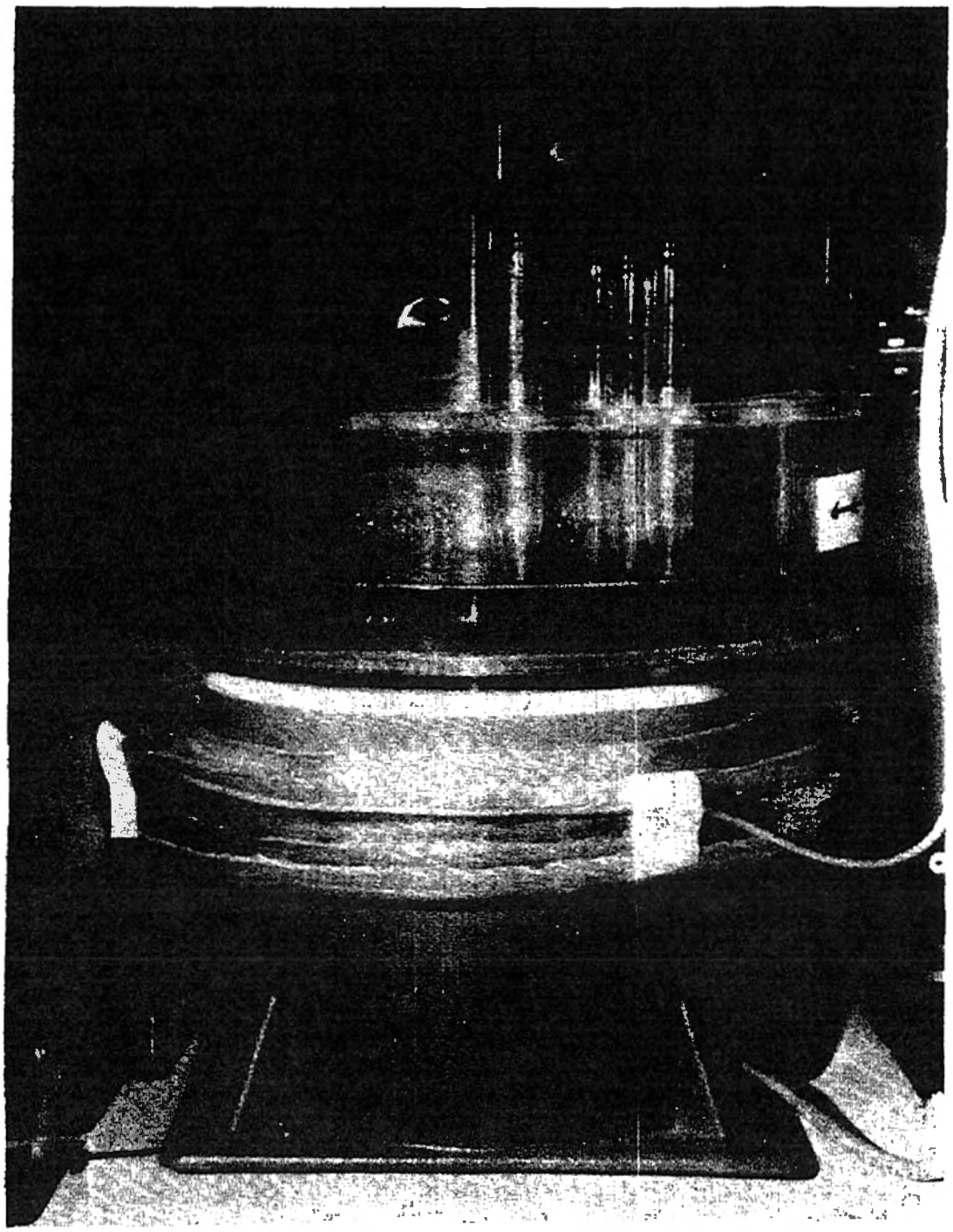

+ 5 VOLTS
22 K
CD4049
U1A
U1B
U1C
1N4148
2N4123
4.7 K
10 K
100 K
.02 uF
10 K
1 K
- 5 VOLTS
U2
X
Y
X0
X1
X2
X3
Y0
Y1
Y2
Y3
INH
A
B
C
CD4052
TO SAMPLE BOTTLE
WATER LEVEL ELECTRODES
FROM HIGH WATER
LEVEL DETECTED
FLIP FLOP
10
14
WATER SAMPLE BOTTLE SELECT LINES

Fig. 21

Fig. 22



position. The computer accomplished this by monitoring the internal, cam-operated microswitch that closed each time the collector moved into one of the 45 original positions. This was monitored over the digital input channel zero of the MIO120 card (Table 1) and three microswitch closures were counted for each new sample position. The 110 volt AC motor that rotated the collector was isolated from the computer by a solid state relay (Figure 12). The computer placed a low on channel 21 of the DIO120 which was connected to BB5 of the valve driver board (Figure 12). Stage C of the 4049 inverter (U3) inverted the low voltage and in turn activated a 2N4123 transistor. This closed the solid state relay and the fraction collector motor ran. Normally, the fraction collector was advanced once every one or two hours. The urine samples were maintained about four degrees C by cooling water flowing through a water bath within the carrousel, and were collected daily for storage in a freezer until analysis.

4.2.5 Data Management

After each measurement or sampling operation the computer monitor screen was updated to show the results for that time interval, a continuous hard copy on a Decwriter 3 printer (Digital Equipment Corp., Maynard, Mass.) was appended, and all data were appended to a file residing on the hard disk.

Some of the detailed information about these operations was given above in the software section on subroutines. The printed output consisted of a file header containing information about the fish and test, a header with 16 column labels followed by the data output, and an end-of-file area reserved

for information about dose times for each fish. The column labels and the data they represented included:

1. DATE - the month and day that the sample was taken.
2. FISH - fish number.
3. SNUM - each fish had their own set of sample numbers. Every time a sample or measurement was performed, SNUM was increased by one.
4. ETIME - the elapsed time in hours that the fish had been in the chamber.
5. ApH - pH of incoming exposure water.
6. BpH - pH of expired water.
7. ADO - dissolved oxygen content in mg/L of the incoming exposure water.
8. BDO - dissolved oxygen content in mg/L of expired water.
9. O2UP% - percent oxygen extracted by the fish from the inspired water. Also called uptake efficiency (U_E).
10. VO_2 - oxygen consumed by the fish expressed as mg/g/min.
11. AFLOW - flow rate (ml/min) of water leaving the A compartment drain of fish chamber number one.
12. BFLOW - flow rate (ml/min) of expired water leaving B compartment.
13. ATEMP - temperature of incoming water.
14. CTEMP - temperature of C compartment water.
15. WATS - first column: no sample = 0, water sample = 1. Second column: water sampler port number (1-4).
16. URNS - number of fraction collector advances.

Only raw data were stored on the hard disk file; hence, the disk file contained the DO results shown for ADO and BDO but not VO_2 or O2UP%. However, the disk file also contained a record of the headbox and fraction collector temperatures; these were not shown on the printer output due to lack of space.

Information in the hard disk file was stored under a CCPM format, but the MACSYM 120 operating system had the capability to transform CCPM files into the DOS format. The DOS file was written to floppy disk, and transferred via modem to a VAX minicomputer (Digital Equipment Corp.) where appropriate information was extracted and merged with other files in the FATS database.

4.2.6 Fish Acute Toxicity Syndrome (FATS) Testing

This system was constructed primarily to perform the required sampling and variable measurement to fulfill FATS testing, and the description of FATS testing included here was only to demonstrate how the system was used in our research program and how it behaved during testing. Methods and procedures were as described in Bradbury et al. (1988); a brief description is provided here but will not include toxicant preparation and analysis, water characteristics, or data analysis. However, some non-automated sampling and measurement procedures were included since these were an integral part of the overall system, and some of these may be automated in the future. Also, Bradbury et al. (1988) defined a new FATS for a group of chemicals termed polar narcotics; the five chemicals tested represented about a third of the test runs completed with the system to date. Although some system functions were not used for those tests, the following description includes all that the system was designed to perform.

4.2.6.1 Physiological Monitoring

During each FATS experiment measurements were made on the physiological variables shown in table two. V_G, VO_2, and U_E were monitored automatically while the remainder were done manually. Ventilatory frequency (f_v) and cough frequency (f_c) were determined from portions of strip-chart recordings made of the trout ventilatory patterns. These were monitored from non-contact stainless steel wire electrodes placed in the B and C compartments of each fish chamber. Each electrode pair was connected via patch cords to a junction box mounted above the fish chamber and from there shielded two-conductor cable led to high gain capacity-coupled preamplifiers set to a time constant of 0.3. Frequencies above 30 Hz were filtered from the signal as it passed through channel amplifiers and the ventilatory signals were recorded on a physiograph rectilinear strip-chart recorder (Narco Biosystems, Houston, TX). EKG electrodes were connected through to the recorders similarly to record heart beat (f_H), but EKG recording also required a third electrode to help reduce electrical noise; this was inserted into the dorsal muscle mass of the trout and connected to ground potential on the junction box.

4.2.6.2 Test Procedures

Spinally-transected rainbow trout were surgically prepared as described by McKim et al. (1987). Each fish was fitted with a latex rubber membrane that separated expired water from incoming water, a dorsal aortic cannula for blood sampling, copper wire electrodes for monitoring the EKG, and a urinary catheter. Four trout weighing between 0.6 and 1.0 kg were exposed to each chemical, or were used as controls. After surgery the fish were placed in individual respirometer chambers, the electrode connections made, and the urinary catheter was connected to the C compartment port.

Table 2. Physiological variables monitored in rainbow trout to define the toxic responses associated with fish acute toxicity syndromes (FATS).

Variable		Units
Ventilation Volume	(V_G)	ml/min
Total Oxygen Consumption	(VO_2)	mg/kg/h
Gill Oxygen Uptake Efficiency	(U_E)	%
Ventilation Frequency	(f_V)	no./min
Cough Frequency	(f_C)	no./min
Heart Frequency	(f_H)	no./min
Total Blood Oxygen (arterial)	(TaO_2)	g/100 mL
Total Blood Carbon Dioxide (arterial)	($TaCO_2$)	mmol/L
Blood pH (arterial)	(pHa)	pH units
Hematocrit	(Hct)	%
Hemoglobin	(Hb)	g/100 mL

Program TEST was entered and the starting time for automated monitoring was set. Physiological monitoring was started between 18-20 hours after the fish had been placed in their chambers, usually 0500 h the next morning. Predose values for all physiological variables were obtained during the control period between 0500 and 1200 h. The computer measured and calculated V_G, VO_2, and U_E every 15 min while f_V, f_C, and f_H were determined from recordings made every 30-60 min. One set of blood chemistry variables was collected during the predose period. The fraction collector was advanced hourly and, if needed, automatic water sampling was activated.

Toxicant delivery was started at 1200 h by interrupting TEST and selecting the proper routine. The fraction collector interval was set to two hours at this time but the sampling schedule for other variables remained the same for the test duration. When needed, the water samplers were activated and set for 4 h intervals. During periods of unattended operation, the physiograph recorders ran continuously. Blood chemistry variables were measured in arterial blood samples taken from each aortic canula two to five times during an exposure. Periodically, water samples were taken from each fish chamber B compartments and the A compartment of chamber one and analyzed for DO content by the Winkler titration method to provide a check on automated measurements.

Temperatures were also checked. Whenever a fish died, that chamber was deactivated for monitoring, and the test was terminated whenever all fish died or a 48 h exposure was completed.

SECTION 5

RESULTS AND DISCUSSION

5.1 SYSTEM EVALUATION

To date 17 tests involving 68 fish have been completed using the system. Testing included three freshwater control runs, two control tests on a carrier solvent used to aid dissolution of some test chemicals, and 13 tests with organic chemicals used in describing fish acute toxicity syndromes (FATS). In a sense, all of these tests constituted evaluation and validation of the stated objectives for the system, although only the first three runs, two controls and a previously tested chemical, were used to certify the system as ready. Collectively, the results from these tests showed that the system performed as designed despite some sporadic electronic malfunctions and problems with sensor calibration during some tests.

It seldom occurred that the computer monitored values were exactly the same as the actual value measured for DO, pH, temperature, or ventilation volume (V_G). Tables three and four show the average percentage deviation from actual value at those times where simultaneous sampling and measurement were done manually. Computer monitored values taken from the flow measurement pressure transducers for V_G were consistently close to actual value, generally within five percent (Table 3). The only exception to this was flow sensor 4B during tests four and five in which case the deviation was 17 percent high and 11 percent low, respectively. For 17 tests overall, the deviation for absolute values showed that sensor 2B performed the best, with a mean deviation of 2.3 percent, despite the fact that during test five it was off by 11 percent.

The average deviation for DO measurements was considerably more erratic, ranging from a -54 percent for sensor 1B to + 61 percent for 3B (Table 4). One recognizable problem that occurred during DO monitoring was air bubbles

Table 3. Average deviation (percentage) from actual value for computer monitored water flow rate.

	Flow Sensor				
Test	1A	1B	2B	3B	4B
1	a	-9	a	a	a
2	a	a	a	a	a
3	a	a	4	4	9
4	a	0	4	3	17
5	1	-1	11	-7	-11
6	4	0	0	3	3
7	6	3	-3	-6	4
8	8	6	0	4	0
9	0	3	1	2	-2
10	8	6	1	-3	4
11	3	3	0	2	4
12	-4	-9	-1	-3	-1
13	2	0	0	3	1
14	4	0	-1	2	-1
15	-1	2	-2	6	3
16	5	3	-2	1	1
17	1	-3	-4	4	2

[a] - Actual values not recorded.

Table 4. Average deviation (percentage) from actual value for computer monitored dissolved oxygen (DO) concentration.

	DO Sensor				
Test	1A	1B	2B	3B	4B
1	-3	-7	-1	-5	-7
2	1	-6	-7	-10	-7
3	-1	-6	-3	-9	1
4	3	-2	-8	3	-4
5	4	2	2	-9	6
6	1	-7	6	3	-1
7	0	-10	2	-5	-7
8	5	-8	14	-5	4
9	0	-11	-6	-4	-5
10	2	-3	0	-11	-10
11	2	-2	11	61	2
12	-24	-14	17	-5	27
13	a	-54	18	-37	-8
14	a	0	10	-24	b
15	-10	-4	11	15	-20
16	-11	2	5	16	-37
17	-14	-5	9	-12	-14

[a] - Electrode malfunction; DO monitored manually

[b] - Chamber not used for this test.

getting trapped in the electrode cell holders when saturated water degassed as it flowed past the electrodes. This was an ongoing problem with chamber 1A since the electrode there always measured DO concentration of the incoming water; it was less of a problem in the other electrode cells where fish were extracting oxygen from the test water before it flowed into the electrode cell. Lightly tapping the electrode cell or forcing water through them with a syringe bulb dislodged the air bubbles and alleviated the problem. Also, the electrode cell holder from chamber 1A was replaced with a redesigned electrode holder constructed so that the water flowed into the bottom of the cell and flowed vertically upwards past the DO electrode rather than horizontally through the cell.

For the stated objectives, a $\pm$ 5 percent deviation was within acceptable limits, but when computer monitored values varied by more than about two percent, a separate computer program was used after a test was over to readjust monitored values obtained for those particular sensors to correspond more closely with actual values. For example, DO electrode and meter output was checked periodically by collecting water samples simultaneous to automated monitoring and DO concentration was determined using the Winkler titration method. If the monitored results from a particular electrode and meter showed continual variance compared to the actual DO concentration then it was assumed that the pre-test calibration was incorrect or that DO electrode performance was faulty and the automated measurements were accordingly in error. Usually, the actual DO concentration was consistently higher or lower than the automated result by a certain amount, but sometimes the variance would gradually increase either high or low as the test continued indicating that the digitized output was drifting. In either case, ratios of actual DO concentrations/computer monitored concentrations were

regressed against the corresponding times in hours from the beginning of the test that the actual DO samples were taken. The slope (m) and the intercept (b) were used as coefficients in the linear equation y = mx + b where x = elapsed time in hours from the test beginning and y = a correction factor used to estimate the actual DO concentration for any test time. The recalculation program read the old data file record by record, recalculated each DO value as DO = y times (monitored DO), and rewrote all data into a new data file in exactly the same format as the old. Table 5 shows the average deviation (percentage) from actual values after DO concentrations were corrected by the recalculation program.

Errant values for water flow measurements by any one pressure transducer were handled similarly except that actual flows versus computer monitored flows were regressed from data sets obtained just before and after a test was performed; flows were not measured manually during a test. Temperature and pH values were not readjusted, although a hard copy record was desirable to ensure that they were close to expected values during periods of unattended operation.

5.2 FATS TESTING

Using strictly manual data gathering methods, McKim et al., 1987b,c, defined FATS associated with narcosis-inducing chemicals, oxidative phosphorylation uncouplers, acetylcholinesterase inhibitors, and respiratory membrane irritants. The first chemical tested with the automated system, 2,4-dinitrophenol, was in the original group of uncouplers, and the results obtained using the automated system were consistent with those obtained manually. This verified that the automated system was suitable for FATS testing as well as showing that the responses used to define a FATS were reproducible.

Table 5. Average deviation (percentage) from actual value for corrected computer monitored dissolved oxygen (DO) concentration.

	DO Sensor				
Test	1A	1B	2B	3B	4B
1	0	0	0	0	0
2	0	0	0	0	2
3	0	0	1	0	6
4	0	0	0	0	0
5	0	0	0	0	0
6	4	5	6	3	1
7	0	0	2	0	0
8	3	7	16	4	11
9	0	0	0	0	0
10	0	0	2	0	0
11	1	6	0	0	0
12	0	2	3	5	0
13	a	8	4	3	5
14	a	0	0	0	b
15	0	0	0	1	0
16	1	6	2	4	7
17	1	4	12	11	5

[a] - Electrode malfunction; DO monitored manually.

[b] - Chamber not used for this test.

The main advantage gained by using the automated system for FATS testing was that four fish could be monitored simultaneously for their cardio-respiratory responses. This was possible because some of the labor intensive manual methods were simply replaced by the system, saving considerable time and effort. When done by the automated system, measurements of V_G and DO were rendered effortless and it became possible to monitor more than two fish per test. This at least doubled the number of FATS tests that could be done in the same time frame.

Also, round-the-clock monitoring was now possible and this greatly increased the number of measurements done for V_G, U_E, and O_2. This ensured data gathering throughout a test for every fish and increased the confidence that data were not missed for periods of critical change.

Another advantage to using the automated system was that certain judgements concerning the course of an experiment could be made while it was in progress. For instance, it is characteristic of narcosis-inducing chemicals that their effects on an organism are reversible even at the point of apparent death, usually defined as respiratory arrest in aquatic toxicology, whereas effects induced by chemicals with more specific modes of toxic action are irreversible. It was necessary to prove that phenol was a narcotic in the sense that its effects were reversible before a new FATS could be defined for the suspected polar narcotics (Bradbury et al., 1988). By following V_G and VO_2 on the computer printout as well as locomotor activity, ventilation, and the EKG on those recordings, the fish could be revived at various stages of intoxication with toxicant-free water and recovery

monitored. Primary toxic responses by phenol were found to be reversible, even when the fish were brought to the point of complete cessation of ventilation.

All of the automated system features were not required for FATS testing. Because most tests ended within 24 hours, automated water sampling for chemical analysis was seldom necessary. Also, urine analysis was not performed since the only variables used in defining FATS at this time were associated with cardio-respiratory functions. Hence, the fraction collector was idled during FATS testing. However, both of these features would be useful for certain pharmacokinetic studies where chemical analysis of incoming and expired waters is critical to understanding uptake by the gills and where metabolites present in the urine would shed light on internal physiological and metabolic processes. Other types of physiological testing may require additional automated features, blood pressure or the electroencephalogram for example. The system was built to allow for expansion should such measurements become necessary.

There are some functions that should be automated to make FATS testing more efficient. More time and effort could be saved if f_V, f_C, and f_H were automated. Manual counts and data input for these would be eliminated, and it would be useful to observe these in real time. Other physiological processes simply defied automation at this time such as blood sampling and analysis.

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FIGURES

14. Water flow measurement device. Two-way valve was normally open; when closed, rising water level in tube at left increased pressure on transducer connected to lower part of tube.

15. Block diagram of pre-test sensor calibration used to supply coefficients (slope and intercept) to linear equations in program TEST.

16. Schematic of one temperature meter circuit.

17. Water sampler consisting of a five-way valve with actuator and four sample bottles. Three-way valve in upper part diverted water from drain line into one of the sample bottles.

18. Schematic of water sampler control board. There was one control board for each sampler in the system.

19. Schematic detail of the bottle selection CD4052 multiplexer. Sample bottle select lines received the AB binary code for bottle number from the digital multiplexing board.

20. Schematic detail of the start sample and water level detect circuit. Start sample pulse was fed through 4001 flip-flop. Detector circuit sensed oscillator signal when bottle filled and shorted electrodes. Detected signal cleared flip-flop and inhibited CD4052 multiplexers.

21. Schematic detail of oscillator used to provide low level ac signal to water level detect electrode in sample bottle.

22. Modified fraction collector for urine collection. Centrifuge tubes extend down into cooling bath within the fabricated carrousel.