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Photon Counting System for the Determination of Creatine Phosphokinase

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PHOTON COUNTING SYSTEM FOR THE DETERMINATION OF CREATINE PHOSPHOKINASE

by

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INTRODUCTION

Background

Acute myocardial infarction

An acute myocardial infarction (AMI) is the gross necrosis of tissue in the myocardium or heart muscle (1). The death of this tissue results from an interruption of its blood supply. This interruption frequently occurs as a result of a clot in the heart. This condition is referred to as coronary thrombosis or heart attack. The dead tissue is irreplaceable with the condition frequently becoming more serious. Survivors of an AMI must take extreme precautions since the inflamed surrounding tissue has a high probability of splitting and causing instant death.

Heart disease is a condition where the blood supply to the heart is limited, frequently causing a coronary thrombosis. Heart disease is rated as this nation's number one killer which amounts to over 600,000 deaths a year (2). Statistics show that one in three patients die during an AMI (3). Treatment generally consists of three to six weeks in bed with limited exercise and a highly restricted diet.

Several tests are available to screen a patient for heart disease. One of the tests is the angiogram which is very painful and has caused death in certain cases. Another test, which is much safer but not totally conclusive, is the stress test. In a

recent development, a radioactive liquid is injected into a vein which produces an image of the heart on a video screen illustrating the presence of any obstructions in the heart (2). Methods of diagnosis following an AMI for a living patient are few and in general rather unreliable.

Creatine phosphokinase and muscle

The enzyme creatine phosphokinase (CPK) has an enzyme classification (EC) os 2.7.3.2. CPK has three isomers, called isoenzymes, which are dimers composed of either muscle (M) or brain (B) subunits. Table I shows the concentration of the isoenzymes in the cells of various organs in percentage of total CPK for that cell (4).

Table I

Distribution of the Isoenzymes of CPK Found in the Cells of Various Tissues

Tissue	% MM	% MB	% BB
Brain cells	--	--	100
Skeletal muscle cells	98	2	--
Myocardium cells	70	30	--

In the event of damage to these cells it is probable that they will rupture and release the isoenzymes into the blood stream. Damage as slight as a bruised muscle can elevate the MB concentration.

in the blood. Skeletal muscle, however, cannot be responsible for MB concentrations greater than three per cent of the total CPK. Thus concentrations higher than three per cent would be indicative of myocardial damage. An AMI will cause the MB concentration to rise sharply, peaking 12 hours after the infarction, and finally return to normal.

CPK enzyme analysis may also be used to help diagnose other disorders. BB isoenzyme concentrations in the spinal column can be used to show the extent of brain damage in cases of head injury (5). The BB isoenzyme has also been found in the blood serum of a patient suffering from a gastric carcinoma one or two days before death (6). CPK assays may also be used to screen male infants for Duchenne muscular dystrophy as well as screen surgery patients for a propensity to develop malignant hyperthemia (7). Many sports trainers are interested in their athletes' muscle condition and are using MM assays to help determine this.

Current Methodology

Electrocardiogram

Doctors frequently order an electrocardiogram (ECG) to be administered to patients entering the hospital with chest pains. This procedure measures the electrical current produced by the heart muscle as it tenses and relaxes. Unfortunately an ECG can be misleading. It may begin to show abnormal variations resulting from an AMI anywhere from a few hours to several days after the

onset of the attack. This abnormality can also be masked by various other conditions such as arrhythmia, or thrombosis. A majority of AMI's can be diagnosed by an ECG but the uncertainties associated with the test usually requires confirmation by other methods.

Electrophoresis

The method most often used to verify an AMI makes use of the enzyme lactate dehydrogenase (LDH EC 1.1.1.27) which has five isoenzymes. Two of these isoenzymes are found in the heart muscle tissue. However, their concentrations require 48 hours to peak after the onset of an AMI. Separation of these isoenzymes can be accomplished in an electrophoretic unit in about two hours. The bands are transferred to an acetate sheet, dyed, and measured using an optical densitometer. If the first band is darker than the second band, the patient has probably suffered an AMI.

CPK is probably a more accurate indicator than LDH in the electrophoretic assay since the MB band is not detectable without myocardial damage. Unfortunately, CPK does not have a usable dye to complete the assay. The CPK bands must be removed and measured fluorometrically.

Liquid chromatography

A recently developed method which is still the center of much controversy among clinical chemists, is the column chromatography or Worthington method of separation of CPK (8,9). The original proponents of this method for clinical use are Mercer and Varat (10).

The Worthington Biochemical Corporation has subsequently developed a kit to determine CPK. Mercer (8) feels that the possibility of incomplete separation or carryover of the isoenzymes is too large with this kit. This particular separation requires 15 to 30 minutes. The eluates are then assayed for CPK with the Rosalki assay which follows NADPH, the reduced form of nicotine dinucleotide phosphate (NADP), using the glucose hexakinase (HK) coupled reaction. The reaction also involves creatine phosphate (CP), creatin (C), adenosine diphosphate (ADP), and adenosine triphosphate (ATP) and is measured at a wavelength of 340 nm.

$$CP + ADP \xrightleftharpoons{CPK} C + ATP \qquad (1)$$

$$ATP + glucose \xrightleftharpoons{HK} glucose\text{-}6\text{-}phosphate + ADP \qquad (2)$$

$$glucose\text{-}6\text{-}phosphate + NADP \xrightleftharpoons[dehydrogenase]{glucose\text{-}6\text{-}phosphate} 6\text{-}phosphogluconate + NADPH \qquad (3)$$

Coupled assay

Several instruments are now commercially available for the analysis of ATP using a luminescent firefly extract which is partially comprised of luciferase and luciferin. The amount of light emitted is proportional to the ATP concentration. The reaction of CPK with its substrates, CP and ADP, yields ATP at a rate proportional to the CPK concentration. This allows the CPK concentration to be determined.

In a study on the CPK isoenzymes' rates, large differences were found in the activities of each of the isoenzymes (11). The activities also vary, independently of each other, with the substrate concentrations of ADP and CP (12). The concentration of the MB isoenzyme can be determined by using two substrate concentration mixtures. One mixture will have the concentrations of the substrates adjusted to give an MB activity of about four times higher than the MM activity. The other will have an MB activity of slightly less due to slightly higher substrate concentrations. Normal patients will show the same ratios of activities at high and low substrate concentrations as that for normal MM concentrations. A patient suffering from an elevated MB concentration will have a higher activity on the lower substrate concentration than on the higher substrate concentration. This amount can be quantitated by plotting the results graphically. This method assumes that only two of the isoenzymes are present in the sample. Since the BB isoenzyme concentration is normally low this assumption is usually valid.

High pressure liquid chromatography

Schroeder (13) has used high pressure liquid chromatography to separate the isoenzymes of LDH. This procedure requires only ten minutes. After separation the eluate is mixed with nicotinamide dinucleotide (NAD) and the rate of production of NADP is proportional to the LDH concentration. NADP is measured spectrophotometrically. The advantages of this method are its rapidity and its potential

application to automation. The disadvantages are carryover and the fact that the blank is measured by exposing the LDH eluates to the ultraviolet light in a spectrophotometer before mixing the NAD in the mixture. The ultraviolet light tends to denature the LDH.

PRINCIPLES OF AN AUTOMATED PHOTON COUNTING CPK ANALYSIS

Creatin Phosphokinase Catalyzed Reactions

CPK catalyzes the reaction of its substrate creatine phosphate (CP) and adenosine diphosphate (ADP) to form the high energy bond of ATP in muscle tissue. The reaction that it catalyzes is the same for all three isoenzymes.

$$CP + ADP \overset{CPK}{\rightleftharpoons} C + ATP \qquad (4)$$

CPK is activated by metal ions such as Mg(II), Mn(II), and Co(II), but is inhibited by the Cu(II), Ba(II), Sr(II), Be(II), Ni(II), Cr(II), and Zn(II) ions. Also adenosine monophosphate (AMP), adenosine, tripolyphosphate, pyrophosphate, and orthophosphate tend to bind to the enzyme causing inhibition.

As mentioned earlier the activities of the individual isoenzymes are significantly different. Their dependence on substrate concentrations is also different. Figure 1 clearly illustrates this difference at a pH of 7.8 (12). The optimum pH for the MM, MB, and BB isoenzymes are 6.9, 7.0, and 7.1 respectively.

Utilizing these differences should allow the individual isoenzyme concentrations to be determined. Then by knowing the isoenzyme rate constants in each of three reaction mixtures, the determination of the isoenzyme concentrations can be made. Such an analysis obviously necessitates the need for a highly sensitive activity detector system.

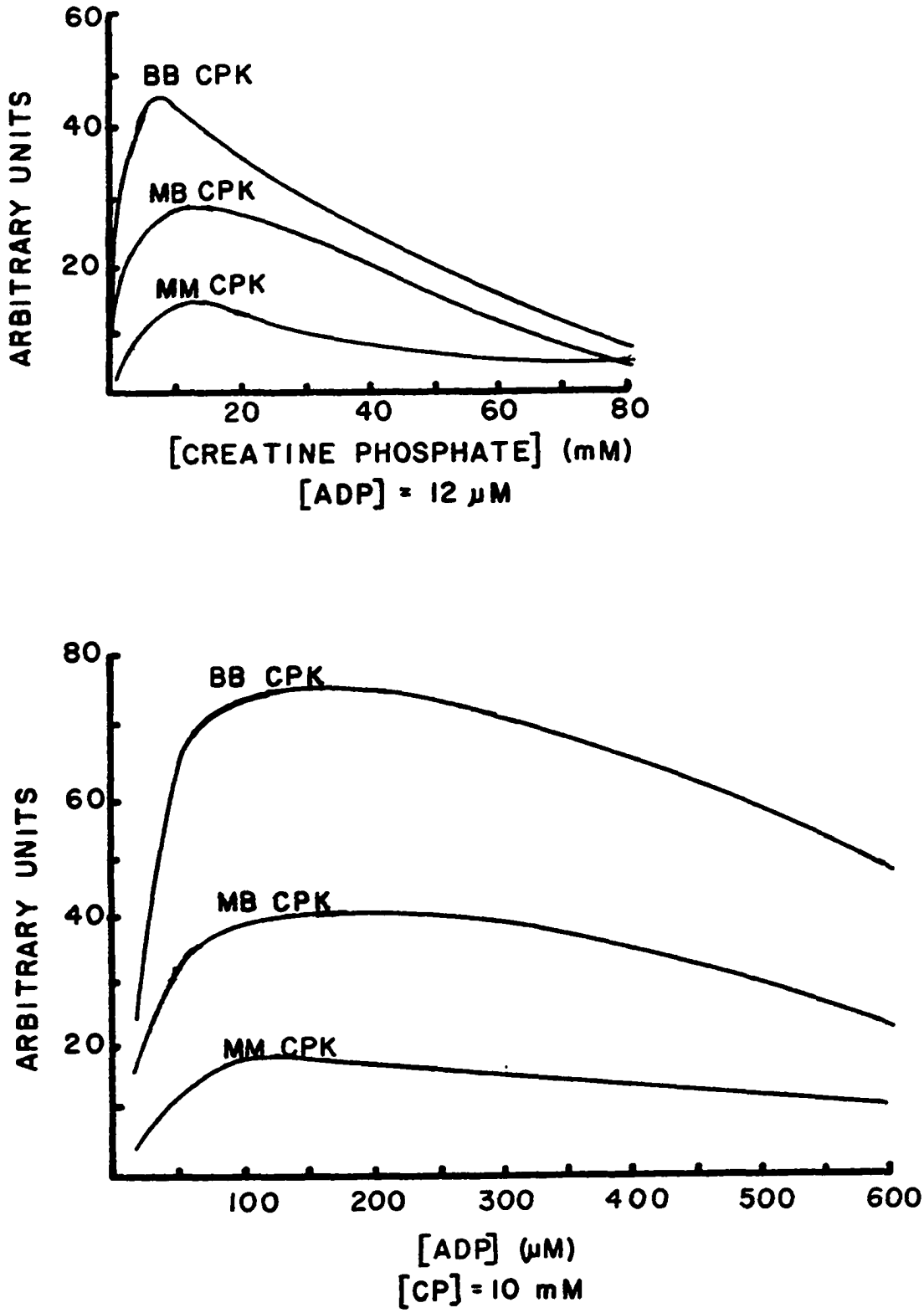

Figure 1. Substrate dependencies for CPK isoenzymes (12).

Luciferase Catalyzed Reactions

Luciferase (EC 1.2.3) is an enzyme produced by the firefly (Photinus pyralis). Firefly luciferase catalyzes the oxidation of luciferin (LH_2) to oxyluciferin (L) in the presence of oxygen, ATP, and Mg(II). During the oxidation, a photon of light with a maximum intensity at 540 nm is emitted.

$$LH_2 + ATP + O_2 \xrightleftharpoons{\text{Luciferase}} CO_2 + AMP + L + light + PPi \quad (5)$$

Metal ion activators for this reaction include Mg(II) and Mn(II) while Cu(II), Zn(II), Cd(II), and Hg(II) behave as inhibitors. AMP is an inhibitor as well as ATP if it is not complexed with Mg(II). The optimum pH for this reaction is 7.8.

Coupling

The rate of the luciferin-luciferase reaction is directly proportional to the concentration of ATP. This reaction has been widely used in commercial ATP analyzers such as Aminco's Chemglow and SAI Technology Company's ATP Photometer. Myokinase, an enzyme found in blood serum will interfere with the luciferin-luciferase reaction since it catalyzes a reaction which produces ATP.

$$AMP + ATP \xrightleftharpoons{\text{Myokinase}} 2\ ADP \quad (6)$$

The Rosalki assay uses AMP to inhibit the production of ATP (14); however, this inhibits the luciferin-luciferase reaction. It has been found, however, that at lower concentrations of ADP, the myokinase does not interfere with a coupled assay (12). The coupled assay only works for a two CPK isoenzyme mixture.

Photon Counting

Photon counting is a means of measuring extremely low light levels. It utilizes a photomultiplier with low dark noise and some type of counter. Since only low light levels will be exposed to the photomultiplier tube, the dynode voltage divider chain of the tube should be wired with higher than normal resistances in order to give greater sensitivity without losing dynode voltage stability. Photons striking the cathode result in the production of a current which can be monitored at the anode. By converting this photon pulse current to a voltage and discriminating against low voltage noise pulses, the photon pulses can be counted. The greater sensitivity of photon counting should be ideal for the quantitation of luciferin-luciferase based ATP or CPK assays. Since it will be necessary to have three different solutions representing different substrate concentrations, three photomultipliers could be used. However, it is more economical to use a single photomultiplier with some type of mechanical shutter system. The counting period will likely be more reproducible if all three measurements are made simultaneously. A mechanical shutter may introduce some error due to a lack of timing reproducibility, but controls in the counters can be used to minimize this effect.

MECHANICAL COUNTING SYSTEM DESIGN

Block Diagram

A block diagram of the mechanical counting system components is shown in Figure 2. The computer is used primarily for data acquisition, computation, and output formating to the teletype. It could be used for the initiation of the counters, thus providing total control from the teletype. However, before the incorporation of such features as this, successful operation of the instrument should be established first. The computer is more thoroughly explained in Appendix A. The interface is actually a part of the computer system and its design and operation is explained in Appendix B. It has been designed to receive and transmit data, to receive a program count, and to transmit instructions from the computer. It has been designed with a five volt power supply having the capacity to accomodate up to four different interfaces on printed circuit cards.

The counter interface controls the timing of the shutters and counters. Since there are three samples to count, the interface must have three banks of counters with the ability to count up and down. The counter interface also transmits the counts to the interface buffer.

The reaction mixtures must be mixed simultaneously. This can be done by mixing the sample serum, ADP, and a luciferin-luciferase solution into a cell. The CP is added with a syringe mechanism which automatically initiates the counter interface.

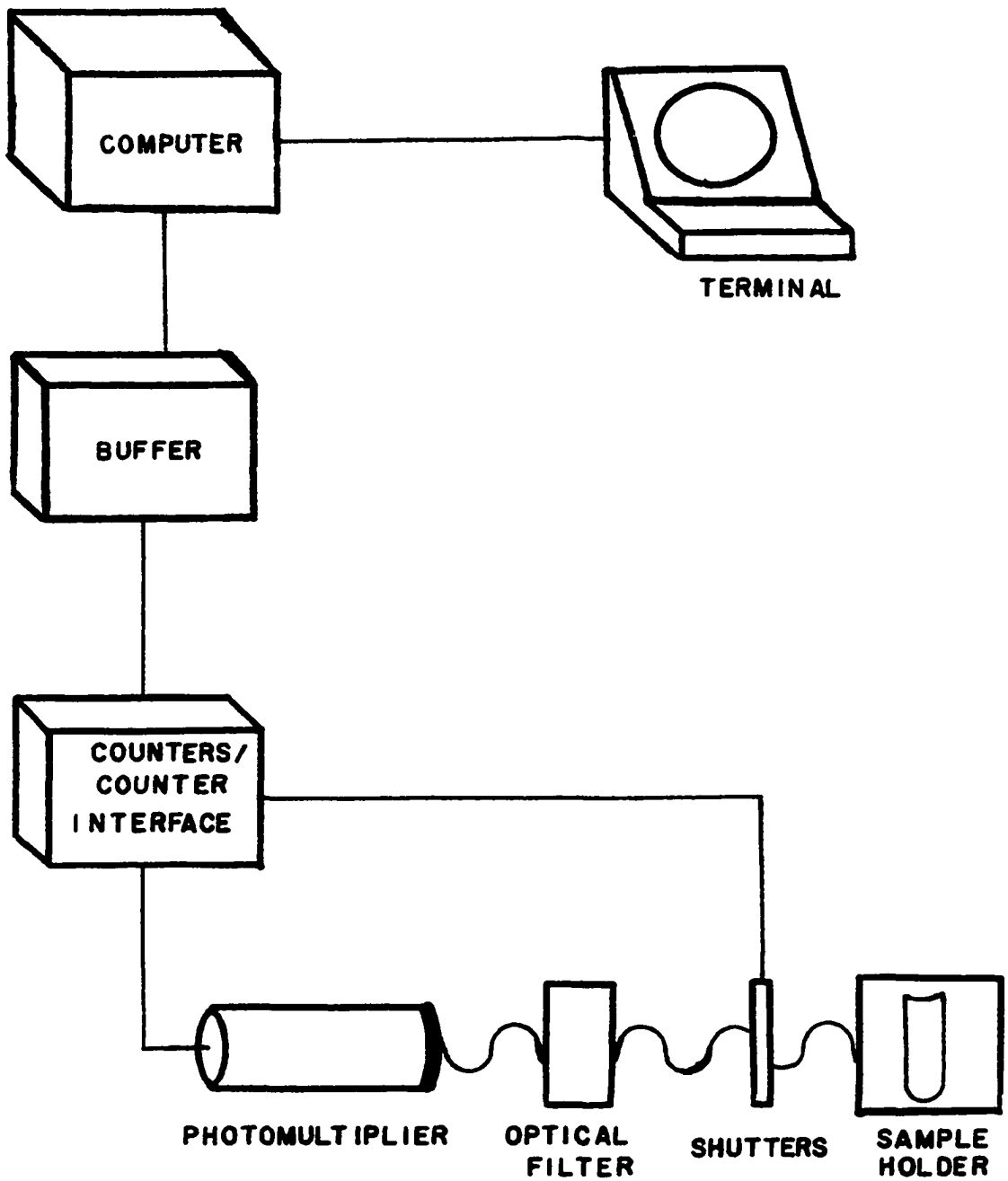

Figure 2. Block diagram for mechanical counting system.

By interconnecting the plungers of three syringes, the CP can be added to all three cells at once. The cells reside in a sample holder which incorporates individual beam attenuators for each cell. The beam attenuators permit balancing the light output to the photomultiplier due to variations in the optical path.

Plastic shutters are placed in the optical path of each sample. These shutters are raised and lowered by 12 volt dc solenoids in order to allow only one sample access to the photomultiplier tube at a time. The solenoids are activated by the counter interface. Immediately in front of the shutters is a 540 nm filter to shield stray light. Any ordinary photomultiplier tube with low dark noise and appropriate wavelength characteristics can be used. The dynode voltage divider in the base is wired for a greater sensitivity and connected to the counter interface.

Sample Holder

The sample holder, depicted in Figure 3, is constructed from a wooden block which has been drilled in such a way as to simultaneously accomodate three cylindrical glass cells of approximately 16 mm diameter. One side of the block is cut away to allow light to escape from the cell into the optical path. A magnetic stirring assembly is located just below the cell to permit each sample to be stirred. The stirring assembly consists of magnets attached to rotating shafts which pass through the bottom of the wood block. On the other end of each shaft is a pulley system. The shafts are interconnected by drive belts with the entire assembly being driven by a single motor.

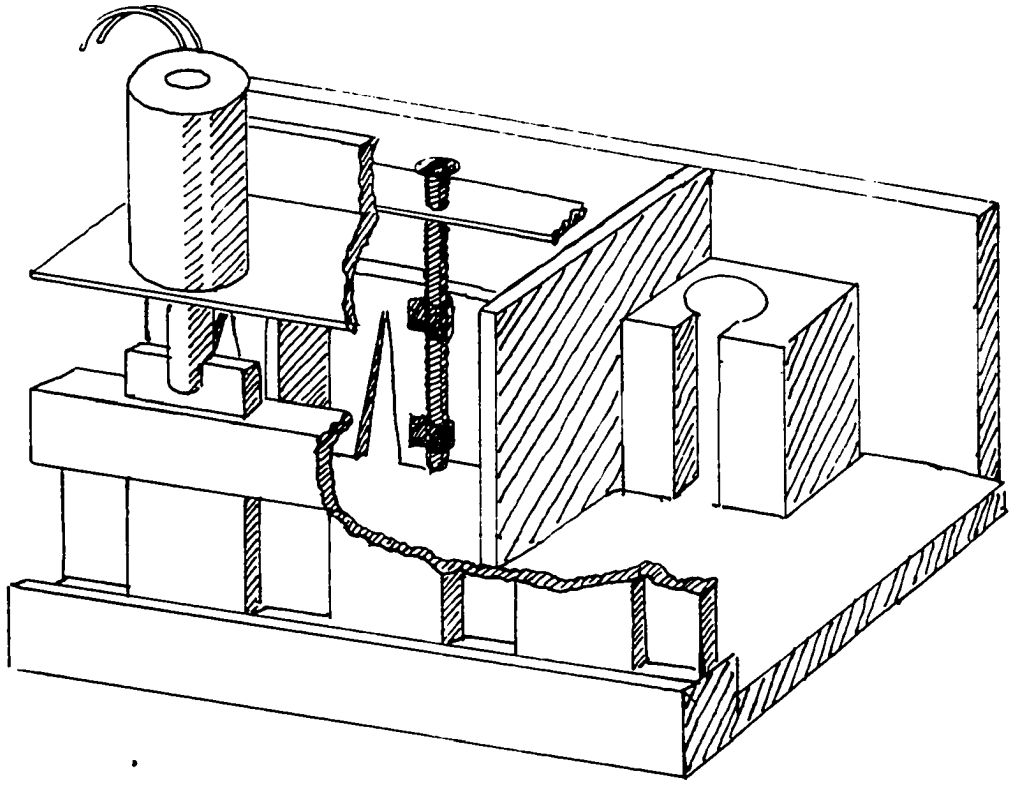

Figure 3. Sample holder and shutter diagram.

The reaction mixtures must be combined simultaneously in order for the reactions to be measured during the same time period of the reaction. Three syringes are mounted over the cells with the plungers interconnected to facilitate simultaneously mixing. The cells contain the mixtures of the blood serum, ADP and luciferin-luciferase while the syringes contain the other substrate, CP. A micro-switch initiates the counters in the counter interface when the syringes have expended their contents.

Two wedge-shaped slots are placed in front of each sample cell. One is stationary facing upward, while the other is facing downward and can move up and down by turning the screw on which it is mounted. This allows the cell photon output to be balanced between the different cells and therefore minimizes the effects of differences in the optical paths.

Shutters

The three plastic shutters which have been placed in front of each cell in the sample holder are actuated with 12 volt dc pull solenoids. These solenoids are controlled by the shutter control on the counter interface board. Figure 4 is a schematic diagram of the shutter control.

The control uses a 100 Hz input signal from a crystal oscillator with an error of no more than 0.1 per cent. Ten pulses from this oscillator are counted in a decade counter which has a chip position of U40 on the circuit board. A list of the electrical components in the interface may be found in Appendix D. U40

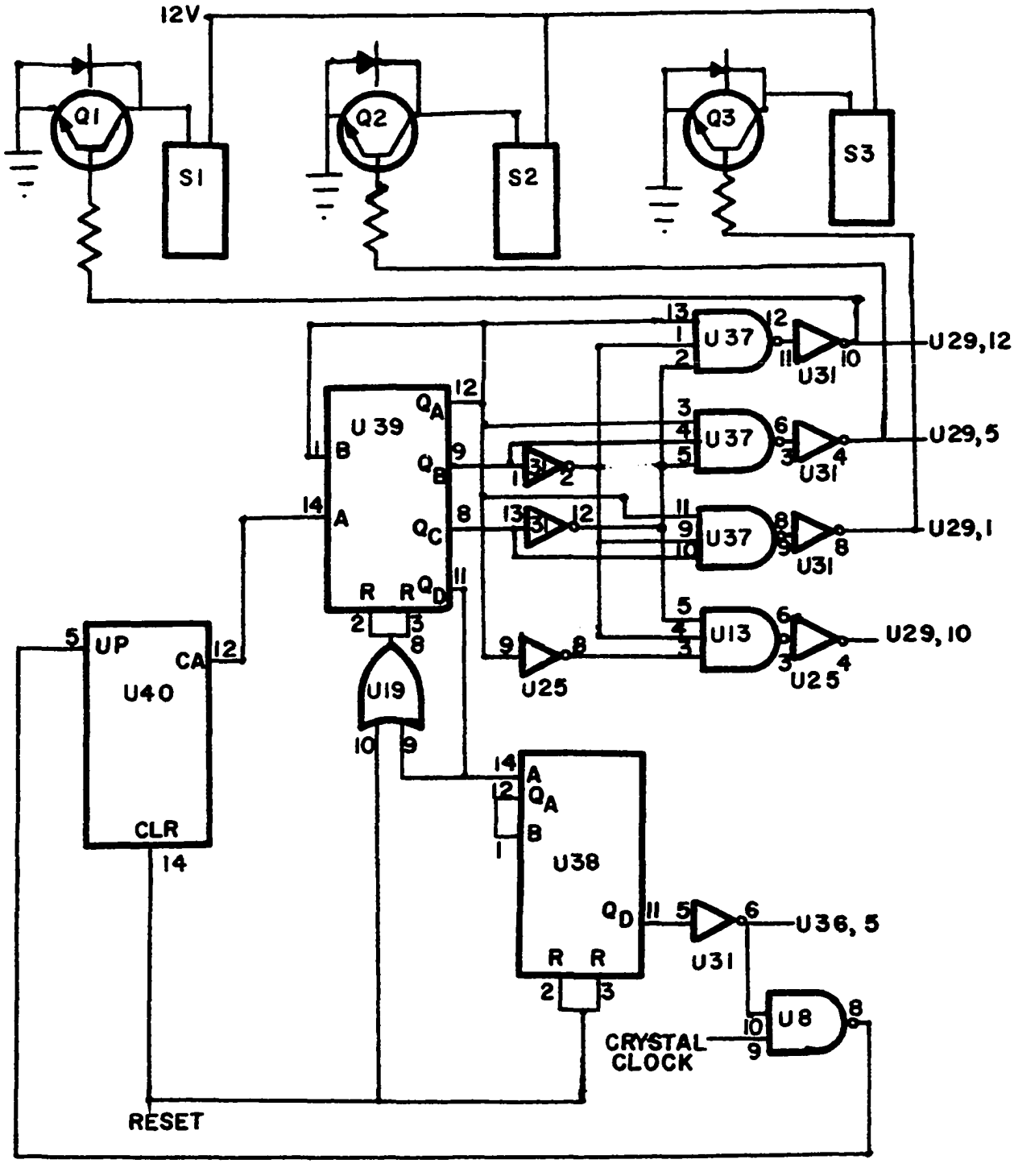

Figure 4. Shutter control schematic.

subsequently outputs a pulse to the shutter control which is itself also another decade counter (U39) and is wired to reset at a count of "8". Ten pulses are counted in U40 in order to average out any variations of the rise time of the oscillator.

When the shutter control counter reaches a count of "1" the first shutter opens for the duration of one count period. When the shutter control reaches a count of "3" and of "5", the second and third shutters open respectively for one count period each. At a count of "7" all the shutters are closed for the dark noise measurement. On the even counts all the shutters are closed to allow the photomultiplier to equilibrate. The counts are decoded in U37 at pins 12, 6, and 8. The decoded counts are inverted at U31 and connected to the base of npn transistors Q1, Q2, and Q3, through a 1 K resistor. These transistors are used as a 12 volt electronic switch to activate the solenoids. The shutter control is also responsible for gating the photon pulses to the appropriate photon counting banks. At a count of "8" the shutter control counter outputs a pulse to the repetition counter, U38. The repetition counter causes the shutter opening sequence to occur eight times. The repetition counter is a decade counter which inhibits the 100 Hz signal (U8 pin 8) and the pulse shaper (U36 pin 5) at a count of "8". Eight was chosen for the ease of design. It stops all counting until it is reset.

Photomultiplier Tube

As previously mentioned, any photomultiplier tube with a sufficiently low dark current at high amplification may be used. The photomultiplier tubes which were available to us for this study are listed in Table II.

While the 6255B tube is superior to the 9558QC, a housing was available for the 9558QC whereas a housing would have had to be constructed for the 6255B. A magnetic lens assembly from EMI is used to reduce the magnetic noise produced by the nearby solenoids.

Table II

Selected Characteristics of Available Photomultipliers

Tube Number	Quantum Efficiency at 540 nm	Dark Current
6255B	10%	1 nA @ 200 A/lm
6217	4%	4 nA @ 20 A/lm
R106 (Hammatsu)	4%	2 nA @ 200 A/lm
R212 (Hammatsu)	4%	2 nA @ 200 A/lm
9558QC (EMI)	10%	2 nA @ 200 A/lm

The calculation of the dynode voltage divider resistances for the 9558QC photomultiplier must first take into account the light flux and the anode current produced by it. If the voltage divider resistors are too small a large dc current will flow

through the dynode voltage divider chain and thus decrease the sensitivity. If the resistances are too large a dc current of the same order of magnitude as the anode current will result and the dynode voltages will become unstable With a high light flux of about 10^8 photons sec^{-1} the anode current for the 9558QC would be about 1.8 uA (15). A dc current of about 100 times greater than the anode current is necessary for the dynode voltage stability (16). The cathode to anode operating voltage of 1200 volts was chosen because it seemed to be an optimal operating voltage for another system (15). This voltage can be varied as long as the maximum recommended voltages are not exceeded in the dynodes. As the voltage is increased the amplitude of the anode current, not to be confused with the dc current, will increase, but there is a point when the signal to noise ratio will no longer increase. This is due to the fact that the noise discrimination voltage is exceeded. The total resistance of the dynode chain becomes 7.5 M with this voltage. The maximum recommended voltage for the cathode to dynode one (D1) is 300 volts. It is general practice to make the value of the cathode-D1 resistor three times larger than the other resistors in the eleven dynode chain. Figure 5 is the schematic for the base configuration for the 9558QC photomultiplier tube. Note that the anode is at a floating ground potential produced by the linear amplifier in the logic section (U42).

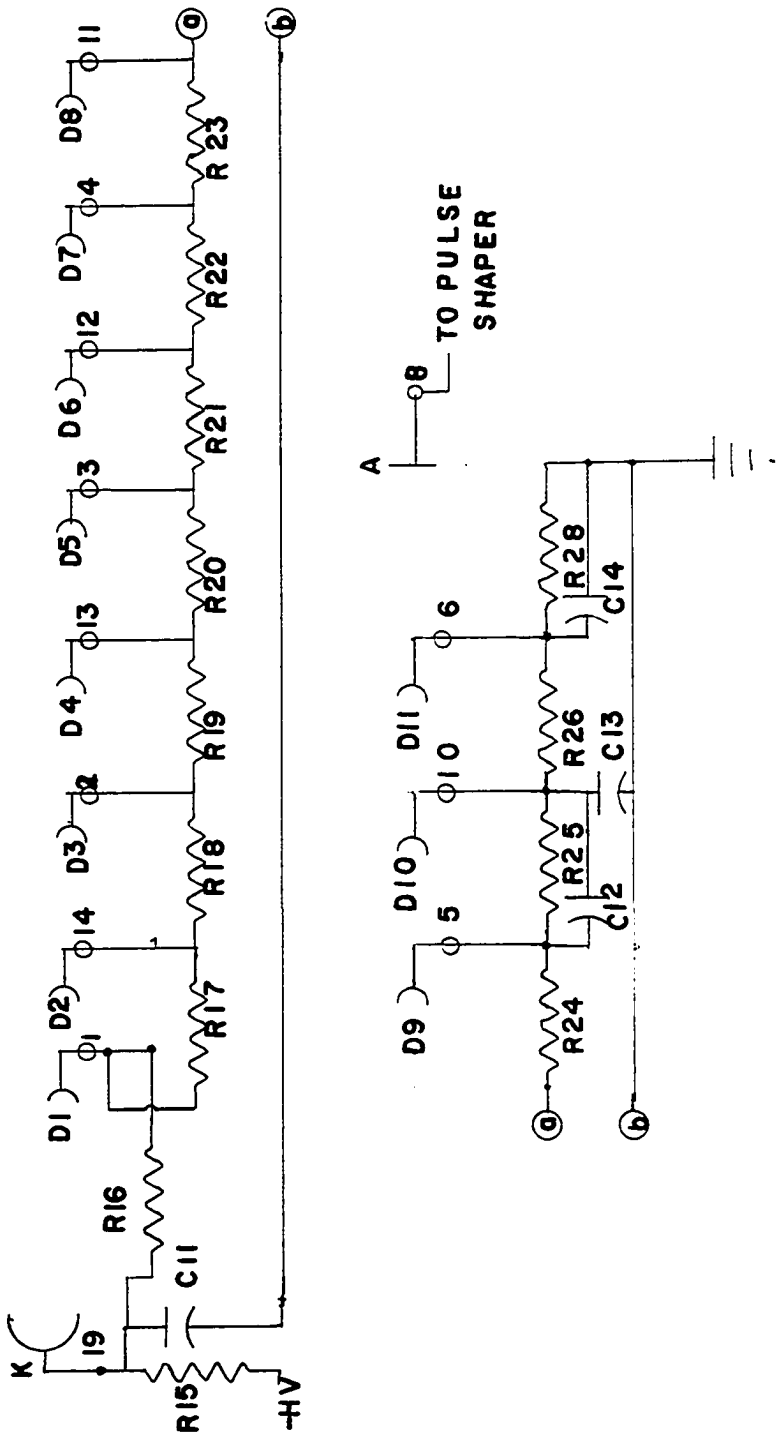

Figure 5. Voltage divider for the dynode chain.

LOGIC SYSTEM DESIGN

Design Guidelines

The logic system must be able to count three banks of counters. These counters should be able to count up for photons and noise as well as being able to count down for dark noise. Each bank must receive the pulses only when its corresponding shutter is open. When the counter cycle is complete it should signal the computer so that the computer can order the transfer of the counts to interface buffer.

Computing Equipment and Peripherals

This project utilized a Pacific Cyber/Metrix (PCM) model 12 computer which was chosen for its relatively inexpensive memory. It has a capacity of 32 K of memory of which only 12 K was filled for this study. Eight K of the memory is volatile while the other 4 K is non-volatile. A parallel input/output buffer whose schematic may be found in Appendix B was constructed for the computer. The PCM-12 emulates the Digital Equipment Corporation's PDP-8 which permits the use of its extensively developed software. A Teletype Corporation's ASR-33 teletype was used to obtain paper tapes and hard copies of program results. A Lear Siegler Adam III Dumb Terminal was used for high speed results without a hard copy. In the current loop mode it has a maximum data transmission speed of 1200 Baud or 120 characters sec^{-1}. A Memodyne 333 Digital Read/Write

Cassette Deck was utilized to record and read the programs into memory at 60 characters sec^{-1} whereas the teletype's paper tape reader reads at only 10 characters sec^{-1}.

Schematics

Decoding system

The decoding system, shown in Figure 6, decodes three input/output (I/O) codes which are given by the octal codes $611x_8$, $613x_8$, and $615x_8$. Combined with an input/output pulse 4 (IOP4), the data are transferred to the interface buffer from the three counter banks. The integrated circuits U1 pin 6, U2 pins 12, 6, and 8 do the octal memory buffer decoding and U8 pins 3, 6, and 11 combine the octal digits to decode the I/O code. An IOP4 with any of these codes denotes a data transfer which necessitates the lowering of the strobe line at the same time data are transferred (U14 pin 11). An I/O code of 6111_8, which is a combination of $611x_8$ and IOP1, checks for the completion of a cycle (U1 pin 2) and lowers the skip line (U14 pin 6).

Counters

The counting system schematic is shown in Figure 7. The integrated circuits used for counting are up/down decade counters each of which outputs a 4 bit binary coded decimal (BCD) number. At a count of ten each counter outputs a carry pulse which can be used at the up-count input of another counter. When the counter

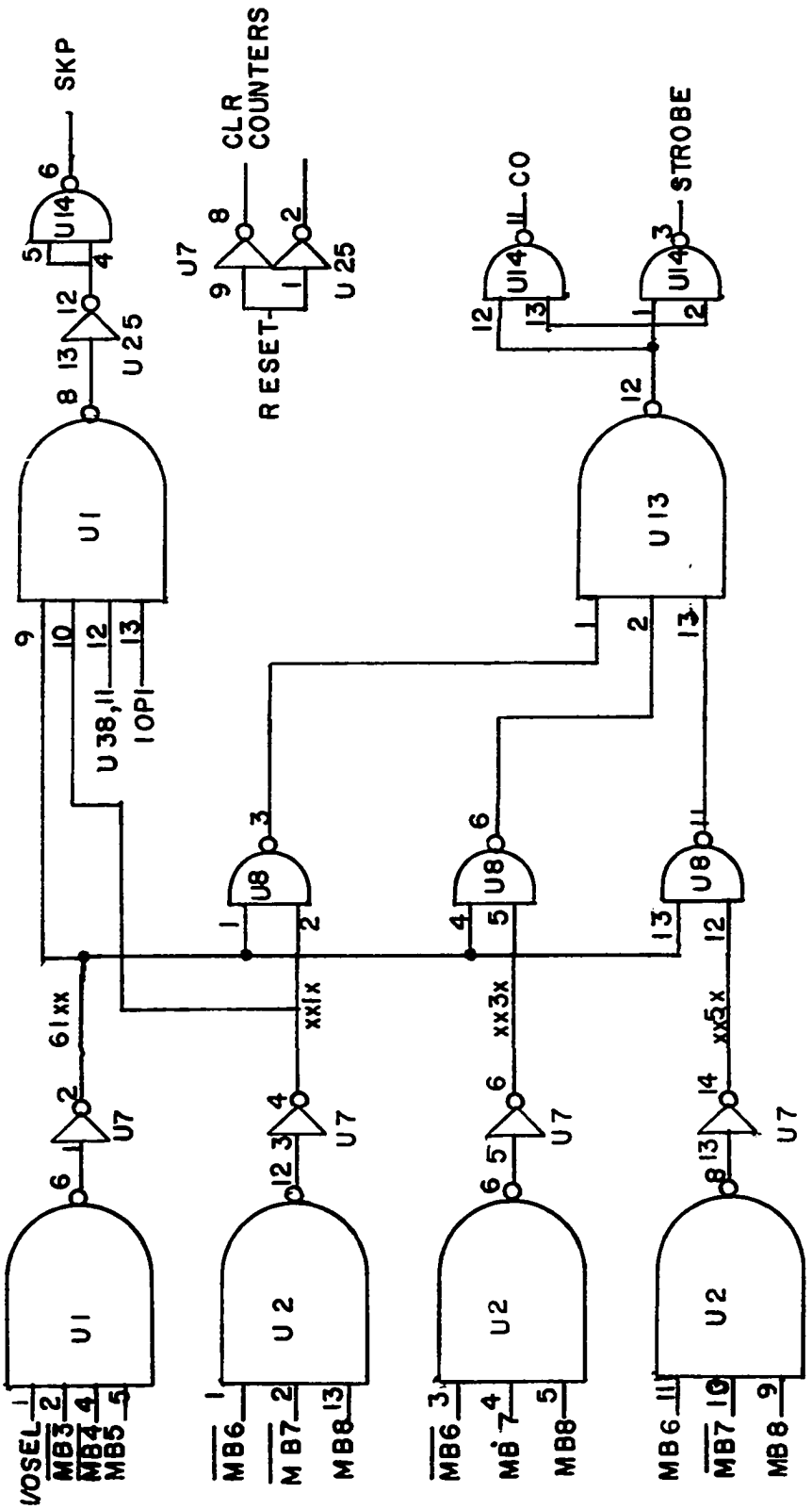

Figure 6. Instruction decoder schematic.

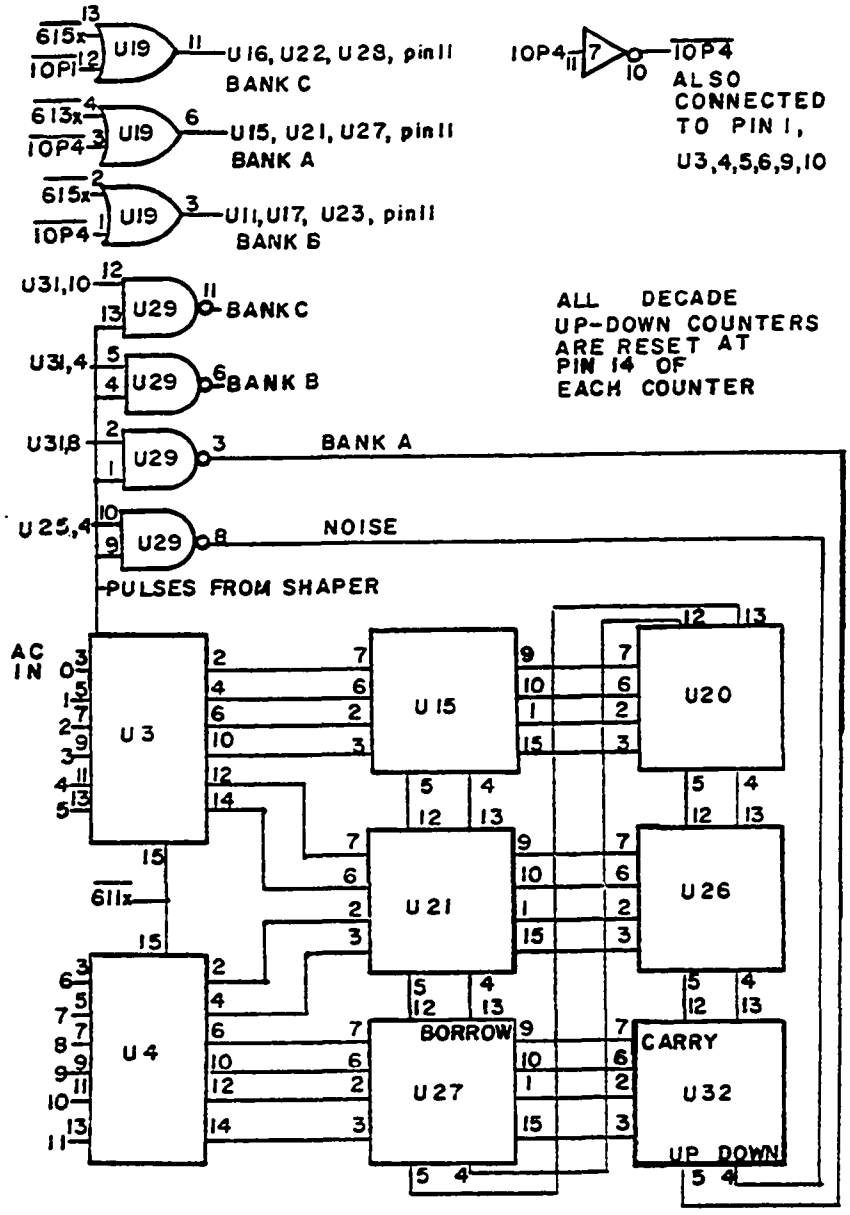

Figure 7. Counting system schematic (continued on page 26).

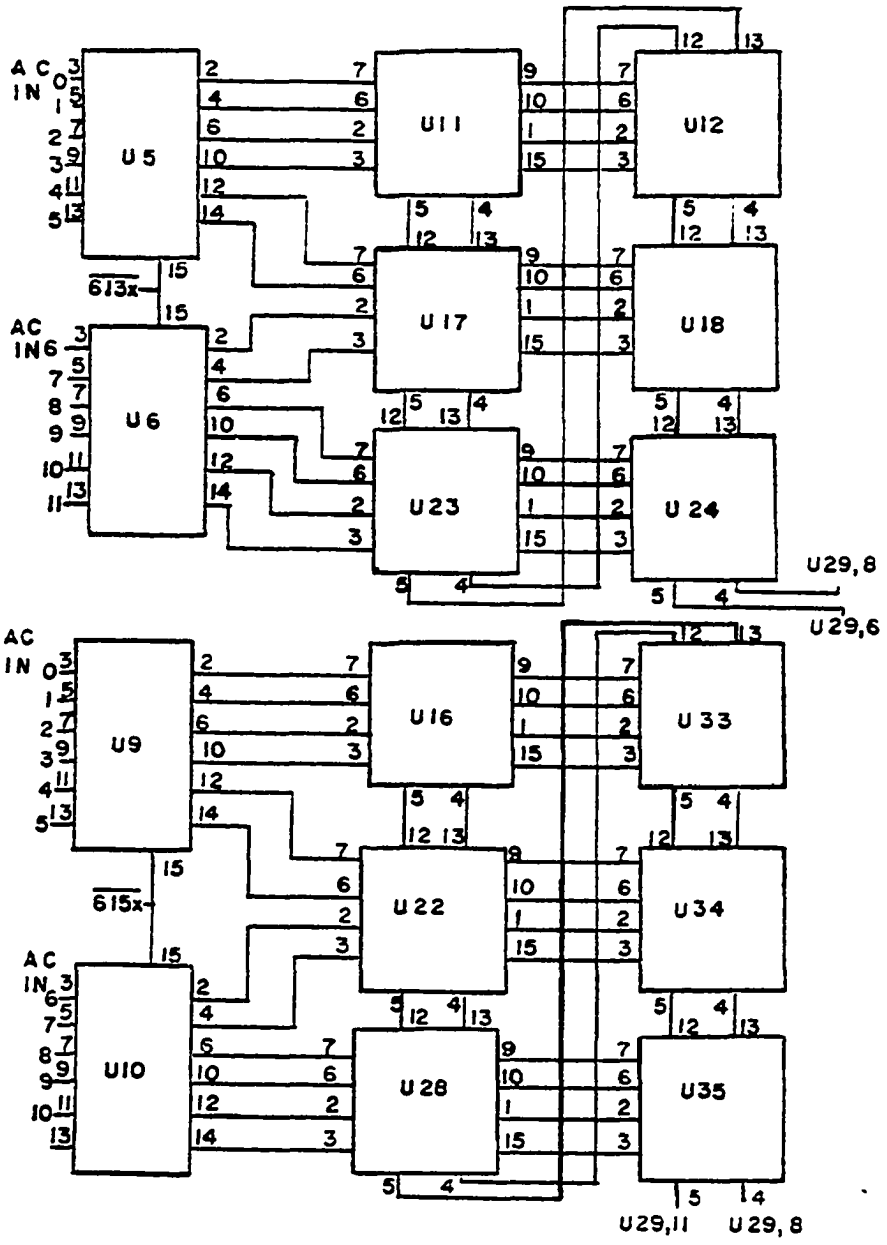

Figure 7. Counting system schematic (continued from page 25).

counts down past zero it outputs a borrow pulse which can be connected to the down-count input of another of these counters. This gives the ability to count up and down when cascading more than one counter. This system cascades six of these counters to give six digits or a maximum count of 999,999 for each of three banks. These counters are found in U11, U12, U14, U15, U16, U17, U18, U20, U21, U22, U23, U24, U26, U27, U28, U33, U34, and U35. The counters receive their up counts when their shutter is open. As mentioned in the shutter section, a shutter is open on the counts of "1", "3", and "5" in the shutter control counter. A gate on U29 is also opened on these counts, gating the photon pulses to the appropriate bank of counters. In each bank of counters the least significant three digits are referred to as the low order digits and the most significant three digits are the high order digits. The outputs of the low order are connected to the inputs of the high order. This allows the high order digits to be transferred to the accumulator after which the low order digits are transferred to the high order counters. The former low order digits which now reside in the high order may then be transferred to the accumulator using the same command as the one which transferred the original high order. This leaves the responsibility of combining the high and low orders to make a six-digit number, to the software or program. The high order bank B transfer command, 6134_8, will load the low order bank A into the high order bank A. The same is true for the bank C transfer command,

6154, loading the low order bank B into the high order. This necessitates the loading of the banks into the accumulator in the order of A, B, and C. Bank C must be transferred with a special command, 6151_8, because the 6114_8, "transfer bank A" command would cause the data in the low order to be transferred before the high order bank C was read by the accumulator. The down-count input pulses are gated by U29 pin 9 which opens when all the shutters are closed at a shutter count of "7".

Each bank of counters uses two hex Tri State drivers to transfer the data to the interface buffer (U3, U4, U5, U6, U9, U10). This transfer takes place when the IOP4 appears at pin 1 and the respective I/O code at pin 15.

Pulse shaper

The schematic for the pulse shaper shown in Figure 8 was designed by Mickey and co-workers (17). The pulses coming from the photomultiplier are negative in value and small. A negative feedback, positive input operational amplifier can be used (U42) giving a gain of about 100. A voltage comparator, U41, can be used to discriminate against the lower non-photon produced noise. When a negative pulse which is more negative than the discrimination voltage reaches the input, the output will drop to ground potential causing a pulse to be produced at the output of the monostable vibrator U36. This five volt pulse then travels to the pulse gates, U29, which gates the pulses to the proper bank. Pin 5 of U36 inhibits the pulses from leaving the pulse shaper. This ensures

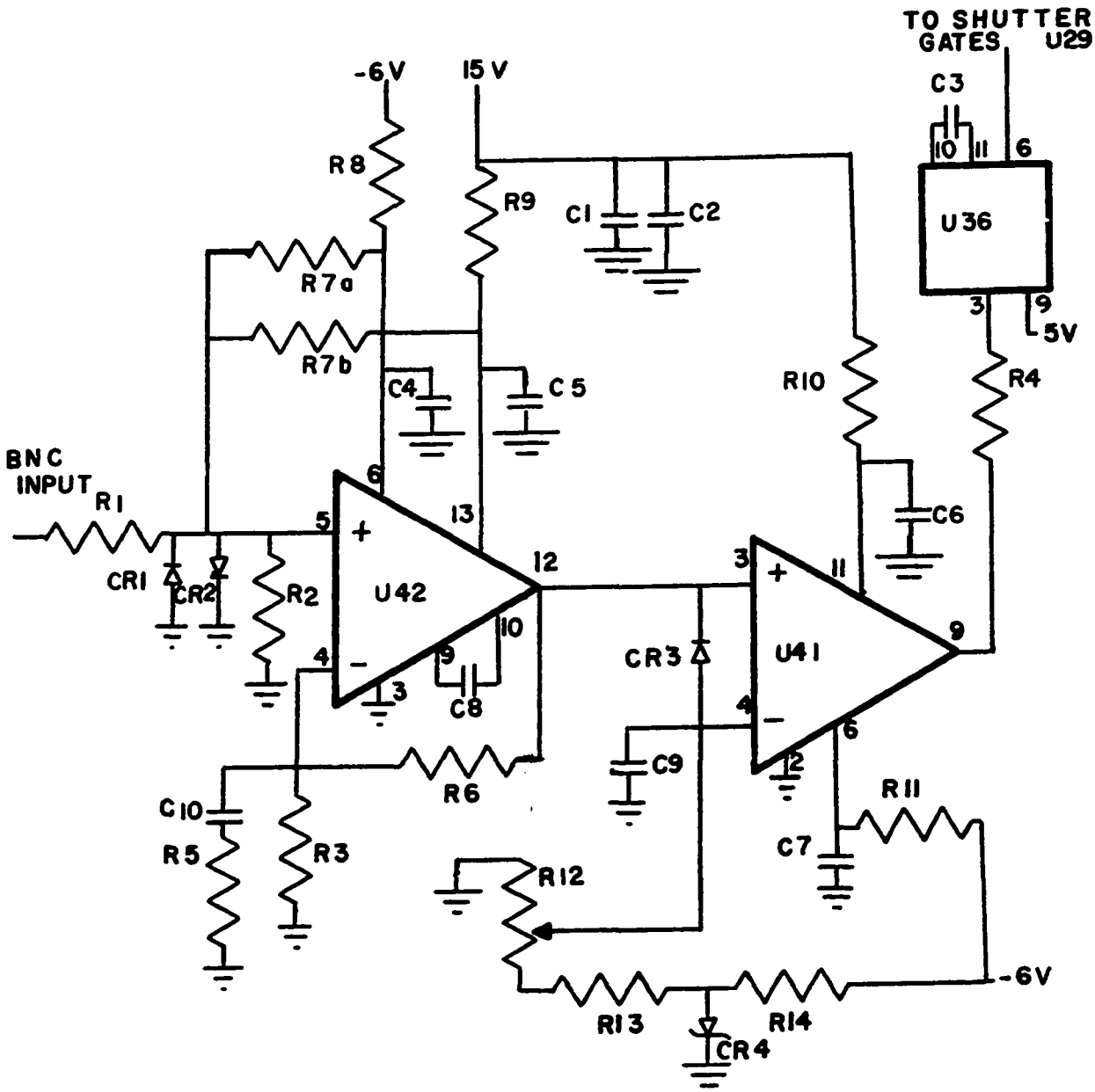

Figure 8. Pulse shaping schematic.

that when the pulse gates are closed, the pulse shaper will not allow noise to pass into the logic system.

SOFTWARE SYSTEM DESIGN

Design Guidelines

The system must be able to read in the rate constants from a terminal. After these have been well established from extensive rate constant studies they may become permanent constants in the software. It is necessary to read in the high and low order words from the three banks of counters and store them in memory. Since each bank requires two words, a total of six storage locations in memory are required. The software must retrieve each of these stored words from memory and convert them from binary coded decimal (BCD) to some usable form of the six-digit number which will be used in the arithmetic software.

When the count cycle is complete it will signal the computer to begin the computations. The calculations will first compute the MM, the MB, and finally the BB isoenzyme concentrations. The per cent of the total concentration of CPK will then be calculated.

Software Utilized

Focal, Fortran, and PAL III are the software languages available for this project. Focal and Fortran both require an extensive amount of time for assembly, however the Computer Science Department at Western Michigan University has a PAL10 cross-assembler which is much faster. This also allows the programs to be edited in TECO rather than using the Symbolic

Editor for the PDP-8. PAL III also has available a 23 Bit Floating Point Routine (DEC-08-YQ1B-PB). All the above languages are available from the Digital Equipment Corporation.

Software Flow Chart

The flow chart in Figure 9 shows the flow of the basic software. If the rate constants become part of the system's constants, the "rate constant input" operation may be deleted. The "rate constant input" operation must be saved, however, if any of the linear electrical components are replaced since the sensitivity of the system may be altered, thus changing the constants.

Implementation

The program listing may be found in Appendix E.

Initially the software reads the constants into the three word locations named KMML, KMBL, KBBL, KMMM, KMBM, KBBM, KMMH, KMBH, and KBBH. These locations are named for the rate constant K of the particular isoenzyme at the low (L), medium (M), or high (H) substrate concentrations. Then a check for cycle completion with a DSF (6111) instruction is carried out. When the cycle is complete the BCD counts are loaded into the high and low orders of the banks. Subsequently the counts are converted for output to the floating point routine which would normally accept ASCII numbers from a terminal. The floating point patch for this is a simple jump to a subroutine which will retrieve the ASCII numbers stored by the "count read in section." The floating point

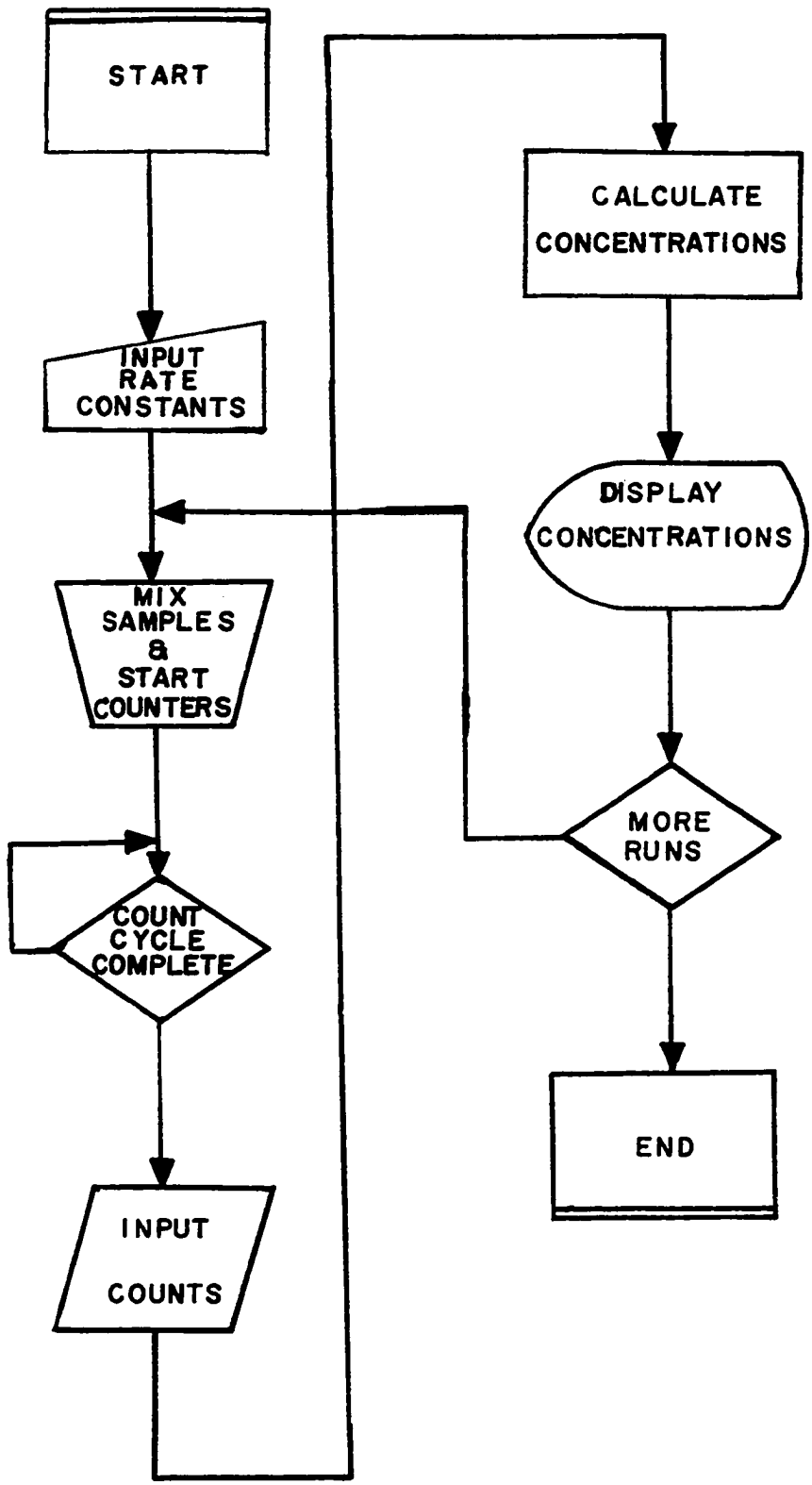

Figure 9. Flow chart for CPK program.

routine converts these values to its standard format and stores them in the locations AL, AM, and AH. "A" is used to denote the activity of the CPK while the remaining letters denote the substrate concentrations.

The enzyme concentrations are then calculated using the following equations:

$$\frac{AH*E + N*KMBL - N*F - AL*G}{E*KMMH + P*F - G*KMML - P*KMBL} = MM \qquad (7)$$

$$\frac{N + P*MM}{D} = MB \qquad (8)$$

$$\frac{AL - KMML*MM - KMBL*MB}{KBBL} = BB \qquad (9)$$

where

$$D = KMBM*KBBL - KBBM*KMBL$$

$$N = AM*KBBL - KBBM*AL$$

$$P = KBBM*KMML - KMMM*KBBL$$

$$E = D*KBBL$$

$$F = KMBL*KBBL$$

$$G = D*KBBH\ .$$

Subsequently the per cent enzyme concentration can be found from which one might deduce that damage to the myocardium has occurred.

DISCUSSION

Reproducibility

The instrument was tested at various discrimination voltages. Since the operational amplifier (U42) has a gain of about 100, the theoretical voltage pulse from the photomultiplier will be approximately 0.17 volts photon^{-1}. The test voltages were set at -0.15, -0.20, -0.25, -0.30, -0.35, -0.40, and -0.45 volts. These values provided a range of discrimination just sufficient for partial noise counting to a level at which only coincidence counts can be measured.

The photon source was comprised of a lamp, Chicago Miniature/ Drake no. 222, powered by four parallel "D" cells. This lamp was chosen for its relatively low light level and its smallness which facilitated its accomodation in the sample holder. A 540 nm glass filter was used to remove stray light in conjunction with an EMI magnetic filter for the removal of magnetic noise.

A 10 Hz signal was input to the shutter control which successively opens each shutter for a period of one second eight times. Hysteresis of the magnetic fields of the solenoids was found to occur, causing erratic behavior of the shutter and thus having a deleterious effect on the precision of the data. Table III lists the results of the reproducibility tests in terms of the coefficients of variation. Although not apparent from the data in the table, the smallest coefficient of variation should occur at a

Table III

Coefficient of Variation at Various Discrimination Voltages for Each Cell

Discrimination Voltage	Cell 1		Cell 2		Cell 3	
	Avg Count(*)	C V	Avg Count(*)	C V	Avg Count(*)	C V
-0.15	4838(6)	6%	12593(13)	5%	38941(8)	3%
-0.20	444142(10)	3%	15454(12)	4%	12068(16)	4%
-0.25	101756(19)	3%	38394(11)	2%	7109(19)	3%
-0.30	459(4)	3%	24359(26)	5%	45160(11)	3%
-0.35	59155(12)	3%	12946(25)	3%	2646(15)	3%
-0.40	45670(12)	3%	6390(11)	3%	16459(15)	3%
-0.45	35637(10)	4%	123834(23)	5%	8915(8)	4%

*Number of determinations

Count period is 10 seconds

discrimination level where a majority of the noise has been removed but just below the value at which coincidence counts would be measured.

Recommendations

Hysteresis

The system has a severe problem with hysteresis in the solenoid, causing inconsistent sample exposure periods. The magnetic fields do not collapse immediately after the solenoids have been deactivated, thus holding the shutters open until a vibration occurs. A vibration usually will occur as a result of the shutter opening for the adjacent cell producing a time period during which both shutters are open. Normally a dark period occurs between shutter openings to allow the photomultiplier to equilibrate. In order to minimize this problem a small wire was placed in the center hole of each solenoid to prevent the solenoid pull rod from completely entering the field. A spring loaded shutter might be used to give a more reproducible stroke of the pull rod. A motorized mirror system might produce even better reproducibility.

Coincidence Counts

The problem of more than one photon striking the cathode simultaneously, a coincidence count, causes a pulse pile up error. This problem can be minimized by using a second set of counters, coincidence counters, with another comparator set at twice the first

discrimination voltage. Only a couple of counters for each bank would be required to count the coincidence counts since the probability of these counts is generally low. These counts could then be added to the first count with either hardware or software techniques. Also, a photomultiplier tube with a faster response time could be incorporated in the system to minimize errors arising from coincidence counts. Errors arising from high energy radiation such as cosmic, gama, and x-rays which cause extremely high voltage pulses could be gated out by using an even higher discrimination voltage than that used for coincidence counts.

Use with chemicals

The reset line of the counters is connected to the reset line on the interface buffer causing initialization of the counters with a "clear all flags" (CAF, 6007_8) command. This reset line should be removed from the buffer and connected to a micro-switch on the syringe plungers to permit initialization when the reaction mixture has been mixed. The "skip on complete count cycle" (DSF, 6111_8) command will be executed even before the plunger has been pushed since the flag for this command remains high until the initialization of the counters. This problem can be overcome by using a second micro-switch on the syringe mechanism which will disconnect this flag until the plungers have been pushed.

Conclusion

The optimization of the chemical reactions has been left for further study. The optics could be improved with the incorporation of more reproducible shutter timing. Problems arising from the angle of the beams entering the glass filter, variations of cell walls, and exceeding the count rate of the pulse shaper, which is about 10^6 counts sec^{-1}, could be handled by the beam attenuator. A slight drift in the operational amplifer (U42) has been observed. The output of this amplifier must be maintained at zero volts dc in order to obtain reproducible results. With the minimization of coincidence counts and the shutter error, the coefficient of variation will probably be less than one per cent.

APPENDIX A

Pacific Cyber/Metrix Model 12 Block Diagram

The PCM-12 is a micro-processor based PDP-8E computer. As its central processing unit it employs an Intersil 6100 micro-processor. This particular unit has a 3.3 M Hz crystal oscillator for its internal clock. The system has a capacity of 32 K words of memory divided into eight fields. In order to utilize more than one field it is necessary to have a memory extender which provides access to the proper field. The computer currently has only two fields of static memory and one field of non-volatile memory all of which are solid state. The control panel, which is connected to the central processing unit, provides manual control of all operations. Also included are 512 words of "read only memory" to execute the manual controls.

The teletype interface makes use of an universal asynchronous receiver-transmitter for the conversion of serial data to parallel data. This interface has both RS-232 and current loop data transmission capabilities. In these studies the 20 mA current loop transmission was utilized. This interface has switch-programmable baud rates of 110, 150, 300, 600, 1200, 2400, 4800, and 9600.

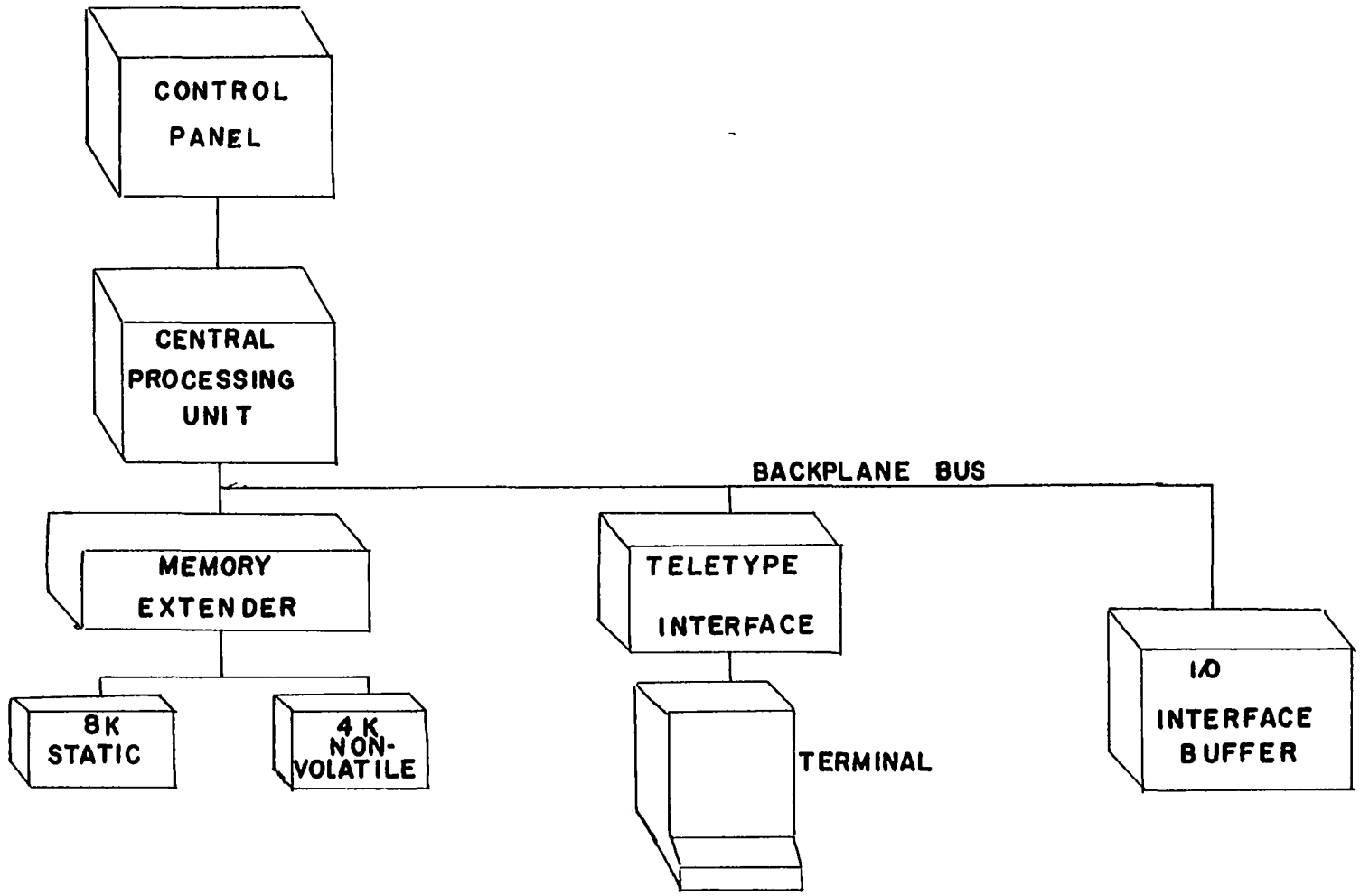

Figure 10. PCM/12 block diagram.

APPENDIX B

Parallel I/O Interface

All the signals coming from and going to the CPU are protected by 74LS04 inverters or 74LS00 NAND gates. For better protection, however, space has been provided for opto-isolators. Time did not permit their addition to the circuit. Like the CPU card (12010) in the PCM-12, the data lines are controlled and protected by the newly developed 8833 Tranceivers by National Semiconductor. They are "third stated" when not transmitting in either direction.

The memory buffer register (MBR) is first driven from the tranceivers. The receive side, which transmits to the CPU, is disabled by the XTB* (U8 pin 4). The transmitter side is enabled by the XTB* (U8 pin 11) and either MEMSEL or CPSEL (U24 pin 3). MB 0 - MB 2 are decoded by U9 pin 12 and latched by both U12 pin 13 and U13 pin 3. This decoded $6xxx_8$ is cleared from U12 after the first I/O cycle and U13 remains unchanged until the next instruction is latched. This aids in I/O pulse timing. MB 3 is on a 7474 (U13 pin 12) for convenience of space. Using the $6xxx_8$ signal from U12, the I/O pulses for IOP1 and IOP2 are simultaneously generated only during the first I/O cycle when MB 11 or MB 10 are high respectively. Each is approximately 580 ns. U9 pin 6 and pin 8 control the timing position of IOP4 so that it appears in the first I/O cycle for a read and the second I/O cycle for a write.

IOP4 can be held in the second cycle by a yet to be installed 8L or 8E switch located on the interface buffer box.

The accumulator input register (AC IN) is first activated to latch incoming data from a device by a low strobe pulse. The CPU will call for the data by opening the drivers, U22 and U23 (8095), coupled with the fact that the strobe has been pulsed, DEVSEL* (U11 pin 8) and XTB* (U8 pin 6). This same combination will cause the receiver side of the tranceiver to transmit to the CPU.

The PCM-12 has the capability of controlling the Program Count Register (PC) with control lines programmed at the first DEVSEL pulse using the AC IN lines to transmit the new program count. This is useful for device handling routines.

Another switch, yet to be installed, is the DMAGNT inhibit switch. The line is now inhibited. When it is connected, the buffer can use the Direct Memory Access (DMA) capabilities of the PCM-12.

The accumulator out register (AC OUT) is latched on each DEVSEL pulse in the I/O cycle. Care must be taken to latch the information in the external interface with a second cycle IOP4 pulse or something similar.

The skip line (SKP) is set by a low pulse to the skip flip-flop, U12, and cleared later in the cycle. C0 is also set with a flip-flop since this is the most used control line. C1 and C2 will have to be controlled by the user in the 8E mode and set, if necessary, on the first DEVSEL pulse of the I/O cycle.

All input to the I/O board for signals are transmitted with an open collector device. The current limiting resistors are 2.2 K.

Limitations of the Parallel I/O Interface

1. In the 8L mode bit 8 (MB 8) must be set to write into the accumulator which causes either a first cycle or a second cycle IOP4 respectively to be generated.
2. The following input lines must be transmitted to the Parallel I/O board with open collector circuits using current limiting resistors of 2.2 K ohms: SKP, INTREQ, DMAREQ, WAIT, STROBE, and Control.
3. The accumulator input will not operate without the strobe pulse.
4. The skip line can only be asserted on IOP1 and IOP2.
5. Reads can only be done with IOP4 or after the second DEVSEL.
6. C1 must be manually lowered for $6(x1x)_2xx_8$ or $60xx_8$, even in 8L mode.
7. The DMA cannot be used when other DMA devices are connected.

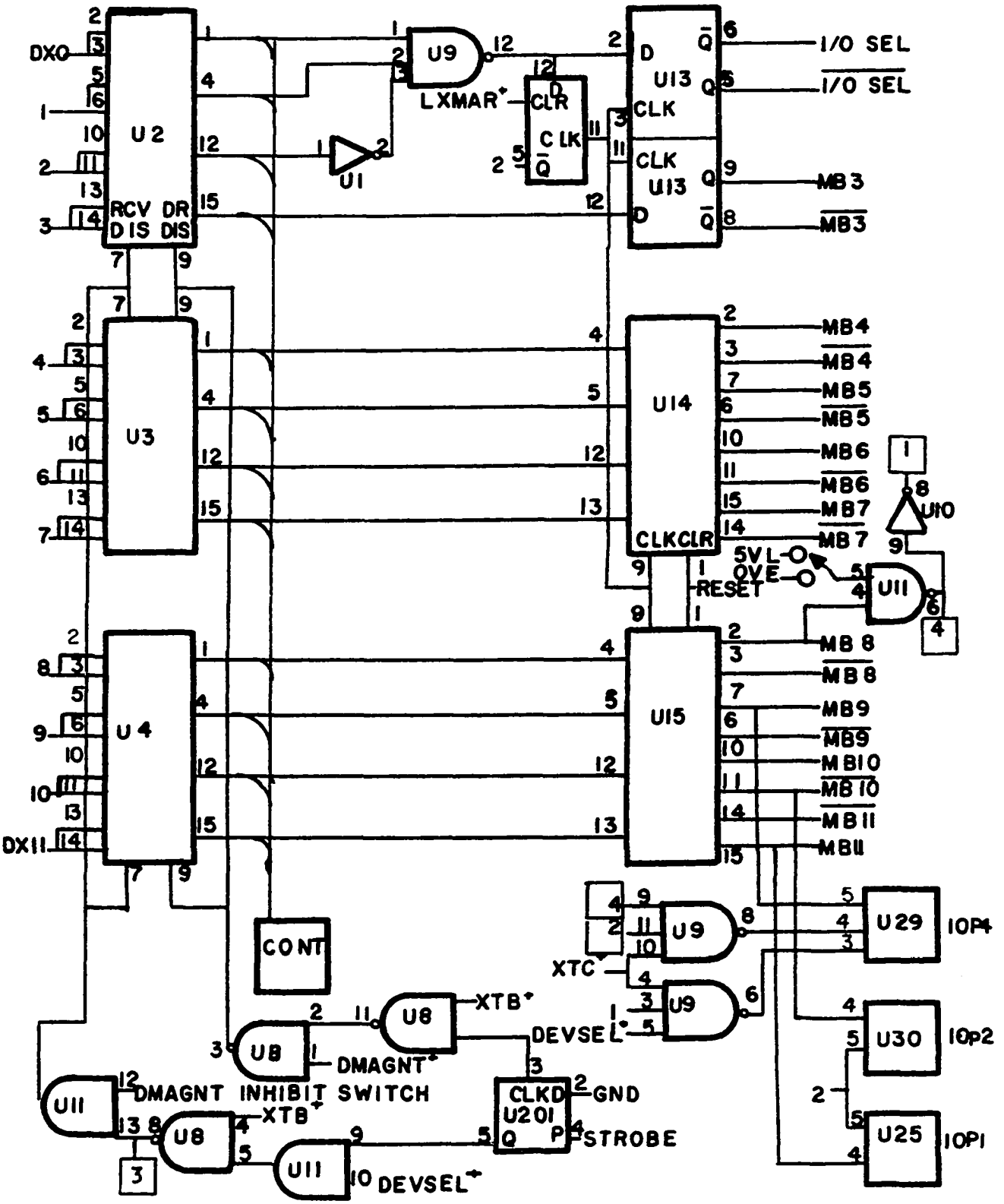

Figure 11. Parallel I/O interface schematic (continued on page 46).

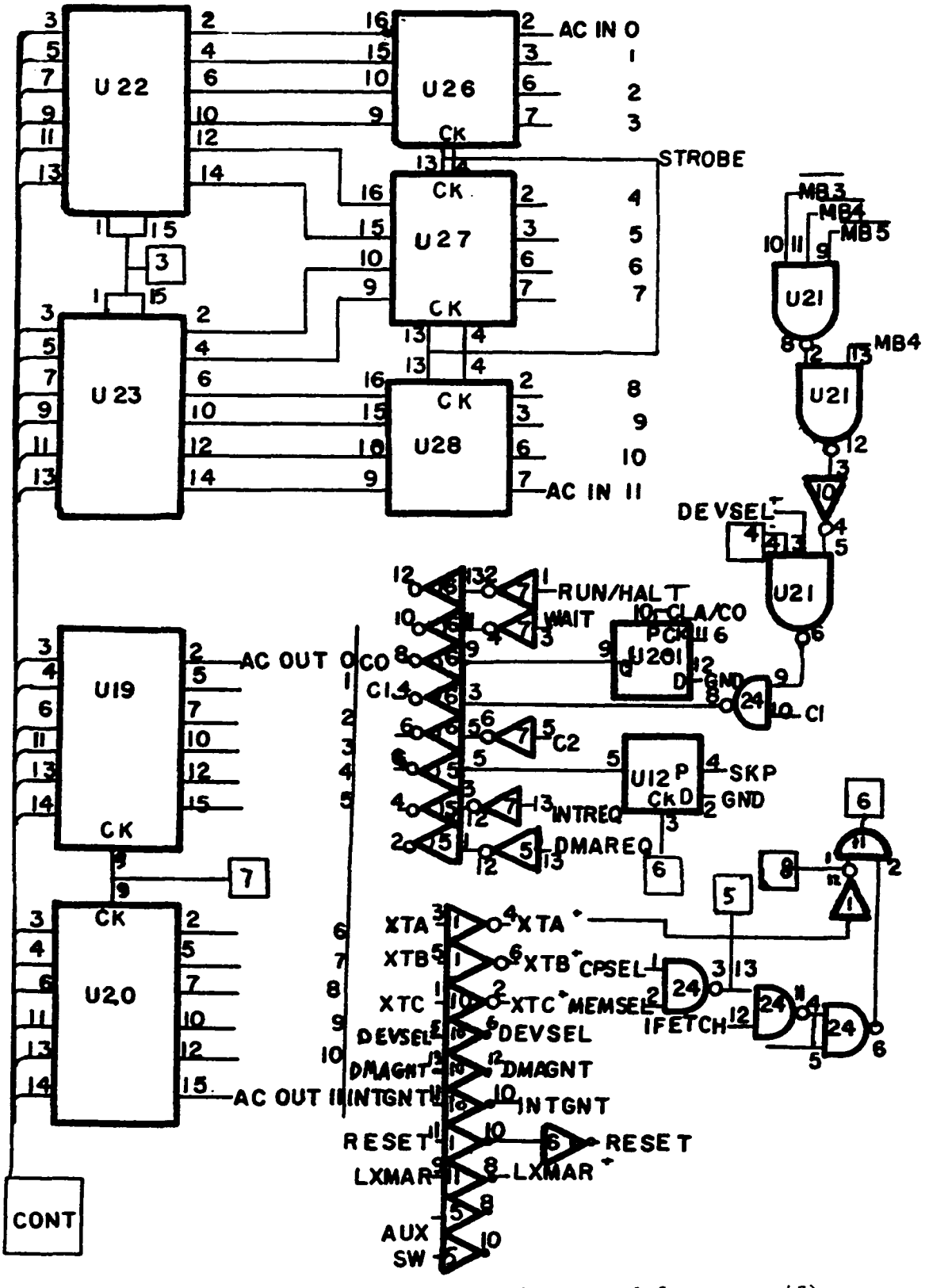

Figure 11. Parallel I/O interface schematic (continued from page 45).

APPENDIX C

Interface of the Memodyne 333 Digital Cassette Reader and Recorder

Introduction

The Digital Equipment Corporation has a complete instruction set for a cassette deck, however the only software on hand was designed for use with a high speed or a low speed paper tape reader. Therefore, the cassette deck interface utilizes the high speed paper tape reader commands. In order to save space and extra integrated circuits, the three octal command codes were incorporated on one interface board. The command codes are $601x_8$ for the Read Mode, $602x_8$ for the Write Mode, and $607x_8$ for for the Error and Reverse Modes.

Read circuitry

The $601x_8$ is decoded on U7 pin 8. Both a "read reader buffer and clear flag" (RRB, 6012_8) and a "clear flag and buffer and fetch character" (RFC, 6014_8) cause the reader flag to be cleared (U16 pin 9). The RFC will subsequently set the reader flag by means of the status line raising the clock input after the tape has advanced. The RFC also causes the monostable multivibrator to fire (U19) transmitting a start pulse to the cassette deck. The read/write/backspace lines to the cassette deck are controlled by the flip-flop, U20. A $601x_8$ lowers the data input to this flip-

flop and is clocked in by the start pulse. An RRB command will strobe the drivers (U4 and U5), connected to the reader buffer, causing the data to be transmitted to the accumulator input register. The "skip if reader flag is set" command (RSF, 6011_8) checks the reader flag and holds the skip line low (U14 pin 8) if it is set.

The interrupt system is enabled on the interface or disabled with the NAND gate flip-flop at U13. A "disable interrupt" command (6020_8) will clear the flag and an "enable interrupt" command (6010_8) will set the flag. When either the read or write flag is set and the interrupt is enabled, an interrupt will occur.

Write circuitry

The $602x_8$ is decoded by U9 pin 4. A "clear write flag" (PCF, 6022_8) will clear the write flag, U16 pin 5, and the status line from the cassette deck will raise the flag after the tape has finished advancing from a "load buffer and write character" (PPC, 6024) command. The PPC command sends a start pulse from the monostable multivibrator, U19. This pulse activates the read/write/backspace flip-flop, U20, which raises these lines to the write mode. A "skip if write flag is set" command (PSF, 6021) checks the write flag for its status. The skip circuitry inhibits the IOP1 pulse if both MB 10 and MB 11 signals are high indicating a load forward command.

Load forward circuitry

The Memodyne 333 cassette deck has a special command, in the read mode, causing it read tape continuously until an unrecorded portion of the tape is found. In the write mode it simply erases a small gap in the tape. This is helpful for searching for data quickly and aligning the tape just before it is read. A 6013_8 causes a "read load forward" and a 6023_8 causes a "write tape gap" command. The read/write/backspace flip-flop, U20, is changed with an IOP1 when a $60x3_8$ appears in the memory buffer register.

Error and reverse mode circuitry

These tape errors are detected by interrupts such as: file protection, head not down, cassette not in place, end of tape, beginning of tape, and parity. If the computer's interrupt system is turned off these errors will probably go undetected. Parity has not yet been connected since the need has not arisen. These errors may be passed through U21 pin 8, however a 6071 command will check the errors and cause the computer to skip.

The Memodyne 333 has a stepper motor which has not been designed to step backward. Nevertheless, the "backspace" command (6074) should cause the cassette deck to step backward but, unfortunately, as a result of inadequate voltages on the motor, this command is unreliable.

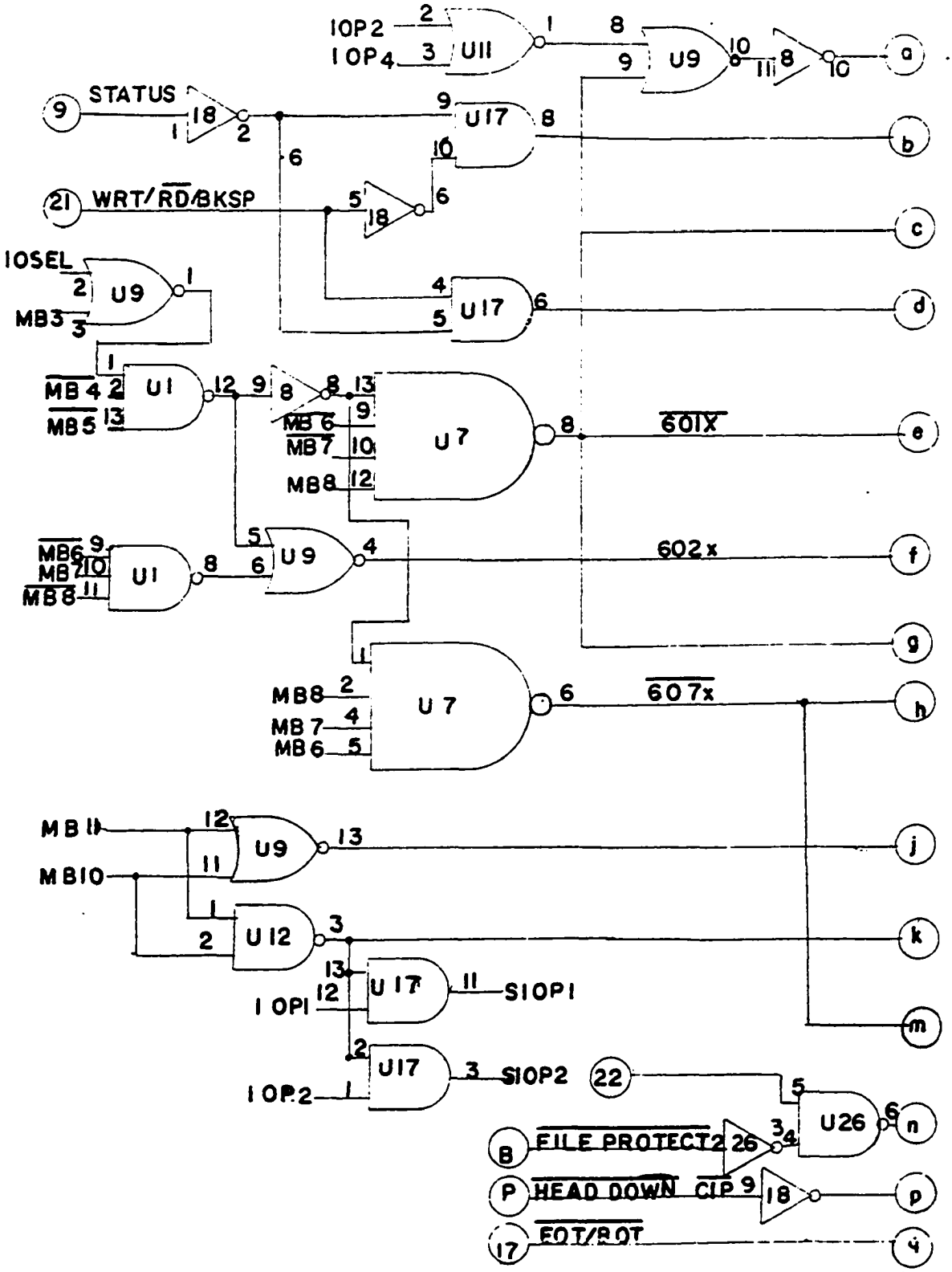

Figure 12. Digital cassette interface schematic
(continued on pages 51 and 52).

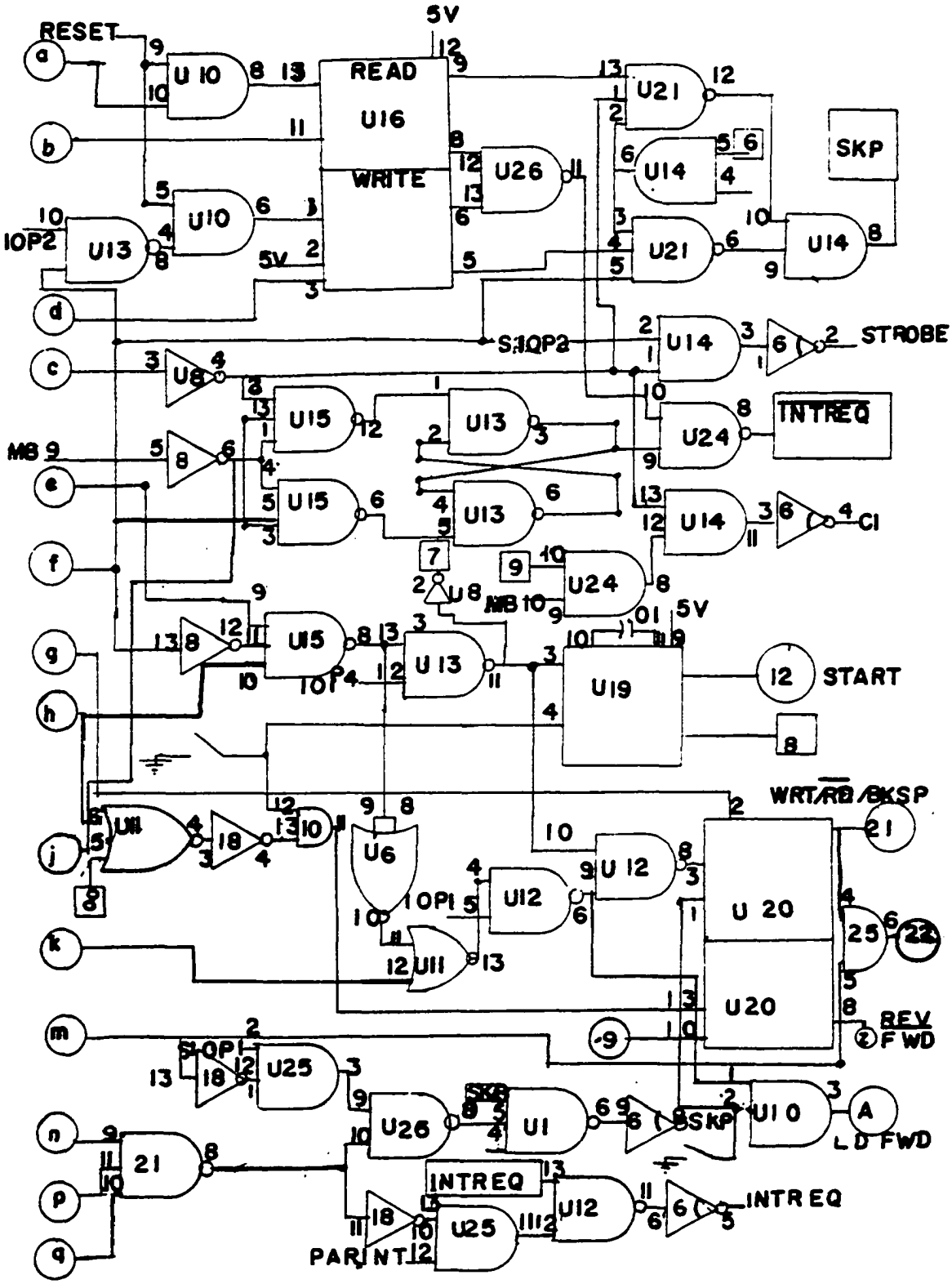

Figure 12. Digital cassette interface schematic
(continued on pages 50 and 52).

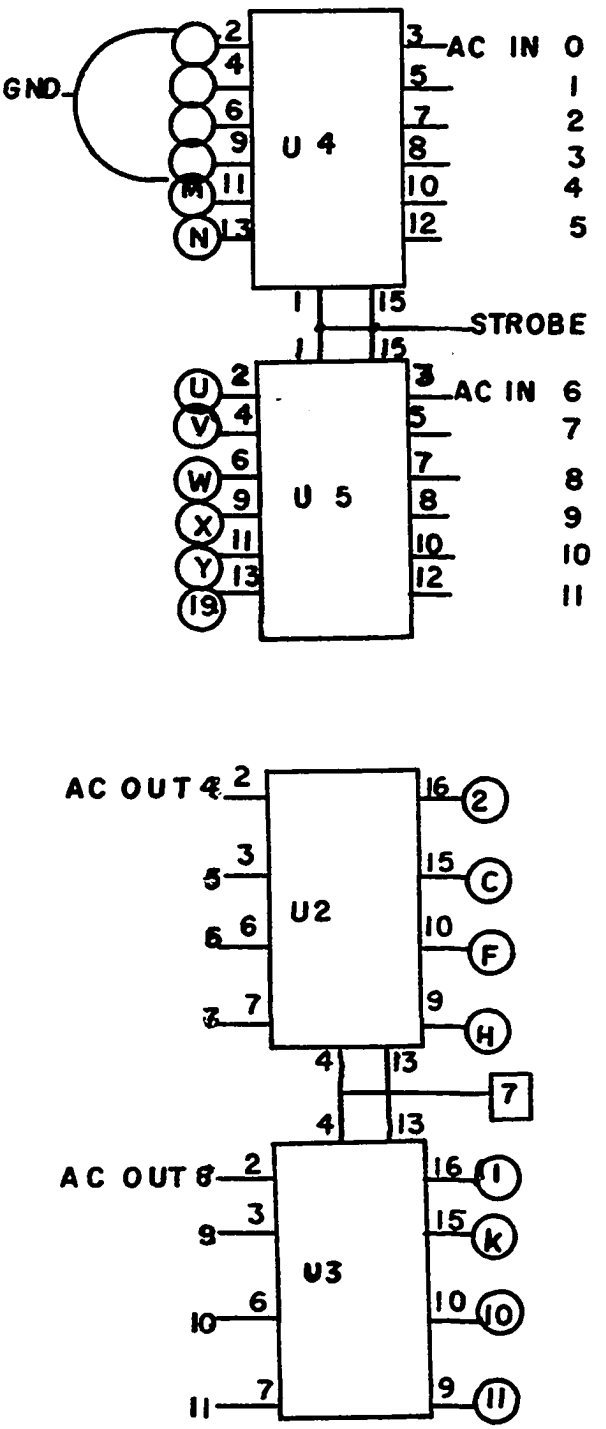

Figure 12. Digital cassette interface schematic (continued from pages 50 and 51).

APPENDIX D

Parts Lists for Circuit Schematic Figures

Figure 4. Shutter Control.

Resistors		
R29-R31	1K	(Ohmite)
Transistors		
Q1-Q3	SK3024	(RCA)
Diodes		
CR5-CR7	420E	(Western Electric)
Integrated Circuits		
U8	DM7400	NAND (National)
U13, U37	DM7410	Three Input NAND (National)
U19	DM7432	OR (National)
U25, U31	DM7404	Hex Inverter (National)
U38, U39	DM7490	Decade Counter (National)
U40	DM74192	Up-Down Decade Counter (National)
Solenoids		
S1-S3	T6X12	12VDC continuous (Guardian Electric)

Figure 5. Voltage Divider.

Resistors

R15	450 K	10 turn potentiometer (Stackpole)
R16	1.2 M	(Ohmite)
R17-R28	470 K	(Ohmite)

Capacitors

C11-C13J	0.01 μf 3 Kv	(Sprague)
C14	0.01 μf 600 v	(Sprague)

Figure 6. Decoder.

Integrated Circuits

U1	DM7420	4 Input NAND (National)
U2, U13	DM7410	3 Input NAND (National)
U7, U25	DM7404	Hex Inverter (National)
U8, U14	DM7400	NAND (National)

Figure 7. Counting System.

Integrated Circuits

U3-U6, U9, U10	DM74365	Tri-state Hex Driver (National)
U7	DM7404	Hex Inverter (National)
U19	DM7432	OR (National)
U29	DM7400	NAND (National)
U11, U12, U15-U18	DM74192	Up-Down Decade Counter (National)
U20-U24, U26-U28	DM74192	Up-Down Decade Counter (National)
U32-U35	DM74192	Up-Down Decade Counter (National)

Figure 8. Pulse Shaper.

Resistors

R1	100 ohms	(Ohmite)
R2-R4	1 K	(Ohmite)
R5	300 ohms	(Ohmite)
R6	100 K	(Ohmite)
R7	2.8 K	trim potentiometer (Stackpole)
R8-R9	680 ohms	(Ohmite)
R10, R11	68 ohms	(Ohmite)
R12	1 K	10 turn potentiometer (Stackpole)
R13	12 K	(Ohmite)
R14	82 ohms	(Ohmite)

Diodes

CR1-CR3	420E	(Western Electric)
CR4	SK3333 4.7 v	Zener Diode (RCA)

Capacitors

C1	22 μf	(Sprague)
C2, C4-C7	0.01 μf	(Sprague)
C3	22 pf	(Sprague)
C8	47 pf	(Sprague)
C9	0.05 μf	(Sprague)
C10	1000 pf	(Sprague)

Figure 8. Pulse Shaper. (continued)

Integrated Circuits

U36	DM74121	Monostable Multivibrator (National)
U41	μA 710 c	Comparator (Fairchild)
U42	MC 1712 L	Operational Amplifier (Motorola)

APPENDIX E

CPK.PAL Program

```
          DSF=6111          /SKIP ON COMPLETE COUNT CYCLE
          DBA=6114          /DUMP BANK                A
          DBB=6134          /DUMP BANK B & LOAD LOW ORDER INTO HIGH
                            /ORDER BANK B
          DBC=6154          /DUMP BANK C & LOAD LOW ORDER INTO HIGH
                            /ORDER BANK B
          DLC=6151          /LOAD LOW ORDER INTO HIGH ORDER BANK C


          *5
0005  7400          7400
0006  7200          7200
0007  5600          5600


          *177
0177    6046        TLS
          *200
0200  4606          JMS I   AINKS   /JUMP TO THE INPUT CONSTANTS ROUTINE
0201  4607  RUN,    JMS I   ACOMB   / JUMP TO THE COUNT COMBINATION
                                    /ROUTINE
0202  4610          JMS I   AINCNT  /JUMP TO THE INPUT TO FLOATING POINT
                                    /FORMAT
0203  4611          JMS I   ACALC   /JUMP TO THE CONCENTRATION CALC.
                                    /ROUTINE
0204  4612          JMS I   AOUTPT  /JUMP TO THE OUTPUT ROUTINE
0205  4613          JMS I   AREPT   /JUMP TO REPEAT ROUTINE
0206  0400  AINKS,  INKS            /ROUTINE TO INPUT THE RATE CONSTANTS
0207  0220  ACOMB,  COMB            /ROUTINE TO COMBINE BCD DIGITS TO
                                    /ASCII DIGITS
0210  2200  AINCNT, INCNT           /ROUTINE TO INPUT ASCII DIGITS TO
                                    /FLTG.PT. FORMAT
0211  0600  ACALC,  CALC            /ROUTINE TO CALCULATE CONCENTRATIONS
0212  2000  AOUTPT, OUTPT           /ROUTINE TO OUTPUT CONCENTRATIONS
0213  2100  AREPT,  REPT            /ROUTINE TO REPEAT ANALYSIS
```

```
                *400
0400    0000    INKS,   0
0401    4405            JMS I   5       /JUMP TO INPUT ROUTINE
0402    4407            JMS I   7       /JUMP TO INTERPRETTER
0403    6311            FPUT    KMML    /RATE CONSTANT MM AT LOW
0404    0000            FEXT
0405    4405            JMS I   5
0406    4407            JMS I   7
0407    6314            FPUT    KMBL    /RATE CONSTANT MB AT LOW
0410    0000            FEXT
0411    4405            JMS I   5
0412    4407            JMS I   7
0413    6317            FPUT    KBBL    /RATE CONSTANT BB AT LOW
0414    0000            FEXT
0415    4405            JMS I   5
0416    4407            JMS I   7
0417    6322            FPUT    KMMM    /RATE CONSTANT MM AT MEDIUM
0420    0000            FEXT
0421    4405            JMS I   5
0422    4407            JMS I   7
0423    6325            FPUT    KMBM    /RATE CONSTANT MB AT MEDIUM
0424    0000            FEXT
0425    4405            JMS I   5
0426    4407            JMS I   7
0427    6330            FPUT    KBBM    /RATE CONSTANT FOR BB AT MEDIUM
0430    0000            FEXT
0431    4405            JMS I   5
0432    4407            JMS I   7
0433    6333            FPUT    KMMH    /RATE CONSTANT FOR MM AT HIGH
0434    0000            FEXT
0435    4405            JMS I   5
0436    4407            JMS I   7
0437    6336            FPUT    KMBH    /RATE CONSTANT FOR MB AT HIGH
0440    0000            FEXT
0441    4405            JMS I   5
0442    4407            JMS I   7
0443    6341            FPUT    KBBH    /RATE CONSTANT FOR BB AT HIGH
0444    0000            FEXT
0445    5600            JMP I   INKS
```

```
                *450
0450  0000  MM,     0
0451  0000          0
0452  0000          0
0453  0000  MB,     0
0454  0000          0
0455  0000          0
0456  0000  BB,     0
0457  0000          0
0460  0000          0
0461  0000  TEMP,   0
0462  0000          0
0463  0000          0
0464  0000  D,      0
0465  0000          0
0466  0000          0
0467  0000  E,      0
0470  0000          0
0471  0000          0
0472  0000  F,      0
0473  0000          0
0474  0000          0
0475  0000  G,      0
0476  0000          0
0477  0000          0
0500  0000  N,      0
0501  0000          0
0502  0000          0
0503  0000  P,      0
0504  0000          0
0505  0000          0
0506  0000  DEN,    0
0507  0000          0
0510  0000          0
0511  0000  KMML,   0
0512  0000          0
0513  0000          0
0514  0000  KMBL,   0
0515  0000          0
0516  0000          0
0517  0000  KBBL,   0
0520  0000          0
0521  0000          0
0522  0000  KMMM,   0
0523  0000          0
0524  0000          0
0525  0000  KMBM,   0
0526  0000          0
0527  0000          0
0530  0000  KBBM,   0
0531  0000          0
0532  0000          0
0533  0000  KMMH,   0
0534  0000          0
0535  0000          0
0536  0000  KMBH,   0
0537  0000          0
0540  0000          0
0541  0000  KBBH,   0
0542  0000          0
0543  0000          0
0544  0000  PMM,    0
0545  0000          0
0546  0000          0
0547  0000  PMB,    0
0550  0000          0
0551  0000          0
0552  0000  PBB,    0
0553  0000          0
0554  0000          0
```

```
                *220
0220  0000  COMB, 0                 /ROUTINE TO COMBINE BCD DIGITS TO
0221  6007        CAF
0222  7200        CLA
0223  6046        TLS
0224  6111        DCF               /IS HARDWARE COUNT DONE?
0225  5224        JMP     .-1       / NO
0226  6114        DBA
0227  3101        DCA     HIGHA     /STORE HIGH ORDER A
0230  6134        DBB
0231  3102        DCA     HIGHB     /STORE HIGH ORDER B
0232  6154        DBC               /READ HIGH ORDER C
0233  3103        DCA     HIGHC     /STORE HIGH ORDER C
0234  6151        DLC               /LOAD LOW ORDER C INTO HIGH ORDER C
0235  6114        DBA               /READ LOW ORDER A
0236  3105        DCA     LOWA      /STORE LOW ORDER A
0237  6134        DBB               /READ LOW ORDER B
0240  3106        DCA     LOWB      /STORE LOW ORDER B
0241  6154        DBC               /READ LOW ORDER C
0242  3107        DCA     LOWC      /STORE LOW ORDER C
0243  1115        TAD     HECT
0244  3014        DCA     WORD
0245  7200  PRINT, CLA
0246  1412        TAD I   ADRH      /
0247  3100        DCA     HIGH      /
0250  1413        TAD I   ADRL      /
0251  3104        DCA     LOW       /
0252  7200        CLA               /CLEAR SPACE OUT OF AC
0253  1100        TAD     HIGH      /LOAD AC WITH HIGH ORDER
0254  7112        CLL RTR
0255  7012        RTR
0256  7012        RTR
0257  7012        RTR
0260  4321        JMS     STORE
0261  1100        TAD     HIGH
0262  7112        CLL RTR
0263  7012        RTR
0264  4321        JMS     STORE
0265  1100        TAD     HIGH      /LOAD AC WITH HIGH ORDER
0266  4321        JMS     STORE
0267  1104        TAD     LOW       /LOAD AC WITH LOW ORDER
0270  7112        CLL RTR
0271  7012        RTR
0272  7012        RTR
0273  7012        RTR
0274  4321        JMS     STORE
0275  1104        TAD     LOW       /LOAD AC WITH LOW ORDER
0276  7112        CLL RTR
0277  7012        RTR
0300  4321        JMS     STORE
0301  1104        TAD     LOW       /LOAD AC WITH LOW ORDER
0302  4321        JMS     STORE
0303  1112        TAD     SP
0304  4326        JMS     STOR
0305  2117        ISZ     CNT       /ARE ALL THREE BANKS PRINTED
0306  5245        JMP     PRINT     /
0307  7200        CLA
0310  1113        TAD     FORT
0311  3012        DCA     ADRH      /RESET HIGH ADDRESS POINTER
0312  1114        TAD     FORFOR
0313  3013        DCA     ADRL      /RESET LOW ADDRESS LOW POINTER
0314  1116        TAD     TWO       / ADD -2 TO RESET CNT
0315  3117        DCA     CNT       / RESET
0316  1115        TAD     HECT
0317  3014        DCA     WORD
0320  5620        JMP I   COMB
```

```
0321  0000  STORE,  0
0322  0110         AND     BCD
0323  1111         TAD     ASCI
0324  3414         DCA I   WORD
0325  5721         JMP I   STORE


0326  0000  STOR,   0
0327  3414         DCA I   WORD
0330  5726         JMP I   STOR


              *100
0100  0000  HIGH,   0
0101  0000  HIGHA,  0
0102  0000  HIGHB,  0
0103  0000  HIGHC,  0
0104  0000  LOW,    0
0105  0000  LOWA,   0
0106  0000  LOWB,   0
0107  0000  LOWC,   0
0110  0017  BCD,    17
0111  0260  ASCI,   260
0112  0240  SP,     240
0113  0100  FORT,   100
0114  0104  FORFOR, 104
0115  0130  HECT,   130
0116  7775  TWO,    7775            /-3
0117  7775  CNT,    7775            /COUNTER FOR THREE INPUTS TO THE
                                    /WORD STORAGE
              *12
0012  0100  ADRH,   HIGH
0013  0104  ADRL,   LOW
0014  0000  WORD,   0
```

```
                *2200
2200  0000  INCNT,  0
2201  1226          TAD     COMCHG  /CHANGE COMMAND TO ACCEPT STORED
                                    /COUNTS
2202  3632          DCA I   ADDCOM
2203  1227          TAD     COMCHN
2204  3633          DCA I   ADCOMM
2205  4405          JMS I   5
2206  4407          JMS I   7
2207  6234          FPUT    AL
2210  0000          FEXT
2211  4405          JMS I   5
2212  4407          JMS I   7
2213  6237          FPUT    AM
2214  0000          FEXT
2215  4405          JMS I   5
2216  4407          JMS I   7
2217  6242          FPUT    AH
2220  0000          FEXT
2221  1230          TAD     COMMCA  /RETURN THE COMMANDS TO ORIGNAL
2222  3632          DCA I   ADDCOM
2223  1231          TAD     COMMCN
2224  3633          DCA I   ADCOMM
2225  5600          JMP I   INCNT
2226  5303  COMCHG, 5303
2227  4170  COMCHN, 4170
2230  6031  COMMCA, 6031
2231  6036  COMMCN, 6036
2232  7501  ADDCOM, 7501
2233  7503  ADCOMM, 7503
2234  0000  AL,     0
2235  0000          0
2236  0000          0
2237  0000  AM,     0

2240  0000          0
2241  0000          0
2242  0000  AH,     0
2243  0000          0
2244  0000          0
```

```
                *600
0600    0000    CALC,   0
0601    4407            JMS I   7       /JUMP TO INTERPRETER
0602    5745            FGET I  AKBBM
0603    3741            FMPY I  AKMBL
0604    6730            FPUT I  AD
0605    5744            FGET I  AKMBM
0606    3742            FMPY I  AKBBL
0607    2730            FSUB I  AD
0610    6730            FPUT I  AD
0611    3742            FMPY I  AKBBL
0612    6731            FPUT I  AE
0613    5745            FGET I  AKBBM
0614    3751            FMPY I  AAL
0615    6734            FPUT I  AN
0616    5742            FGET I  AKBBL
0617    3752            FMPY I  AAM
0620    2734            FSUB I  AN
0621    6734            FPUT I  AN      /STORE THE VALUE FOR N
0622    5747            FGET I  AKMBH   /CALCULATE F
0623    3742            FMPY I  AKBBL
0624    6732            FPUT I  AF      /STORE AF
0625    2741            FSUB I  AKMBL   /CALCULATE F-KMBL FOR DEN OF MM
0626    6737            FPUT I  ATEMP
0627    5743            FGET I  AKMMM
0630    3742            FMPY I  AKBBL
0631    6735            FPUT I  AP
0632    5745            FGET I  AKBBM
0633    3740            FMPY I  AKMML
0634    2735            FSUB I          /CALCULATE P
0635    6735            FPUT I  AP      /STORE P
0636    3737            FMPY I  ATEMP
0637    6737            FPUT I  ATEMP   /CALCULATE THE P(F-KMBL)
0640    5730            FGET I  AD
0641    3750            FMPY I  AKBBH
0642    6733            FPUT I  AG      /STORE THE VALUE FOR G
0643    3740            FMPY I  AKMML
0644    2737            FSUB I  ATEMP
0645    6737            FPUT I  ATEMP   /P(F-KMBL)-G*KMML
0646    5731            FGET I  AE
0647    3746            FMPY I  AKMMH
0650    1737            FADD I  ATEMP
0651    6736            FPUT I  ADEN    /THIS  THE DENOMINATOR FOR MM EQ.
0652    5732            FGET I  AF
0653    3734            FMPY I  AN
0654    6737            FPUT I  ATEMP   /FOR  THE NUMERATOR FOR MM EQ.
0655    5730            FGET I  AD
0656    3750            FMPY I  AKBBH
0657    5733            FGET I  AG      /STORE THE VALUE FOR G
0660    3751            FMPY I  AAL
0661    1737            FADD I  ATEMP
0662    6737            FPUT I  ATEMP
0663    5734            FGET I  AN
0664    3741            FMPY I  AKMBL
0665    2737            FSUB I  ATEMP
0666    6737            FPUT I  ATEMP
0667    5753            FGET I  AAH
0670    3731            FMPY I  AE
0671    1737            FADD I  ATEMP
0672    4736            FDIV I  ADEN    /DIVIDE BY THE DENOMINATOR
0673    6754            FPUT I  AMM     /CONCENTRATION OF MM
0674    3735            FMPY I  AP
0675    1734            FADD I  AN
0676    4730            FDIV I  AD
0677    6755            FPUT I  AMB     /STORE THE MB CONCENTRATION
```

```
0700  3741          FMPY I   AKMBL
0701  6737          FPUT I   ATEMP
0702  5754          FGET I   AMM
0703  3740          FMPY I   AKMML
0704  1737          FADD I   ATEMP
0705  6737          FPUT I   ATEMP
0706  5751          FGET I   AAL
0707  2737          FSUB I   ATEMP
0710  4742          FDIV I   AKBBL
0711  6756          FPUT I   ABB
0712  1754          FADD I   AMM      /CALCULATE PERCENT CONCENTRATIONS
0713  1755          FADD I   AMB
0714  6737          FPUT I   ATEMP
0715  5754          FGET I   AMM
0716  4737          FDIV I   ATEMP
0717  6757          FPUT I   APMM
0720  5755          FGET I   AMB
0721  4737          FDIV I   ATEMP
0722  6760          FPUT I   APMB
0723  5756          FGET I   ABB
0724  4737          FDIV I   ATEMP
0725  6761          FPUT I   APBB
0726  0000          FEXT
0727  5600          JMP I    CALC

0730  0464  AD,     D
0731  0467  AE,     E
0732  0472  AF,     F
0733  0475  AG,     G
0734  0500  AN,     N
0735  0503  AP,     P
0736  0506  ADEN,   DEN
0737  0461  ATEMP,  TEMP
0740  0511  AKMML,  KMML
0741  0514  AKMBL,  KMBL
0742  0517  AKBBL,  KBBL
0743  0522  AKMMM,  KMMM
0744  0525  AKMBM,  KMBM
0745  0530  AKBBM,  KBBM
0746  0533  AKMMH,  KMMH
0747  0536  AKMBH,  KMBH
0750  0541  AKBBH,  KBBH
0751  2234  AAL,    AL
0752  2237  AAM,    AM
0753  2242  AAH,    AH
0754  0450  AMM,    MM
0755  0453  AMB,    MB
0756  0456  ABB,    BB
0757  0544  APMM,   PMM
0760  0547  APMB,   PMB
0761  0552  APBB,   PBB


      *170
0170  0000  LOAD,   0
0171  7200          CLA
0172  1414          TAD I    WORD
0173  5570          JMP I    LOAD
```

```
                *2000
2000  0000  OUTPT, 0
2001  7200        CLA
2002  1256        TAD     CR
2003  6041        TSF
2004  5203        JMP     .-1
2005  6046        TLS
2006  7200        CLA
2007  1257        TAD     LF
2010  6041        TSF
2011  5210        JMP     .-1
2012  6046        TLS
2013  4407        JMS I   7
2014  5660        FGET I  AAMM
2015  0000        FEXT
2016  4406        JMS I   6
2017  4407        JMS I   7
2020  5663        FGET I  AAPMM
2021  0000        FEXT
2022  4406        JMS I   6
2023  4407        JMS I   7
2024  5661        FGET I  AAMB
2025  0000        FEXT
2026  4406        JMS I   6
2027  4407        JMS I   7
2030  5664        FGET I  AAPMB
2031  0000        FEXT
2032  4406        JMS I   6
2033  4407        JMS I   7
2034  5662      FGET    AABB
2035  0000        FEXT
2036  4406        JMS I   6
2037  4407        JMS I   7
2040  5665        FGET I  AAPBB
2041  0000        FEXT
2042  4406        JMS I   6
2043  7200        CLA
2044  1256        TAD     CR
2045  6041        TSF
2046  5245        JMP     .-1
2047  6046        TLS
2050  7200        CLA
2051  1257        TAD     LF
2052  6041        TSF
2053  5252        JMP     .-1
2054  6046        TLS
2055  5600        JMP I   OUTPT

2056  0215  CR,     215
2057  0212  LF,     212
2060  0450  AAMM,   MM
2061  0453  AAMB,   MB
2062  0456  AABB,   BB
2063  0544  AAPMM,  PMM
2064  0547  AAPMB,  PMB
2065  0552  AAPBB,  PBB
```

```
                *2100
2100  0000  REPT,  0
2101  5703        JMP I   ARUN
2102  5700        JMP I   REPT

2103  0201  ARUN,  RUN
```

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