

FINAL PROGRESS REPORT

CONTRACT FOR THE ANALYSIS OF COMPONENTS IN BIOLOGICAL FLUIDS IN A GRAVITY FREE
ENVIRONMENT EMPHASIZING PROCEDURES SUITABLE FOR USE IN AN ORBITING LABORATORY

Contract Number NAS-9-7934

Contract Date 9/1/67-8/31/68

Contracted By:

Michael Reese Hospital and Medical Center
Chicago, illinois 60616

Administered By:

NASA Headquarters
Washington, D.C. 20546

Report Prepared by:

Samuel Natelson, Ph.D.

Thomas C. Puckett, B.S.,M.T. (A.S.C.P.)

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OBJECTIVES

This report is a continuation of the final progress report submitted, covering the contract dates 8-1-66 to 8-31-67, of contract Number NSR -14-015-001.

In the earlier report, feasibility studies were made on the application of a tape system of analysis for the analysis of blood and urine for various components. The objective was to develop a system which used no liquid reagents and thus could be used in a gravity free environment. The objective was to develop a system which was completely automatic and required minimum skill for operation.

The system proposed comprised the following:

1. A receiving tape, on which a measured specimen is to be placed from a precalibrated capillary tube.
2. A porous tape, such as cellophane or cellulose acetate, through which the component to be analyzed diffuses, separating it from protein and cellular components.
For protein assay micropore cellulose acetate is proposed to separate protein from red cells.
3. A reagent tape, impregnated with a reagent, to produce a color or fluorescent spot specific for the component to be analyzed.

In the earlier study the following objectives were achieved.

1. A receiving tape was designed which comprised a mylar tape, on which was disposed circular discs of filter paper (Whatman #3).
This permitted the acceptance of the specimen in a confined

area for analysis.

II. The reagent tape was designed, comprising filter paper backed by Mylar.

III. The intermediate porous tape was cellophane (approx. 1 mil), or cellulose acetate which were both found to be satisfactory.

IV. Instrumentation:

A. An instrument was designed and built for moving the three tapes. This system included:

1. Means for depositing a sample repetitively in a predetermined area. This was done by designing a capillary dispenser.
2. Control means for moving the tapes at predetermined intervals.
3. A wetting zone to add a particular reagent in order to react with the dialyzed specimen.
4. A pressing device where the three tapes are pressed together for a predetermined interval and time.
5. A drive system to move the tapes at predetermined intervals to separate the tapes and move the reagent tape through a densitometer at constant speed for evaluation of the reflectance of the colored spots formed.
6. A drying device to dry and heat the spots before they are read
7. A densitometer, which is light weight, uses a minimum of current and reads the spots by reflectance.

8. Take up reels to remove the spent tapes.
9. In the procedure, a sample is deposited from a capillary tube on the sample tape. This is then moved and inverted to meet the cellophane and wetted reagent tape. The pad so formed is pressed. By dialysis, some of the sample moves through the cellophane and reacts with the reagent on the tape. The reaction is stopped in the dryer (70°C) and the paper is then dried. The intensity of the spots are now compared with proper standards.
10. A smaller instrument was then built which included the tape wetter but which used a roller principle for applying pressure to the tapes.

V. Methodology was then studied for applying these instruments to specific methods of analysis.

Preliminary studies showed that glucose, urea, ammonia, protein and blood pH were applicable. The study then focused down on the sugar and urea methods as suitable methods for more intensive study.

OBJECTIVES OF THE PRESENT STUDY

The original study was divided into three phases.

Phase I comprised the construction of a completely automated system regardless of weight and power requirements. This has been completed and functions well. This system serves to act as a test instrument for developing methodology. It also can be used in the routine laboratory of clinical chemistry on earth.

Phase II contemplated the building of a smaller instrument of light weight but including flexibility for testing different procedures. A form of this instrument was built but it needed to be modified.

Phase III contemplated the building of a practical light weight instrument for use in space. This includes the use of narrower tapes, capable of doing a battery of tests simultaneously and with minimum power and space requirements.

From the above, it is apparent that the smaller instrument of the previous report needed to be modified. In the present study this problem was undertaken. The approach taken included the following:

- I. Return to the press plate of the larger instrument instead of a roller system.
- II. Elimination of the wetting roller, and preparation of dried impregnated tapes.
- III. Eliminating the method of sampling from capillaries which would be a hazard in space flight.
- IV. Further exploration of methodology once the new instrument was built.

How these problems have been solved or partially solved will now be discussed.

CONSTRUCTION OF THE LIGHTWEIGHT INSTRUMENT

The construction of the lightweight instrument followed the lines previously outlined. Major improvements were made as follows:

1. The movement of the press plate was redesigned to the simple movement of a cam which lifts the plate against the pressure of springs. As the cam releases the lifting bar the spring applies the pressure. The tension of the spring is adjustable so that any predetermined pressure can be applied to the tapes.
2. In the original design of the larger instrument there were three motors. In the present instrument there are only two motors. One pulls the tapes forward at predetermined intervals. The other runs continuously. This motor activates the press plate at intervals determined by the speed of the motor. This same motor drives the reagent tape through the densitometer.

Obviously, unless perfect synchronization occurred, the reagent tape would move either too fast or too slowly. In the first case, the tape would become taught, pulling against the closed press plate. This would either tear the tape or stall the motor. In the latter case, a large loop would develop behind the densitometer resulting in the tape eventually touching the metal resulting in contamination.

To resolve this problem, a continuously adjustable speed control system was installed between the motor and the rollers driving the reagent tape. This is adjustable so that the loop behind the densitometer remains constant.

If during a run the loop varies significantly in size, the speed can readily be adjusted to maintain a constant loop size. For this purpose a "Zeromax" adjustable gear train was used. This instrument is commercially available and was used for practical purposes. A continuously varying pulley can easily be designed to replace this instrument. This would weigh much less and yield just as efficient control.

3. Since a wetting system is not used, only the spots are moist as they leave the press plate. The drying system can then be shortened to run of only six inches to achieve complete dryness of the reagent.
4. Since the reagent tape is dry, one can roll it up together with the porous tape. Thus only two payoff reels are needed instead of the three of the original instrument.

How the instrument is actually constructed follows below.

Figure 1

Front view of the Tape Microassay System

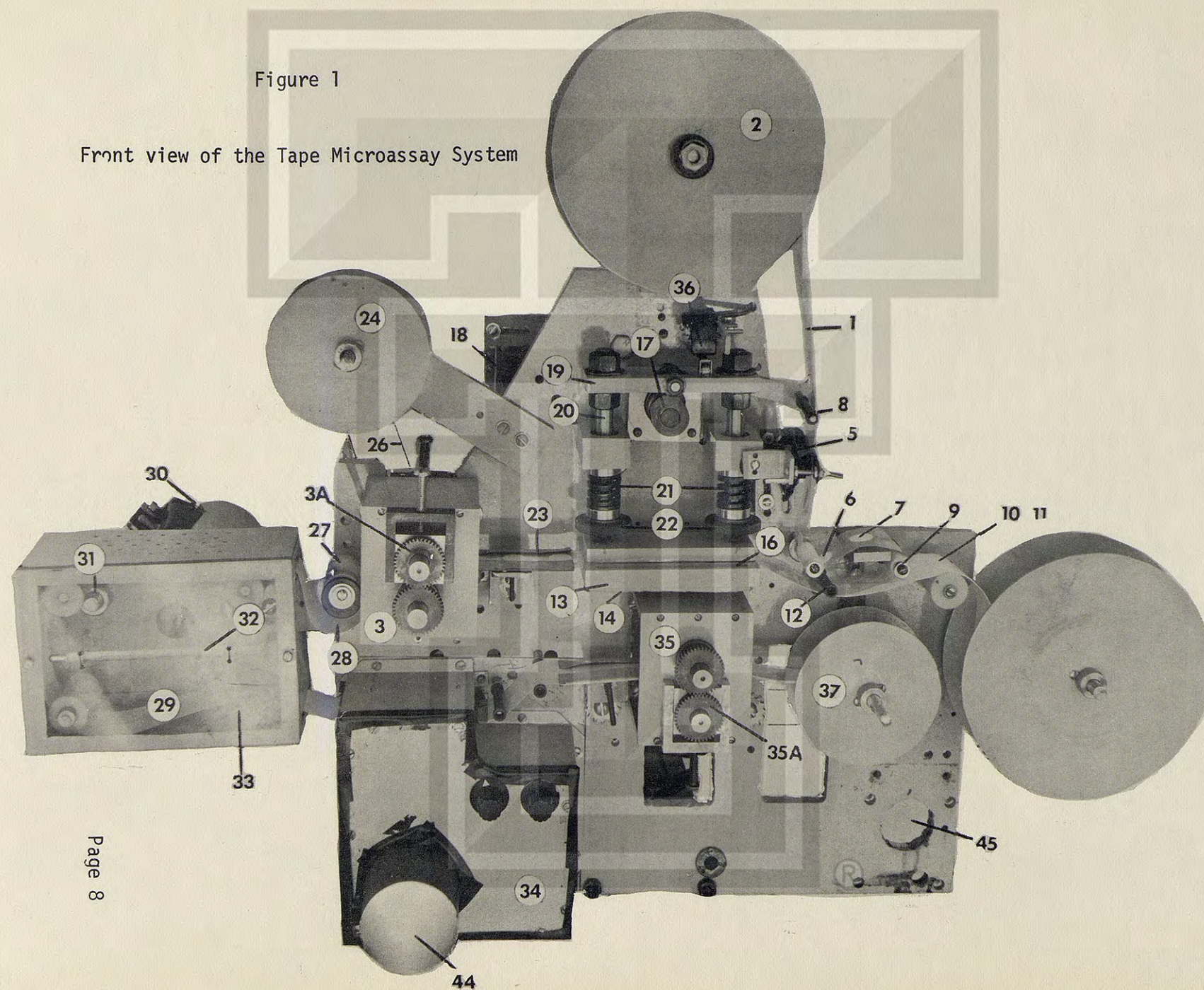


Figure 1 : Front view of the tape microassay system

1. sample tape.
2. payoff reel.
3. tape puller.
- 3a. gears to synchronize motion of both rollers of tape puller.
4. pull motor
5. paper switch.
6. paper due to receive the sample.
7. sampling station.
8. roller to guide sample tape.
9. roller to invert sample tape.
10. reagent tape.
11. porous tape (cellophane)
12. collecting roller,
13. pressplate
14. heating mantle under and adjacent to press plate.
16. thermometer well.
17. cam to lift press plate.
19. lift plate.
20. lift bars.
21. adjustable springs to adjust tension on press plate.
22. adjusting nuts to adjust spring tension.
23. tape guide.
24. take up reel for sample and porous tape.
25. pulley wheels
26. sliding spring belt.
27. separator roller.
28. paper loop
29. constant temperature drier
30. heater blower.
31. adjustable nut to locate heater blower.
32. thermometer to measure temperature in drying box.
33. Lucite window,
34. densitometer.
35. tape puller, pulls tape at constant speed through densitometer.
- 35a. Gears to synchronize tape roller(35).
36. switch, activated when lift plate is raised.
37. take up reel for reagent tape,

INSTRUMENT DESIGN

Referring to Figures 1,2,3,4,5, and 6.

The sample tape (1), on payoff reel (2), is pulled forward periodically by tape puller (3), which is activated by pull motor (4). When the tape is pulled to paper switch (5), it trips the switch by the thickness of the paper disc (6). This stops motor (4), and permits adding the sample at sample position (7).

The sample tape now moves under roller (8), over the sampling position and is inverted on roller (9). It now meets the combined reagent tape (10), and porous tape (11), at collecting roller (12).

The three tapes now move to pressplate (13). Pressplate (13) is kept at constant temperature by a heating coil (14), under the press plate. The temperature is read on thermometer (15), in the thermometer well (16). The pressplate is raised and lowered periodically by the cam (17), which is driven continuously by main motor drive (18). This cam rotates on wheel on lift plate (19) secured to the lift bars (20) which are attached to the press plate. Springs (21) and adjusting nuts (22) permit adjustment of the tension on the pressplate. Switch (36) is normally open. When the lift plate elevates, it closes, permitting pull motor (4), to pull the tapes forward. The tapes cannot be therefore pulled forward unless the pressplate is in the up position.

The tapes now move to the tape puller (3), through the guide (23).

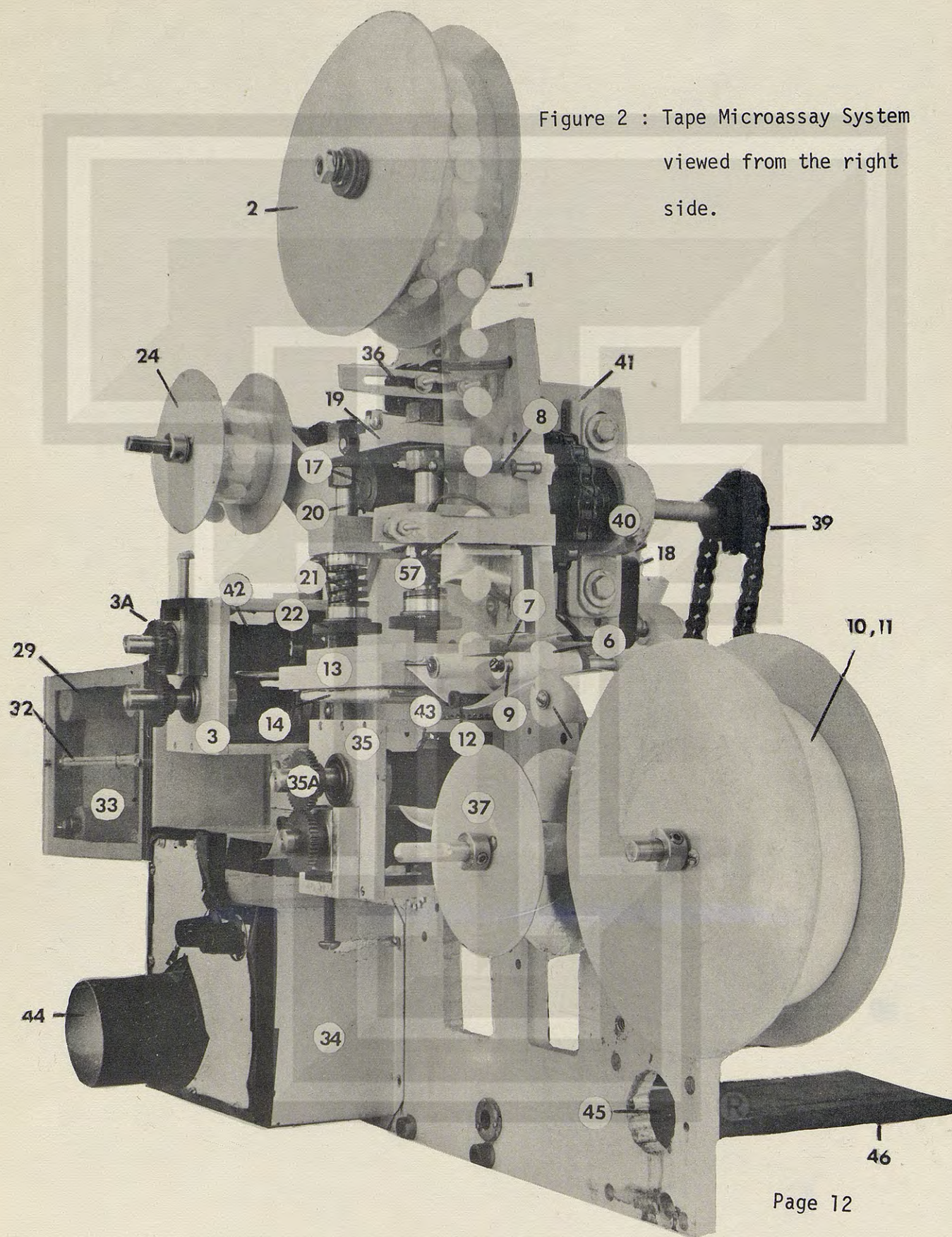
Take up reel (24) which is activated by the pull motor, through pulleys (25) and sliding spring belt (26) takes up the sample tape and porous tape from a separator roller (27).

A loop (28) now forms, of the reagent tape, which contains the spots. The reagent tape now moves through drying box (29), and is guided around by rollers. Heater blower (30), maintains the temperature in the drying box by adjusting the distance from the mouth of the blower to the opening in the heating box by adjustable nut (31). Temperature is read on the thermometer (32) through Lucite window (33). The temperature is maintained at 70°C in the drying box.

The tape is now pulled through the densitometer (34), by tape puller (35) activated by the main drive motor (18), through Zeromax adjustable speed control (52), by chain and sprocket drive (36). This maintains a constant loop (28) and at the same time maintains a constant speed through the densitometer.

The densitometer output is read off on a recorder, not shown, as a series of peaks which are compared to standards. The reagent tape is now rolled up on the take up reel (37), which is activated by pulley and sliding drive to the take up reel (38).

Figure 2 : Tape Microassay System
viewed from the right
side.



Legend for Figure 2 : Tape microassay system viewed from the right side.

1-38. Numbers are the same as in the legend for Figure 1.

39. sprocket and chain drive main motor to Zeromax speed control.

40. sprocket and chain drive to main motor.

41. main motor mount

42. rollers of tape puller, for the three tapes.

43. rollers of tape puller for the reagent tape.

44. heat sink for lamp of densitometer.

45. space and hole allowed for tape wetter if desired.

Legend for Figure 3 : Tape microassay system viewed from the left side.

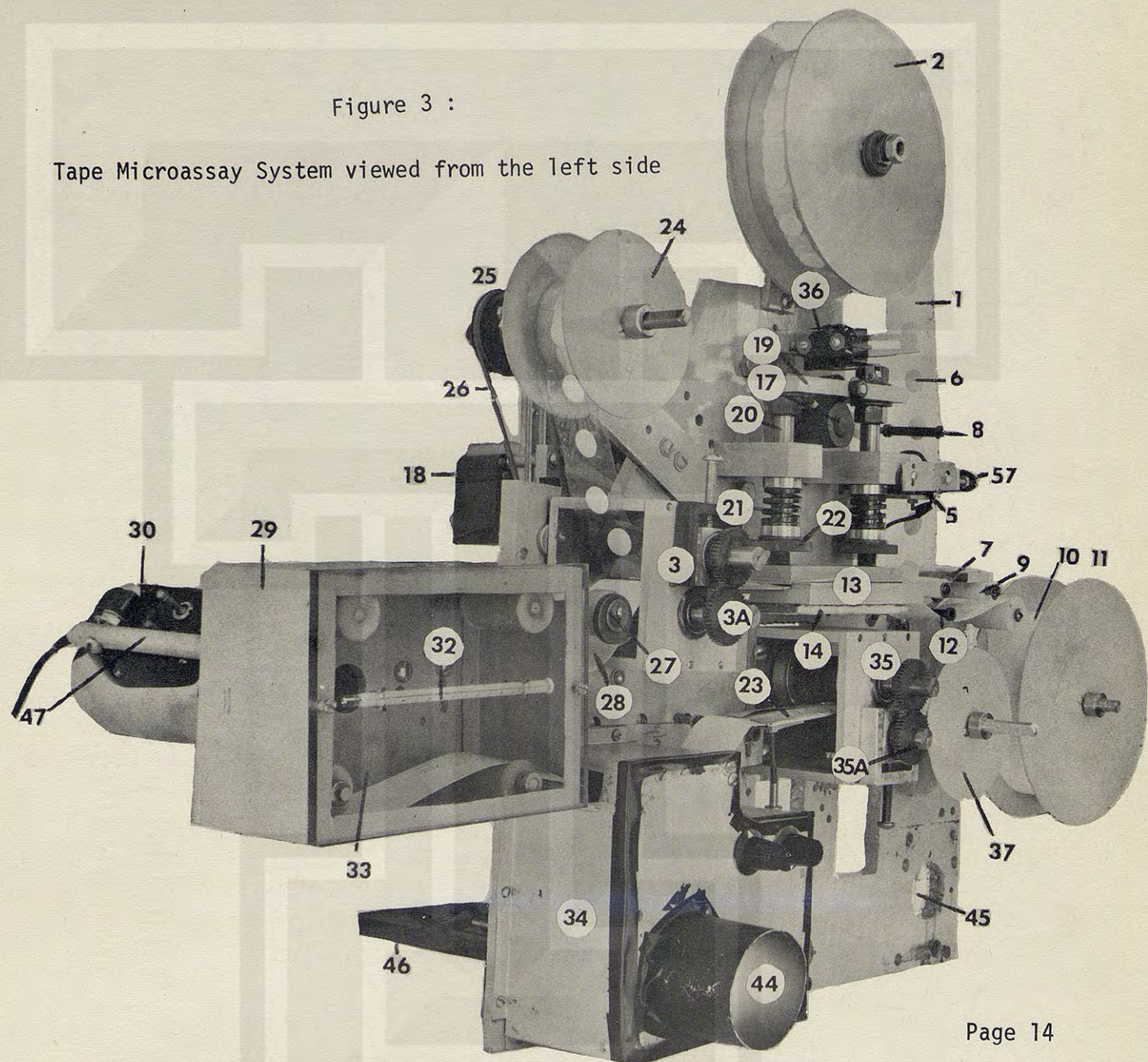
1-45 same as figures 1 and 2.

46. supporting plate for instrument.

47. adjustable rod holding heater blower in place.

Figure 3 :

Tape Microassay System viewed from the left side



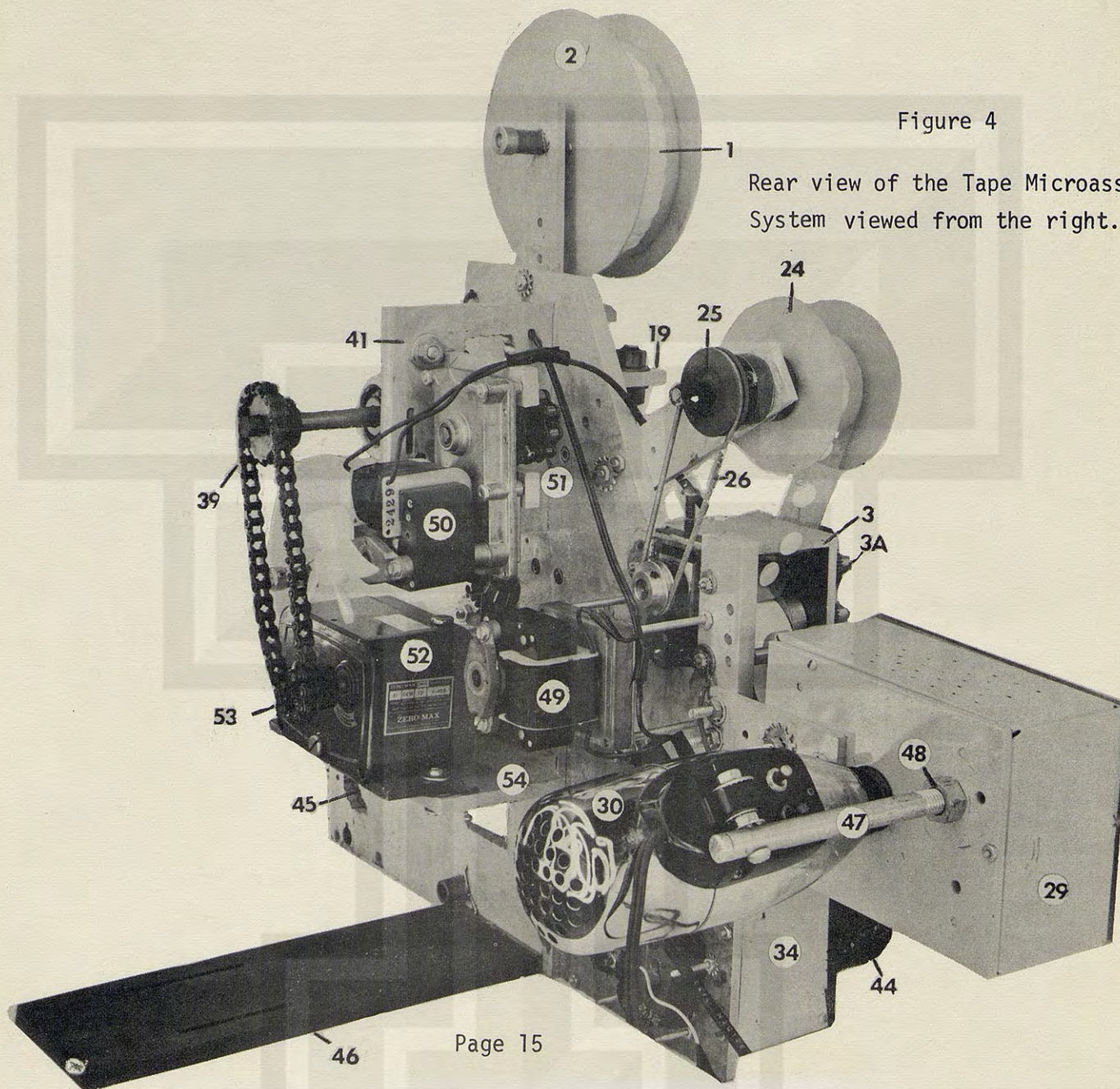


Figure 4

Rear view of the Tape Microassay System viewed from the right.

Figure 5 : Rear view of the Tape
Microassay System
viewed from the left.

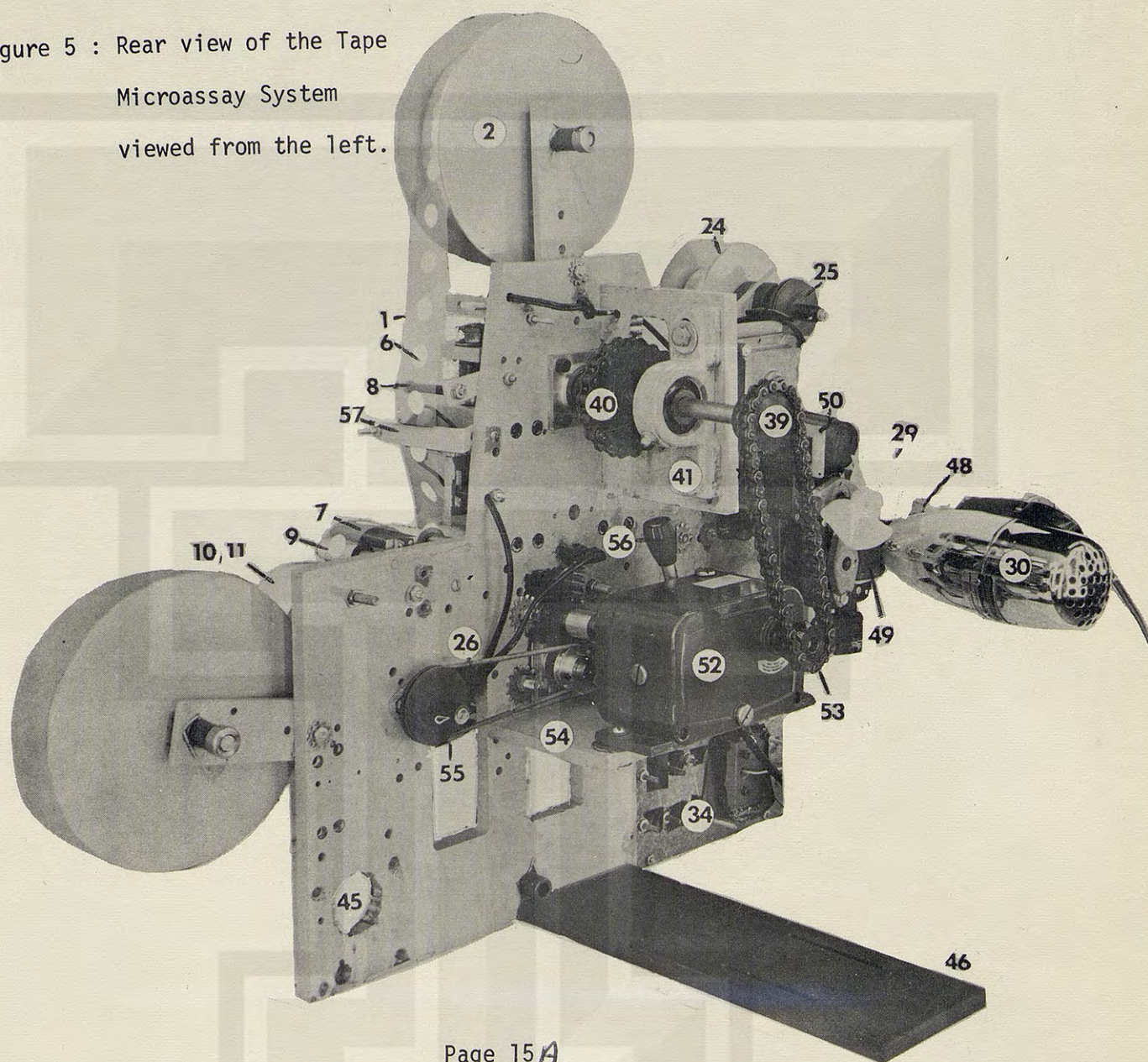


Figure 4 : Rear view of the tape microassay system viewed from the right .

1-47. same as Figures 1,2, and 3.

18. main drive motor.

48. adjusting nut to adjust blower

49. tape puller motor activating take up reel for sample and porous tape.

50. main motor and cooling fan, driving cam, and tape puller, operating at constant speed, through the densitometer. Tape puller is activated through the Zeromax gear chain.

51. sprocket driving tape puller mentioned in (50).

52. Zeromax gear chain which allows adjustment of rate at which reagent tape moves thorough the densitometer.

53. sprocket and chain drive at Zeromax gear chain.

54. support for Zeromax gear chain.

Figure 6 : Close up of pressplate system and control switches

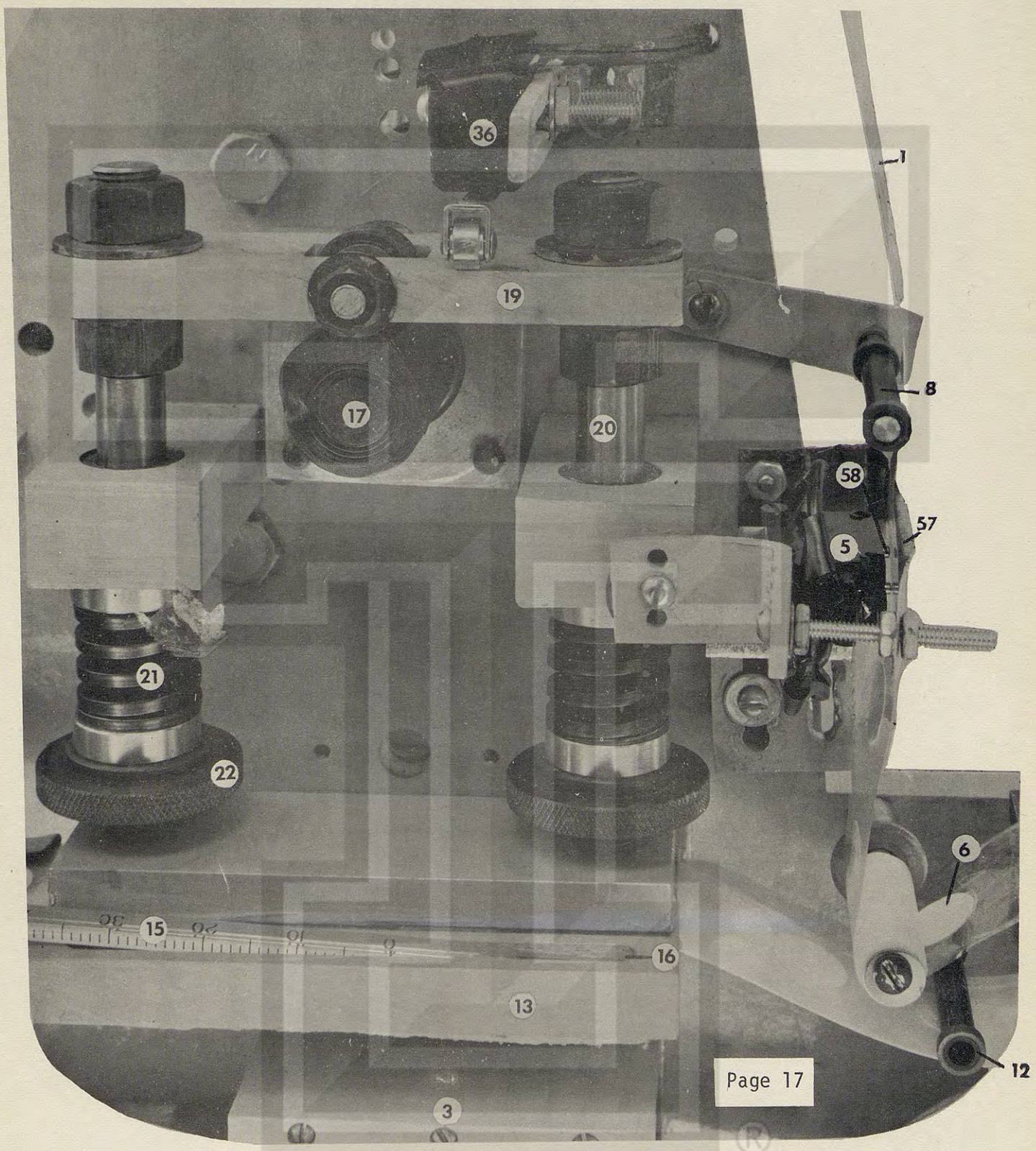


Figure 5 : Rear view of the tape microassay system viewed from the left.

1-54 same as Figures 1,2,3,4, and 5.

- 55. pulley driving take up reel for reagent tape after it moves through the densitometer.
- 56. control handle on Zeromax gear train for continuously adjusting the speed of the puller acting on the reagent tape through the densitometer.
- 57. adjustable spring clip to hold sample tape to switch

Figure 6 : Close up of press plate system and control switches.

1-57 same as figures 1,2,3,4, and 5.

- 15. thermometer to measure temperature of press plate.
- 58. pins of paper switch. Both pins are compressed to stop movement of paper switch and center sample disc on sampling station.

List of all numbers used in Figures 1-6

1. sample tape.
2. payoff reel.
3. tape puller.
- 3a. gears to synchronize motion of both rollers of tape puller.
4. (49) pull motor.
5. paper switch.
6. paper disc to receive specimen.
7. sampling station.
8. roller to guide sample tape.
9. roller to invert sample tape.
10. reagent tape.
11. porous tape (cellophane).
12. collecting roller.
13. press plate.
14. heating mantle adjacent to press plate.
15. thermometer to monitor temperature of press plate.
16. thermometer well.
17. cam to lift press plate.
18. main drive motor.
19. lift plate.
20. lift bars.
21. adjustable springs to adjust tension on press plate.
22. adjustable nuts to adjust spring tension
23. tape guide.
24. take up reel for sample and porous tape.
25. pulley wheels.
26. sliding spring belt.
27. separator roller.
28. paper loop
29. constant temp. drier.
30. heater (drier) blower.
31. adjustable nut to locate blower.
32. thermometer to monitor drier temperature.
33. Lucite window.
34. densitometer .
35. tape puller : pulls tape at constant speed through densitometer.
- 35a. gears to syn. tape roller in (35).
36. switch, activated when plate is lifted.
37. take up reel for reagent tape
38. pulley & spring drive to take up reel
39. sprocket and chain drive from main motor to Zeromax speed controller.
40. sprocket and chain drive to main motor.
41. main motor mount.
42. rollers of tape puller, for the 3 tapes
43. rollers of the tape puller for the reagent tape.
44. heat sink for lamp of densitometer.
45. space allowed for tape wetter if desired.
46. supporting plate for instrument.
47. adjustable rod holding blower for drier.
48. adjusting nut to adjust blower
49. tape puller motor activating take up reel.
50. main motor driving cam, tape puller, and Zeromax drive.
51. sprocket, driving tape puller to pull tape through densitometer.
52. Zeromax gear chain- allowing adjustment to speed through densitometer.
53. sprocket & chain drive at Zeromax gear chain.
54. support for zeromax gear chain.
55. pulley driving take up reel for reagent tape.
56. control handle of zeromax,
57. adjustable spring clip to hold sample tape to switch.
58. pins of paper switch (both must be compressed to stop movement of tapes through the machine and sampling station).

THE CONTROL BOX

Referring to Figure 7 and 8, the pilot light goes on when the power switch (S-1) is turned on. This feeds current to the heating coil of the press plate when switch (S6), is turned on, through a continuously variable voltage transformer (T-1). In this way the temperature of the pressing zone is controlled.

When the switch (S5) is turned on, current is fed to the lamp of the densitometer. The continuously variable autotransformer (T2) permits control of the intensity of the light. The step down transformer (T3) sets a limit of 12 volts so that the lamp cannot be overloaded.

When switch (S4), is turned on, the heater blower is started in the drying chamber.

When Switch (S3) is turned on, the main motor, attached to the cam, which lifts the press plate, is activated. The cam is in the lifting position for 25° of its motion. The motor used was a 6 r.p.m. shaded pole motor of 100 inch lbs. lift.

With power switch (S1) closed and switch (S3) closed, the machine will begin to operate. Let us assume that the paper disc is in the position to close both switches of double switch (S2). These switches are placed close to each other to accurately position the disc. They are in series, and as the disc moves forward it closes the first and then the second. Both need to be down simultaneously. This trips the relay (R), which is pulled down, cutting off any source of current to the pull motor but starting the timer motor, since section (A) of the relay has made contact.

The timer motor (Industrial Timer Corp. Series 90, Time Delay Switch) is adjustable and can take anywhere from 3 to ten seconds depending upon where the stop for the flag is located. This is usually set for 8 seconds depending upon where the stop for the flag is located. This is the time given for applying the sample on the paper disc in the sample area of Figure 1.

After the sample has been deposited, the timer motor flag presses pin (P) and trips the microswitch. This yields a current source, bypassing the paper switch, and starting the pull motor. Switch (S-1) is closed at this point because the main motor cam is adjustable and when the paper disc closes the paper switch, the cam is in an up position, lifting the press plate and closing switch. (S-1).

As the sample tape is pulled, the disc is pulled out of the paper switch (S-2), causing the relay (R), to be inactivated. The relay springs back, opening section A and closing Section B. The timer motor is inactivated, and springs back to the flag stop. The pull motor would now stop, except for the fact that a bypass of current is supplied by section (B) of the relay.

The pull motor continues to move the sample tape forward until the paper switch is closed by the next paper disc. This starts the timer motor by closing section (A), but the pull motor stops because section (B) is now open.

For the interval of time that the timer motor takes to close the pin (P), the pull motor remains inactivated. When the pin is pressed, the cycle starts all over again. The switch (S-1) insures that the pull motor will not operate when the press plate is in a down position.

Figure 7 : Housing containing the
electrical control system.

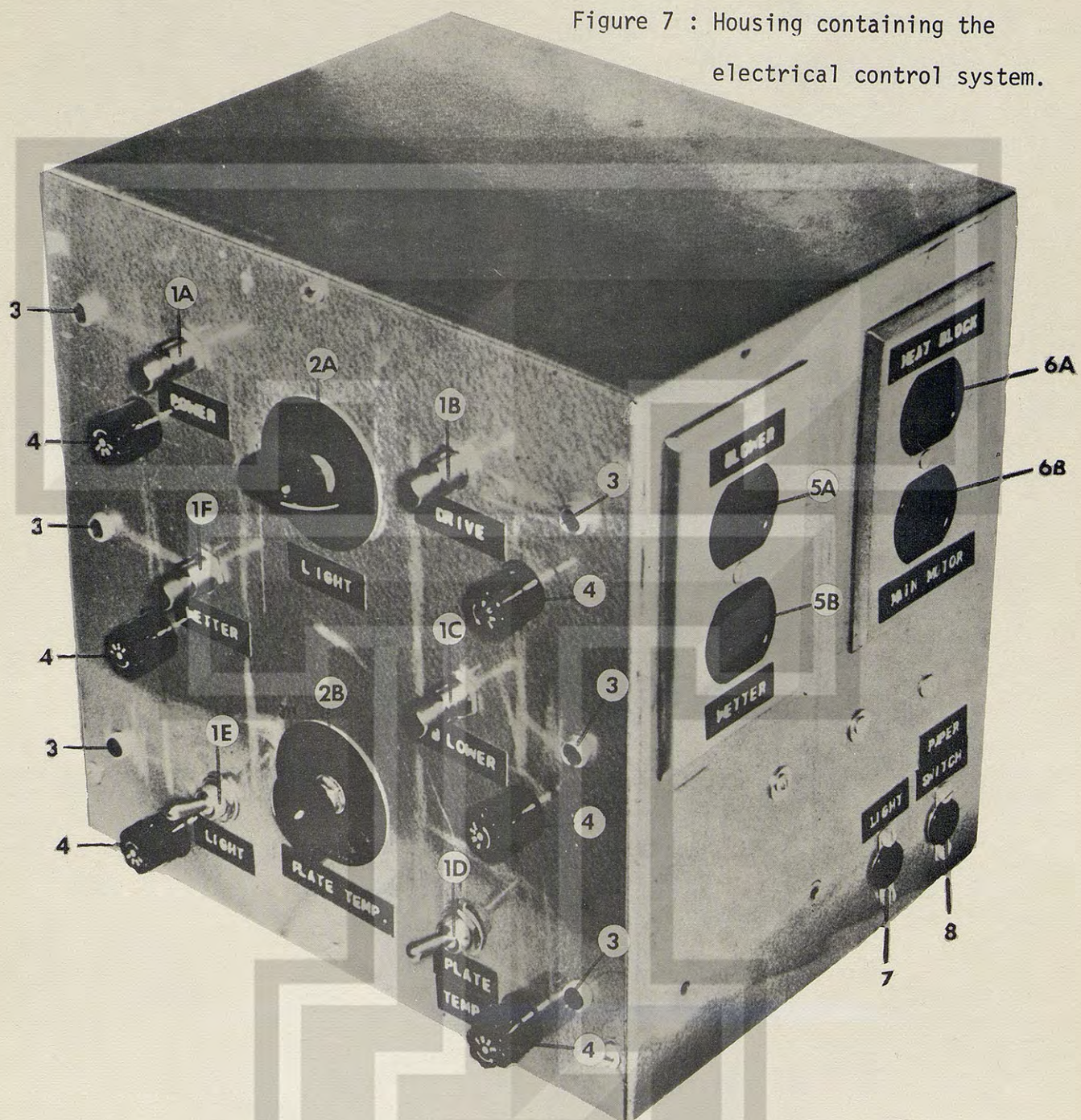


Figure 7 : Housing containing electrical control system. 1. Switches to turn on: a) power, b) drive, c) blower, d) heating plate, e) light of densitometer, f) wetting roller if required. 2. Controls : a) to regulate light intensity of densitometer, b) to control press plate temperature. 3. Bezel lights to indicate which function is on. 4. Fuses for the various functions. 5. Plug to feed current to : a) blower, and b) wetting motor if used. 6. Plug for : a) heating block, b) main motor. 7. Plug for light in densitometer. 8. Plug for paper switch.

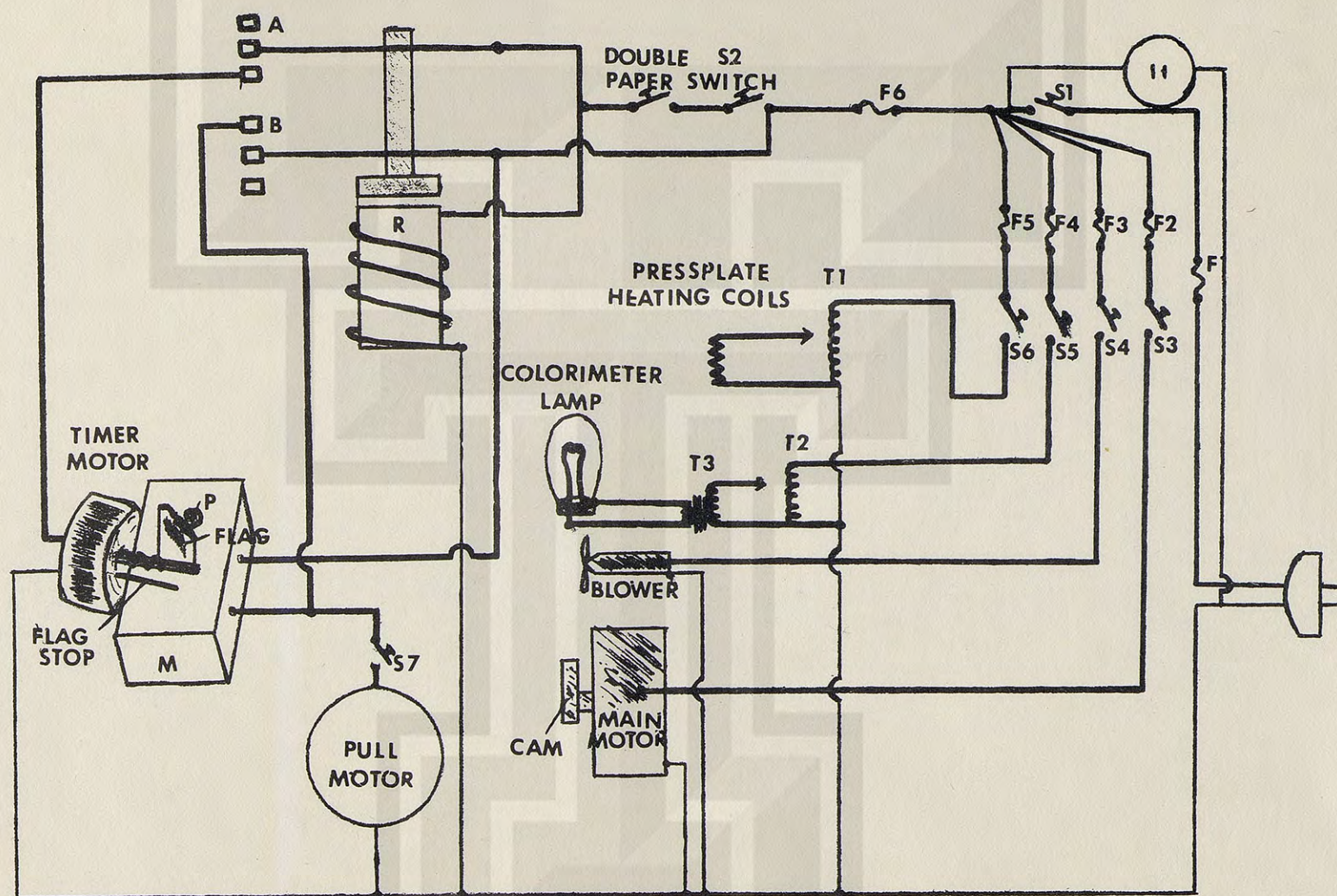


Figure 8: Circuit for electrical system. S-1,2,3,4,5,6 are main switch, paper switch, and switches to turn on main motor, heater blower, densitometer lamp, and press plate. S-7 is switch on lift plate. T-1 & T-2 are variable transformers to control press plate and colorimeter lamp. T-3 is a fixed 12 Volt transformer to control light intensity in densitometer. F is the main fuse and F-2-F-6 are fuses to the different components. P is the pin on microswitch M. R is the double pole double throw magnetic relay. A and B are the contact points of this relay.

IMPREGNATING THE REAGENT TAPE

In order to operate the instrument, reagent impregnated dried tapes needed to be developed. These tapes had to contain an active reagent and remain active for extended periods of time. Previous experimentation, described in the earlier report, had indicated that this could be accomplished if the tapes were kept thoroughly dry after preparation. To prepare such tapes, an instrument had to be devised, whereby we could experiment on the best method for their preparation. No such instrument could be purchased and we therefore devised one. Figures 9, 10, and 11 show the design of this instrument.

A 1/4 inch aluminum plate (1), is supported by two 1/2 inch aluminum rods (2). Four rubber stoppers (3), are affixed, as shown, to serve as feet. On the plate is mounted two pay-off reels, one to hold the reagent tape (4) and the other to hold the dialysis membrane (5). A porcelain roller (6) in a wetting tray (7) is coupled with an 18 RPM shaded-pole motor (W.W. Grainger, Inc., Chicago, Illinois). The porcelain roller rotates counter to the movement of the tape.

The reagent contained in the wetting tray is picked up by the roller and transferred to the tape. The tape, which comprises Whatman #3 paper laminated to 1 mil Mylar, moves with the paper downward so that the paper side is held tightly to the porcelain roller as it rotates.

The wetted tape now moves through a drying chamber (8), comprising a container with a Lucite window (9), on which is mounted a thermometer (10), to record the temperature in the dryer. A blower and heater combination

(Oster Hair dryer model 202, Milwaukee, Wisconsin) (11), is mounted on an adjustable mount (12), so that it can be moved toward or away from the drying chamber opening (13). In this manner the temperature is adjusted in the drying chamber. For enzyme reagents, which are unstable to heat, the blower can be operated at room temperature.

A pay off reel (4), contains the tape to be impregnated (14). The tape used is Whatman Chromatography paper, 3 MM, 1-1/2 inches wide and 300 feet long, with a thickness of 0.33 mm. These tapes have been backed on the rough side with a 1 mil thickness grade of Mylar, (Alco Press, Inc., Chicago, Illinois). The chromatography paper tapes were obtained from W.E R Balston Ltd., England by Standard Scientific Supply, Chicago, Illinois

A second payoff reel (5), contains a roll of porous tape or dialyzing membrane (15).

The dialyzing membrane chosen was a 0.5 mil Cuprophane of the medical grade 150PT-M, 38 mm wide and 150 mm long (Bemberg Company, Wuppertal, West Germany). The porous membrane is prepared by the cupric ammonium acetate method (Celanese) as opposed to the Viscose Method used by the DuPont Corporation. As a result it is contaminated with ammonia and copper. This can be washed out by rolling the membrane from one reel to a second, under water containing 5% acetic acid. For some procedures such as glucose, by glucose oxidase, the membrane can be used directly. For other determinations it needs to be washed. The Bemberg Co. claims that they can supply NH_3 and Cu free membranes. DuPont 0.8 mil Viscose prepared cellophane can be used for sugars by the glucose oxidase method.

The tape puller comprises two rollers which are recessed in the center so as not to touch the tape where the reaction will take place, eventually. The construction of the tape puller is the same as that described for the instrument for analysis. The rotation of the tape puller rollers is activated by a 2 R.P.M. AC-synchronous motor (17), (Holtzer-Cabot, Boston Massachusetts). This motor also activates a take up reel (18) by means of a sliding spring pulley (19).

The tape is impregnated in the following manner. Pay off reel (4), feeds the reagent tape to the wetting roller. The tape is impregnated with the reagent from the wetting tray (7) and moves through the drying chamber and to the tape puller (16).

The partially dried reagent tape now meets the porous tape and both are rolled up together on the take up reel. The combined tapes on the spool are now cooled in the deep freeze and transferred to a lyophilizer. In the lyophilizer the last traces of moisture are removed during a period of 48 hours. The roll is now transferred to a vacuum dessicator and stored in the deep freeze until ready for use.

Figure 9 : Reagent tape impregnator (view from right)

1. Aluminum backing plate, 12x26" x 1/4".
2. Aluminum supporting rods.
3. Rubber stoppers used as feet of the instrument.
4. Pay off reel for reagent tape.
5. Pay off reel for porous tape.
6. Porcelain wetting roller in Lucite tray containing the reagent.
8. Drying chamber.
9. Lucite window of drying chamber.
10. Thermometer to measure the temperature in the drying chamber (not shown but in same position as in Figure 1).
12. Adjustable mount for heater.
13. Opening in drying chamber to allow dry heated air to enter.
14. Tape to be impregnated.
15. Dialyzing membrane.
16. Tape puller, 16A. Meshed gears synchronizing top and bottom rollers.
18. Take up reel for combined dried tapes.
19. Sliding spring pulley to reel up the tape

Figure 10 : Reagent tape impregnator (view from left).

- 1-19. Same as Figure 9.
20. Rubber recessed roller pulling the tape.
21. Turn over roller leading tape to take up reel.

Figure 11 : Reagent tape impregnator (view from the rear)

- 1-21 Same as Figures 9 and 10.
7. Motor, 18 R.P.M. to drive porcelain roller.
11. Heater- blower.
17. 2 R.P.M. Tape puller motor.
19. Sliding spring pulley to reel up the tape.

Figure 10: Figure 10 : Reagent tape impregnator, view from left front

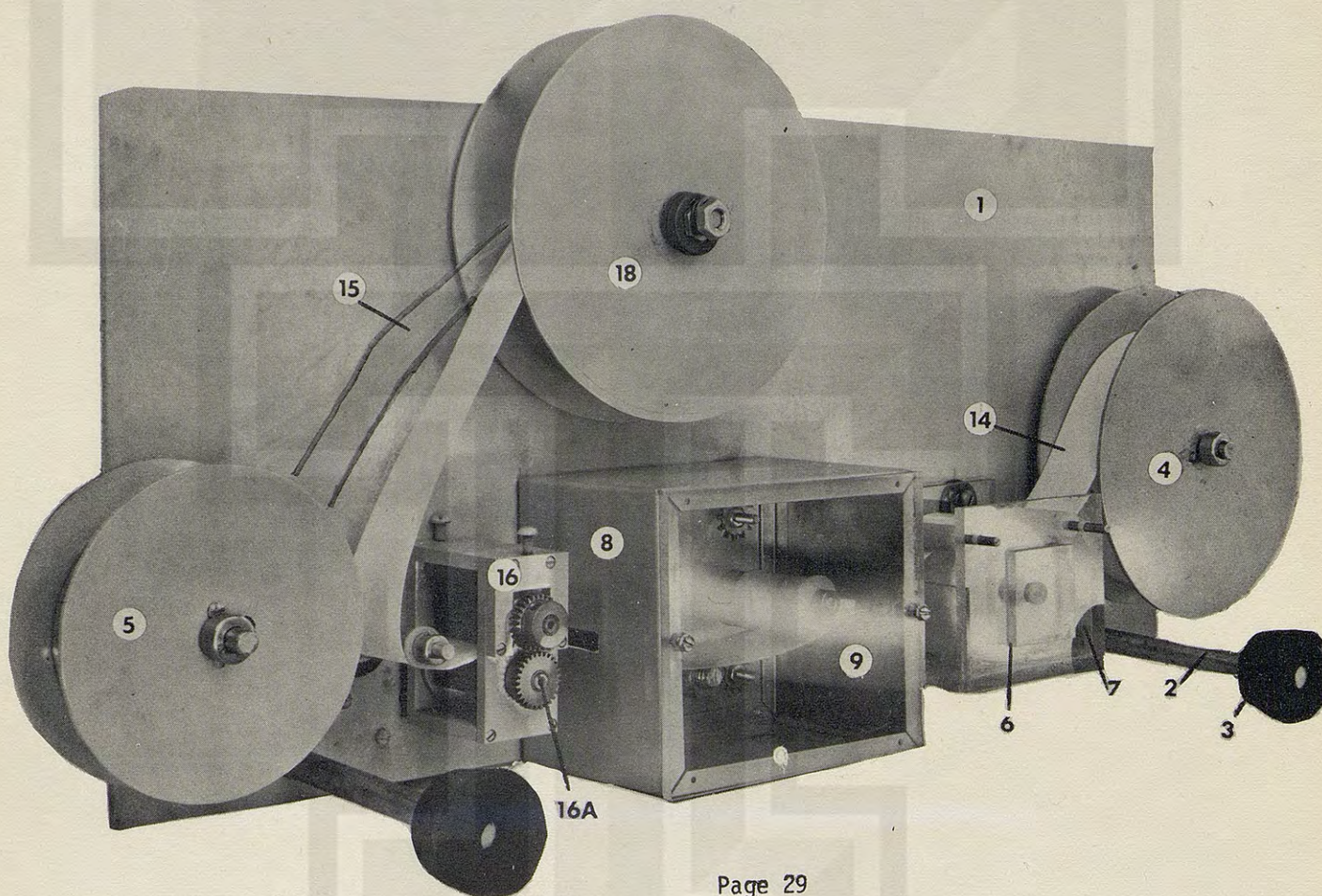
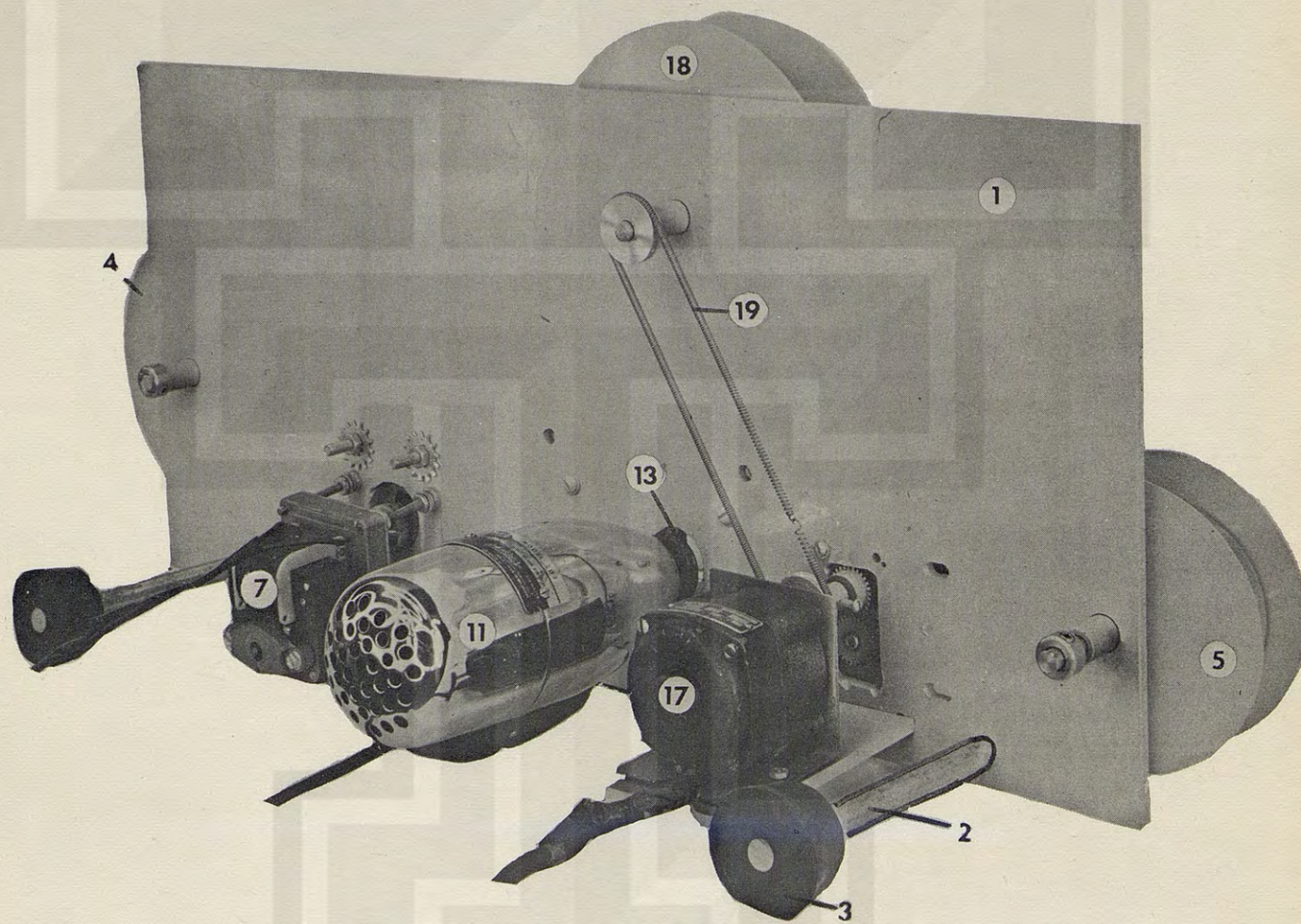


Figure 11 : Reagent tape impregnator, viewed from right rear.



THE REFLECTANCE DENSITOMETER

The reflectance densitometer is essentially the same as has been described in the earlier report. Only a second lens has been incorporated to sharpen the focus. The description is included in this report to completely describe the system.

Referring to Figure 12, light from the tungsten source (1), is brought to a focus by means of the double convex lens (2), and the single convex lens (3), at a small hole drilled in a selenium photocell (3) type A5PL, I.R.C., one inch in diameter. A light filter (5) is interposed to obtain monochromatic light. The beam spreads, once it passes the hole in the photocell, creating a disc of light which falls on the spot (6), on the reagent tape (7).

Reflected light now activates the selenium photocell. The electrical output of photocell (4), is balanced by the electrical output of another selenium photocell (8). This photocell is illuminated from the same light source (1) through a single convex lens (9), a light filter (10), and a 5 mm diaphragm (11). The output of the two photocells is balanced by the 5K ohm, 10 turn potentiometer (12). The 10K ohm, 10 turn potentiometer (13) adjusts for full scale deflection of the recorder from leads (14) and (15). Potentiometer (12) is used to zero the recorder.

The selenium photocells, type A5PL, were obtained from International Rectifier Corporation of El Segundo, California, the lenses from American Science Center of Chicago, Illinois, and the filters are from the Klett Manufacturing Company, Inc.

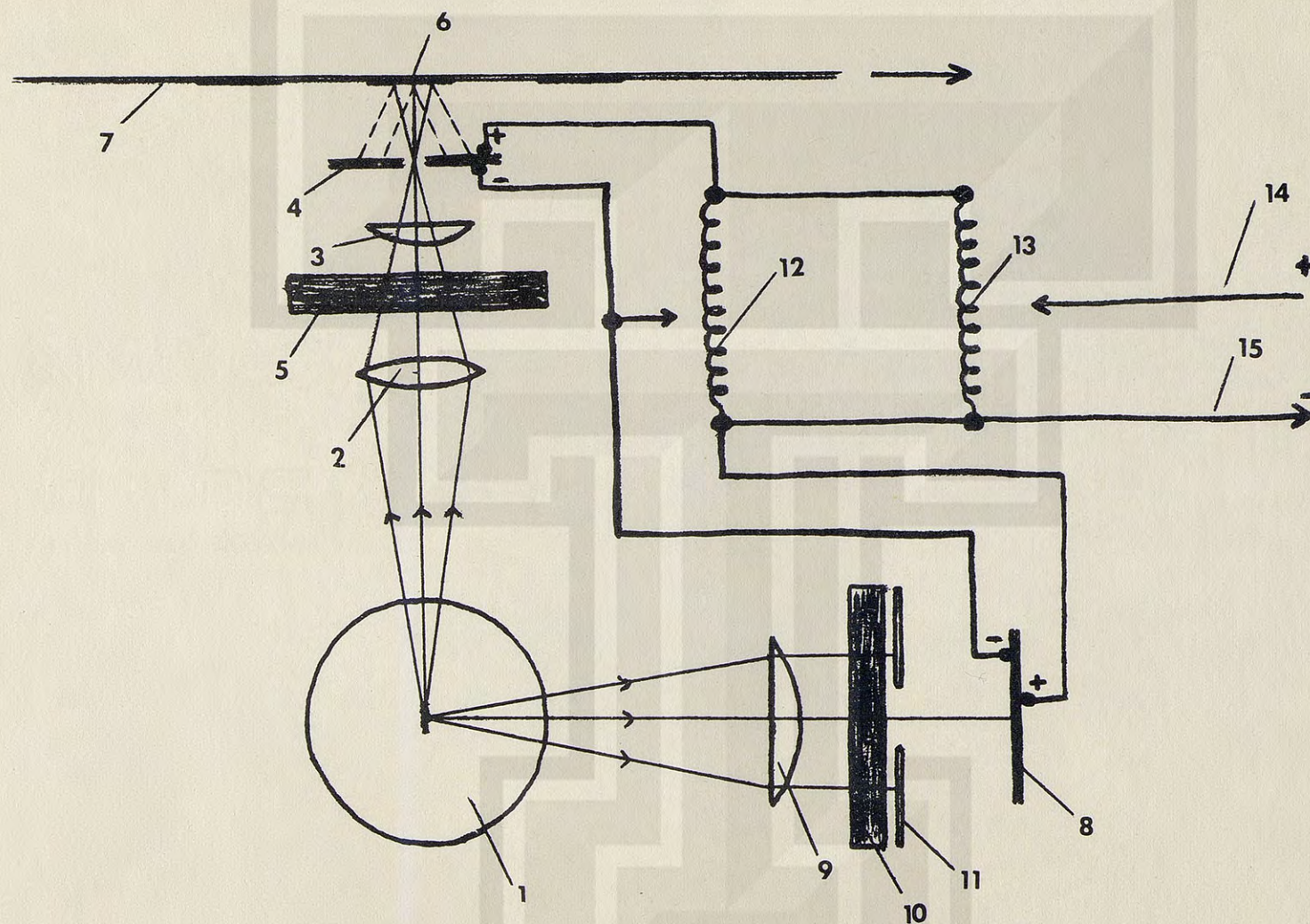


Figure 12 : The Reflectance Densitometer

1. Light source. 2 and 3. Focussing lenses. 4. Photocell with small aperture in center. 5. Light filter. 6. Paper disc reflecting light to photocell. 7. Sample tape 8. Reference photocell. 9. Collimating lens 10. Light filter 11. Diaphragm 12. 10 turn potentiometer 5000 ohms. 13. 10 turn potentiometer 10000 ohms. 14 & 15 lead to recorder.

SOLUTIONS USED TO IMPREGNATE THE TAPES

Urea or NH_3 by Dimethylaminobenzaldehyde:

Two grams of methyl cellulose (15 centipoise USP, Fisher Scientific Company) are dissolved with stirring in 200 ml of warm distilled water. Five grams of oxalic acid (Analytical Reagent grade) are dissolved in this solution while stirring continues. Two grams of dimethylaminobenzaldehyde are now added while stirring continues keeping the solution at 50° on a hot plate for one hour. The solution is cooled to room temperature and filtered through fluted filter paper. This solution is used within a few hours for impregnating the tapes.

Glucose by Glucose Oxidase:

A 1% gelatin solution (Knox unflavored gelatin, carbohydrate and fat free, Knox Gelatin, Inc., Johnstown, N.Y.) is prepared by dissolving one gram of gelatin in 100 ml of warm water with vigorous stirring.

A buffer, pH 7.0 is prepared by dissolving 27.6 g of sodium dihydrogen phosphate ($\text{NaH}_2\text{PO}_4 \cdot \text{H}_2\text{O}$, Mallinckrodt, A.R.) in 500 ml of water. NaOH (10%) is added drop by drop with stirring until the pH of 7.0 is reached. The solution is now diluted to 1 liter with water.

At room temperature, 200 ml of the buffer is placed in a flask. Eight ml of the 1% gelatin solution is added, followed by 400 mg of glucose oxidase (Type II, Sigma Chemical Company, St. Louis, Missouri, 13000 units/g) with stirring. After the solids are dissolved 12 mg of horseradish peroxidase (Type I, 63 Purpurogallin units/mg, Sigma Chemical Company) is added, stirring the mixture. Forty milligrams of ortho-dianisidine, dihydrochloride (3,3'

dimethoxy benzidine, Di-HCL, analytical reagent grade) are now added with stirring, until dissolved.

The reagent is used within 3 hours of preparation for impregnating the tapes. When not in use it is kept refrigerated.

PREPARING THE REAGENT TAPE

The tapes are impregnated with the instrument described above. The tape moves at the rate of 13.8 cm/minute through the instrument. For glucose oxidase, the drying chamber is maintained at 40°C. The tapes are in the drying zone for 65 seconds. For the urea reagent, the drying chamber is maintained at 70°C.

After the tapes are prepared and rolled up with the cellophane, they are lyophilized for 48-72 hours to insure dehydration. The stability of the glucose oxidase reagent will not be maintained unless lyophilized for at least 48 hours because of the difficulty in removing residual water from a rolled up tape. Once the tape is properly prepared they are stable indefinitely if kept in a plastic bag which also contains a silica gel drying agent. They must also be kept away from light. They are best kept in this manner in the deep freeze.

Tapes, with glucose oxidase, prepared in March of 1967 still have the same activity in January of 1969. These have been kept in plastic bags in the refrigerator. Even at room temperature, when kept away from moisture stability has been observed over a nine month period provided the tapes are kept away from direct light and kept anhydrous.

METHODOLOGY

Sampling:

In the earlier report the sample was placed on the spot by means of a pipet or a capillary sampler using precalibrated capillaries. It was noticed that reproducible results could be obtained if the paper discs, on the sampling tape, were saturated with the sample. This mode of sampling was therefore explored.

In effect, in this mode of sampling, the paper disc is the measuring device, by means of which the samples are to be added to the instrument. This would have the advantage that a drop of blood, which gathered on the finger of the astronaut, after puncture, could be held to the paper disc. The paper disc would absorb the sample until it was saturated. In this manner no capillaries or pipets would be necessary.

A study was therefore undertaken to evaluate this method of sampling. Since water evaporates, it is difficult to reproduce weighings unless they are performed rapidly. Ethylene glycol was therefore chosen as the test material.

Squares were cut from the sampling tape containing a single disc. A capillary containing excess ethylene glycol was held at an angle of 45° barely touching the disc. After eight seconds (the time used for sampling on the automatic instrument) the capillary was removed. The paper was actually saturated in less than four seconds. The section of sampling tape containing the disc was weighed before and after the sample had been applied.

Table I indicates the reproducibility obtainable with this method of sampling:

TABLE I
PRECISION OF THE METHOD OF SAMPLING WITH
PAPER DISCS

Sample	dry wt.	wet wt.	net
1	57.3	115.7	58.4
2	56.7	116.0	56.3
3	55.1	110.3	55.2
4	56.6	112.4	55.8
5	57.6	113.7	56.1
6	56.4	114.5	58.1
7	55.3	110.9	55.6
8	56.1	112.1	56.0
9	55.0	111.1	56.1
10	54.8	110.8	56.0

Mean = 56.6 mg., Standard Deviation= ± 1.1 mg.
% Error (95% probability)= $\pm 3.6\%$

From Table I it can be seen that this method of sampling has a precision of $\pm 3.6\%$. Considering that approx. 50 μ l are being sampled it is apparent that this method of sampling is satisfactory for the purposes intended.

RATE OF EVAPORATION OF BLOOD FROM THE SAMPLE DISC

In order to study the effect of evaporation rate on the tape system of analysis, 50 μ l samples of blood were pipetted on to single discs supported on the Mylar tape. The discs were weighed promptly and at timed intervals thereafter. The results are shown in Table II for ten consecutive specimens.

TABLE II
Weight loss (mg) from 50 μ l of blood by evaporation

Elapsed Time (Minutes) \ Sample #	1	2	3	4	5	6	7	8	9	10	Mean
1	0.9	0.8	0.9	1.0	1.0	0.8	0.9	0.9	1.0	0.9	0.9
2	1.4	1.6	1.5	1.6	1.5	1.4	1.5	1.4	1.3	1.6	1.5
10	8.8	8.8	8.9	8.7	8.8	8.8	8.7	8.8	8.8	8.8	8.8

The sample disc is in place for sampling for 8 seconds. An additional eight seconds elapses before the sample tape reached the pressing zone. Evaporation in one minute is $0.9/50 \times 100 = 2.5\%$. Evaporation for sixteen seconds calculates to approximately 0.7%.

The evaporation rate is not quite linear but very little error is attained by assuming that it is linear for the first minute. The error of 0.7% is consistent for each specimen. Since standards are run under the same conditions and results are compared to these standards, this error becomes insignificant.

APPLICATION OF THE INSTRUMENT TO SPECIFIC PROBLEMS

I. Instrument Evaluation:

In order to evaluate the performance of the instrument a red dye was chosen which would pass the cellophane membrane. Samples were administered to the sample tape using the paper discs to sample the dye solution. In this manner the following factors were being checked:

1. The uniformity of the spots formed as the dye passed the tape.
2. The reproducibility of the intensity of the spots.
3. The performance of the instrument in repetitive analysis.
4. The performance of the densitometer and readout system.
5. The performance of the instrument at a rate of 180 specimens/hr.
6. The uniformity of the cellophane.

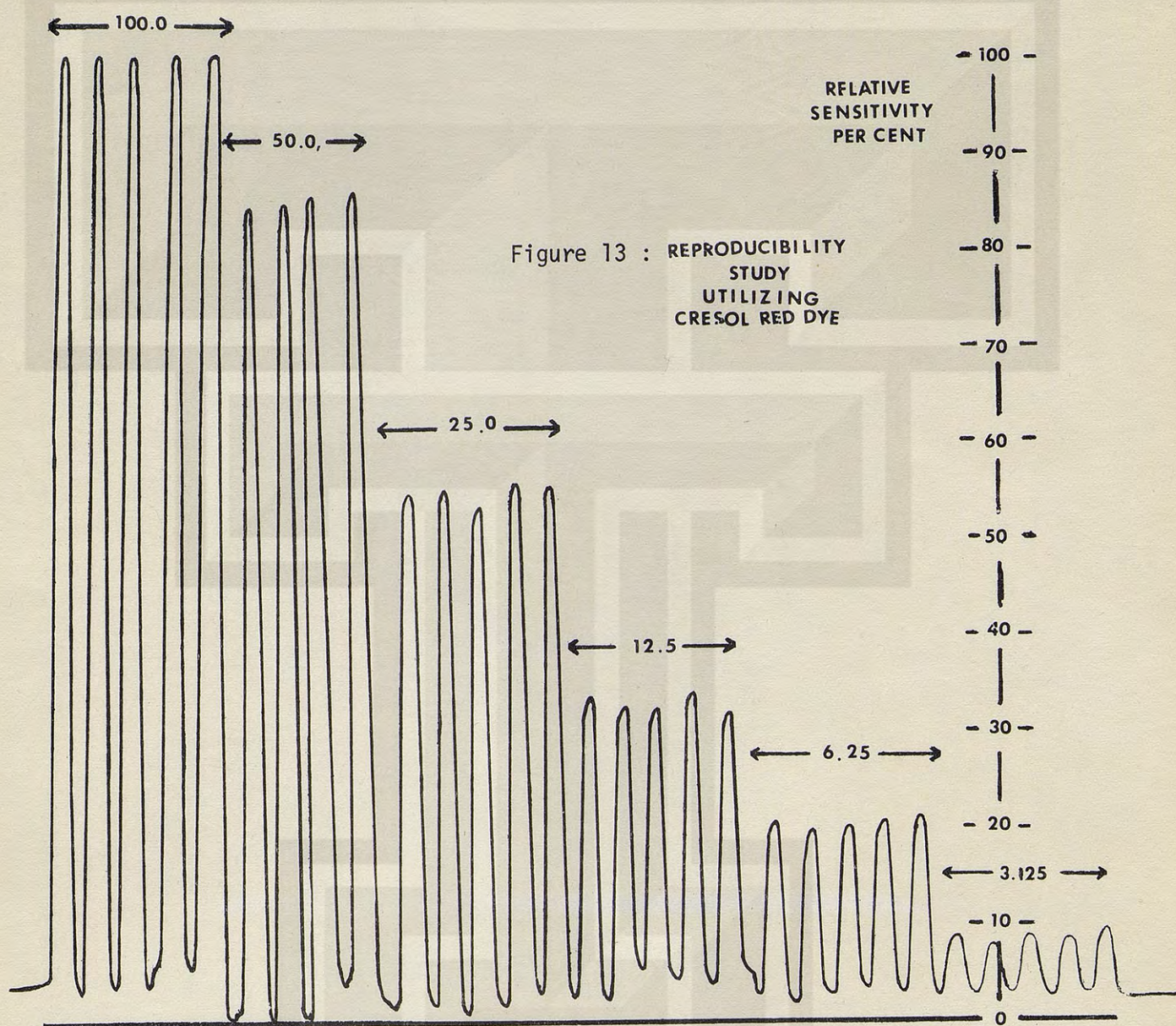
Figure 13 shows the curves obtained in this study. The dye was successively diluted, and sets of five were run on the same dilution. For example, the dye was diluted to half its concentration. This was again diluted to half its concentration etc.

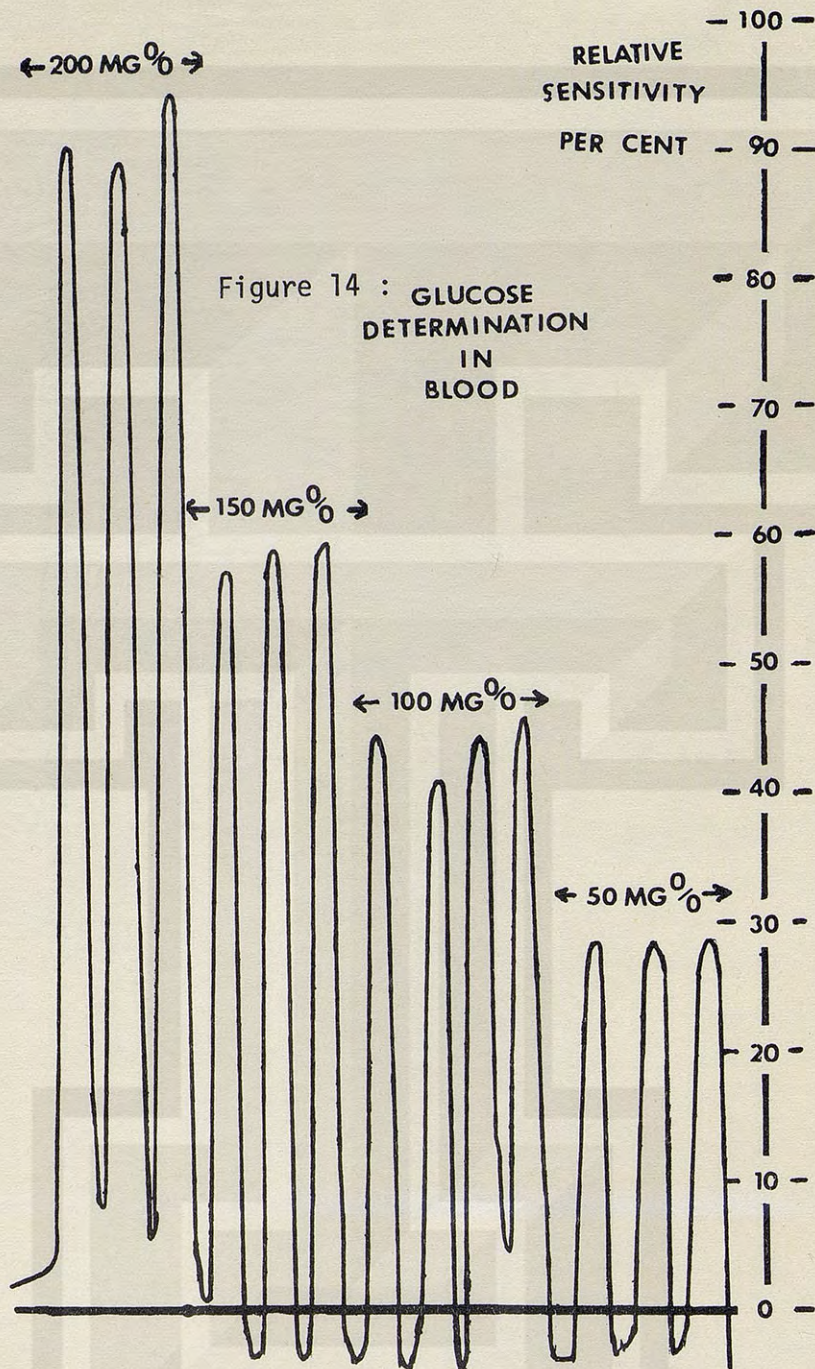
Analysis of the data indicated that reproducibility was of the order of $\pm 3\%$ (2 Sigma) for the sets.

From this study one may conclude that the performance of the instrument and the uniformity of the cellophane is satisfactory for our purposes. The basic principle of this method of analysis, is thus proven to be sound.

II. Glucose Determination

Figure 14 shows typical tracings obtained for the standard curve





for glucose determination using the dry impregnated tapes and approximately 50 μ l on the sample disc.

Analysis of the results obtained on repetitive analysis indicated that more reproducible results could be obtained when the tapes were impregnated from a wetting roller than when the dry tapes are used. The reason for this may be that uniform distribution of the reagent on the dried tape had not been achieved. This probably occurs during the drying procedure.

The sensitivity of the dried tape, using the glucose oxidase reagents described above, remained the same from week to week.

In comparing these results with those obtained by the routine laboratory, on 400 specimens, correlation was observed between this procedure and that obtained by ferricyanide reduction, with the autoanalyzer ($\pm 3\%$). However one case was found where the autoanalyzer gave a value of 550 mg/100ml., and the glucose oxidase method gave 50 mg/100 ml. The test on this specimen was then repeated by the Folin-Wu method and the glucose oxidase method in a test tube. The Folin-Wu method gave 600 mg/100 ml. The glucose oxidase procedure gave 50 mg/100 ml.

The patient whom this blood was drawn was in diabetic coma. In this condition, glucose oxidase inhibitors accumulate in the plasma which inhibit the glucose oxidase method. Glucose added to normal plasma is readily recovered by the glucose oxidase method but not in these abnormal sera.

This phenomenon has been observed before with glucose oxidase procedures and this method is undesirable as a test procedure for several reasons.

1. Glucose oxidase is inhibited by substances, which pass through a

cellophane membrane and which accumulate in the body in diabetic coma. It is also inhibited by certain drugs like the sulfonamides.

2. Glucose oxidase reagent stability can be achieved but accidental subjection of the tapes to moderately elevated temperatures, to moisture or to air could inactivate the tapes.

3. The reagents are very expensive as compared to the conventional chemical reagents.

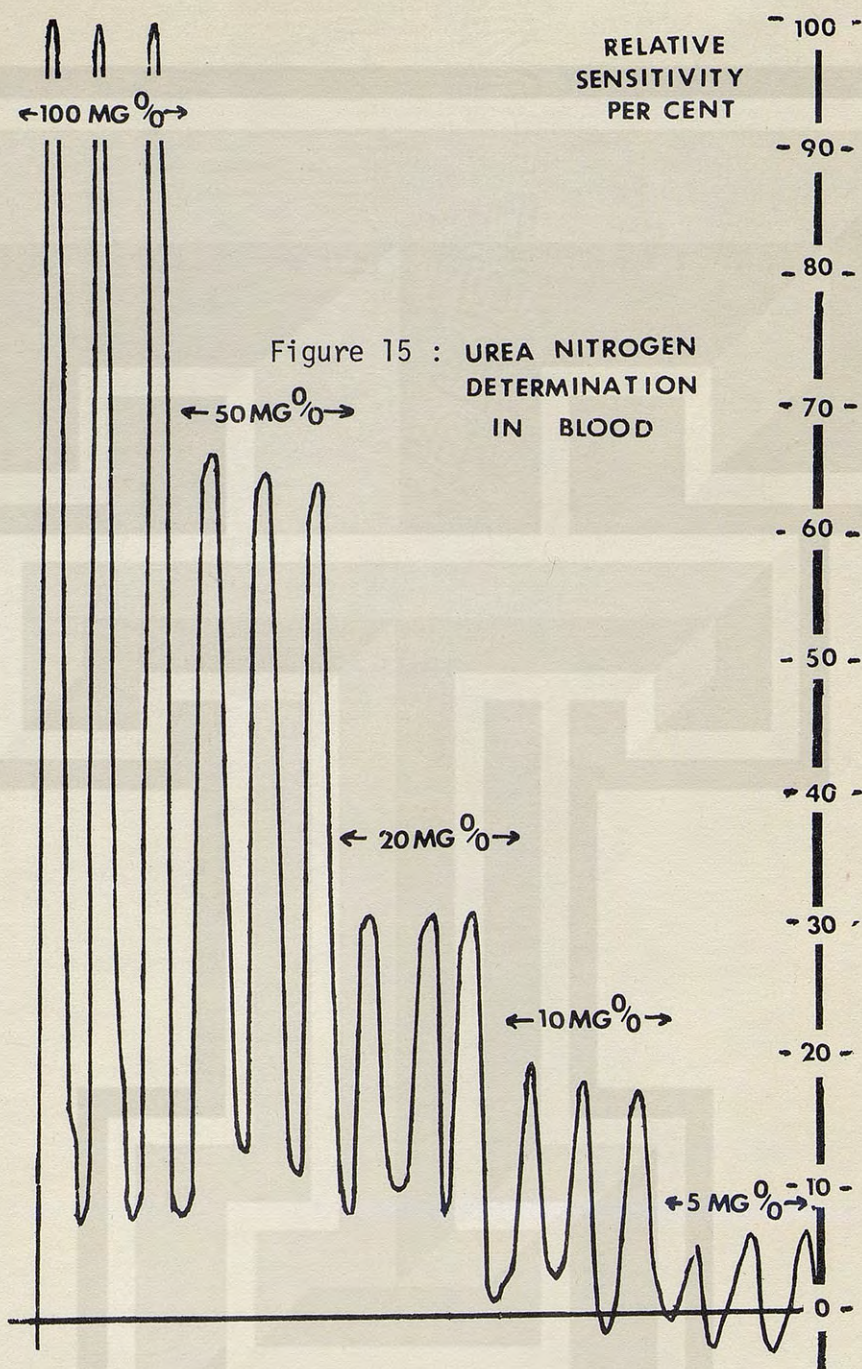
In conclusion, while the method employed yields reproducible results it is now felt that a restudy of simple reducing agents would be desirable for the tape system. The o-toluidine method is under study.

III. Urea Determination

The urea method chosen for study was the reaction of dimethylaminobenzaldehyde to produce a yellow color.

Figure 15 demonstrates the standard curve obtained with this method using the dry reagent tape. The tape contains dimethylaminobenzaldehyde, methyl cellulose and oxalic acid. Similar observations were made here, as were observed with the glucose method. The wet tape system, using a roller to moisten the tapes with reagents was more reproducible than that when the reagent was predried on the tapes. Nevertheless the results, as indicated by Figure 20 were satisfactory ($2\sigma = \pm 5\%$) for practical purposes.

When this procedure was compared to determinations performed by the routine laboratory correlation was generally within 2-3%. However, an occasional serum yielded high results. In these cases it was found that the patient had been given Gantrisin (a sulfonamide). Sulfonamides were



demonstrated to interfere in this procedure.

In conclusion, by this procedure, the instrumentation was shown to yield reproducible results in all cases. Interference was observed in the presence of certain drugs. For this reason this procedure could not be used in the routine laboratory without modification. For specialized purposes with individuals who are known, not to be on drug therapy this procedure would be of value.

IV. Routine Data

Routine data is presented in Tables III, and IV for both glucose and urea analysis, comparing the results with that obtained by the autoanalyzer procedure. The glucose method of the tape system uses glucose oxidase, that of the autoanalyzer uses ferricyanide reduction.

The urea method of the autoanalyzer uses the monoxime of diacetyl, in the presence of ferric salts. That of the tape system uses dimethylaminobenzaldehyde in the presence of oxalic acid.

In both instances it is apparent that these procedures would be acceptable, provided that the patient were not on drugs. In the urea method, the interference from gantrisin is noted. In the case of glucose oxidase, some patients, not on drugs but in diabetic coma will yield low results.

This data however clearly shows that the basic principle of the instrument is sound and that reproducibility is readily achieved. Analysis is rapid. Instrumentation is simple and essentially foolproof.

TABLE III

Comparison of Values Obtained for Blood Glucose By the Autoanalyzer
(Potassium Ferricyanide Method) and the Tape System (Glucose Oxidase Method)

Sample #	Autoanalyzer (Mean Value)	Tape System	Sample #	Autoanalyzer (Mean Value)	Tape System
1	96	89,89	34	118	116,117
2	86	89,89	35	124	115,115
3	90	90,92	36	126	130,131
4	128	128,120	37	85	82,86
5	88	85,88	38	108	106,110
6	86	85,85	39	80	76,76
7	278	280,278	40	106	108,106
8	276	250,250	41	178	176,175
9	142	138,139	42	164	168,168
10	226	199,184	43	139	154,150
11	210	180,182	44	88	98,96
12	114	112,114	45	81	90,88
13	92	90,86	46	88	92,92
14	118	117,114	47	100	94,87
15	144	142,138	48	105	110,107
16	90	87,96	49	137	124,127
17	143	158,158	50	1176	1120,1150
18	112	110,108	51	130	127,127
19	98	96,96	52	167	165,162
20	112	115,115	53	92	89,95
21	120	110,112	54	86	80,80
22	102	100,97	55	96	90,90
23	80	84,80	56	116	117
24	118	120,116	57	174	180,170
25	90	88,86	58	96	90,90
26	107	112,112	59	228	212,207
27	98	88,91	60	140	148,140
28	106	108,106	61	110	110,110
29	106	112,111	62	164	150,153
30	102	96,90	63	236	225,217
31	122	130,134	64	102	106,110
32	120	120,120	65	138	138,129
33	196	190,187	66	87	86,85

TABLE IV

Comparison of Values Obtained for Urea Nitrogen By the Autoanalyzer (Diacetyl Method) and the Tape System (Dimethylaminobenzaldehyde Method). Circled Samples Indicate Patients on Sulfa Drugs. Note Interference in These Specimens

Sample #	Autoanalyzer (Mean Value)	Tape System	Sample #	Autoanalyzer (Mean Value)	Tape System
1	18	18,18	34	16	18,18
2	16	12,14	35	17	14,16
3	29	23,29	36	9	7,7
4	12	11,10	37	13	9,9
5	26	24,25	38	62	59,59
6	23	19,19	39	61	59,60
7	12	10,10	40	10	10,11
8	19	67,65	41	10	8,9
9	21	23,24	42	15	15,15
10	46	47,46	43	16	67,68
11	13	13,13	44	17	17,18
12	46	50,51	45	16	18,18
13	8	6,7	46	18	16,17
14	90	90,90	47	15	12,12
15	14	16,15	48	13	14,17
16	17	18,18	49	14	14,15
17	30	30,30	50	4	8
18	14	15,14	51	4	7
19	8	8, 8	52	17	14,14
20	12	12,12	53	100	98,100
21	8	9,9	54	15	16,16
22	10	12,12	55	10	8,9
23	12	30,30	56	40	44,44
24	196	200,200	57	13	38,37
25	17	19,18	58	11	12,13
26	95	95,95	59	17	19,20
27	71	75,76	60	10	11,10
28	15	18,14	61	19	17,17
29	21	22,26	62	46	50,51
30	13	13,13	63	21	20,22
31	15	16,21	64	15	18,18
32	126	120,121	65	16	18,17
33	13	11,12,13	66	7	7,7

ACHIEVEMENTS UNDER THIS CONTRACT

The major achievements under this contract were as follows:

1. A practical instrument was designed and built suitable for automatic analysis of blood for various components in a weightless environment at a rate of speed not equalled by any automatic equipment available today.
2. This instrument when tested for reproducibility on thousands of specimens showed a precision of $\pm 3\%$ (2σ).
3. A system of sampling was devised where no measuring device is required by the operator. The sample disc absorbs a reproducible amount of sample. In space flight this means that the astronaut could prick his finger, allow a drop of blood to accumulate, touch this to a piece of paper and withdraw the finger with excess blood on it. This method of sampling can be used for other purposes of analysis such as hemoglobin assay, ratio of oxyhemoglobin to hemoglobin etc.
4. A practical system was devised for impregnating tapes and drying them (lyophilization) in a compact form for large scale production.
5. A reflecting densitometer was developed on the basis of a new principle where high stability comparable to that obtained with photomultiplier tubes is obtained, using inexpensive selenium cells. This eliminates the need for high power consumption.
6. Since completing this report a study has revealed that a reaction between sulfanilic acid in the presence of succinic acid and arsenic acid, as an oxidizing agent, shows strong promise of being a specific method for glucose estimation.

PROBLEMS STILL TO BE SOLVED

For the present, instrumentation problems have been solved satisfactorily. The focus on continued study is the search for improved methodology for specific procedures.

Glucose oxidase activity is inactivated by certain drugs. The dimethylaminobenzaldehyde method for ureas required that sulfa drugs be absent. Diacetyl is more specific for urea but sensitivity is low when the reaction is carried out at lower temperatures.

Commercial sources need to be found for the tapes. The cellophane we have been using is approx. 1 mil in thickness. This is made by the Viscose process and contains no significant impurities. Thinner cellophane 0.5 mil obtained from Bemberg Co., permits more rapid dialysis. However it is contaminated with copper and ammonia since it is made by the cupra-ammonium process. The securing of this cellophane washed free of impurities is now under study.

The sample tape presents a problem in that placing discs or rectangles at regular intervals presents a manufacturing problem with high initial investment. Study of the reagent tape made of materials other than paper is also under study. Fiberglass is one example.

All of these problems suggest their own solution. Studies need to be performed to select the most practical procedures.