

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

FINAL PROGRESS REPORT

Contract Number NAS-9-7934, Supplemental Agreement No. 2

Contract Date 9/1/68 - 8/31/69

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.

Bruce MacCallum, B.A.

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This report is a continuation of the final progress report covering the contract dates 8/1/67 - 8/31/68 of contract # NAS-9-7934.

Background

In the earlier report, an automated system was described for the analysis of blood and urine for various components. The system was designed so that no liquid reagents were employed.

The general system proposed is to apply a sample of blood on a paper disc. The paper disc adsorbs a measured amount of blood. This eliminates the need for a measuring device for measuring out the sample.

This paper disc is disposed on an elongated flexible plastic support, such as a mylar tape. This is called the sample tape. A porous tape such as cellophane is also part of the system. This porous tape permits the blood filtrate to pass but retains protein and erythrocytes. After passing the cellulose tape, the sample comes in contact with a reagent tape. The reagent on this tape produces a color with the unknown component sought.

In the earlier study, an automated system was built. In this system, the three tapes are fed from pay off reels. On activation of the instrument, the paper disc, on the sample tape moves to a sampling station and stops. The blood is added. The disc carrying the blood now moves around a roller to meet the cellophane tape and the reagent tape. The sequence is now, sample tape, cellophane and reagent tape, with the sample disposed on the sample tape face to face with the cellophane.

The three tapes now move to a press plate, which applies pressure and raises the temperature to 37°C. Dialysis of the blood filtrate takes place from sample tape, through cellophane to reagent tape. The tapes are separated and the reagent tape is heated, to dry, and the colored spots are read on a densitometer, by reflectance.

Objectives of the Present Study

After examination of the automated system, it was felt that the power requirements for such a system would be excessive, if used in a space laboratory. It was further anticipated that switching from one procedure to another, with the automated system of analysis, would require excessive storage space in that a separate roll of reagent tape would be required for each type of analysis. It was also felt that the weight of the automated instrument would be excessive.

For these reasons, the individuals engaged in this study were asked by the contractor to concentrate on hand system. It was visualized that,

1) For each type of test a pad would be prepared containing the three tapes. These pads would be stored in a small cupboard or similar arrangement. Thus, any number of different types of pads could be stored.

2) The press plate should be hand operated, using a minimum of current. It would preferably be operated at ambient temperature. Using a longer pressing time would obviate the need for a higher temperature. If the temperature is elevated it should not exceed 50°C.

(3) A densitometer would be developed, hand operated, for read out of the pads which could read several specimens. The only current consumed would be that of a flashlight bulb which could be battery operated.

In summary, it was required that a new set of instrumentation be developed for a hand operated machine. In addition, a means had to be developed for a system, applicable in space, for sampling a specimen of blood and diluting it for analysis. This also had to be hand operated. How these problems were approached and solved will be discussed below.

The Test Pad

The upper layer, where the sample is deposited is called the sample paper. The lowest layer, which contains the reagent is called the reagent paper. These will now be discussed, along with the porous layer, which in this case is cellophane.

It was decided to use a circular arrangement for several reasons.

1) A multiplicity of specimens could be disposed radially at regular intervals.

2) A mechanism was commercially available for moving a circular card. This is called the View Master, and is used, generally, as a stereopticon.

3) An instrument for reading the reagent circular pad could be readily designed based on principles developed earlier for the automated machine.

The Sample Paper

The sample paper is shown in Figure 1. It comprises a circular piece of cardboard on which is pasted, by means of rubber cement, Whatman #3 filter paper discs 15.5 mm in diameter. These are disposed equidistant from each other, with the disc edge 5 mm from the edge of the cardboard. The cardboard is ordinary poster board 0.5 - 0.6 mm in thickness. The rubber cement acts as a barrier to the passage of water in the sample to the cardboard.

The sample paper is notched. At the center of the circular cardboard, a hole (3/16") in diameter is punched. This helps in lining up this section of the pad with the other layers of the pad. This size disc (15.5 mm) measures approx. 50 μ l of sample when saturated.

The Cellophane

The cellophane used may be 0.5 - 1 mil in thickness. The 0.5 mil cellophane is obtained from the Bemberg Co., in Wuppertal, West Germany. Unfortunately, this cellophane contains ammonia which has not been completely washed out. The 1 mil cellophane is available from the DuPont Co., and is made by the viscose process. With the heavier cellophane, one merely presses the pad longer to achieve the same results in the pressplate. For this reason, the use of the heavier cellophane is not a significant disadvantage. The cellophane is cut in circles, slightly in excess of 9 cm. The orientation of the cellophane is not of significance. It is placed and centered between the sample and reagent circles. For this purpose, it has a 3/8" diameter hole which is punched out in the center.

Index for centering
in colorimeter

Whatman #3
15.5 mm.
filter paper discs

3/16" hole

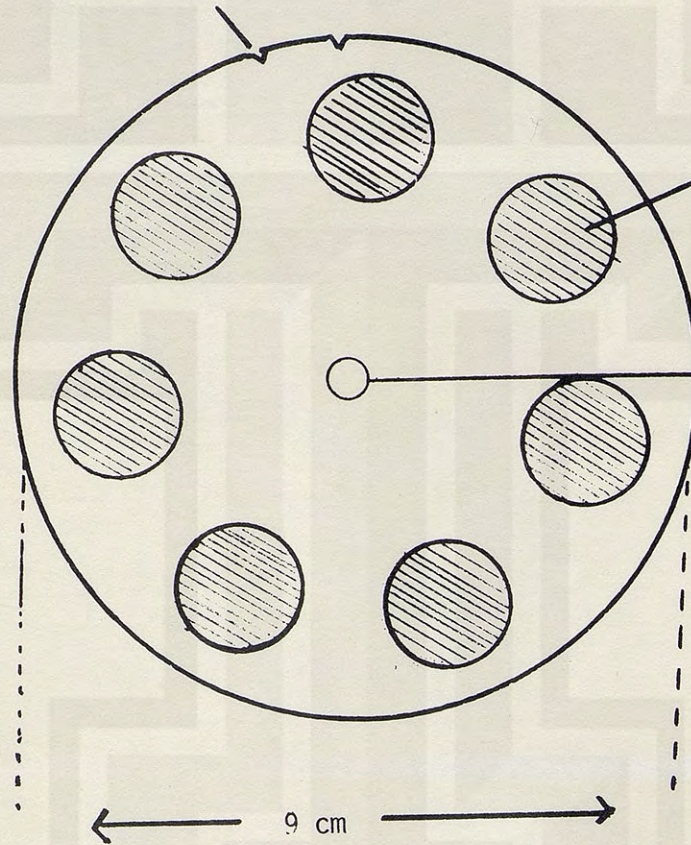


Figure 1. Sample Circular Pad

The Reagent Paper

The reagent paper comprises 9 cm Whatman #3 circles of filter paper which have been glued to cardboard by means of rubber cement. They are punched with rectangular indexing holes. A hole is punched in the center and the edges are notched so as to line up the reagent paper in the densitometer for readout (See Figure 2).

The reagent to be used is impregnated in the filter paper circles before mounting on the cardboard. This is done by dipping in the reagent, removing the excess by shaking, and placing the filter paper in the support shown in Figure 3. The filter paper on its support is placed in a stainless steel container and lyophilized. For this purpose, the Vacu-Plate lyophilizer of Refrigeration for Science, Oceanside, N.Y. is used.

After lyophilization, the filter paper is mounted on the cardboard. The holes are punched out and the reagent paper so prepared is ready for assembly in the pad.

The support shown in Figure 3 comprises a series of plastic shelves on a plastic support. A stainless steel container 8" deep and 6" wide will serve to hold the container designed to hold up to 20 filter paper circles, during lyophilization. The support shown in Figure 3 was made by cutting circles in the covers of plastic Petri disposable dishes and gluing them to a Lucite rod for support as shown in the figure.

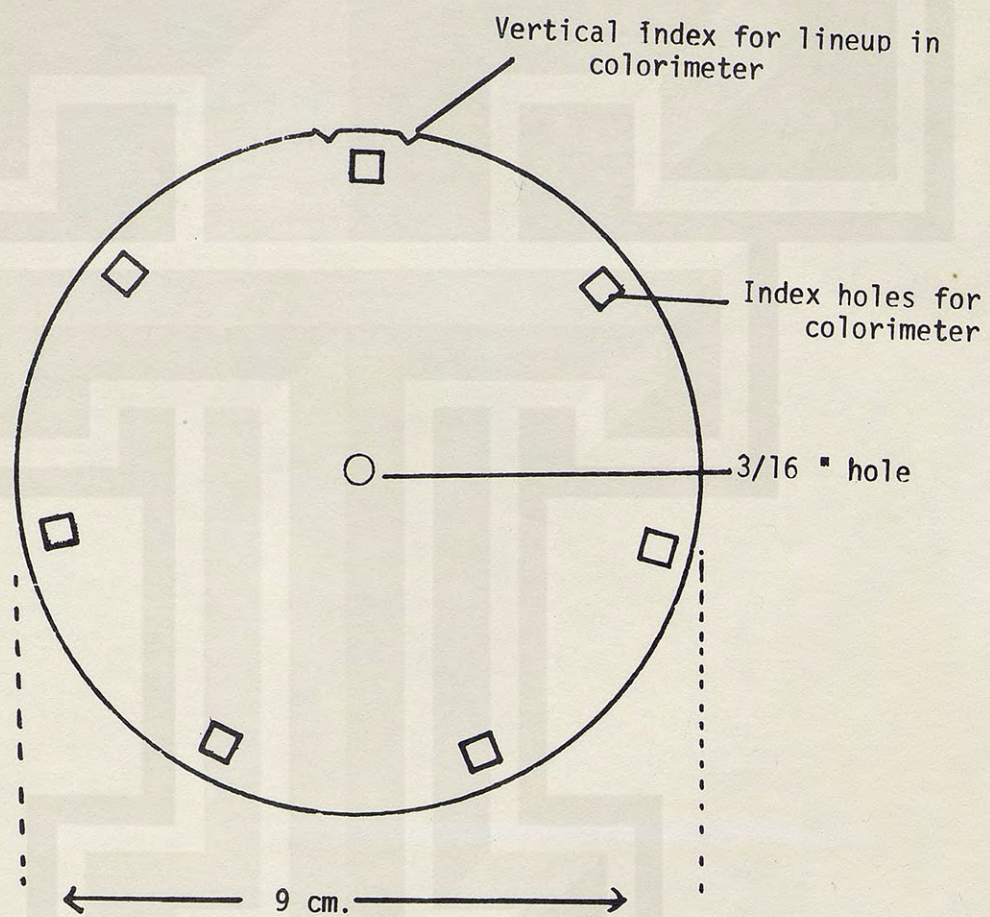
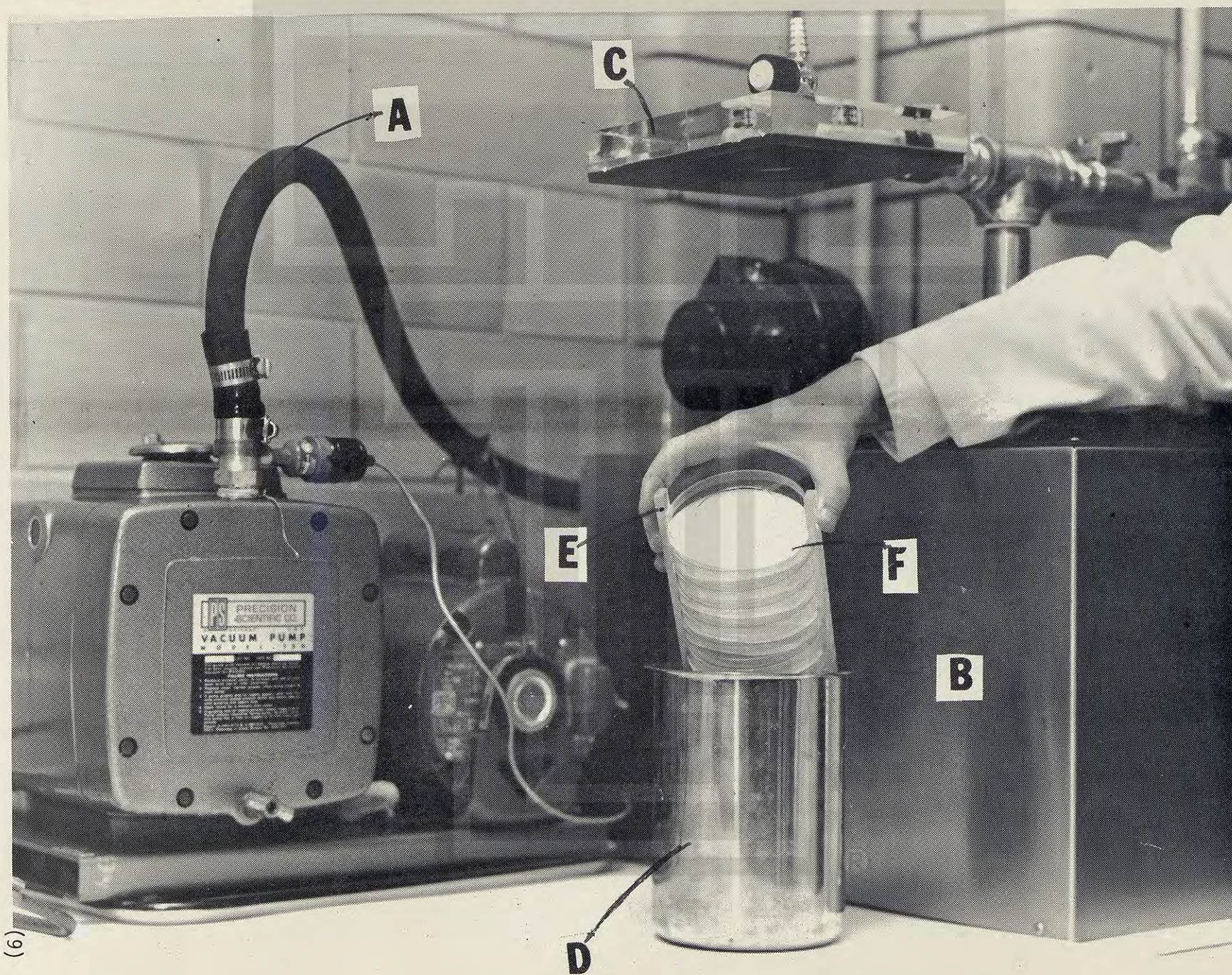


Figure 2. Reagent Circular Pad

Figure 3: Arrangement for lyophilization of the reagent paper. A is the connection to the Vacu-Plate(C). B is the lyophilizer (Refrigeration for Science, Oceanside, N.Y.). D is the stainless steel beaker for holding the plastic rack. E is the plastic rack made out of disposable Petri dishes and lucite rod holding the reagent papers F.



The Assembled Pad

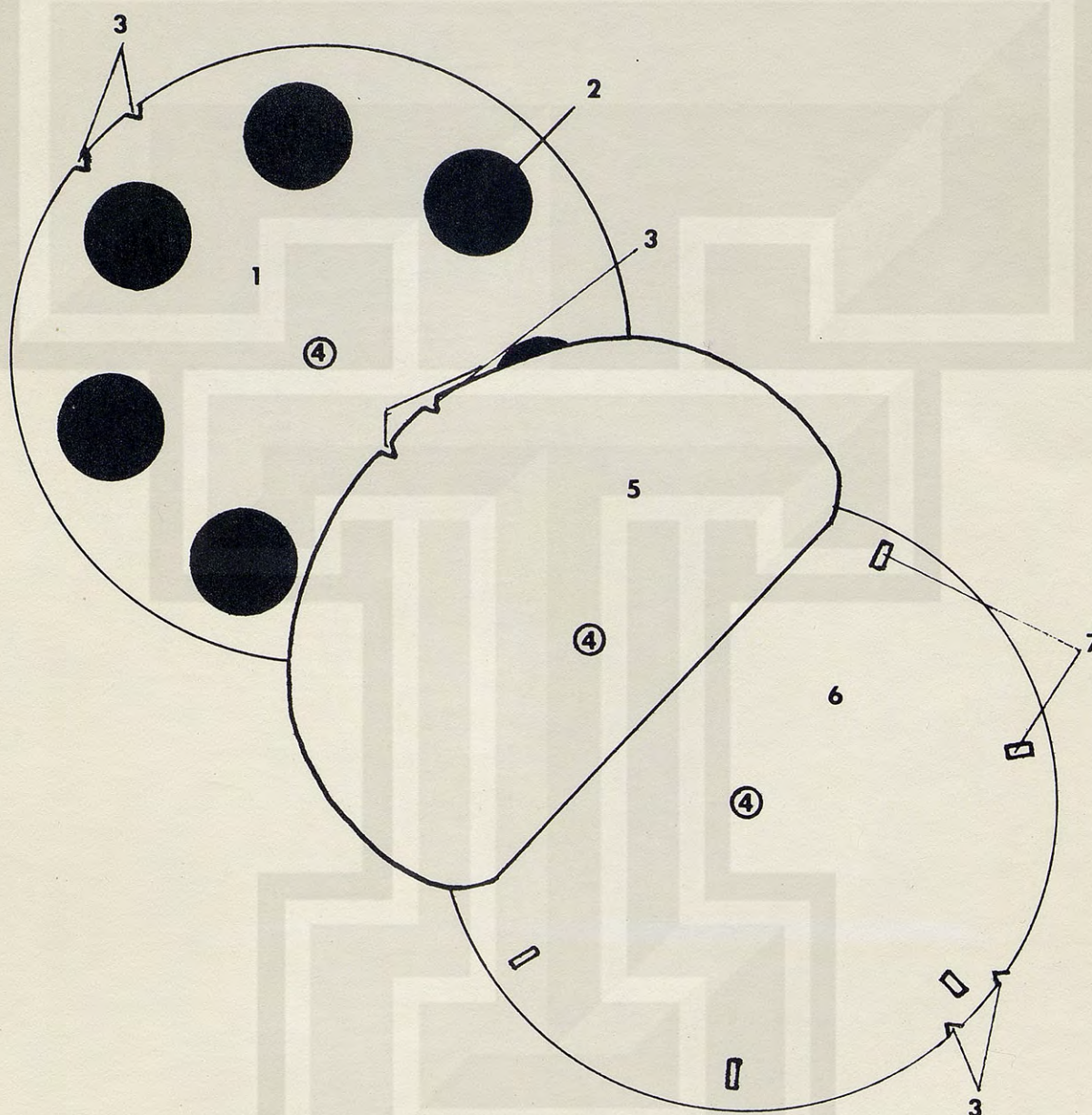
The three circles containing the specimen discs, cellophane and reagent, respectively are aligned by means of the hole in the center and wedges at the edge. The specimen discs face downward so as to face the cellophane. The set up is shown in Figure 4.

The Pressplate

The pressplate is so designed that hand closure will cause the plates to snap together. The metal plates are circular discs of aluminum, attached together by a clamping arrangement as shown in Figure 5. When the handles are grasped, and pressure is applied, the plates move together. A final squeeze of the hand grip causes the second lever to move down and force the top plate down. This locks the plates together, under pressure. The plates are released, by holding one handle down and pulling up on the second handle.

The lower plate is heated by an electrical Nichrome tape. A Variac transformer controls the current going through the heating wire so that the temperature can be set at a predetermined value. A thermometer rests on the upper plate and measures the temperature at which the pressing is carried out.

Figure 4. The assembled pad.



1. Sample circular pad. 2. Whatman #3 15.5 mm filter paper discs. 3. Indexing for line up in colorimeter. 4. Center hole. 5. Cellophane. 6. Reagent circular pad. 7. Index holes for colorimeter.

Figure 5 A: The pressplate. A is the heating coil, B is the aluminum block on which rests the assembled pad C. D is the top plate and E is the thermometer for measuring the temperature of the top plate. F is the spring for applying pressure to the plate. G is the lock hinge which closes and locks as pressure is applied at H by bringing it together with J.

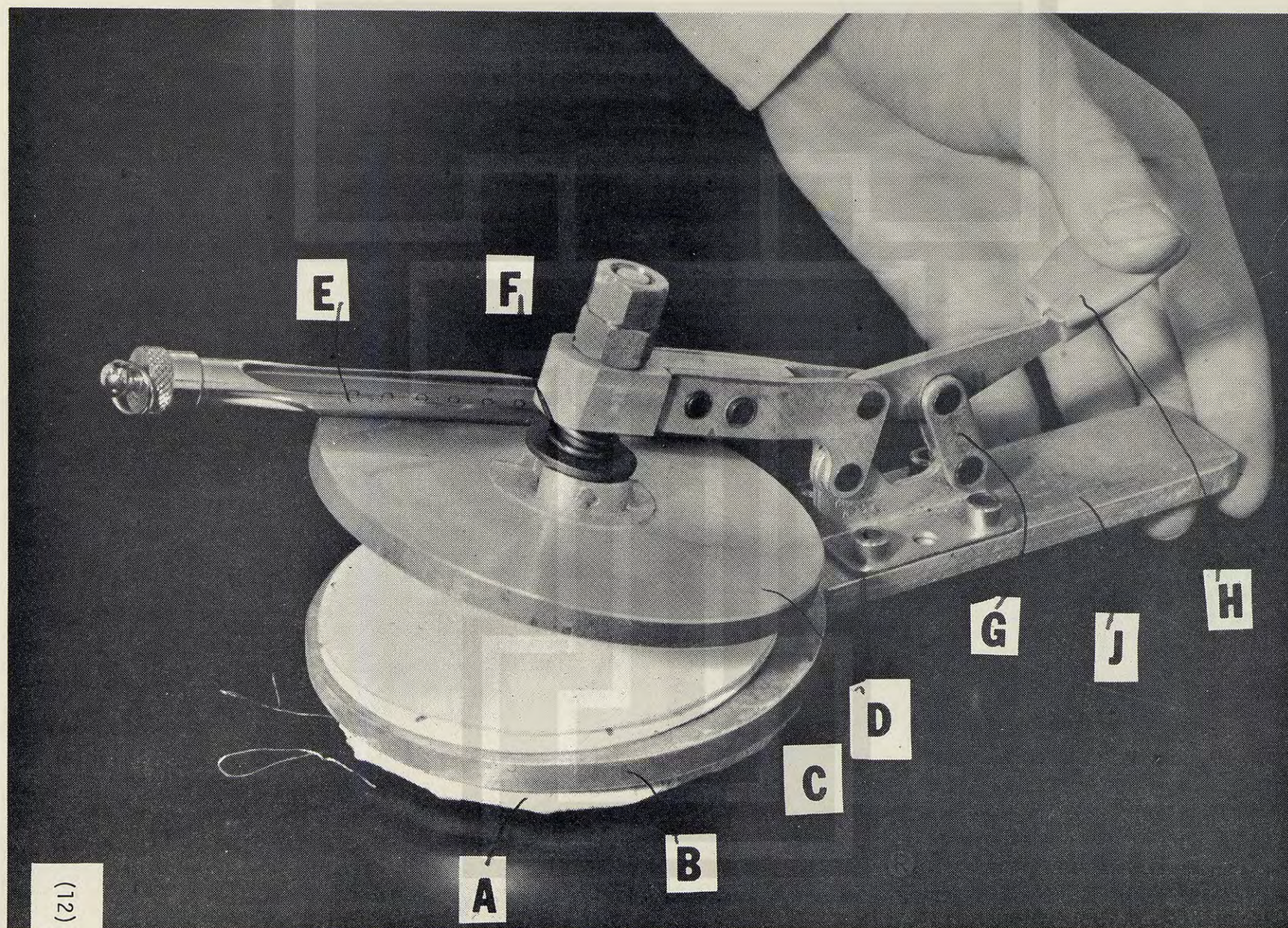
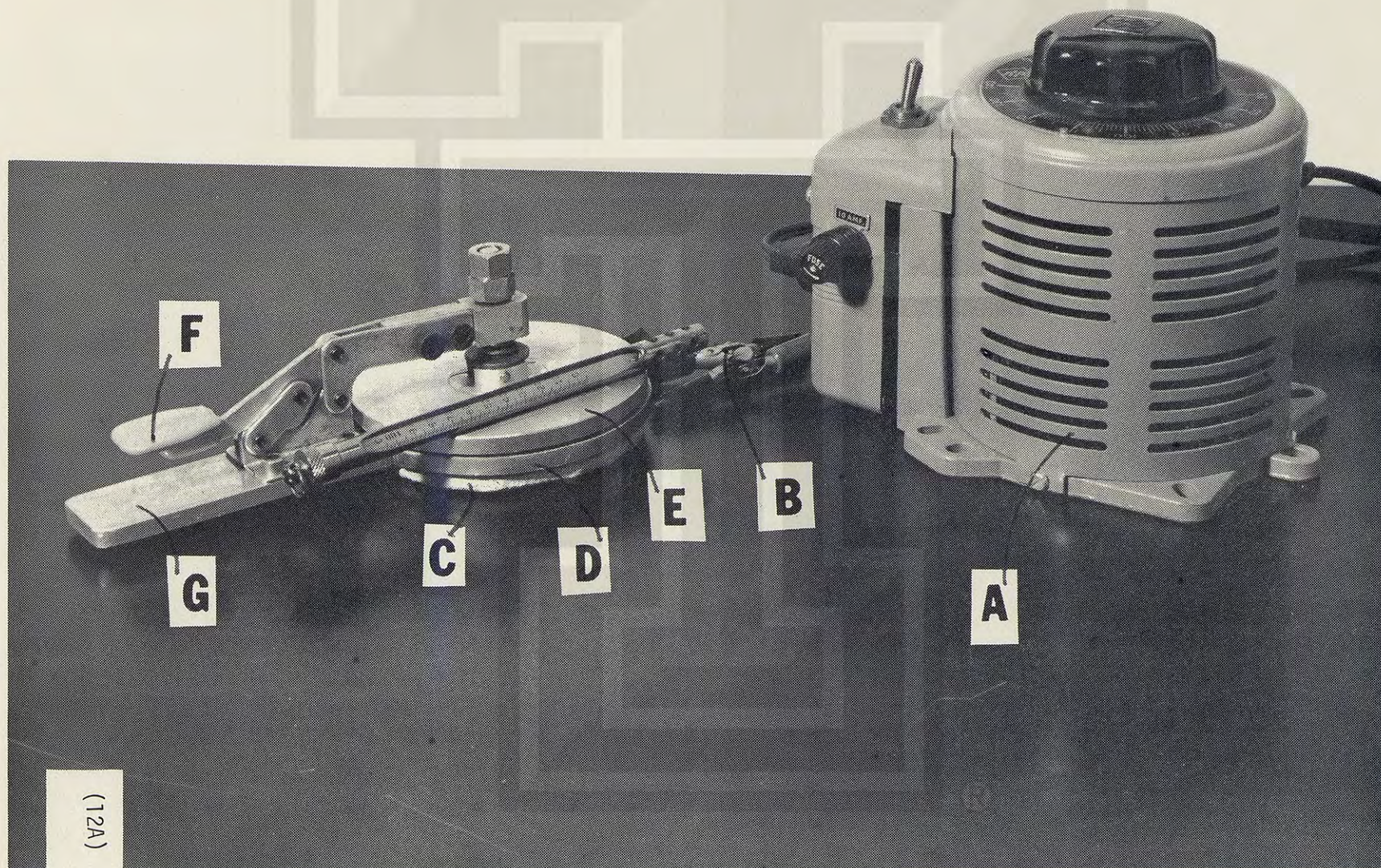


Figure 5 B: The pressplate closed showing the variable transformer for adjusting the temperature. A is the Variac transformer connected to the heating coil at B. C is the heating coil which heats aluminum blocks D and E. The pad is retained between them. Separating F and G by lifting F from G causes the aluminum blocks to separate so as to load or remove the pad. See Figure 5 A.



The Colorimeter

The colorimeter operates on the same principle described previously. A beam of monochromatic light is brought to a focus at the back of an aperture in a photocell. The light passes the aperture and irradiates the colored spot to be evaluated. The light which reflects from the spot activates the photocell. The current flowing in the photocell is evaluated by means of a voltmeter or recorder or printout device.

For the present application, a View-Master stereopticon is mounted in such a manner that the light beam irradiates the spot mounted on the reagent circle. The reagent circle when placed in the View-Master instrument can be made to change positions by pressing the lever, as for changing a picture. Thus the seven spots are presented in sequence to the beam of light. The operator then records the response for each specimen.

Provision is made to set the instrument to zero when irradiating a blank spot. Switching to a series of standards permits the setting up of a standard curve. Switching to the unknown yields a value which may be compared to the standards, in order to estimate the concentration of the desired component in the blood.

Readout on the instrument is with a high impedance millivoltmeter. The output depends upon the lamp intensity. With the 6 volt lamp, as shown, the output is approx. 2 mv. With a 12 volt lamp the output is approx. 4 mv. The circuit is shown in Figure 6. ®

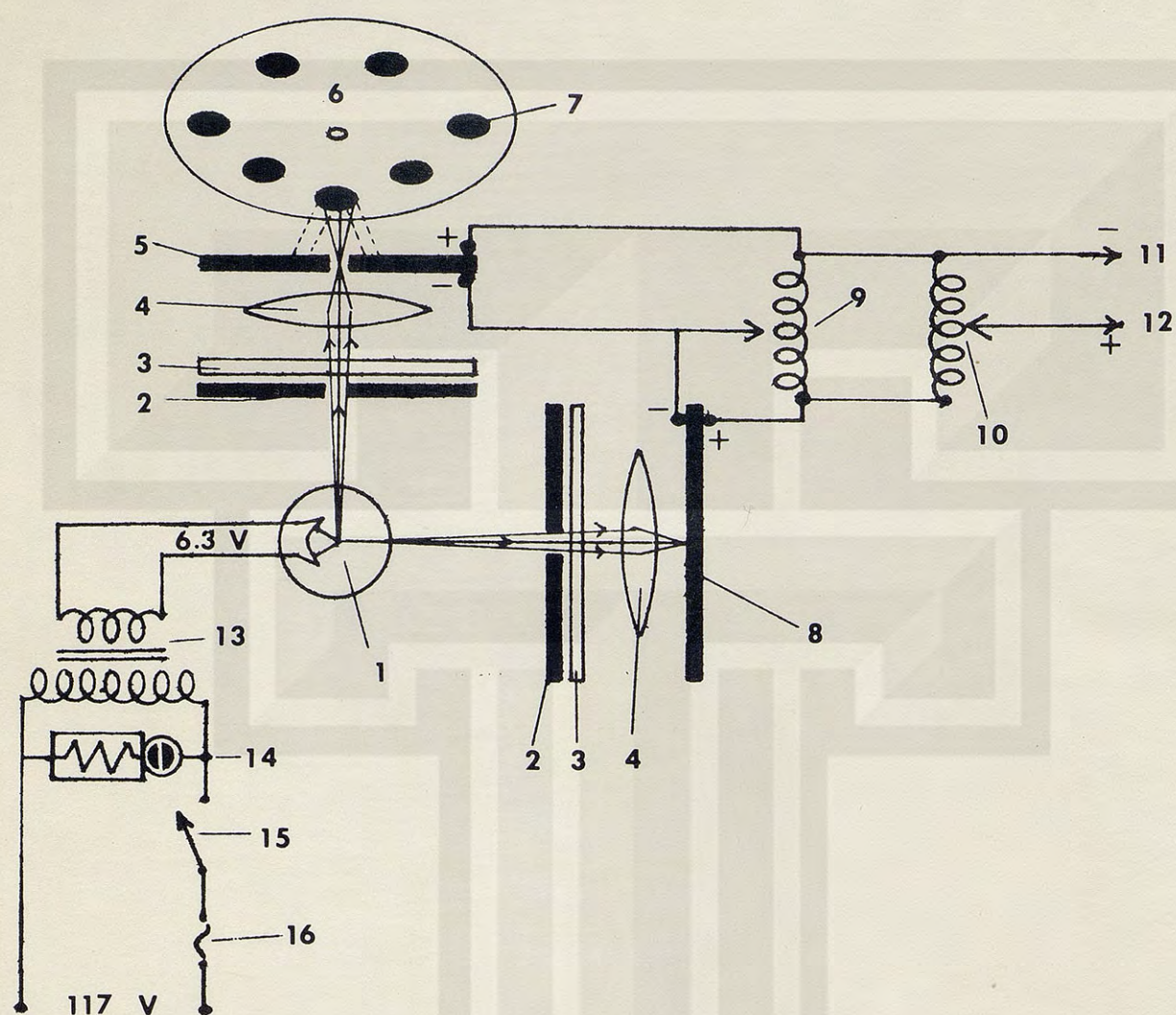
Figure 7 is an overall view of the instrument with the reagent card in place. Figure 8 is the same instrument with the cover removed so as to demonstrate the positioning of the view master.

The card is self centering. With the punched edges held in the vertical position, the reagent card is inserted in the instrument. As one presses the lever, each specimen comes in to place. After reading all seven specimens, they can be reread to check or stored and reread later.

The voltmeter is adjusted to read zero when the blank is in place. This reflects the most light. The voltmeter is therefore set in reverse so that a negative voltage is being measured.

A standard, higher than the unknown is inserted, or a black flag may be used, to set the millivoltmeter at some convenient reading such as 200 (-2 mv). The logarithm of the values read are taken and a standard curve made, evaluating the equivalence of the logarithm of the unknown values found. As an alternative, a digital logarithmic voltmeter may be used.

Figure 6: The colorimeter. The reagent circle moves around the center to place the discs sequentially in the optical field.



1. Light source. 2. Diaphragm. 3. Light filter. 4. Focussing lenses. 5. Photocell with small aperture in center. 6. Reagent disc. 7. Reaction spot. 8. Reference photocell. 9. 10 turn potentiometer 5000 ohms. 10. 10 turn potentiometer 10,000 ohms. 11 and 12. leads to voltmeter. 13. Stepdown transformer. 14. Neon indicator light. 15. On-off switch. 16. Fuse.

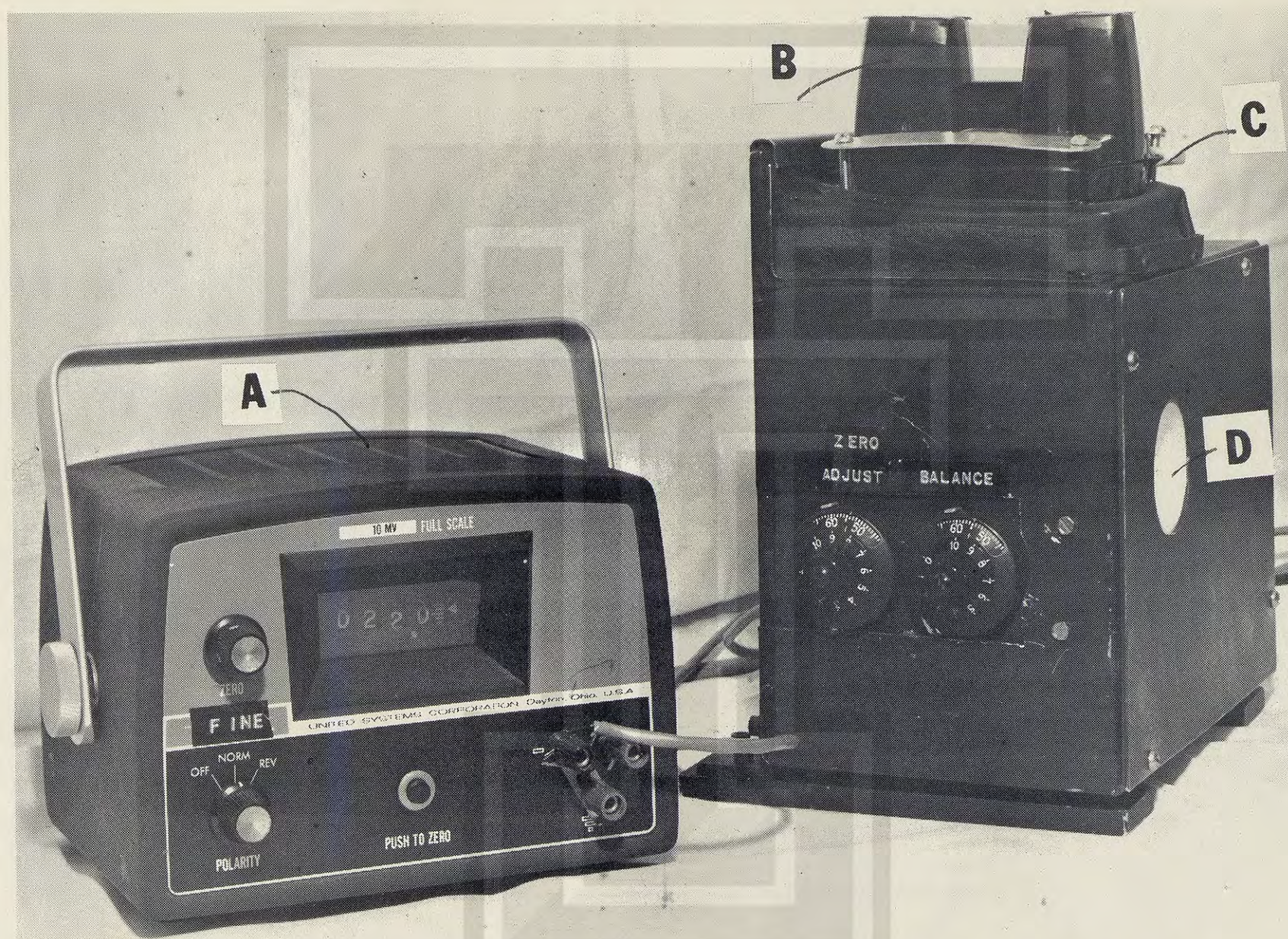
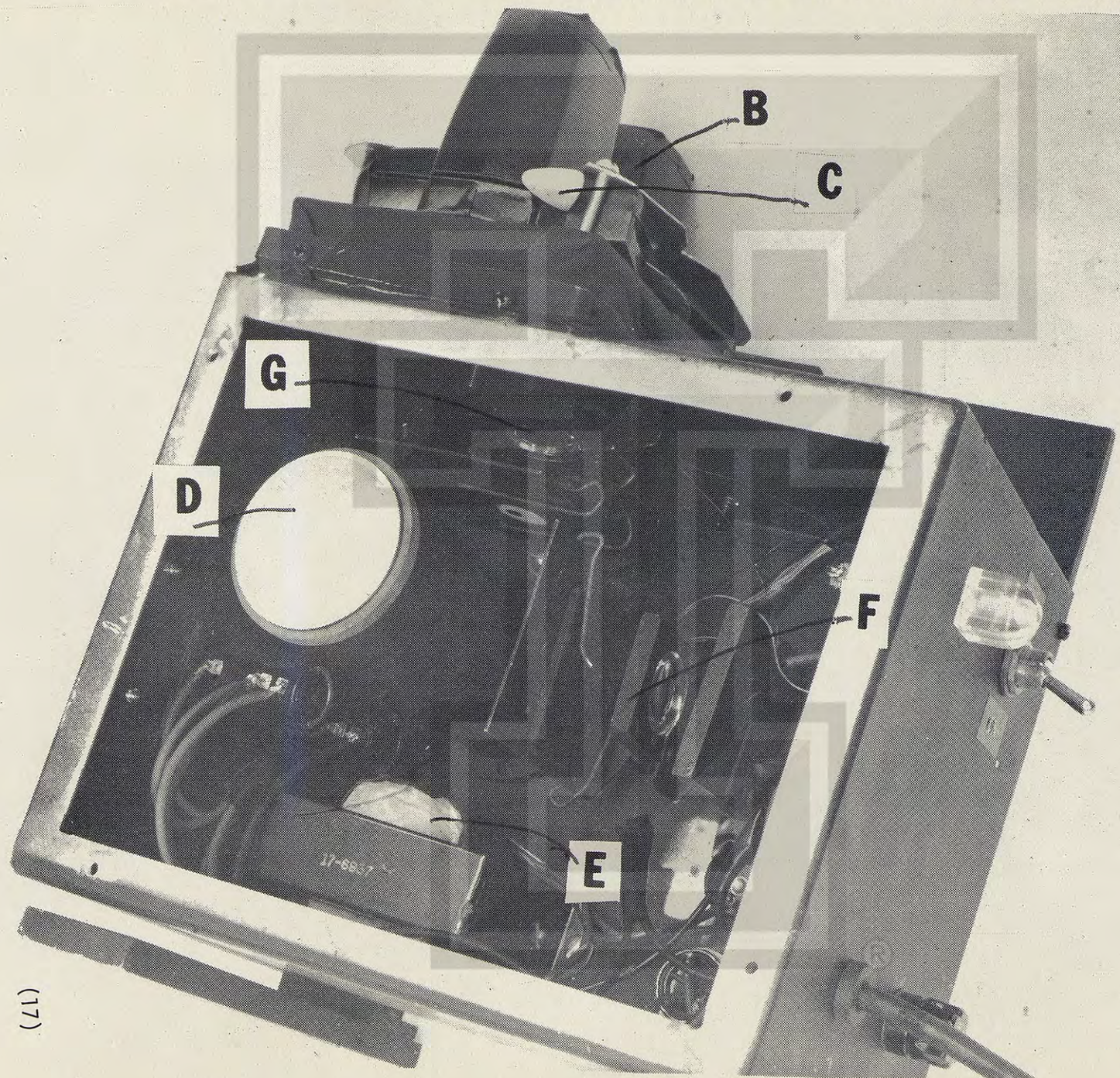


Figure 7: The reflecting densitometer. The digital voltmeter readout is shown at A. The Viewmaster is mounted at B as shown, and the handle C permits the change of spot in the field. The heat sink for the lamp is shown at D. The adjustment controls are also visible.

Figure 8. The colorimeter with cover removed. B is the View Master. C the change position handle and D is the light chimney as in Figure 7. The 6 volt step down transformer is shown at E. The reference beam assembly is shown at F. The light focussing on the hole in the photocell is shown at G.



The Hand Autodilutor

The objective was to produce an autodilutor which could be contained in the hand and be hand operated. The purpose is to sample a specimen of blood from the fingertip and then expel the specimen with the aid of water so as to hemolyze the blood and dilute it. This instrument is needed to operate in a weightless environment.

This problem was finally solved by means of the device shown in Figure 9. A roller(B) passes over a piece of flexible tubing(F) in order to aspirate the sample by peristalsis. A second roller passes over a second tubing or wider bore to eject the sample plus some diluent. The two tubes are connected but move in opposite directions. Operation of a single wheel with two rollers on opposite sides then serves to alternately pass over one and then the other tubing.

The tubing rests on a platform (G) which is hinged near one end(E) and is spring loaded (I). The rollers pass over the tubing and push back the platform. In this way excessive pressure is not brought to bear on the tubing. This movement of the platform also serves a second function. When neither roller rests on the rubber tubing, the liquid could siphon out. This is prevented by the arrangement shown in Figure 9. When the rollers are not on the tubing, the platform is in the up position held there by the spring (I).

By passing the connecting tubing through the platform and back, a segment of the tubing (H) is underneath the platform. The platform is supported off center so that it may move up and down around a pivot (E). As the platform moves up, when the rollers are off the tubing, the spring pushes the opposite end of the roller down. This causes the loop of tubing (H) under the rocker arm to be pressed against a projection (C). This acts as a valve to stop the flow of liquid. When the rollers are off the rubber tubing, the valve is thus closed. When one of the rollers presses the rocker arm down, the side opposite moves up. This releases the pressure on the loop of tubing (H) under the rocker arm and the liquid may now flow.

The instrument may operate in any position. The reservoir of diluting fluid is kept in a plastic bag. The plastic bag is protected by a plastic container around it. This container has a hole punched in it to equilibrate with ambient atmospheric pressure. As liquid is pumped out of the plastic bag, the plastic bag collapses. In this way liquid will flow regardless of the orientation of the plastic bag or whether it is in a weightless environment. Figure 10 is a photograph of the instrument used.

The tubing in the hand autodilutor may be replaced to change the volumes being sampled and ejected and the ratio of each to the other. For this purpose, Table I calculates the volume of fluid delivered per cm of Tygon clear tubing. The color code is that of the Technicon Co.

In the instrument, the roller moves 1.4 cm and the figures in the last column of Table I need to be multiplied by 1.4 to obtain the volume delivered.

By changing tubing, one controls the ratio and amount delivered. For example, referring to Table I, if one wishes to sample 5 μ l and deliver 25 μ l, one would use black tubing for the sampling tube and green tubing in the diluting line. Note that the diluting line must contain the full volume to eject the sample and also the diluent behind it. Note that only nominal values are used such as 4.56 μ l. One is limited in this procedure by the tubing available. Since the same volumes are used for the standards and unknowns, the exact volume is of no consequence. It need be only in the range to produce adequate sensitivity.

Table I

Tube selection for autodilutor

Values for volumes delivered by clear plastic Tygon tubing

Color(Technicon)	I.D. (inches)	I.D.(cm)	Volume per cm(μ l)
ORANGE-BLACK	.005	.0064	0.13
ORANGE-RED	.0075	.0095	0.28
ORANGE-BLUE	.010	.0127	0.51
ORANGE-GREEN	.015	.0191	1.14
ORANGE-YELLOW	.020	.0254	2.03
ORANGE-WHITE	.025	.0318	3.17
BLACK	.030	.0381	4.56
ORANGE	.035	.0445	6.20
WHITE	.040	.0508	8.10
RED	.045	.0572	10.26
GREY	.051	.0648	13.17
YELLOW	.056	.0711	15.88
YELLOW-BLUE	.060	.0762	18.23
BLUE	.065	.0826	21.40
GREEN	.073	.0927	26.99
PURPLE	.081	.1029	33.23
PURPLE-BLACK	.090	.1143	41.02
PURPLE-ORANGE	.100	.1270	50.65
PURPLE-WHITE	.110	.1397	61.28

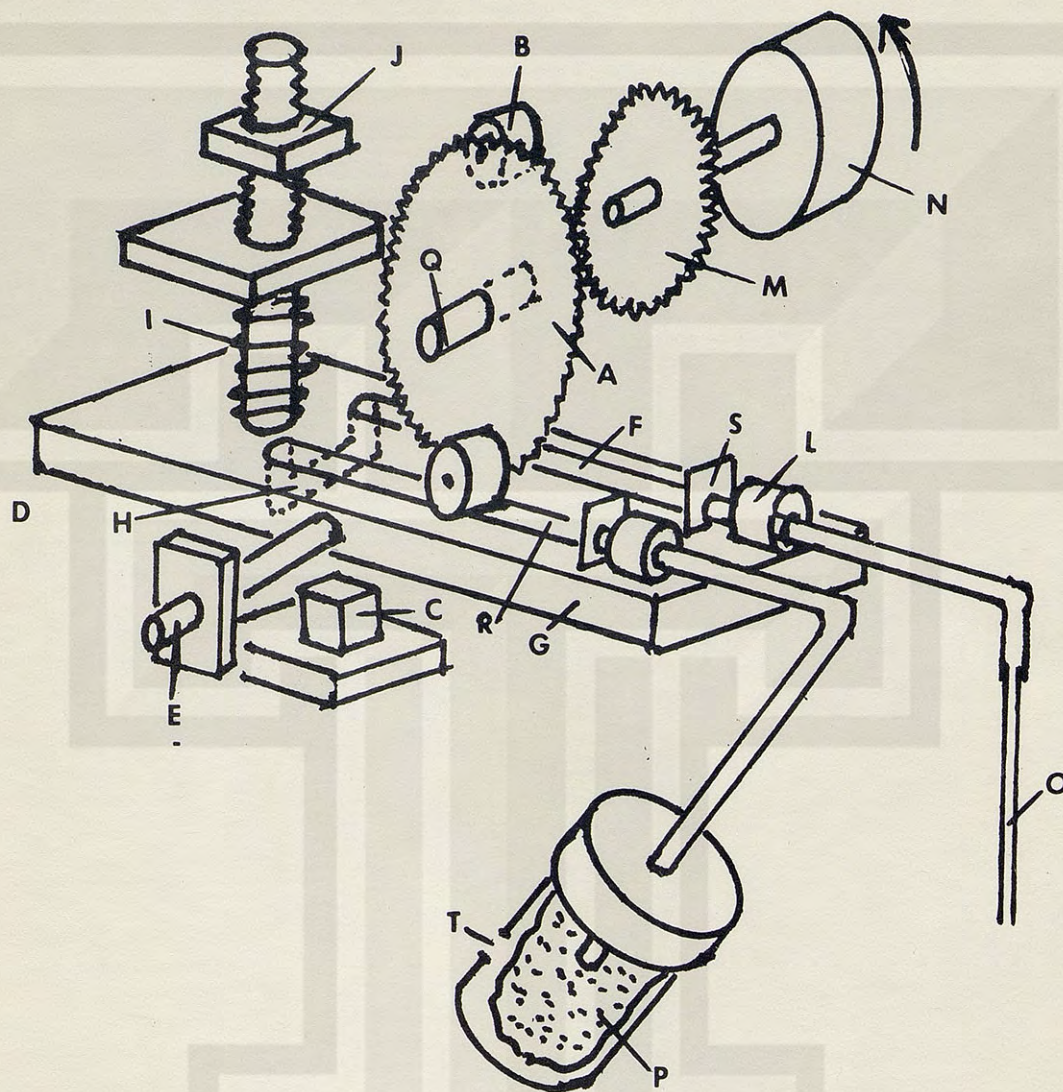


Figure 9: The Hand Autodilutor , schematic representation. See page 22 for identification of the parts.

Legend for Figure 9

The Hand Autodilutor. (A) is a gear which rotates on an axle (Q). B is one or two rollers on opposite sides of the gear A. The rollers pass alternately over rubber tubing (F) and (R). When passing over tubing (F) the sample is picked up at plastic tip (O). When passing over wider tubing (R), diluent from container (P) is pumped out so that the sample at O is delivered along with the diluent. The tubing rests on platform (G) which rocks on pivot (E). A spring (I) whose tension is adjustable by nut (J), keeps the end of the rocker down except when the rollers press on the tubing. The tubing is threaded underneath the platform at H. When the rollers are off the tubing, the spring pushes down to bring tubing H in contact with rigid surface (C). This presses on the tubing at H and acts as a valve to seal the tube. This prevents liquid flow when the rollers are off the tubing. In order to keep tubing in place, stops (L) are placed around the tubing so that tubing can not be pulled beyond the stop point at S. The diluting fluid is retained in container (P) which is a plastic bag inside a plastic bottle with a hole to the air at (T). The bag collapses as diluent is removed, this occurs because the hole at (T) permits air pressure to push on the outside of the plastic bag. This permits the operation of the instrument in any position and makes it independent of gravity. Rotation of gear A is simplified by means of gear (M) and wheel (N) arrangement. Wheel (N) is operated by the thumb while the operator holds the barrel of the instrument containing the container (P).

Figure 10: Model of the hand autodilutor. The plastic bag is held in the plastic container at A. The container is threaded to screw into cap B. Tube C dips into the diluent in the plastic bag. The spring to press on the tubing and act as a valve is shown at D. The tubing being pressed is at E. The gear train is operated from knob F to finally rotate gear G, carrying the roller H for milking the tube. A similar roller is on the opposite side at I. The base pivots at J. Sampling is with the plastic tube at K supported with plastic at L.

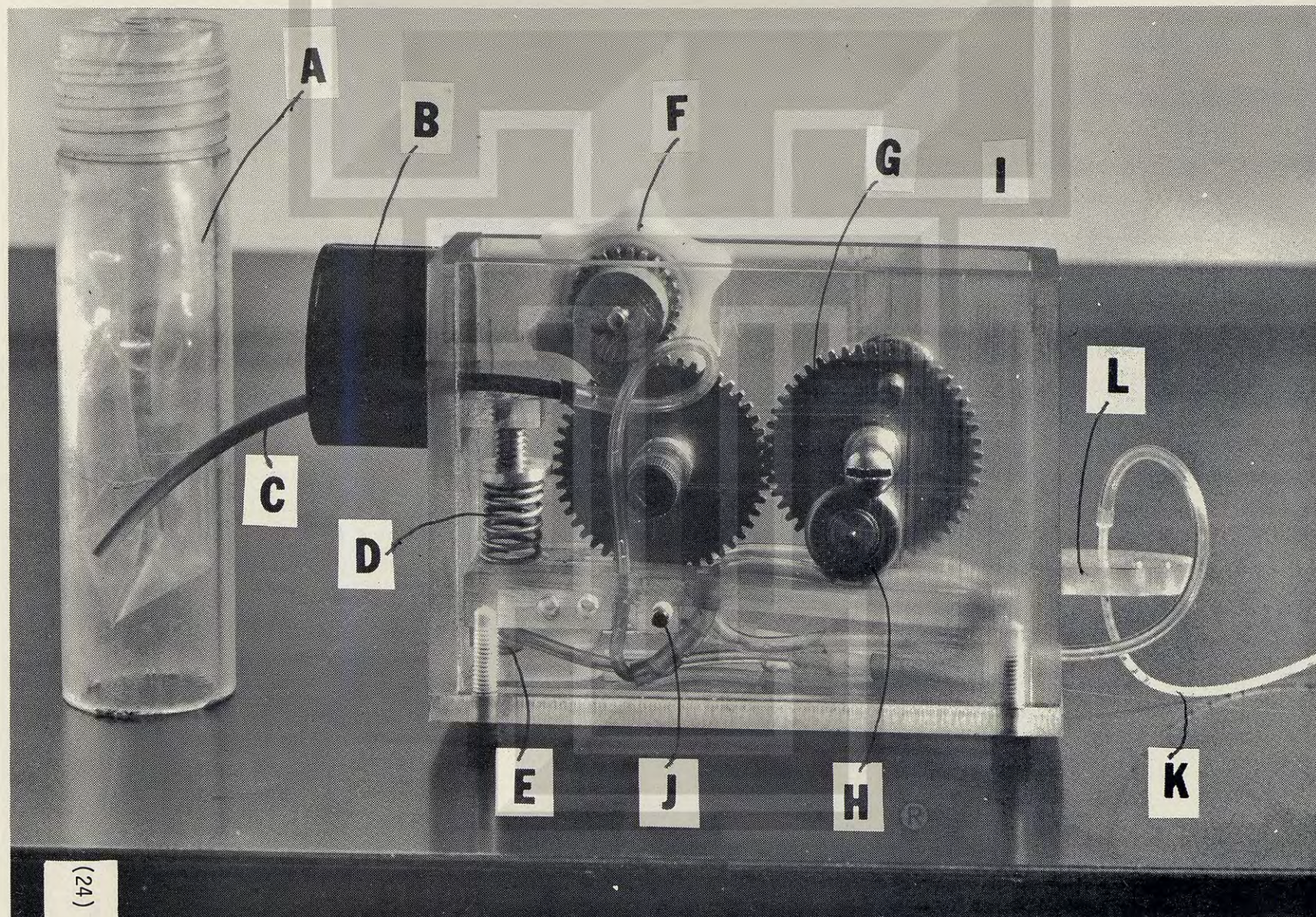


Figure 11: Sampling with the hand autodilutor. The letters are the same as in Figure 10. The finger from which the blood is drawn is shown as M.



The Overall Procedure

The overall procedure may now be summarized:

1. The pad is removed from the plastic bag in which it is preserved.
2. The top layer of the pad is bent back and blood or standards are applied to the circles by means of a hand autodilutor.
3. The pad is reassembled and pressed in the pressplate for, usually, 15 minutes at 37°C.
4. The pad is removed from the press plate and the reagent circle removed. The rest of the pad is discarded.
5. The intensity of the colored spots on the reagent paper are read in the colorimeter as compared to standards in order to evaluate the level of the unknown component sought.

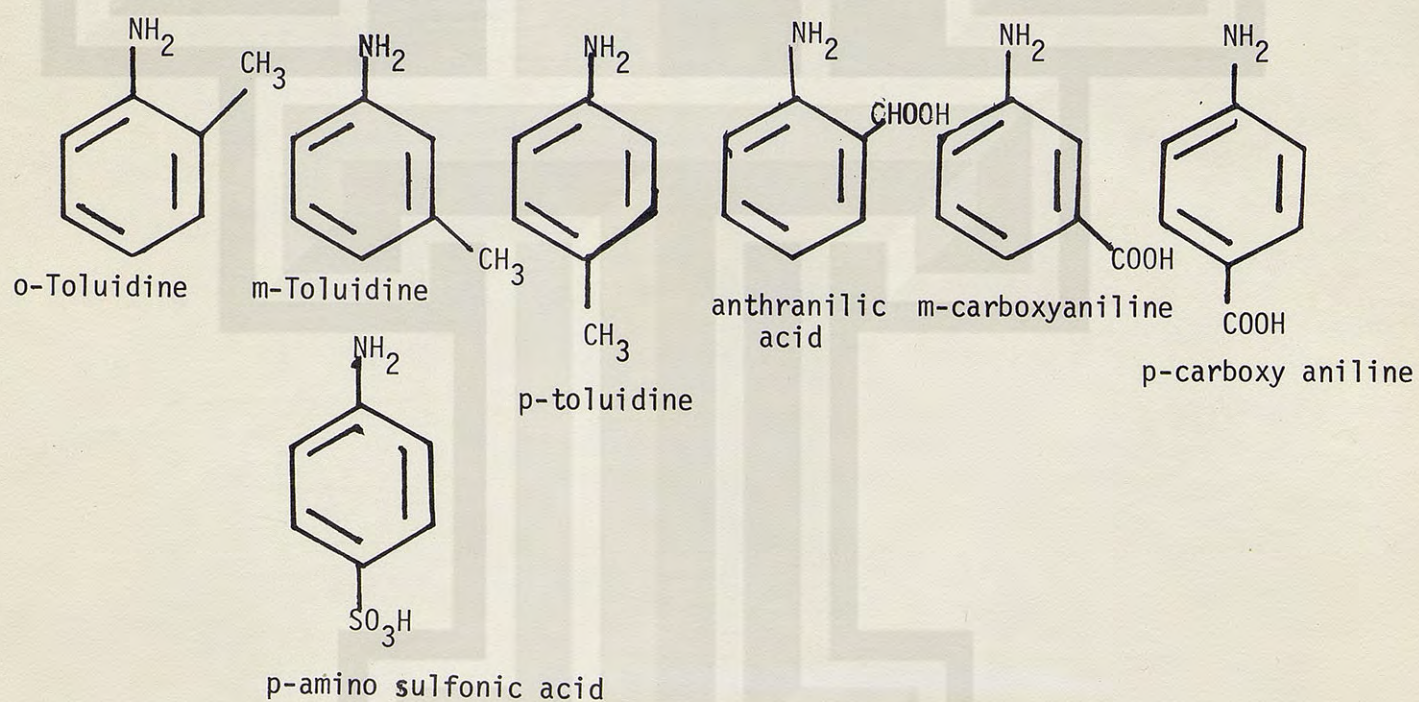
The procedure has been applied to the determination of glucose and description of this procedure will serve to illustrate the method.

The Determination of Blood Glucose with the Hand Operated Pad System

In the earlier study it was found that the glucose oxidase procedure could be used for analysis by the tape system. Paper, impregnated with glucose oxidase, could be retained for several years without significant change in its activity provided moisture and light were excluded. However, it was noted that certain inhibitors may be present in blood which inhibit the glucose oxidase reaction.

For this reason, in the previous report, it was pointed out that the o-toluidine method was being explored.

When this procedure seemed to be satisfactory in principle, an extensive study was undertaken for finding the best conditions for preparing impregnated reagent paper and selecting the proper reagents. The compounds explored in addition to o-toluidine were p- and m-toluidines, p-Amino sulfonic acids and the anilino-carboxylic acids, o, p, and meta- amino benzoic acids.



Two basic observations were made, which limited the areas of study. First, that air, for oxidation, was needed in order to produce the color and second that the reaction had to be carried out in a strongly acid medium essentially anhydrous. In solution, the reaction is carried out at 100°C in glacial acetic acid in the presence of thiosemicarbazide..

On paper, the thiosemicarbazide seemed to inhibit color formation and was omitted. The anhydrous acidic medium was achieved by using succinic acid. After exploring numerous oxidizing agents, it was found that arsenate was satisfactory, and this was then finally utilized.

In order to avoid washing out of the reagent to the periphery of the spot, it is necessary to include some substance in the solution to prevent this process. In the past, (see previous report) methyl cellulose, albumin, gelatin and polyvinyl pyrrolidone have been used for this purpose. In this study, it was found advantageous to use polyvinyl alcohol.

The reagent paper circles were then impregnated with the following solution.

Glucose Reagent Paper Circles

To 100 ml of warm deionized water add 2 gm of polyvinyl alcohol and stir until dissolved. Now add 5 gm. of succinic acid followed by 100 mg of arsenic acid and 1 ml. of o-toluidine. Stir until dissolved. Whatman #3 filter paper circles (9 cm) diameter is added to this solution in a wide mouth bottle. The bottle is evacuated, to insure removal of any air within the circles. The circles are removed, excess solution shaken off, and blotted lightly with filter paper.

The impregnated filter paper circles were placed on the shelves of the rack shown in Figure 3 and lyophilized overnight. They were then sealed to the cardboard backing (Figure 2) by means of a light coat of ordinary rubber cement.

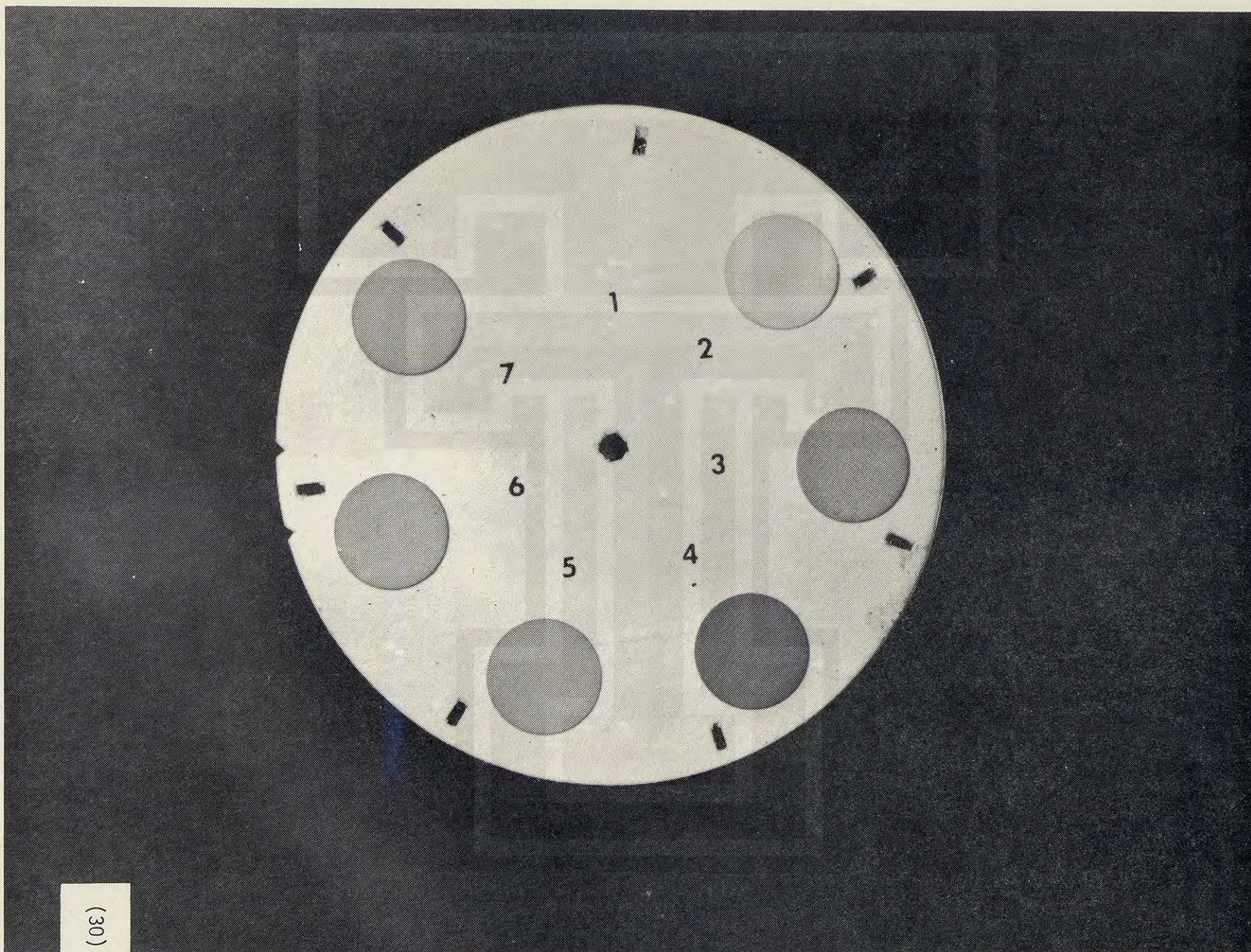
Blood Analysis for Glucose

The sample of whole blood (25 μ l) were diluted twofold and placed on the sample disc. Three samples were measured in this manner. Thus the volume placed on the disc was 50 μ l. On one paper disc, 50 μ l of water was added as the blank. On the remaining three positions the 50, 100 and 200 mg/100 ml glucose standards were placed. In place of the aqueous standards, standardized control sera were also used in certain experiments. These had a value of 84.4, 143.2, and 202 mg/100 ml. They were Versatol, Versatol A diluted with an equal volume of versatol and versatol A, respectively. These values were obtained by assay of the glucose content of the prepared sera. In preparing these sera 1% NaF solution was used as diluent and glucose preservative. In addition, the diluting fluid was 1% with respect to a non-ionic detergent (Brij-35) for all specimens.

The pad was assembled and pressed in the press plate for 10 minutes, at 50°C. The reagent paper was removed and placed in an 80°C oven and heated for 10 minutes. The reagent paper was now read on the colorimeter. Figure 12 is a photograph of a typical reagent circle with the developed spots.

It is to be noted that in this procedure the color develops mainly while being heated at 80°C for the ten minutes.

Figure 12: Appearance of reagent paper after the color has been developed prior to reading. The blank is 1, the standards are 2,3,4 representing 50, 100 and 200 mg/100 ml of glucose and the serum is at 5,6, and 7.



Analysis of the Data

Figure 13 is a typical standard curve obtained, using aqueous standards and standards prepared from standardized control sera. It is to be noted that good correlation exists between the two standard curves. This was unexpected since dialysis from a protein containing solution is not the same as dialysis from an aqueous solution. The results were obtained probably, because of the prolonged dialysis time (10 min) permitting a condition closer to equilibrium to be established. For purposes of analysis the serum standards were chosen as more closely resembling the whole blood.

The data for 30 different bloods analyzed by the two procedures is listed in Table II. The standard deviation is given as ± 3.04 for this procedure and ± 3.39 for the autoanalyzer. This difference is significant at the 5% level and barely significant at the 1% level, when the F test, to compare precision of the methods, was applied. The reproducibility was therefore satisfactory.

When the mean values are compared, using the t test, it is apparent that the results obtained with the autoanalyzer are different. This is reflected in the difference of the means. Comparing the means from Table II, by applying the t test, a significant difference between values exists at the 1% level. We can then say that this method will yield results which are somewhat higher than that obtained with the autoanalyzer. The values, however, by inspection appear to be satisfactory for the purpose of monitoring changes in blood glucose levels.

Figure 13: Comparison of standard curves obtained for glucose by the o-toluidine method. Aqueous standards are compared to standardized serum controls.

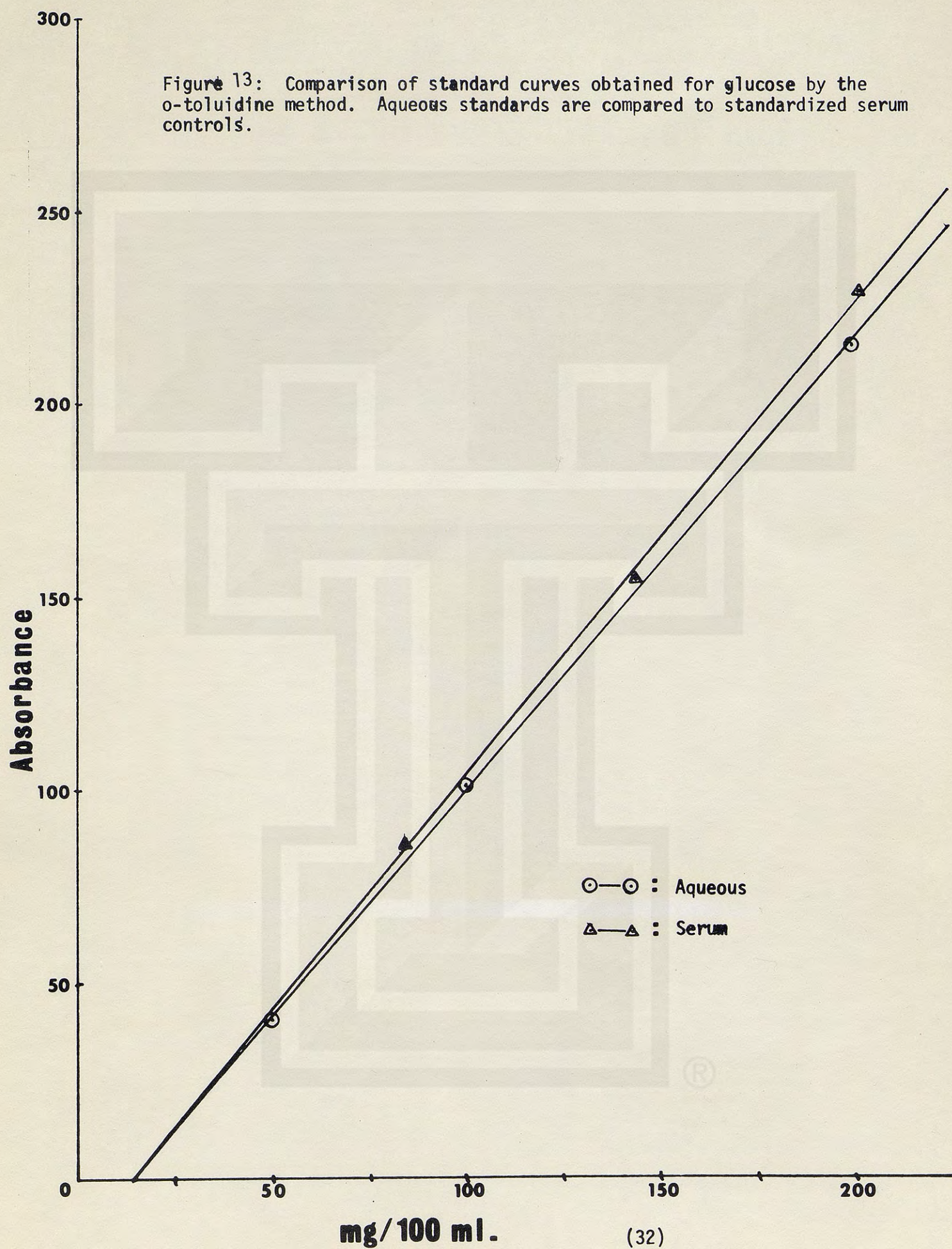


Table II

Comparison of the results obtained by the hand pressplate system and Technicon's Autoanalyzer for blood glucose levels. The sample was diluted 1:8 before placing in the autoanalyzer cups. Blood was diluted 1:2 in the pad system. Results on serum also given for comparison by the autoanalyzer.

SAMPLE #	PAD SYSTEM			AA WHOLE BLOOD			AA SERUM		
	RESULT	MEAN	% DEV	RESULT	MEAN	% DIFF	RESULT	MEAN	% DIFF
1	76 84	80	5.0	92 83	87.5	5.1	84 80	82	2.4
2	160 165	162.5	1.5	183 180	181.5	0.8	187 183	185	0.1
3	107 107	107	0.0	80 87	83.5	4.1	115 109	112	2.7
4	218 212	215	1.4	178 190	184	3.3	228 216	222	2.7
5	101 105	103	1.9	83 84	83.5	0.6	105 99	102	2.9
6	89 84	86.5	2.9	79 73	76	3.9	94 90	92	2.2
7	90 95	92.5	2.7	79 75	77	2.6	112 104	108	3.7
8	84 78	81	3.7	84 72	78	7.7	99 93	96	3.1
9	110 110	110	0.0	78 80	79	1.3	102 98	100	2.0
10	180 172	176	2.3	159 149	154	3.2	175 165	170	2.9
11	143 152	147.5	3.0	159 157	158	0.6	175 165	170	2.9
12	152 149	150.5	1.0	168 163	165	1.8	186 186	186	0.0
13	160 155	157.5	1.6	169 173	171	1.2	189 180	184.5	2.4
14	102 102	102	0.0	71 65	68	4.4	96 92	94	2.1
15	104 104	104	0.0	71 65	68	4.4	96 92	94	2.1

Table II continued:

SAMPLE #	PAD SYSTEM			AA WHOLE BLOOD			AA SERUM		
	RESULT	MEAN	% DEV	RESULT	MEAN	% DEV	RESULT	MEAN	% DEV
16	84 75	79.5	5.6	60 60	60	0.0	95 85	90	5.6
17	73 78	75.5	3.3	61 59	60	1.7	92 88	90	2.2
18	75 80	77.5	3.2	85 81	83	2.4	85 79	82	3.7
19	84 91	87.5	4.0	81 83	82	1.2	88 80	84	4.8
20	168 163	165.5	1.5	150 136	143	4.9	163 157	160	1.9
21	84 81	82.5	1.8	99 95	97	2.1	98 94	96	2.1
22	172 172	172	0.0	151 147	149	1.3	156 152	154	1.3
23	90 94	92	2.2	85 83	84	1.2	93 87	90	3.3
24	114 114	114	0.0	99 95	97	2.1	101 99	100	1.0
25	84 89	86.5	2.9	88 78	83	6.0	85 79	82	3.7
26	102 102	102	0.0	96 98	97	1.0	104 100	102	2.0
27	184 194	189	2.6	167 169	168	0.6	203 209	206	1.5
28	84 100	92	8.7	104 96	100	4.0	127 123	124	2.4
29	110 113	111.5	1.3	110 104	107	2.8	140 132	136	2.9
30	107 100	103.5	3.4	70 82	76	7.9	95 89	92	3.3
Std. dev. %			± 3.0			± 3.53			± 2.83
Mean	116.85			106.67			122.85		
Std. dev. of mean			± 3.04			± 3.39			± 3.27

In Table II, values obtained with the autoanalyzer on the serum of the blood are also listed. These values are significantly higher than the values obtained for whole blood with the autoanalyzer or the pad system. They resemble more closely the blood values by the pad system.

It is to be expected that in the presence of solids from the hemolyzed erythrocytes, less would dialyze in the autoanalyzer system than would dialyze from serum. This is reflected in the mean values obtained in Table II for the autoanalyzer. With the pad system longer dialysis time and a different reagent were used. The fact that the results on whole blood more closely resemble those obtained with serum in the autoanalyzer is actually an advantage.

The bloods used in Table II were taken from a run of bloods in the routine laboratory. Using bloods for normal individuals the normal range was determined as 72 to 96 by the pad system and 55 - 101 by the autoanalyzer for whole blood and 64 to 112 for serum. These results are summarized in Table III.

Table III
Normal values for blood glucose levels with the pad system as compared to the autoanalyzer on whole blood and serum.

# cases	PAD SYSTEM		AA WHOLE BLOOD		AA SERUM	
	(mean)	(+std dev)	(mean)	(+std dev)	(mean)	(+ std. dev)
12	84.4	5.9	80.6	12.08	93	12.34

Problems Still Extant

(1) A major problem is a source for the preparation of the pads, especially the reagent circle. We have had to prepare these ourselves. We are in communication with Pioneer Office Forms Inc. 6121 W. 35th Street, Cicero, Illinois, concerning the preparation of the sample circle by applying pressure to a filter paper to elevate the circles so as to form discs. The rest of the paper would be impregnated with low molecular weight polyethylene. Only the discs would remain porous. This is equivalent to producing raised letters on calling cards.

(2) Methodology is available at present only for glucose and urea. Other methods would have to be worked out. For this purpose a large volume of prepared pads needs to be available for experimentation.

(3) The autodilutor needs to be prepared in its final form. The present model is operative and efficient but bulky.

(4) The readout device (Vacuum tube digital voltmeter) needs to be miniaturized. It is too bulky and heavy.

(5) The 0.5 mil cellophane available commercially is contaminated with urea and ammonia. A special analytical grade needs to be obtained from the manufacturer.

Summary of Achievements in This Study

(1) A practical reflecting densitometer has been prepared for reading colors developed on a circular reagent paper by reflectance. The instrument is convenient and practical for routine use.

(2) A hand autodilutor has been developed which will work in any position in a weightless atmosphere or on the ground. It samples a specimen and ejects the specimen mixed with a measured amount of diluting fluid. It may also be used as a micro sampling device.

(3) A press plate was developed, practical for use in a weightless environment. Gripping the handles and applying pressure permits the closing of the press plate on the pad. A clamp assignment is provided which keeps the tension between the plates. This is adjustable by means of tightening nuts, so that the pressure on the pad may be varied.

(4) A circular pad system was set up for analysis in a weightless environment. The sample (25 μ l) is taken with the autodilutor, diluted to twice its volume, and placed on one of the sample discs on the sample circle. Standards are also placed on similar discs. A pad is then made with the samples facing a layer of cellophane below which is the reagent circle. Pressure is applied with the pressure plate. The reagent paper is removed and heated to develop the color.

The optical density of the colored spots developed on the reagent circle are evaluated by reflectance with the densitometer.

(5) A method for glucose estimation in whole blood using the o-toluidine procedure was developed as a more practical method for the purposes described above. This was applied successfully to the pad system.