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HEMOGLOBIN FUNCTION IN STORED BLOOD. (U)
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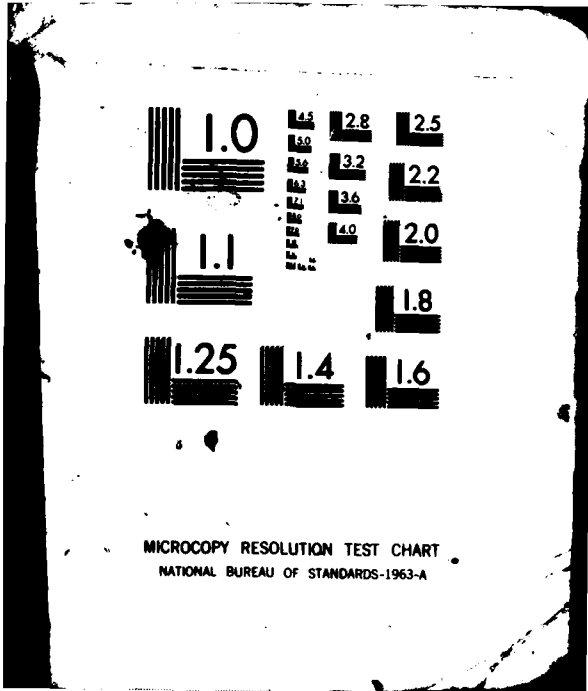
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HEMOGLOBIN FUNCTION IN STORED BLOOD

Annual Report

R. Ben Dawson, M.D.

August 1974

Supported by

US Army Medical Research and Development Command
Washington, DC 20314

Contract No. DADA17-72-C-2005

University of Maryland School of Medicine
Baltimore, MD

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20. ABSTRACT (Continue on reverse side if necessary and identify by block number) Blood storage experiments, 4°C, were carried out for the purpose of improving preservative solutions for maintaining ATP (for red cell viability) and 2,3-DPG (for hemoglobin function). Blood banking conditions were used for selecting, processing and storage of blood in all experiments. Fluorimetric techniques were used for determining ATP and 2,3-DPG concentrations.		

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Each group of experiments will be summarized by one or two short statements of the most important conclusions.

1. Adjusting the pH of the preservative to between 5.4 and 7.0 in ACD and CPD solutions containing adenine and inosine with or without methylene blue, showed that the pH 6.4 to 7.2 preservatives afforded the best DPG maintenance.
2. Experiments with CPD-adenine-inosine with and without methylene blue indicate that the methylene blue effect is dependent on the presence of inosine for maintenance of 2,3-DPG.
3. Improved maintenance of 2,3-DPG in CPD-adenine preservatives with the metabolic nutrient dihydroxyacetone and the metabolic regulator pyruvate were studied and the conclusion was that an additive effect is apparent when both of these agents are used. These findings which are not surprising since their mechanisms of action are different.

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ANNUAL PROGRESS REPORT ON CONTRACT DADA 17-72-C-2005

U. S. Army Research & Development Command

Washington, D. C.

TITLE:

Hemoglobin Function in Stored Blood: In Vitro Studies

DATE:

August 1974 (Dates covered: August 1, 1973 - July 31, 1974)

INVESTIGATORS:

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INTRODUCTION

a. The shelf life of whole blood stored at 4° C can be extended from 21 days (ACD) or 28 days (CPD) to 35 days with adenine. This important advance in blood banking marked the acceptance of additives which influence the control of metabolic energy in the red cell. Adenine enters the cell, is incorporated eventually into adenosine triphosphate (ATP), which is required for phosphorylation of glucose, the first step in the energy yielding glycolytic pathways which are responsible for maintaining a viable cell. (1, 2)

Studies with adenine and the two basic preservatives, ACD and CPD, have shown post-transfusion survival of over 70% after storage periods of 35 days. Thus, adenine exerts its effect in either ACD or CPD. (3) Adenine has been used successfully and without harm for nine years in one country and for less time in others.

CPD was approved for blood bank use by the military and Red Cross in 1972 and was generally adopted by over 90% of blood banks in the United States during 1973. Several advantages over ACD are important. Blood stored in CPD maintains higher levels of 2,3-DPG (2,3-diphosphoglycerate) and a higher pH than ACD stored blood. (4,5,6) Dependence of the higher 2,3-DPG on the higher pH was confirmed. (7) These differences in 2,3-DPG and pH are also apparent when either, adenine, inosine, or both are present in the two basic preservatives. (6)

b. The purpose of transfusing blood is to provide for the transport of oxygen to the body tissues. This is the function of hemoglobin. However, stored red cells which have a normal survival after infusion may be unable to deliver as much oxygen as fresh red cells. (8) This abnormality may persist for 24 hours or longer in a patient who has received 2-3 units or 7-14 day old ACD blood. (9,10) This defect may be critical for any seriously ill medical or surgical patient who requires transfusion therapy (more than one unit of blood).

During the first week of storage in ACD or ACD-adenine the oxygen affinity increases and remains abnormal throughout the period of storage. Inosine, added to ACD-adenine blood at collection slows this increase in oxygen affinity. Also, inosine added to ACD or ACD-adenine blood after 20 days of storage causes a return toward normal of the oxygen affinity. (8,11)

Inosine exerts its effect by supplying ribose, which is phosphorylated without requiring ATP. Ribose phosphate produces energy via linking reactions between the pentose phosphate pathways and the Embden-Meyerhof pathway. These reactions are important late in storage when glucose utilization has diminished. It seems clear that inosine can greatly potentiate the beneficial effects of adenine during storage, resulting in better maintenance of ATP. Also, inosine by providing three carbon substrates to glycolysis for metabolism to 2,3-DPG preserves the ability of red cells to transport oxygen.

c. The correlation between red cell survival and ATP levels in stored blood is explained by the several functions of ATP which are necessary for cell viability. However, ATP levels do not correlate with oxygen affinity during storage. Levels of 2,3-DPG determine oxygen affinity and thus hemoglobin function. (12,13)

When normal levels of 2,3-DPG are present, oxygen dissociation is normal. But when 2,3-DPG falls, as during storage at 4° C, the oxygen affinity of hemoglobin increases. This results in poorly functional red cells. Maintaining levels of 2,3-DPG near normal is therefore important for maintaining functional red cell hemoglobin. Inosine helps to maintain 2,3-DPG by contributing a ribose to the pentose phosphate pathway which allows a 3 carbon sugar to enter glycolysis below the two main rate-limiting reactions and contributes to synthesis of 2,3-DPG.

It was established in this laboratory that, at the pH of CPD, the amount of phosphate present in CPD is optimal for maintaining 2,3-DPG, without being detrimental to ATP maintenance. (14)

e. Other studies established that the optimal pH for maintaining both 2,3-DPG and ATP is 5.6, without metabolic additives or regulators. (15,16) The optimal pH was found also with 0.25 mM adenine--this is one half of the concentration previously used and the concentration recently adopted by the Swedish government. (17) A second study which looks at a narrower pH range with adenine and confirms the above is reported below. A pH study in CPD-adenine-inosine is also reported in summary below and the ATP data are attached because they show an important finding.

f. The effects of various phosphate concentrations in the presence of adenine and adenine plus inosine on the concentrations of ATP (viability) and 2,3-DPG (hemoglobin function) were investigated as joint projects with Dr. Walter F. Kocholaty, Biochemist, USAMRL. In these experiments it was shown that concentrations of phosphate higher than that present in CPD (2 mM) do not seem to improve the maintenance of ATP and 2,3-DPG when adenine is present. However, with adenine and inosine, higher concentrations of phosphate--at least 6-8 mM--seem to be better. (18)

g. The suggestion that methylene blue (19) might be an important metabolic regulator in red cell storage by Dr. Walter Kocholaty, Biochemist, USAMRL, working with the principal investigator, resulted in a series of promising studies. (20) CPD-methylene blue with adenine and inosine will maintain normal 2,3-DPG and p50 (hemoglobin function) values for five to six weeks, the optimal period of viable storage for liquid blood banking. The methylene blue concentration used was very small, a catalytic amount which is considerably less than the amounts that are given in the clinical treatment of the condition, methemoglobinemia. The concentrations of adenine and inosine are similar to the concentrations of adenine and inosine which have been used by other investigators in laboratory and clinical research in this country and in transfusion practice in several countries in Europe for a number of years. It is believed that the work involving methylene

blue represents an important advance in blood preservation research and its further study is an important part of the work of this laboratory. Preliminary data from a current study are reported here.

h. Dihydroxyacetone serves as a 3 carbon metabolic nutrient for red cell metabolism and results in better maintenance of 2,3-DPG. (21) Its effects on ATP have not been well studied. Preliminary data from a current study are reported below.

i. The pyruvate effect--improved 2,3-DPG maintenance by oxidation of NADH (22)--is being studied in pilot experiments in this laboratory.

j. Packed red cells with hematocrits up to 94% (23) are being studied in CPD-adenine to ascertain if more glucose might not be needed. The first two studies are reported here.

SPECIFIC METHODS AND MATERIALS

(1) Oxyhemoglobin dissociation curves, traditionally analyzed by the Van Slyke gasometric apparatus are also analyzed by the spectrophotometric apparatus, the CO-Oximeter. Both methods, which represent different approaches and measure different aspects of oxygen affinity, require the use of a tonometer and a pH blood gas meter.

(2) Concentrations of 2,3-DPG and ATP, traditionally measured by manual spectrophotometry or fluorometry, are also measured by the automated method of Prins and Loos (26) which has been adopted and developed under the direction of the principal investigator.

(3) Measurements of pH are made anaerobically by the blood gas meter on the sample directly aspirated from the storage bags. Thus pH measurements are made before the blood has been exposed to the atmosphere and allowed to change by evolution of CO₂ and other gaseous exchange.

(4) Blood Cell Morphology is being studied in certain of the preservative experiments. Some preliminary scanning electron microscopy (SEM) was done on red

cells in an experiment with adenine and dihydroxyacetone.

RESULTS AND DISCUSSION

A. Summary Interpretation of Three, Ten Unit Studies

Using Adenine and Inosine, and Adenine, Inosine and Methylene Blue

1. The first study here is one in which adenine-inosine and methylene blue were used in minimal effective concentrations in ACD, with various pHs being the parameter studied. The study was carried only through three weeks for ATP and DFG analyses but it is clear that ATP is better maintained in the low pH preservative and DFG better maintained in the higher pH preservatives. This is not a surprising finding but we have not tested the pH effect before in the presence of methylene blue. Part of the experimental sampling was carried through 42 days for the analyses of osmotic fragility or percent of cells hemolyzed by hypertonic saline. The osmotic fragility curves shown are from two units at 42 days. The obvious difference between preservatives is the greatly increased fragility of the high pH preservative especially the 7.2 preservative. Looking back at the osmotic fragility curves done at each of the other weeks in storage the increased fragility with the 7.2 pH preservative began to appear at day 14. Copies of the fragility, ATP and DFG data are given in graph form as the first part of the appendix 2d.

2. This experiment, another study in which 10 units were studied from normal donors so that such differences as might be observed between preservatives could be analyzed statistically, was an investigation of the pH effect in CPD with adenine .25 mM and inosine 10 mM. pH range was 5.4 to 7.4, a range which from previous experiments should include or contain the optimal pH for maintaining both ATP and DFG. This seems to be the first experiment in which the pH effect is obvious throughout the whole 42 day storage period. The ATP values in the high pH, 7.0, preservative were still above 50% of normal at 42 days, consistent with an expected adequate survival. Also, shown in appendix 2C are some data from analyses of DFG levels. Unfortunately, there was some difficulty in storing these

samples and the analyses were incomplete. However, with the exception of the 7.0 preservative it appears that the other pHs are satisfactory for maintaining DPG above 50% of day zero values for as long as 21 days.

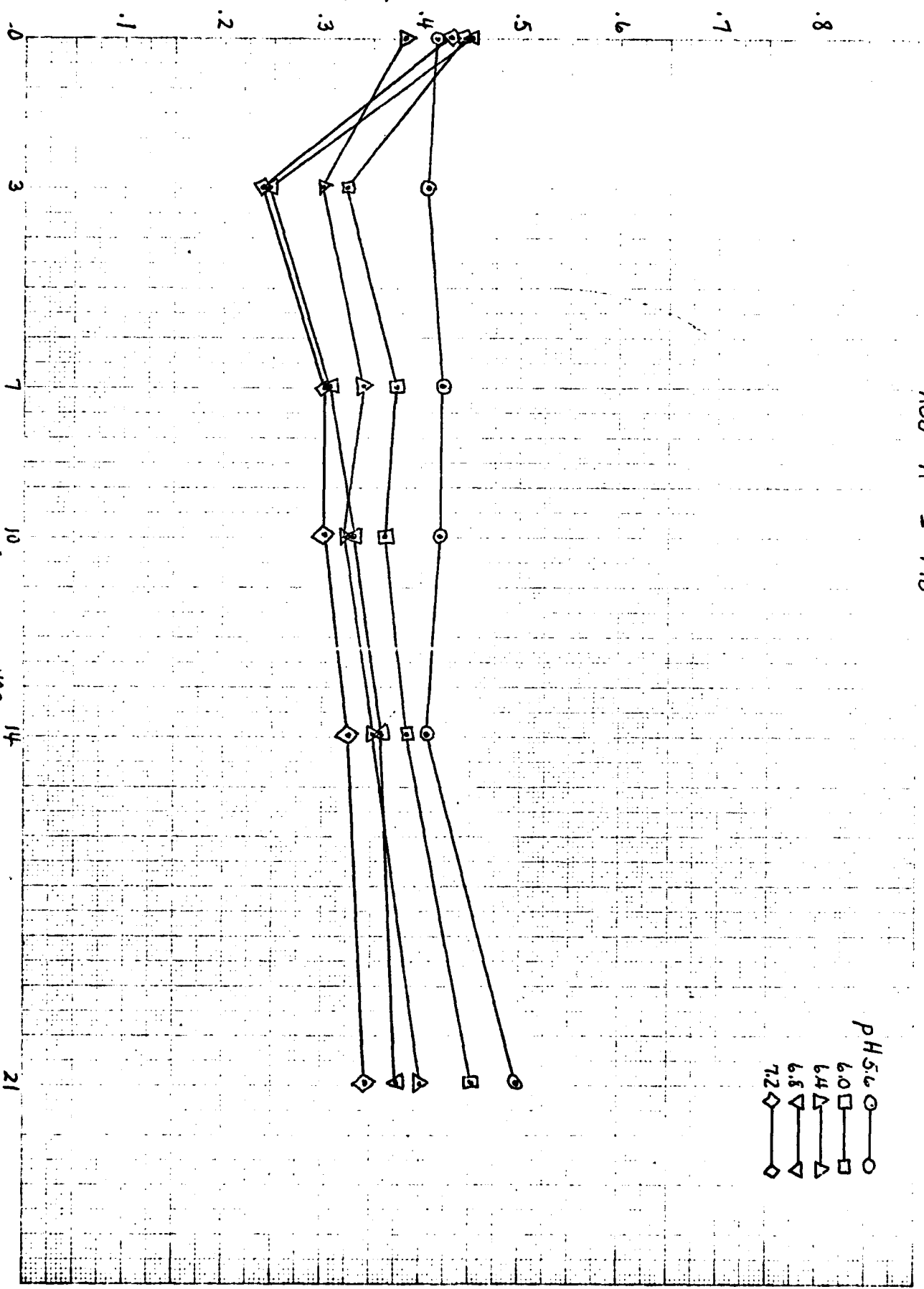
3. In this 10 unit study the averages are shown on the tables and graphs in Appendix 2D. This is a CPD-adenine-inosine-methylene blue study whereas the first study in Appendix 2D is an ACD study. Also, the pH range here avoids the higher pH of 7.2 which was found to be unsuitable in the ACD study included here in Appendix 2D. Also, this study avoids the low pHs near 5.0 which are characteristic of ACD and no longer considered suitable for blood storage because of their deleterious effect on DPG and thus hemoglobin function. In this study the ATP values do not seem to be maintained well after the first two weeks of storage such that in most of the preservatives the values are less than half normal at 14 days. This poor maintenance of ATP is unexpected and unexplained. It will have to be explained by repeating parts of the experiment. The basic part of the preservative, CPD-adenine, should provide good maintenance of ATP at the lower pH values studied, 5.4 and 5.8; the pH of natural CPD is 5.67 so that preservatives with pH values close to this should maintain ATP quite well. Also, inosine has been shown in the past to improve or assist adenine in the maintenance of ATP. Further, methylene blue has not been shown to have a deleterious effect on ATP maintenance. In fact, in a preliminary experiment reported here (~~Appendix 8~~) methylene blue seems to have a slight beneficial effect for maintenance of ATP in the presence of CPD-adenine-inosine. The DPG analyses for this experiment are currently being run and the data from the first unit seems to show fairly good maintenance of DPG concentrations in the second three week period of storage with some of the preservatives.

ATP (μ M/ml. W.B.)

DAYS OF STORAGE AT 4°C

ACD - A - I - MB

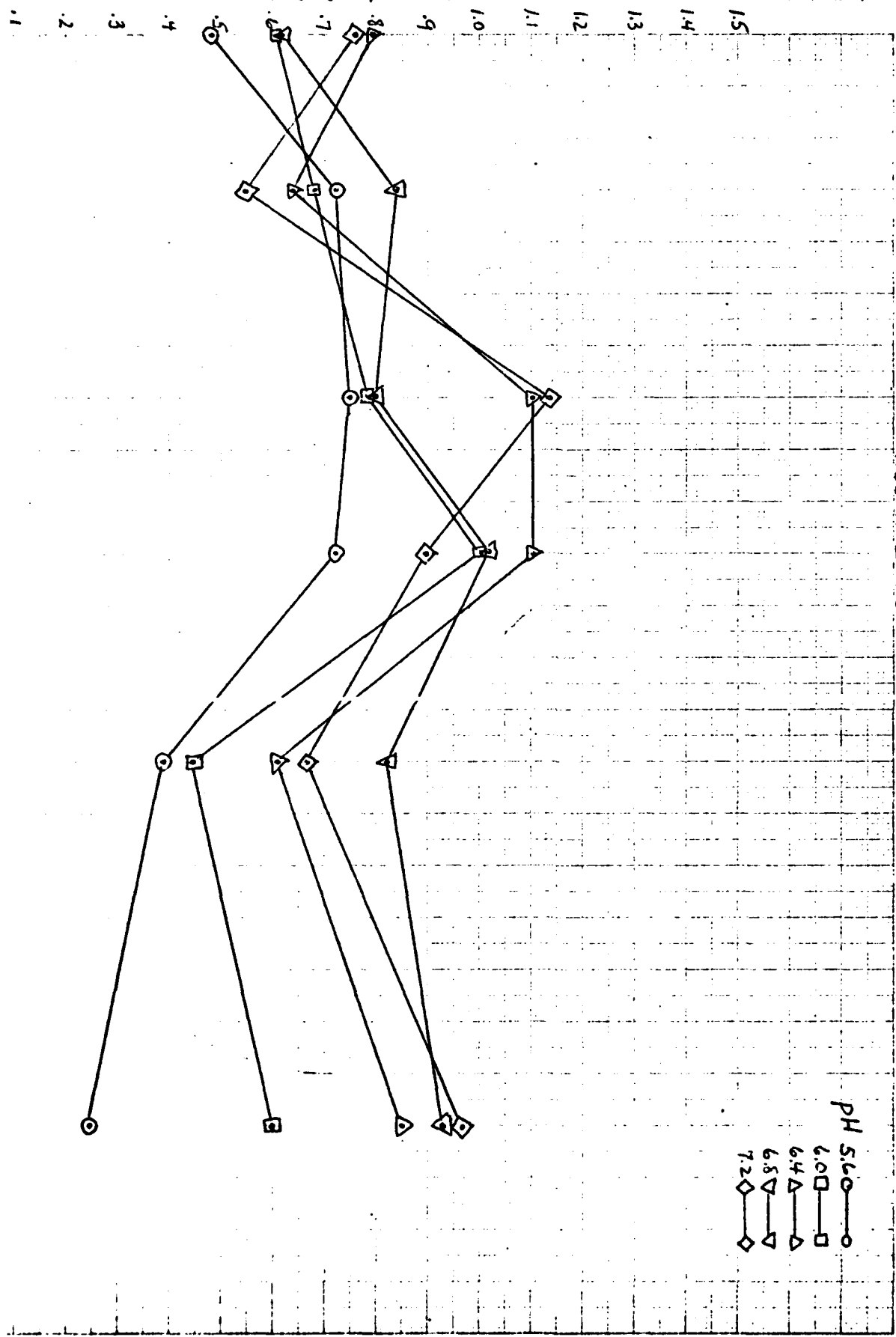
PH 5.6 ○
 6.0 □
 6.4 △
 6.8 ▽
 7.2 ◇



10 Millimeter to the Centimeter

2d

DPG (MM/ML WB)



ACD-A-I-MB

2c

12-108

9

ACD - Adenine - thio - Methylene Blue
Osmotic Fragility
10 Aug 71 42 Days

9, 5.6
9, 6.0
9, 6.4

9, 6.8
9, 7.2

X

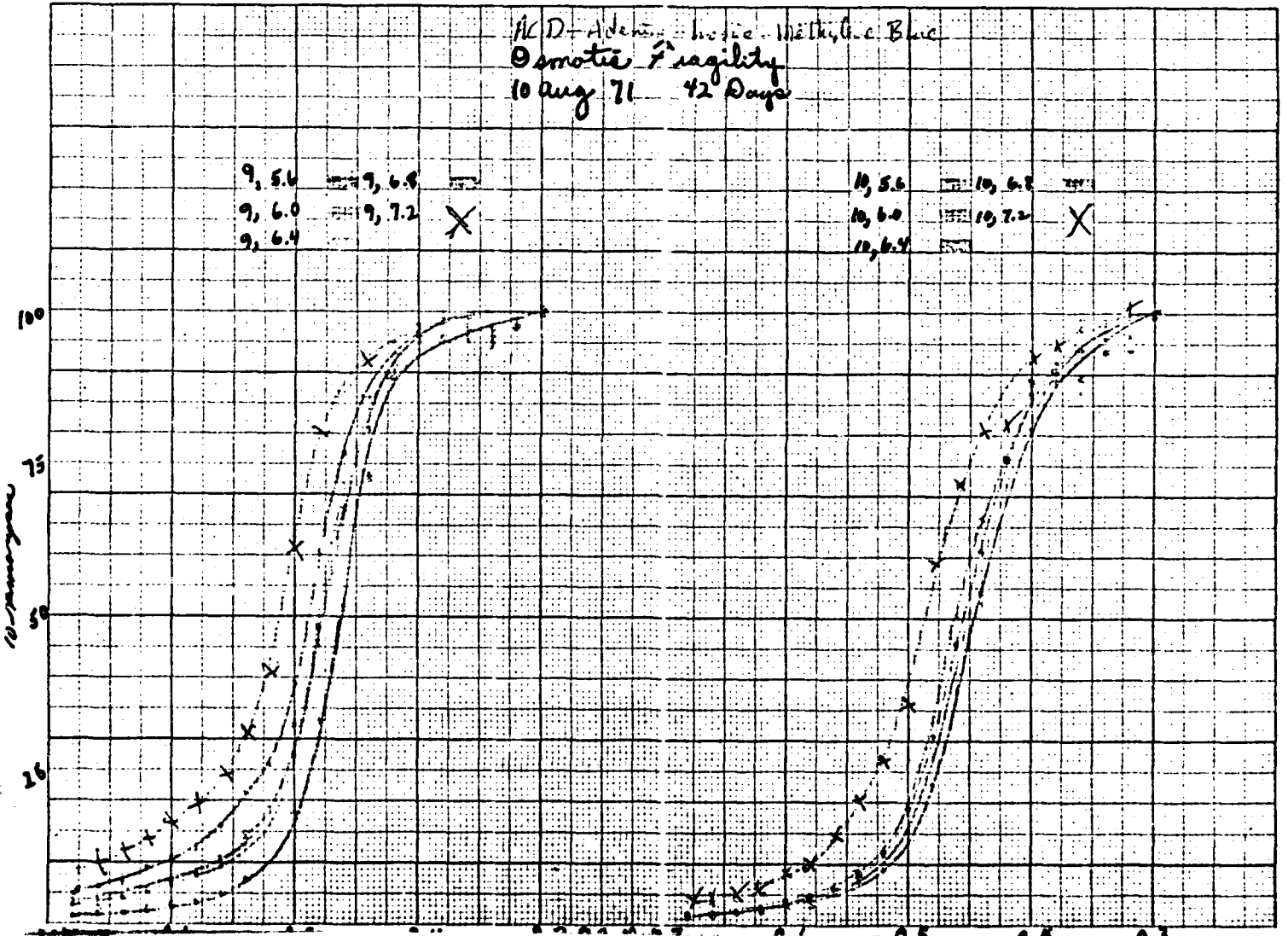
10, 5.6
10, 6.0
10, 6.4

10, 6.8
10, 7.2

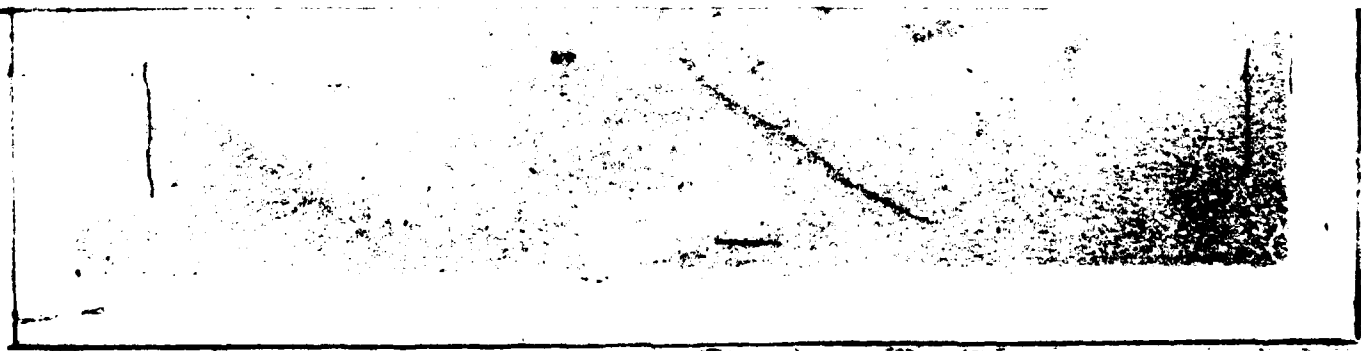
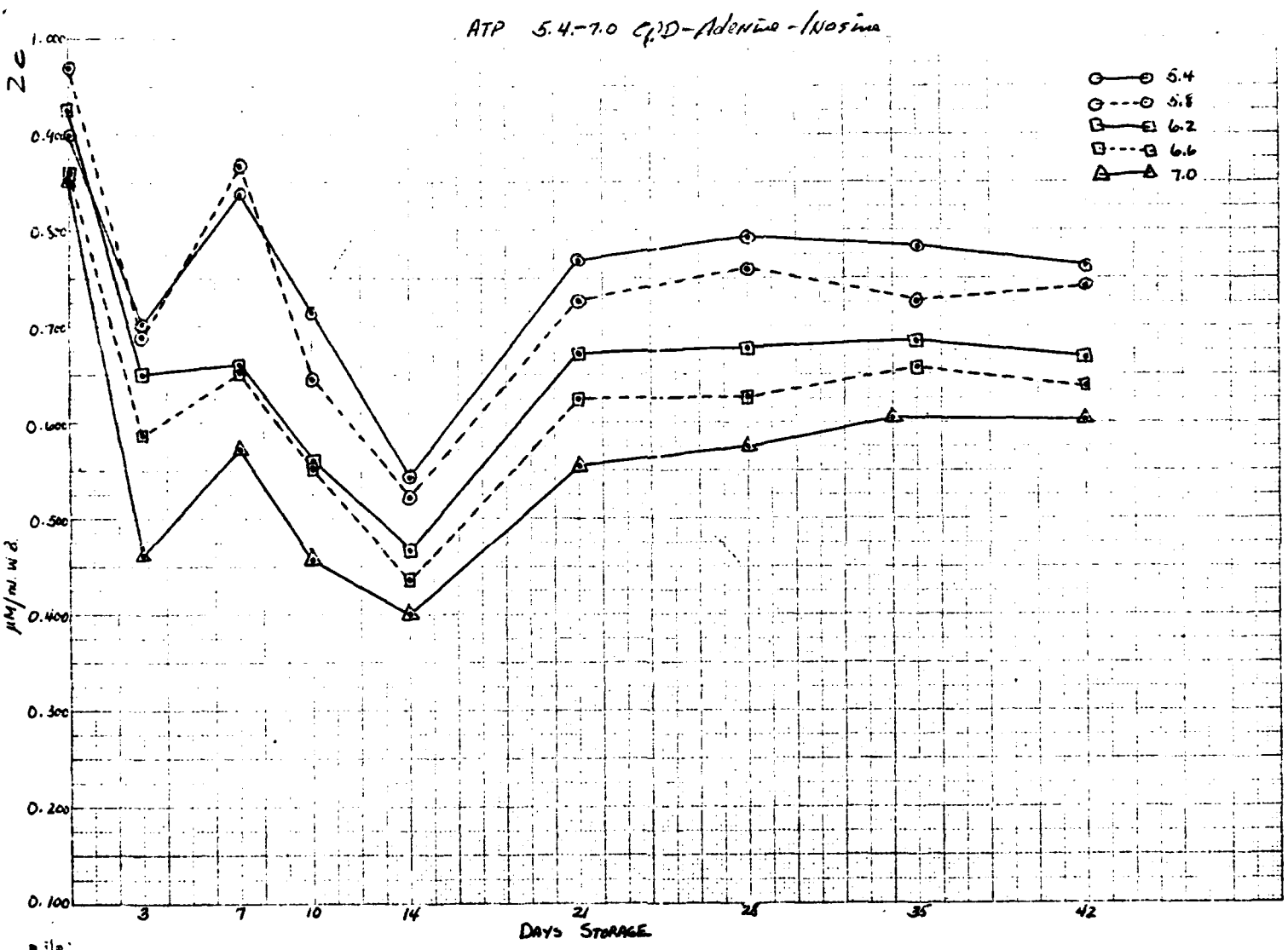
X

p2

3.2

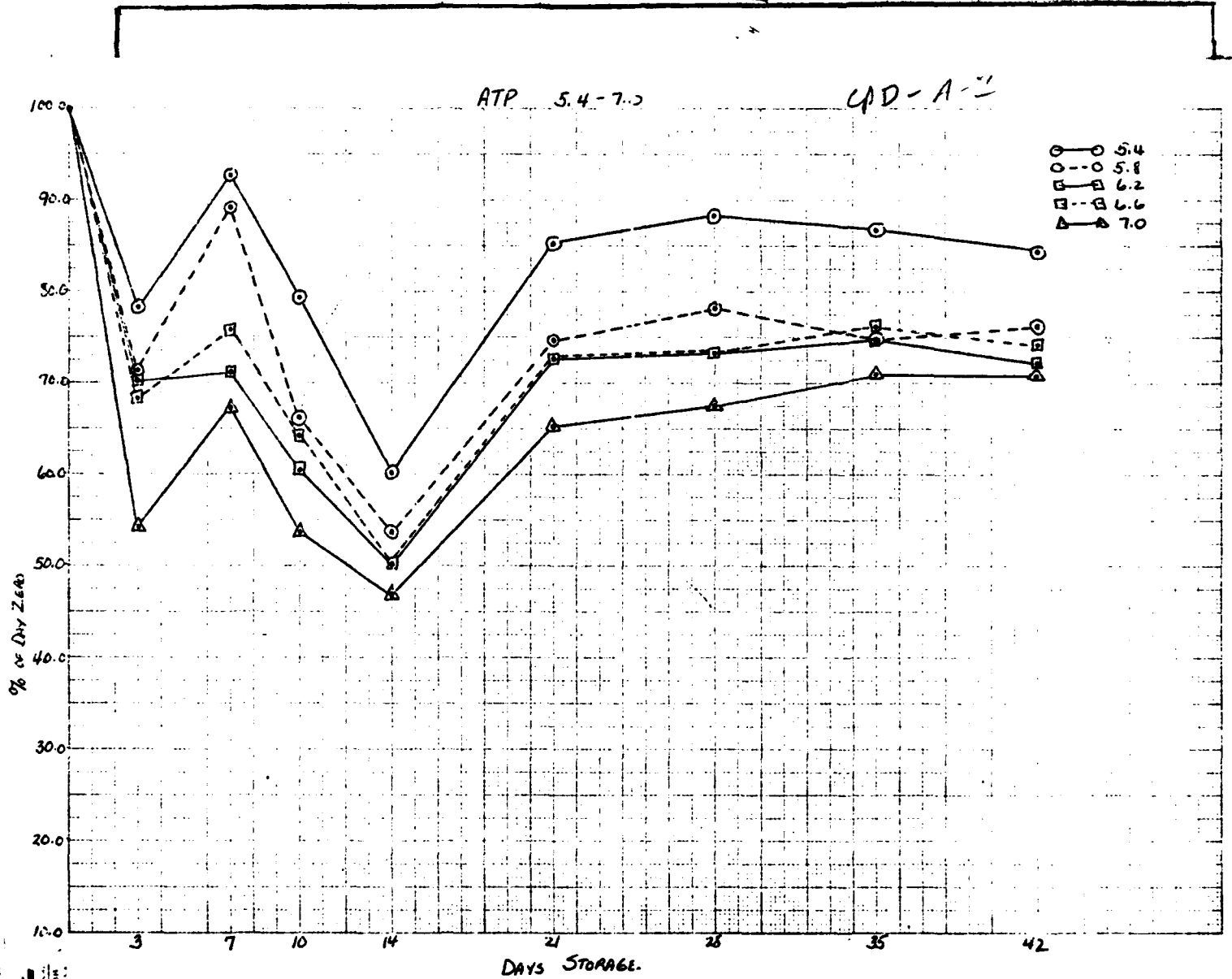


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ATP 5.4-7.0

CPD-A-11



ATP 5.4-7.0
 March 1975

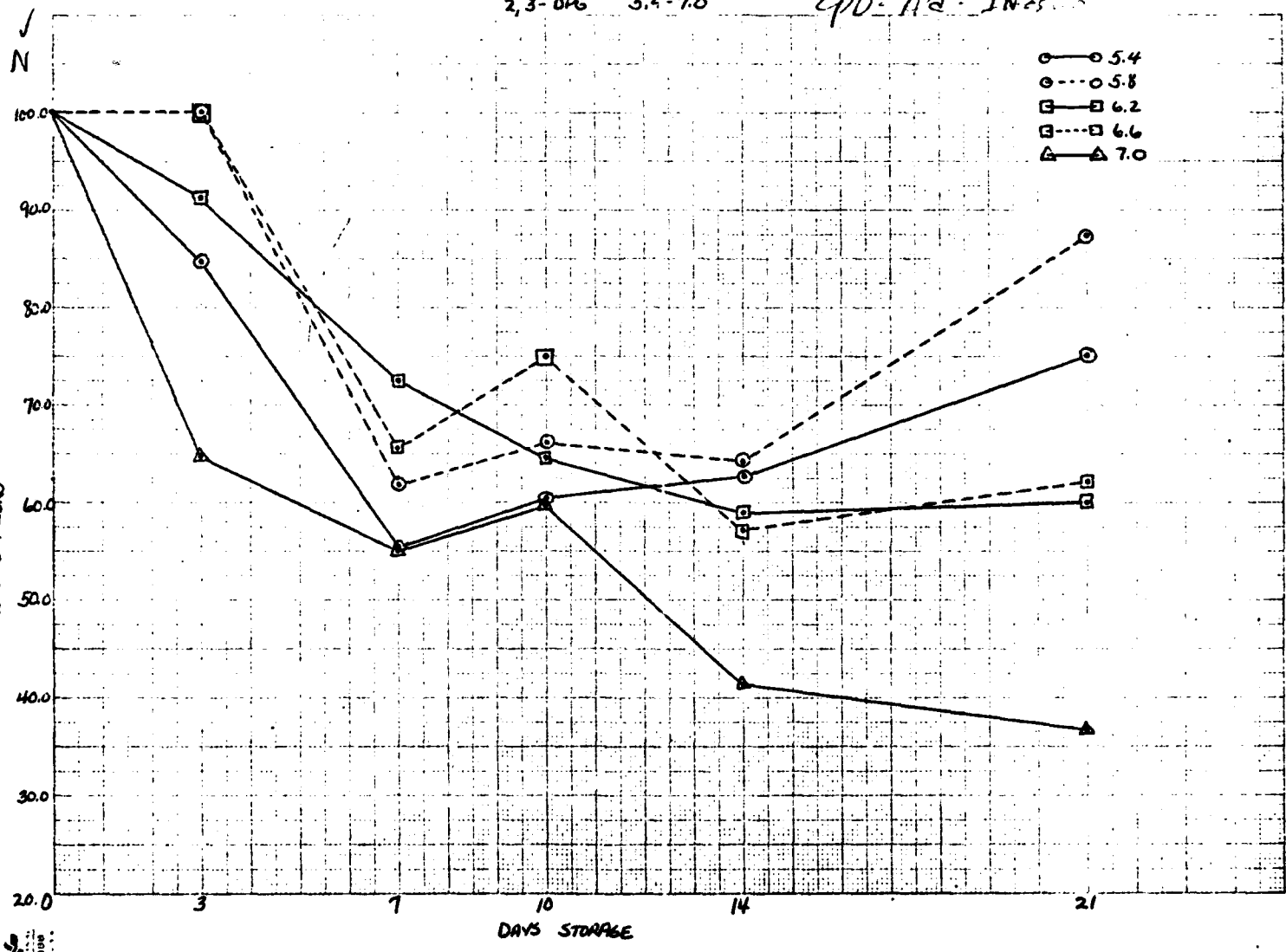
MM/M/WB

	0	3	7	10	14	21	28	35	42
5.4	.900	.704	.836	.715	.541	.766	.794	.781	.761
5.8	.970 .807	.690 .830	.867	.645	.522	.726	.760	.727	.740
6.2	.925	.650	.660	.560	.467	.673	.677	.685 .757	.667
6.6	.860	.586	.652	.552	.436	.625	.627	.657	.638
7.0	.851	.462	.572	.458	.400	.555	.575	.605	.604

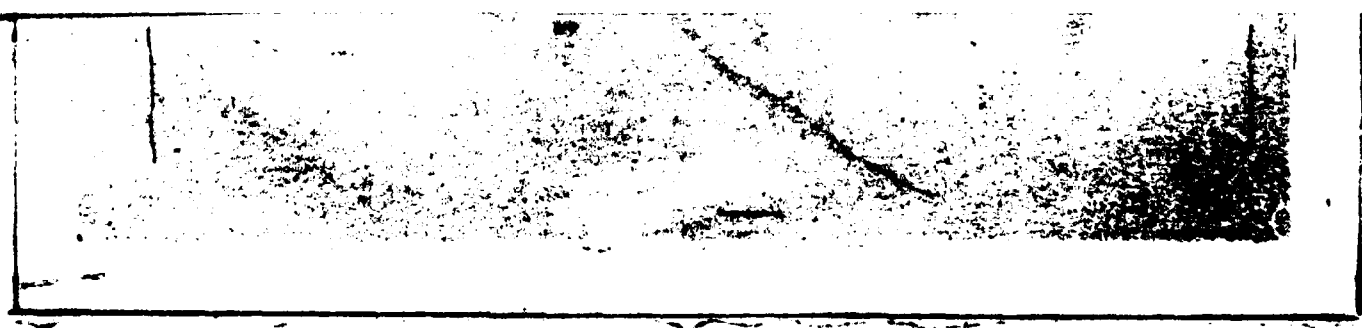
% OF Day Zero

5.4	100.0	78.2	92.8	79.4	60.1	85.3	88.2	86.7	84.5
5.8	100.0	71.1	89.3	66.4	53.8	74.8	78.3	74.9	76.2
6.2	100.0	70.2	71.3	60.5	50.4	72.7	73.1	74.0	72.1
6.6	100.0	68.1	75.8	64.1	50.6	72.6	72.9	76.3	74.1
7.0	100.0	54.2	67.2	53.8	47.0	65.2	67.5	71.0	70.9

2,3-DAG 5.4-7.0 CPD-Ad-Inc



12 108



2.3-DPG

5.4-7.0

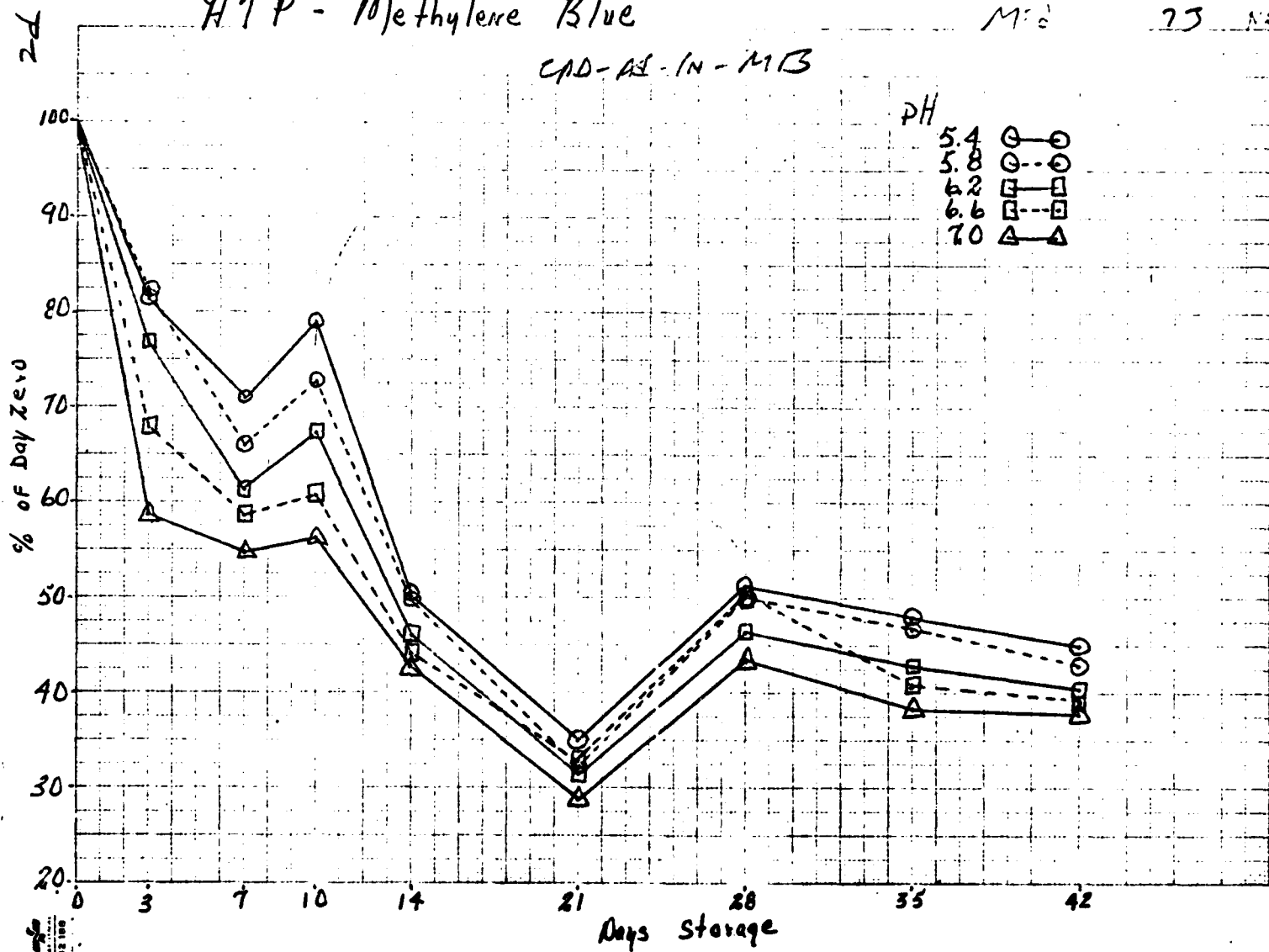
	0	3	7	10	14	21	28	35	42
5.4	.851	.721	.478	.517	.533	.640	.540	-	-
5.8	.914	.915	.568	.606	.588	.800	.300	-	-
6.2	1.000	.913	.725	.616	.590	.600	-	-	-
6.6	1.040	1.040	.681	.750	.593	.648	-	-	-
7.0	1.280	.829	.706	.764	.530	.470	-	-	-
			% OF DAY ZERO						
5.4	100.0	84.7	55.5	60.5	62.5	75.1	63.1	-	-
5.8	100.0	100.1	61.9	66.2	64.2	87.5	32.8	-	-
6.2	100.0	91.3	72.5	64.6	59.0	60.0	-	-	-
6.6	100.0	100.0	65.5	75.0	57.2	62.1	-	-	-
7.0	100.0	64.8	55.1	59.8	41.4	36.7	-	-	-

H₂P - Methylene Blue

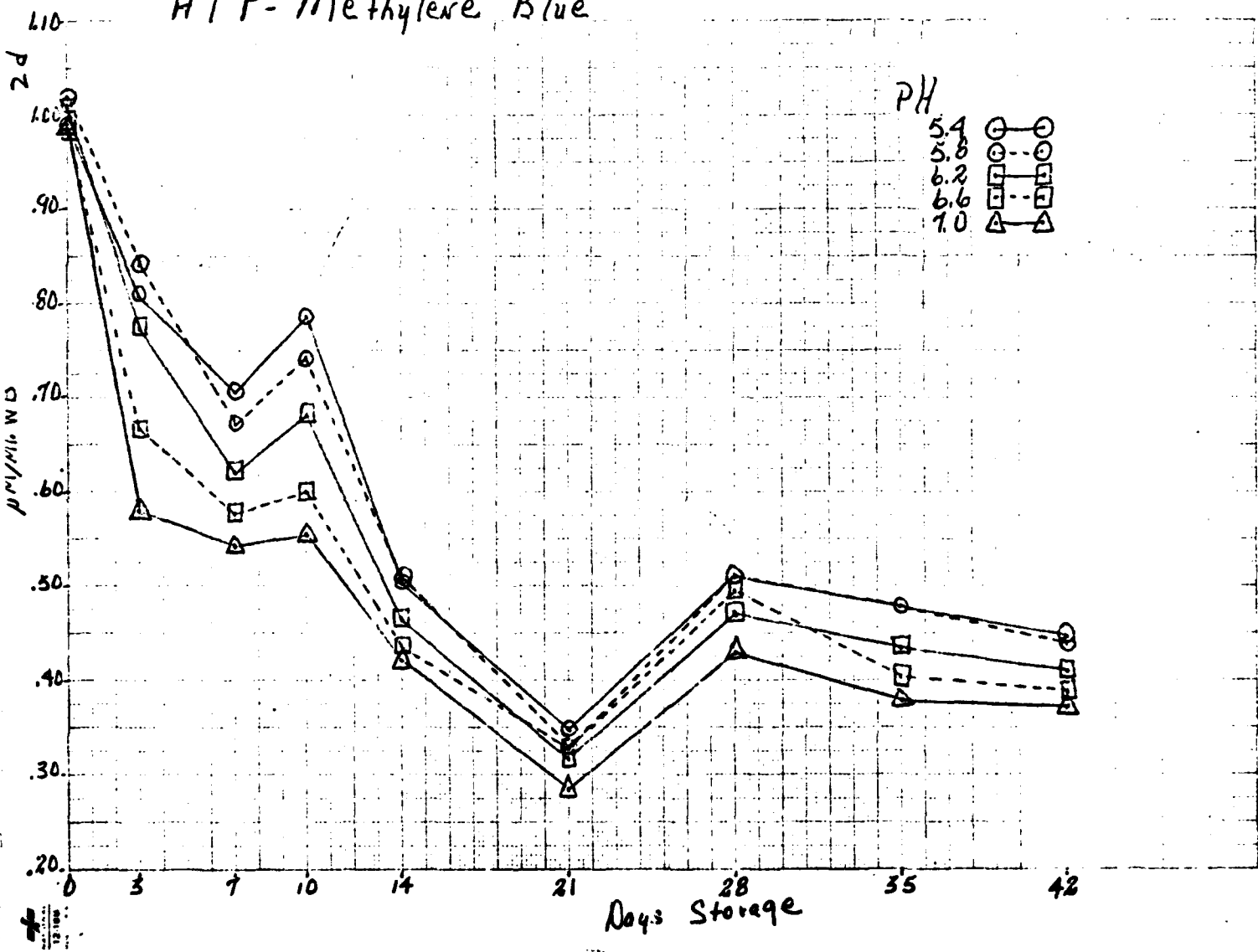
M: 2

73 New

CAD-AS-IN-MB



ATP-Methylene Blue



ATP Methylene Blue (5.4 - 7.0)

June 1973

µM/ml NB

	0	3	7	10	14	21	28	35	42
5.4	.994	.808	.706	.786	.501	.348	.510	.478	.448
5.8	1.020	.841	.674	.743	.506	.330	.510	.476	.438
6.2	1.010	.774	.620	.681	.465	.318	.470	.434	.408
6.6	.985	.668	.578	.600	.435	.323	.495	.402	.356
7.0	.988	.580	.543	.555	.423	.285	.430	.378	.373

% OF Day Zero

5.4	100.0	81.2	71.0	79.0	50.4	35.0	51.3	48.0	45.0
5.8	100.0	82.4	66.0	72.8	49.6	32.3	50.0	46.6	42.9
6.2	100.0	76.6	61.3	67.4	46.0	31.4	46.5	42.9	40.3
6.6	100.0	67.8	58.6	60.9	44.1	32.7	50.2	40.8	39.1
7.0	100.0	58.7	54.9	56.1	42.8	28.8	43.5	38.2	37.7

only two samples on day 28

Methylene Blue (PH 5.4-7.0)

Sample	0	3	7	10	14	21	28	35	42
1	5.4	.92	.72	.72	.40	.29			
	5.8	.80	1.05	.51	.77	.48	.31		
	6.2	.79	.92	.43	.68	-	.29		
	6.6	.82	.81	.46	.63	.37	.28		
	7.0	.87	.72	.41	.55	.34	.25		
2	5.4	.90	.97	.58	.71		.29	.78	
	5.8	1.02	1.13	.62	.70		.26	.73	
	6.2	.98	.90	.54	.63		.27	.66	
	6.6	.90	.76	.48	.55		.26	.61	
	7.0	.87	.64	.42	.52		.24	.56	
3	5.4	1.00		.82	.90	.58	.42	.47	.44
	5.8	1.04		.73	.83	.58	.40	.46	.39
	6.2	1.01		.70	.77	.46	.37	.41	.37
	6.6	.98		.62	.67	.48	.42	.42	.34
	7.0	.99		.64	.64	.40	.36	.35	.34
4	5.4	.89		.76	.93	.82	.28		
	5.8	.97		.71	.85	.77	.27		
	6.2	1.05		.69	.78	.73	.28		
	6.6	1.02		.62	.73	.67	.27		
	7.0	1.04		.57	.65	.60	.26		
5	5.4	.85		.64	.70	.71	.26		
	5.8	.85		.59	.60	.67	.25		
	6.2	.93		.57	.60	.63	.25		
	6.6	.89		.50	.48	.54	.22		
	7.0	.75		.46	.45	.51	.20		
6	5.4	.92		.69	.74	.61			
	5.8	.88		.67	.69	.68			
	6.2	.87		.62	.60	.59			
	6.6	.80		.57	.53	.65			
	7.0	.94		.65	.55	.73			

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~~4-10~~
~~87~~
~~79~~
~~91~~
~~76~~

6
~~6~~
~~67~~
~~59~~
~~65~~
~~73~~

METHYLENE BLUE (pH 5.4-7.0)

	0	3	7	10	conv. 14	20	28	conv. 35	42
#7 5.4	1.35	.87	.93	1.12	.36			.37	.83
5.8	1.41	.86	.85	1.02	.36			.39	.82
6.2	1.46	.75	.74	1.01	.34			.33	.73
6.6	1.39	.63	.70	.86	.29			.31	.63
7.0	1.33	.53	.58	.71	.34			.26	.58
#8 5.4	1.34	.72	.62		.28			conv. .34	.64
5.8	1.30	.71	.71		.26			.38	.63
6.2	1.34	.67	.63		.26			.37	.61
6.6	1.30	.61	.65		.25			.33	.61
7.0	1.30	.54	.59		.24			.30	.54
#9 5.4	.88	.71	.61		conv. .31				.62
5.8	.94	.62	.66		.31				.62
6.2	.85	.38	.64		.31				.54
6.6	.90	.53	.62		.27				.54
7.0	.77	.51	.60		.20				.53
#10 5.4	.82	.66	.71	47	44	55	55	46	37
5.8	.95	.68	.69	47	43	49	56	49	41
6.2	.85	.63	.60	41	40	45	50	44	39
6.6	.85	.36	.56	35	38	49	57	42	36
7.0	.85	.54	.57	37	37	40	51	43	37

NA Machine

sample is missing

#8 + #17 Day 0 are correct for M.B. (labeled on Auto read chart)

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B. RESULTS OF PACKED CELL EXPERIMENTS WITH CPD-ADENINE

First Packed Cell Experiment

Figure 1 marked PC₁ shows maintenance of ATP during storage for 42 days in whole blood and packed cells. Units are grouped and the units having hematocrits of 57 and 72% are considered together and the two units having hematocrits each of 95% are considered together. The whole blood units had hematocrits of 33 and 34% and their values have been averaged for presentation in this graph. This study has been previously presented to the Blood Research Group at its meeting in January in Chicago, and the graph showing values after 35 days for all six units was published as an addendum I believe on the last page of the proceedings of that meeting. In this presentation, the data has been replotted by averaging the groups of whole blood and two types of hematocrits and using a uniform ordinate of percent of day zero. Looking at the right hand extreme of the graph, it is obvious that all ATP values stay above 40% of normal, even at day 42. At day 35 they are above 60% of normal. Seeing this another way the hard packed units' ATP values almost reach 40% of normal at 42 days whereas at day 35 they almost reach 60% of normal. The unpacked units maintained an ATP concentration above 100% of normal throughout the 42 day storage period. An additional graph on this experiment, PC_{1X}, shows percent of day zero ATP values for the four units of most interest, the two 95% units and the 33 and 34% units. Also, the actual values of ATP in micromoles per gram of hemoglobin and percent of day zero are included in tables 1a and 1b.

Packed Cell Experiment No. II

PC_{2A} is a graph of the average values from the two groups of units, the packed units of 88% hematocrit and the whole blood units. At 35 day storage the packed units had dropped their ATP concentrations to 54% of normal whereas the whole blood units averaged 94%. The difference between the packed cell and whole blood units appears to be less at 42 days both in this averaged graph and the graph PC_{2B} in

which individual values are shown for each of the four units. After 35 days there is a fairly rapid decrease in ATP concentrations, the rate of fall being greater for the whole blood units. I would predict that these packed cell units with adenine, having ATP values greater than 50% of day zero, will provide 24 hour post-transfusion survival considerably higher than the minimum 70% required. The aim, of course, would be 35 days for routine storage and 42 days emergency storage for rare units or times of shortage.

Adenine Packed Cells with Double Glucose Experiment No. I

We and others have noticed in blood storage experiments, especially with adenine, the ATP concentrations will frequently increase to 110 to 130% of day zero values at some point during the first three weeks of storage. However, in this double glucose experiment the ATP in one unit increased to 170% and in another to 284% of the day zero values. These erratic increases were seen only in the whole blood units however, there were slight increases, similar to what we usually see, in the packed cell units. As with the other two experiments, graphs are attached of the average of the pairs of units marked as DG for Double Glucose I, I-A for averages and I-B for values of the six units. Looking at graph DG₁B showing all six units, one can see that ATP concentrations do not fall below 50% at day 35 or 40% at day 42 in any of the units. This percentage of day zero graph is included because one of the whole blood units apparently starts out with a very low actual ATP value. If this unit were eliminated from the study then the average ATP values during the 42 day storage period for the whole blood units would not appear to be higher than the values for the two groups of packed cell units. Although on realizing this I will retest the sample and track down the donor to see if the low value is real. No matter how that turns out it appears that with double glucose the differences between the groups of units seems to have been minimized. For example, from day 14 through day 42 in the double glucose experiment the average difference between light and hard packed units was less than 10%; whereas, in the PC₁ experiment from day 14

ough 42 the average difference between these two groups was closer to 20%. The actual values are 8.6% average difference in the double glucose experiment and 18.2% average difference in PC_1 from 14 to 42 days. Tables 3A and 3B from the double glucose experiment are included.

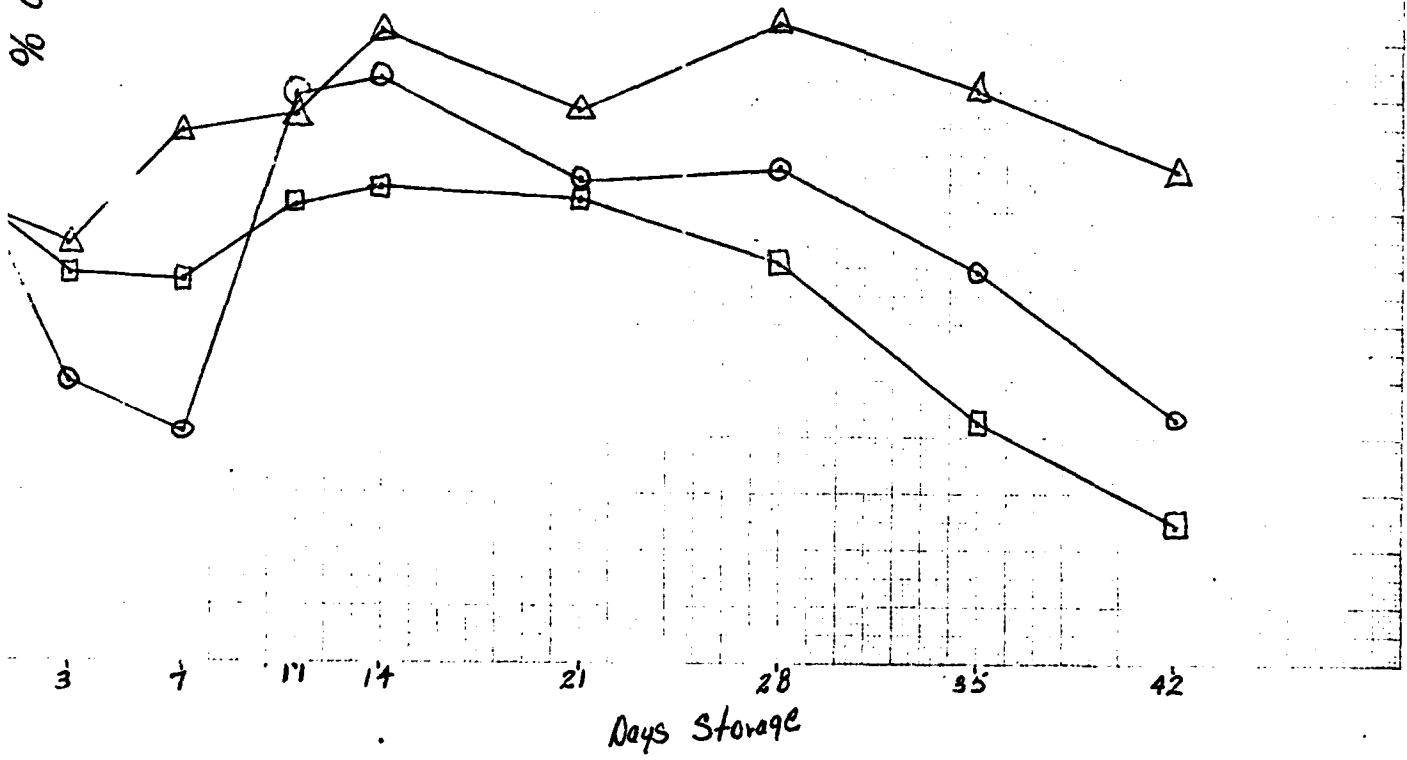
In summary, these three experiments done with whole blood units demonstrate that packed cells do not maintain their ATP concentrations as well as whole blood units, especially during the 4th through 6th weeks. Since it is desirable to store packed cells for five to six weeks in CPD-adenine it seems important to determine whether or not an adequate amount of glucose was present in CPD-adenine as presently constituted for maintenance of ATP during this prolonged storage period. A lot of inferences might be made from the small amount of data contained in these three experiments. Some inferences and suggestions have already been made but there is the obvious danger of making too much out of too little. At the risk of approaching that point and I hope I would only be approaching it, let me conclude my summary saying that if we were planning to store blood for 42 days in CPD-adenine with the present amount of glucose we might be in trouble. Notice in Table 2-B that columns 3 and 4 at day 42 show 28% ATP. This experiment was done at the same time and under the same conditions as the double glucose experiment which shows in table B for 42 days again the last two columns hematocrits of 89% ATP, values of 60% and 45.8%. Now, in those same tables go up one line and see higher than 50% ATP concentrations without glucose and around 70% with double glucose. In final summary then it appears that double glucose might be helpful for 42 days but it might not be necessary for a 35 day packed cell unit.

ATP- Adenine Packed Cells I

% of Day Zero

PC-I

- | | | |
|----|-----|-------|
| | | Hct. |
| 1. | ○—○ | 57+73 |
| 2. | □—□ | 95+95 |
| 3. | △—△ | 34+33 |



ATP Ad. PC-1

PC Ix

2-00	-	Hct.
4-00	-	95
5-00	-	34
6-00	-	33

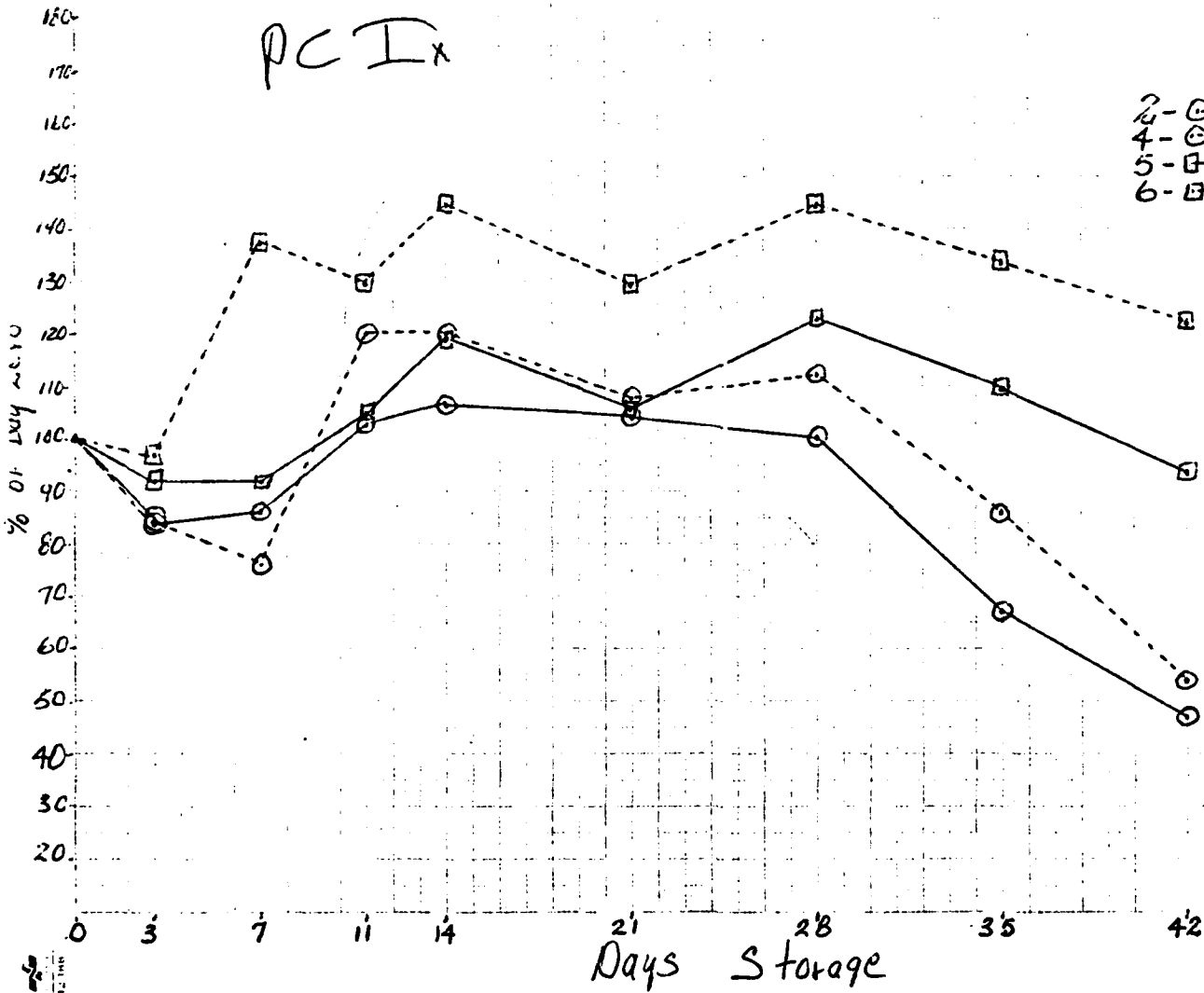


Table 1.2

ATP - Adenine Packed Cells I
uM/gm. Hgb

Days Storage	Bag No. (Hct.)					
	1 (57)	2 (95)	3 (73)	4 (95)	5 (34)	6 (33)
0	6.1	4.9	5.0	5.0	6.2	5.4
3	3.4	4.1	4.2	4.7	5.7	5.2
7	2.7	4.2	3.8	4.5	5.7	7.4
11	7.4	5.0	6.0	5.0	6.5	7.0
14	7.7	5.2	6.0	5.1	7.4	7.8
21	6.3	5.1	5.4	5.0	6.6	7.0
28	6.3	4.9	5.6	4.1	7.6	7.8
35	5.7	3.3	4.3	2.9	6.8	7.2
42	4.5	2.3	2.7	2.1	5.8	6.6

1000 10

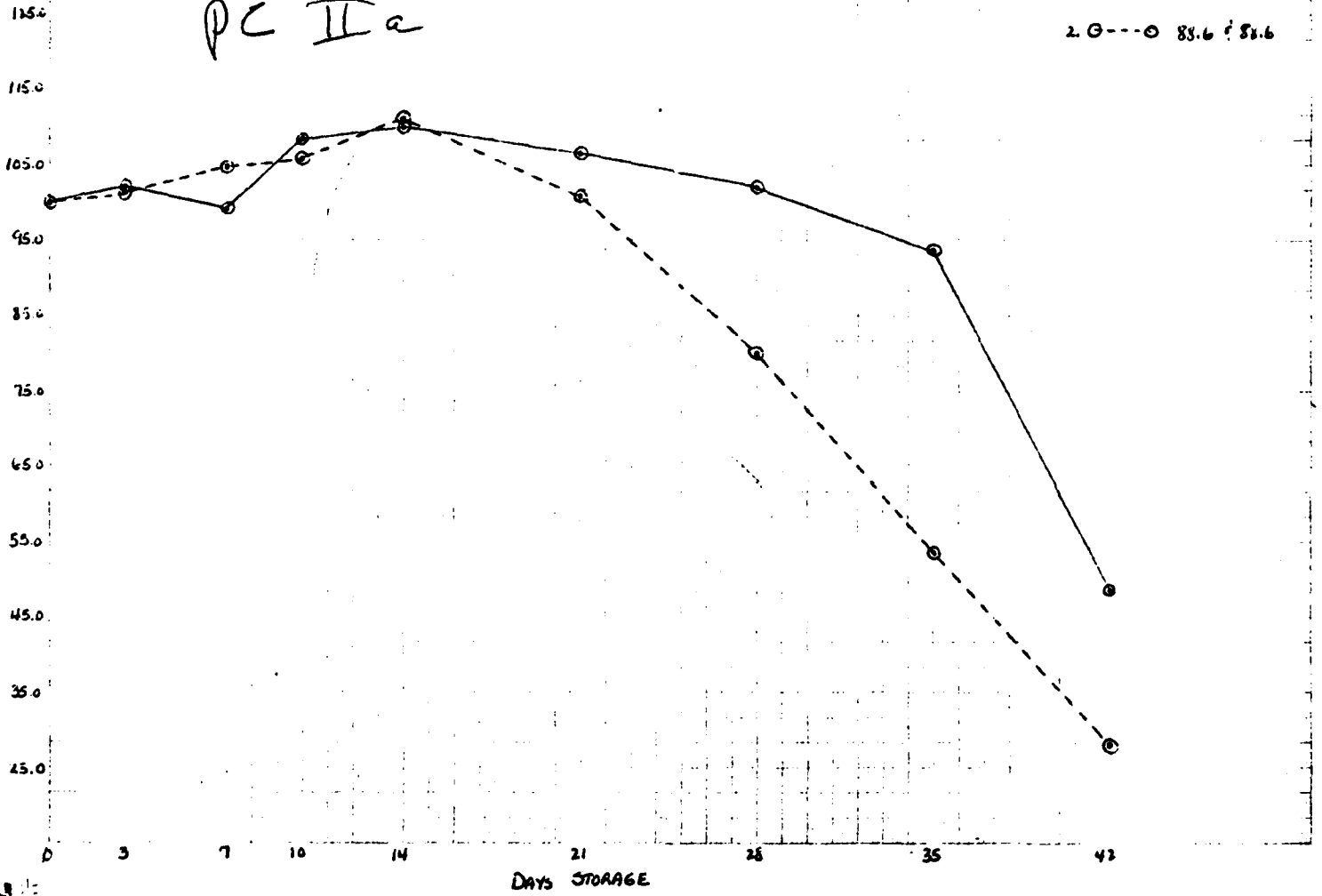
ATP - Adenine Packed Cells I
% of Day Zero

Days Storage	Bag No. (Hct.)					
	1 (57)	2 (95)	3 (73)	4 (95)	5 (34)	6 (33)
0	100.0	100.0	100.0	100.0	100.0	100.0
3	55.7	83.7	84.0	94.0	91.9	96.3
7	44.3	85.7	76.0	90.0	91.9	137.0
11	121.3	102.0	120.0	100.0	104.8	129.6
14	126.2	106.1	120.0	102.0	119.4	144.4
21	103.3	104.1	108.0	100.0	106.5	129.6
28	103.3	100.0	112.0	82.0	122.6	144.4
35	93.4	67.3	86.0	58.0	109.7	133.3
42	73.7	46.9	54.0	42.0	93.5	122.2

ATP- ADENINE PACKED CELLS II

PC IIa

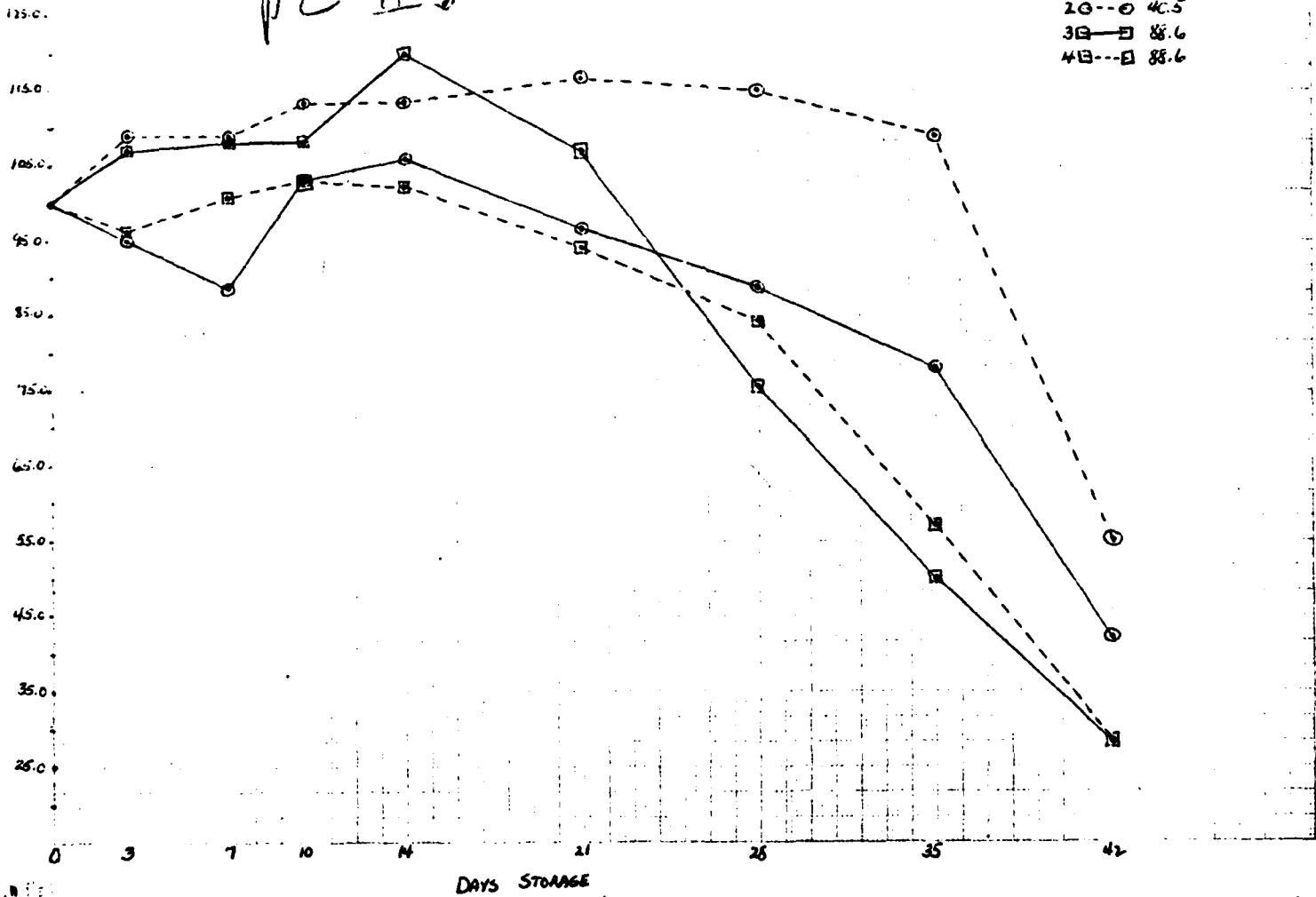
HCT
1. \bigcirc — \bigcirc 31.4 & 40.5
2. \bigcirc --- \bigcirc 83.6 & 88.6



ATP-ADENINE PACKED CELLS II

PC II ↓

HCT
 1 ○—○ 39.4
 2 ○--○ 40.5
 3 □—□ 88.6
 4 □--□ 88.6



-29-

10 Millimeters to the Centimeter

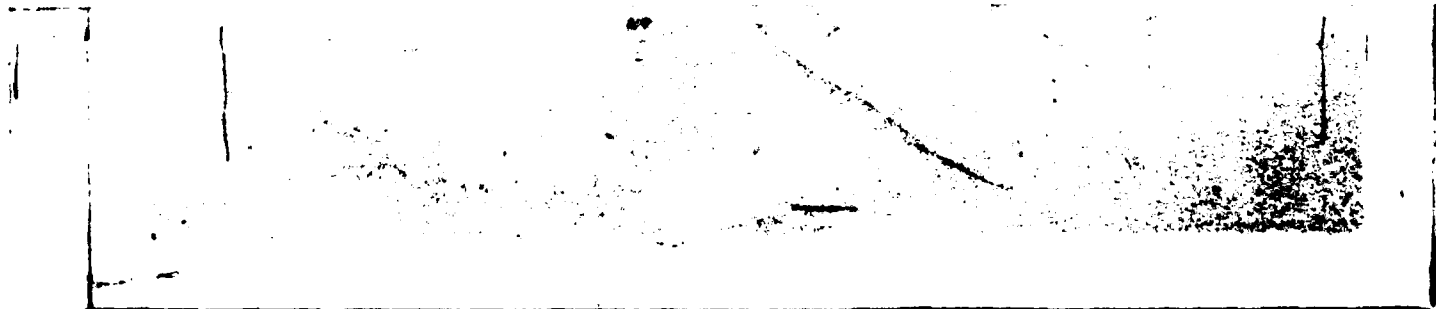


Table 2.

ATP - Adenine Packed Cells II
uM/gm. Hgb

Days Storage	Bag No. (Hct.)			
	1 (39.4)	2 (40.5)	3 (88.6)	4 (88.6)
0	5.20	5.20	5.53	7.01
3	4.96	5.68	5.93	6.46
7	4.63	5.68	6.01	7.09
10	5.37	5.92	6.01	7.24
14	5.53	5.92	6.64	7.17
21	5.04	6.08	5.93	6.61
28	4.63	6.00	4.19	5.91
35	4.07	5.68	2.77	4.02
42	2.20	2.88	1.58	1.97

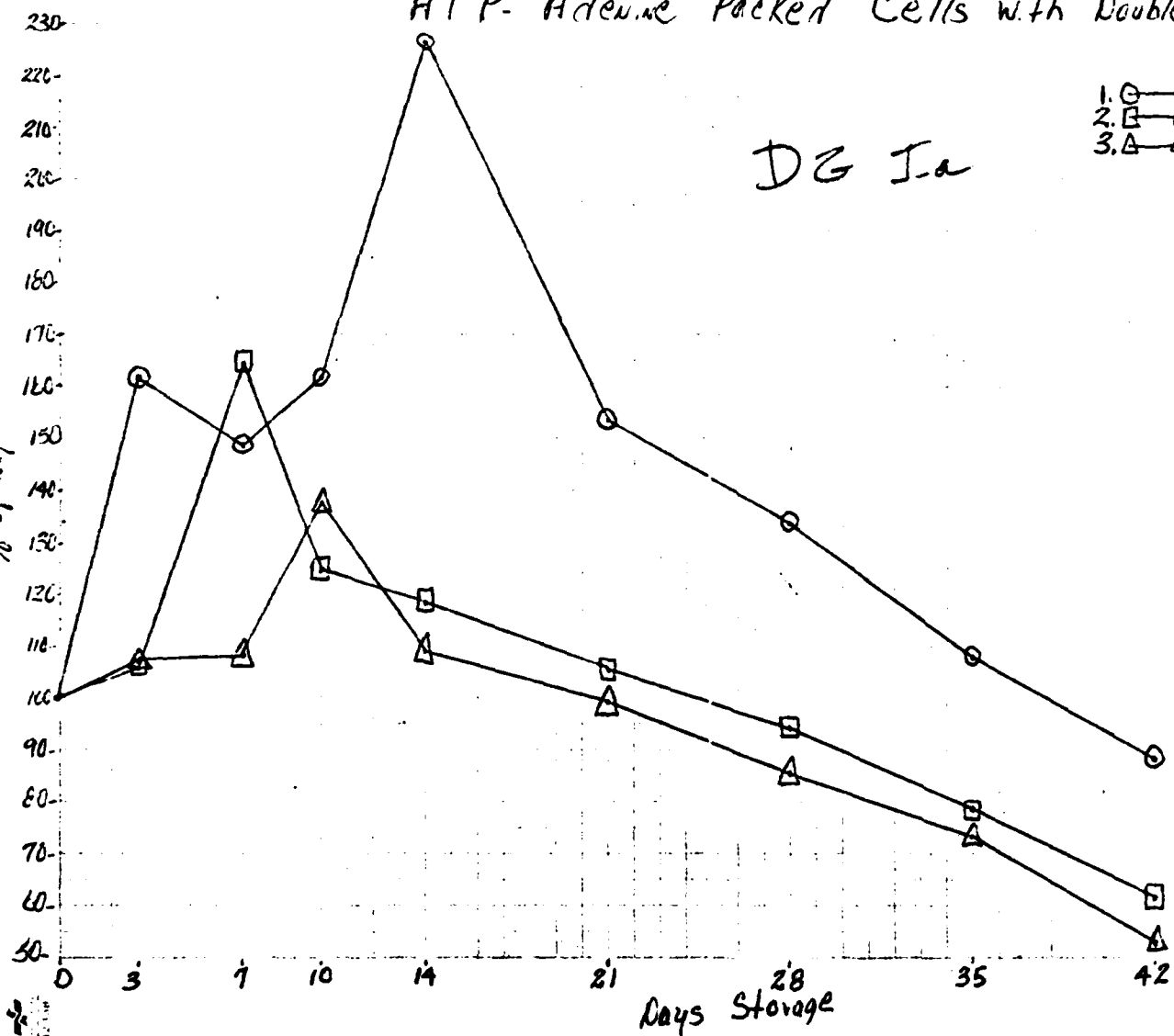
ATP - Adenine Packed Cells II
% of Day Zero

Days Storage	Bag No. (Hct.)			
	1 (39.4)	2 (40.5)	3 (88.6)	4 (88.6)
0	100.0	100.0	100.0	100.0
3	95.3	109.2	107.2	96.1
7	89.0	109.2	108.6	101.1
10	103.2	113.8	108.6	103.2
14	106.3	113.8	120.0	102.2
21	96.9	116.9	107.2	94.2
28	89.0	115.3	75.7	84.3
35	78.2	109.2	50.0	57.3
42	42.3	55.3	28.5	28.1

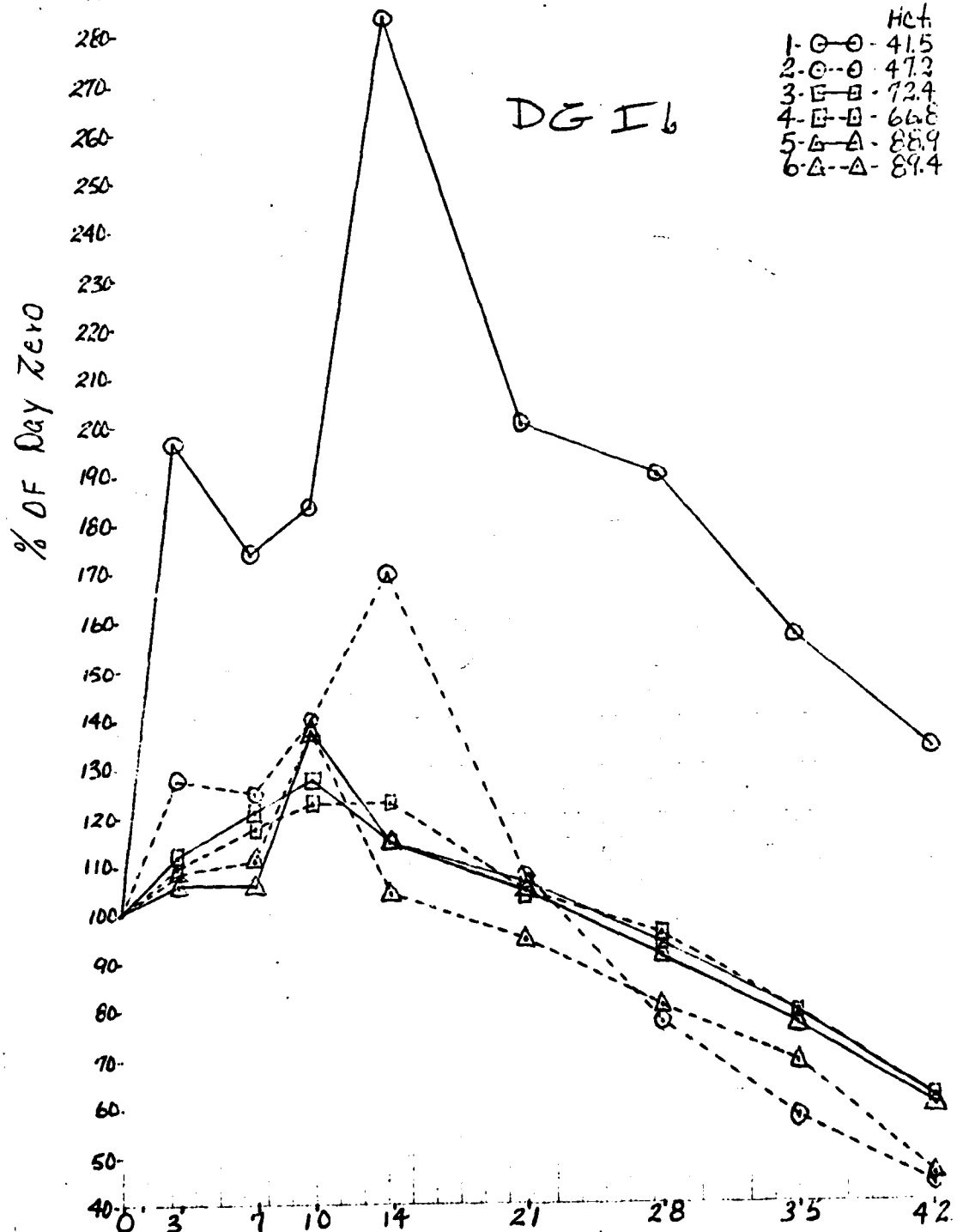
H1 P- Adenine Packed Cells with Double Glucose I

DG Ia

	Hct.
1. ○—○	41.5+47.2
2. □—□	72.4+66.8
3. △—△	88.9+89.4



ATP Adenine Packed Cells with Double Glucose I



Hct.

1	○-○	41.5
2	○-○	47.2
3	□-□	72.4
4	□-□	66.8
5	△-△	88.9
6	△-△	89.4

DG I 6

% OF Day Zero

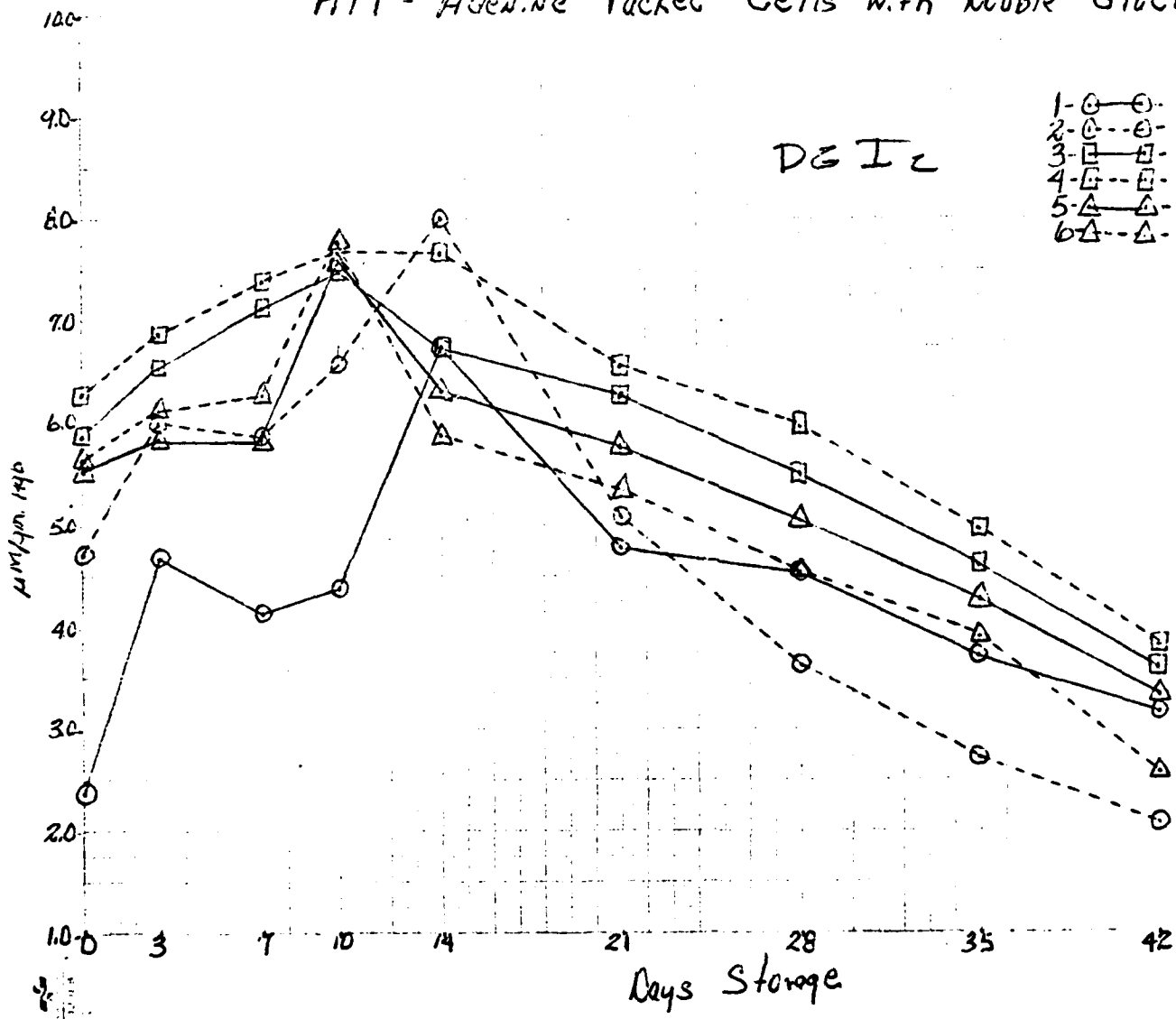
10 Millimeters to the Centimeter

Notes Slaves -33-

HYP - Adenine Packed Cells with Double Glucose I

DG I 2

	Hct.
1-○-○-	41.5
2-○-○-	47.2
3-□-□-	72.4
4-□-□-	66.6
5-△-△-	86.9
6-△-△-	89.4



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ATP - Adenine Packed Cells with Double Glucose I
uM/gm. Hgb

Days Storage	Bag No. (Hct.)					
	1 (41.5)	2 (47.2)	3 (72.4)	4 (66.8)	5 (88.9)	6 (89.4)
0	2.38	4.71	5.88	6.26	5.51	5.65
3	4.68	6.00	6.54	6.87	5.83	6.12
7	4.13	5.86	7.11	7.37	5.83	6.27
10	4.37	6.57	7.49	7.68	7.56	7.76
14	6.75	8.00	6.73	7.68	6.30	5.88
21	4.76	5.07	6.26	6.57	5.75	5.33
28	4.52	3.64	5.50	5.96	5.04	4.55
35	3.73	2.71	4.64	4.95	4.25	3.92
42	3.17	2.07	3.60	3.84	3.31	2.59

ATP - Adenine Packed Cells with Double Glucose I
% of Day Zero

Days Storage	Bag No. (Hct.)					
	1 (41.5)	2 (47.2)	3 (72.4)	4 (66.8)	5 (88.9)	6 (89.4)
0	100.0	100.0	100.0	100.0	100.0	100.0
3	196.6	127.3	111.2	109.7	105.8	108.3
7	173.5	124.4	120.9	117.7	105.8	110.9
10	183.6	139.4	127.3	122.6	137.2	137.3
14	283.6	169.8	114.4	122.6	114.3	104.0
21	200.0	107.6	106.4	104.9	104.3	94.3
28	189.9	77.2	93.5	95.2	91.4	80.5
35	156.7	57.5	78.9	79.0	77.1	69.3
42	133.1	43.9	61.2	61.3	60.0	45.8

METABOLIC ADDITIVES, NUTRIENTS AND REGULATORS:

Adenine-DHA-Pyruvate; Inosine-Methylene Blue

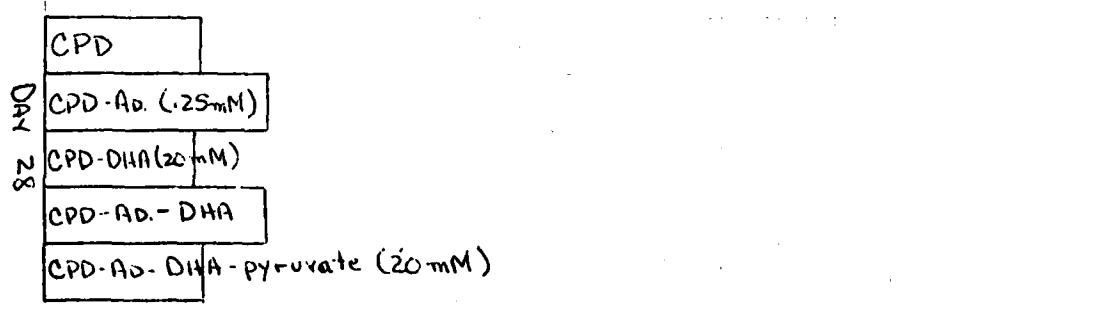
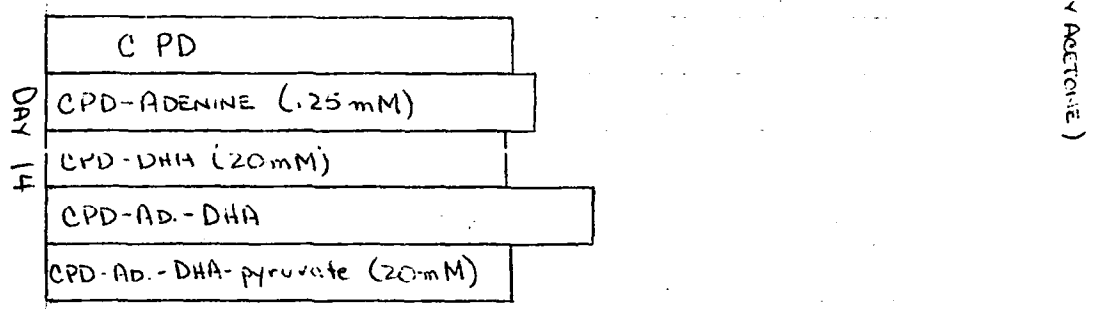
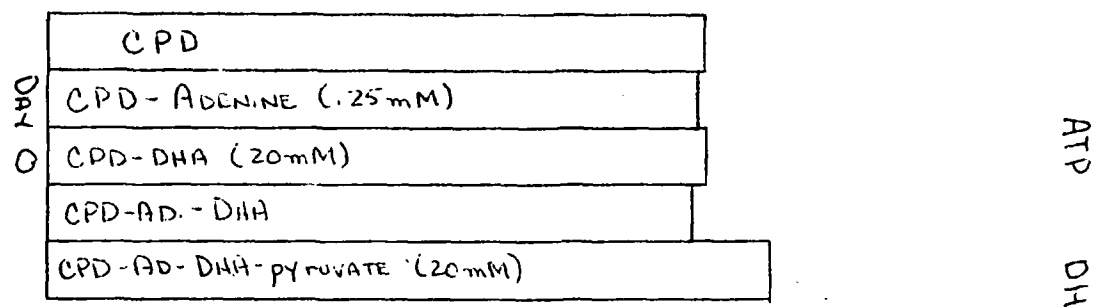
1. Adenine-DHA-Pyruvate

The ATP maintenance in this experiment is apparently a little better in the adenine containing preservatives at day 14 and day 28. However, the differences between preservatives are small and no conclusion should be made. The DPG concentrations are clearly better maintained in the presence of DHA, whether adenine is present or not. At 21 days of storage with DHA the 2,3-DPG concentrations were essentially normal or equal to day zero values. Normal 2,3-DPG values at 21 days of storage had henceforth only been obtained in this laboratory with inosine present in the preservative. Further at 35 days of storage with DHA, the 2,3-DPG values are still approximately 50% of day zero. At day 42 with DHA and pyruvate the 2,3-DPG value is slightly above 50% of normal.

2. Inosine-Methylene Blue

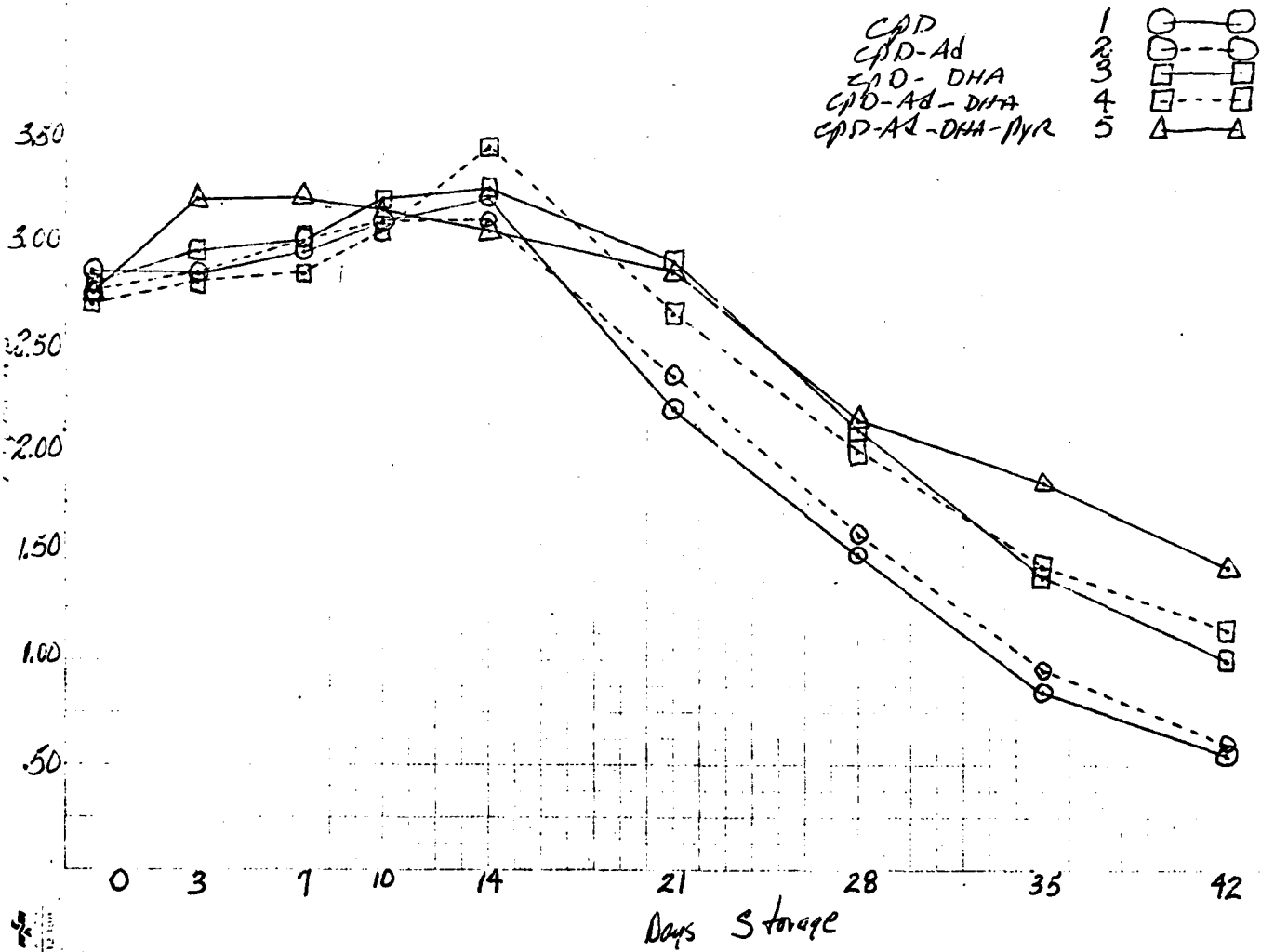
In this experiment the optimal concentration of inosine is being evaluated in the presence of methylene blue in a CPD-adenine preservative. ATP concentrations are shown in a bar graph for days 3, 7, and 35. Variations in inosine concentrations in the presence of methylene blue do not seem to make a significant difference. This is not surprising since inosine or methylene blue would not be expected to have much effect on ATP maintenance. 2,3-DPG concentrations are maintained at at least normal levels for 35 days of storage in the presence of 10 or 15 mM inosine. It is of note that the low concentration of inosine, 5 mM, preserves DPG concentrations at day zero levels or better for 28 days. Methylene blue does not have a striking effect on maintenance of 2,3-DPG in this experiment. The units with methylene blue seem to have slightly better DPG maintenance during the first two weeks of storage and this difference will have to be restudied to see if it is significant.

3.60
3.40
3.20
3.00
2.80
2.60
2.40
2.20
2.00
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1.60
1.40
1.20
1.00
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.60
.40
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0



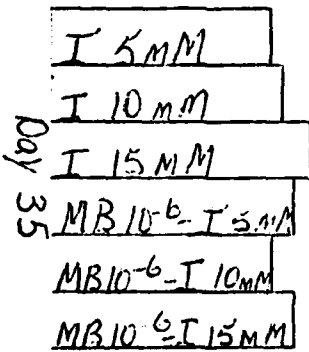
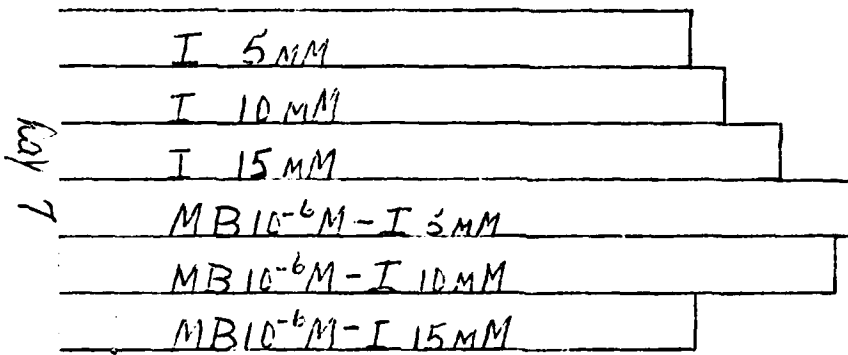
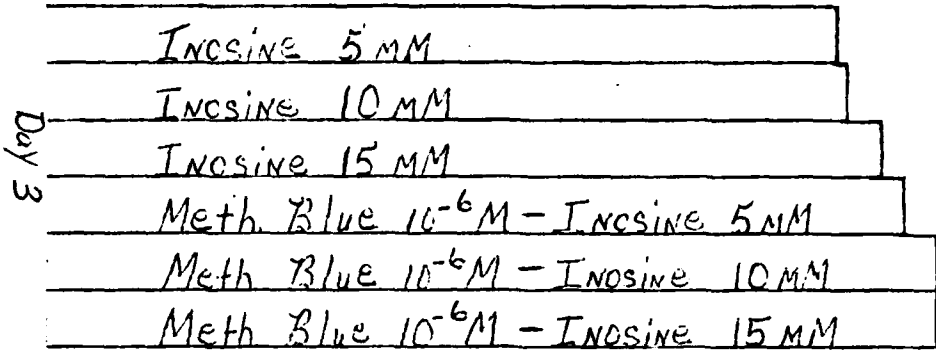
10 Millimeters to the Centimeter

23 UPG- D-1 (N:hydroxyacetone)



µM/ML. WADIC 15000

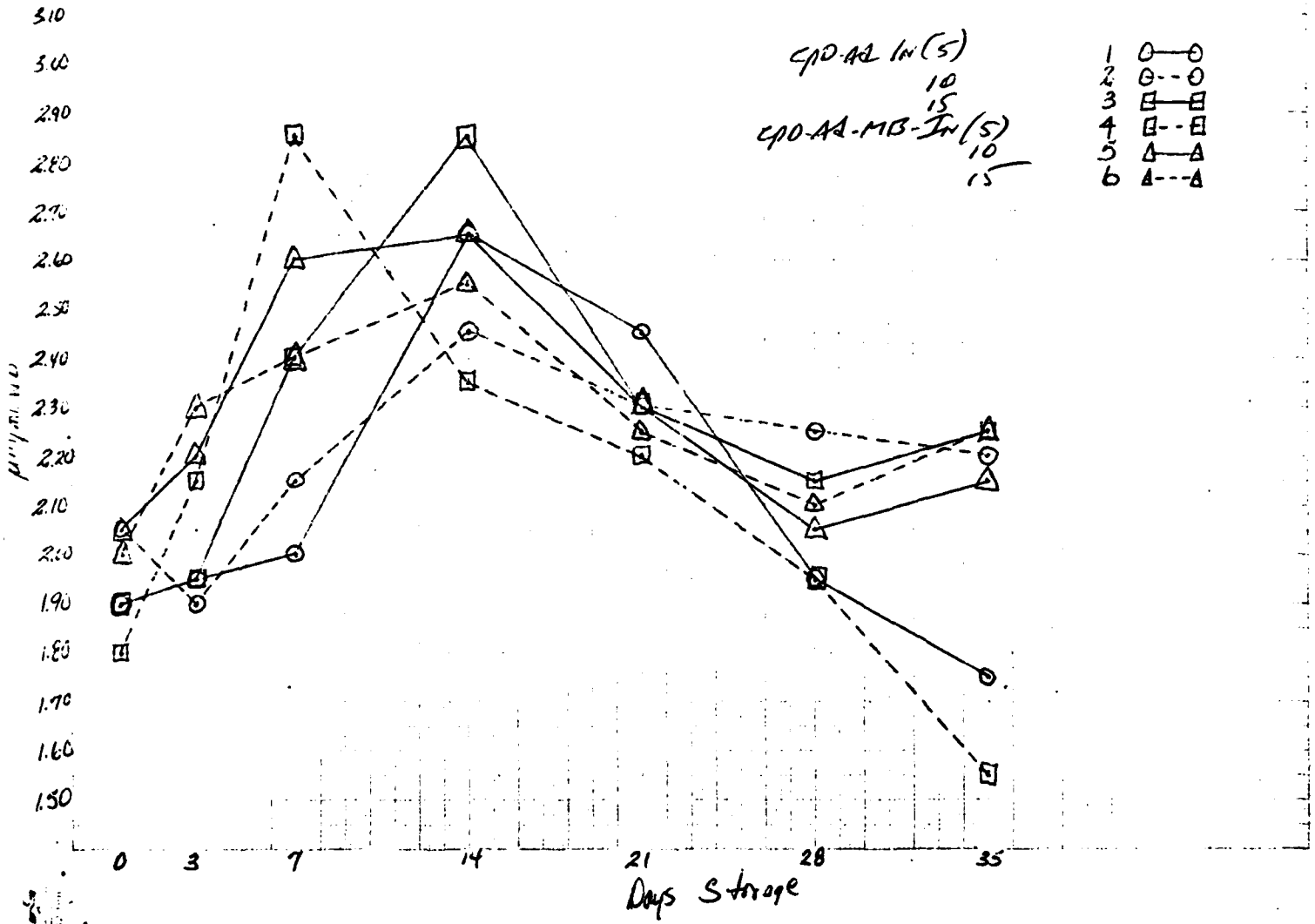
200
190
180
170
160
150
140
130
120
110
100
90
80
70
60
50
30



HTP. C.P.U. Adenine (1.25 mM)

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2.3 NPG - M-1 (Proof of Methylene Blue)



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