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DIMENSIONAL, OSMOTIC, AND CHEMICAL CHANGES OF ERYTHROCYTES IN STORED BLOOD. III. COMPARISON OF 3 DILUTIONS OF ACID CITRATE-GLUCOSE SOLUTION (ACD)

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Research Article

DIMENSIONAL, OSMOTIC, AND CHEMICAL CHANGES OF ERYTHROCYTES IN STORED BLOOD. III. COMPARISON OF 3 DILUTIONS OF ACID CITRATE-GLUCOSE SOLUTION (ACD) [1]

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Previous studies have indicated that acid glucose-citrate solutions provide the best preservation available at the present time (1). The mixture proposed by Loutit and Mollison (2, 3), conventionally called ACD–I in the United States, has been most extensively studied and appears unsurpassed in its preservative qualities. It is added in amounts of 25 ml. per 100 ml. of blood. The study to be reported was undertaken with the aim of defining the minimal amount of acid citrate-glucose diluent compatible with optimal preservation of blood. With this in mind a comparison was instituted among 3 acid citrate-glucose solutions so adjusted as to produce the same degree of acidity and the same citrate concentration as is achieved with ACD mixture, even though they were added in amounts of 15, 20, and 25 ml. per 100 ml. of blood (originally designated ACD–G, H, K). On the basis of previous data which indicated that only about 0.2 gram of glucose were glycolyzed by 100 ml. of blood during the usual storage periods, the glucose content of these solutions was reduced to ½ of that in ACD–I. Since the anticipated differences between the preservatives were small compared with the variability between individual bloods, the experiment was conducted with special attention to randomization of the variables concerned. By its arrangement this study afforded an opportunity to assess the variability among different bloods treated in the same way and to compare it with the variability among several samples of one blood treated in a different way.

EXPERIMENTAL

From each of 6 healthy male volunteers samples of blood were drawn and collected aseptically in 3 different acid citrate-glucose solutions. The samples were then distributed into vials and stored in a previously described manner (1). The 18 blood samples available were tested over a period of 22 days (from the twenty-second through the forty-third day of storage), each blood sample being examined twice during the period.

The analytical methods used have been described previously, except for the procedure for estimating glycolyzing power of blood, which was modified with a view toward simplifying the measurement by determining glucose disappearance instead of lactic acid accumulation and rendering it more sensitive by increasing the pH of the blood sample during incubation.

The solutions used, the composition of which is listed in Table I, were made up on the basis of the following considerations: The concentration of glucose was adjusted so as to give a final constant increment of 0.3 gram per 100 ml. of blood mixed with the preservative solution. The concentration of sodium citrate was calculated in a similar way with the design of keeping the final concentration of citrate ion constant. In order to obtain the same pH value of about 7.0 in all bloods, the concentration of citric acid was varied so as to make the amount added to 100 ml. of blood constant. This adjustment was based on the consideration that the amount of citric acid required to bring a blood to a desired pH value is dependent on the buffering properties of blood, principally of its erythrocytes, but is unrelated to the final volume of dilution.

TABLE I

Composition of preservative solutions

Mixture	Na citrate*	Citric acid*	Glucose*	pH	Volume added to 100 ml. of blood
	grams per 100 ml.	*grams per 100 ml.*	*grams per 100 ml.*		*ml.*
ACD-15 (ACD-G)	1.98	0.78	2.30	4.90	15
ACD-20 (ACD-H)	1.58	0.59	1.80	4.96	20
ACD-25 (ACD-K)	1.33	0.47	1.50	5.03	25

* Tri-sodium citrate $\cdot 2H_2O$, citric acid $\cdot H_2O$, glucose anhydrous.

[1] The work described in this paper was done under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and the Children's Hospital of Cincinnati.

TABLE II

Osmolarity of preservative solutions and resulting changes in the osmotic and electrolyte concentration of blood

Solution	ACD–15	ACD–20	ACD–25
Sodium citrate, *mM. per liter*	67	54	45
Sodium citrate, *m. osm. per liter*	270	215	180
Citric acid, *m. osm. per liter*	37	28	22
Glucose, *m. osm. per liter*	128	100	83
Total osmolarity of solution, *m. osm. per liter*	435	343	285
Total osmolarity of solution, *per cent of initial blood tonicity*	140	111	92
Effective tonicity,* *m. osm. per liter*	307	243	203
Effective tonicity, *per cent of initial blood tonicity*	99	78	66
Osmolarity of blood after mixing,** *m. osm. per liter*	330	317	304
Osmolarity of blood after mixing, *per cent of initial*	106	102	98
Electrolyte concentration of blood after mixing, *per cent of initial*	100	96	92

* Effective tonicity is designated the osmotic concentration of substances which permeate the cell membrane very slowly, if at all. In this instance its value was calculated on the assumption that the electrolytes, but not glucose, conform to this condition.

** Calculated on the basis of an initial concentration of 310 milliosmols per liter of water in blood, and of a water content of 0.80 gram per gram of blood.

TABLE III

Corpuscular volume and hemoglobin concentration of erythrocytes stored in 3 dilutions of ACD solution

Preservative solution	Corpuscular volume		Corpuscular hemoglobin	
	μ	*per cent of "normal"**	*grams per 100 ml.*	*per cent of "normal"**
Heparin	89.0±1.4		34.0±0.4	
ACD–15	95.3±0.4	107	31.8±0.4	94
ACD–20	97.9±0.8	109	31.3±0.4	92
ACD–25	99.5±0.8	112	30.6±0.2	90

* The values in heparinized blood samples have been designated as "normal."

The osmotic characteristics of the solutions employed are given in Table II. In Table III are summarized data on the initial corpuscular volume and hemoglobin concentration of erythrocytes in the 3 preservatives. The results are in good agreement with the hypothesis previously discussed (1).

During storage the corpuscular volumes remained unchanged at their respective values, despite increasing acidification of the blood, a finding which, if interpreted in a manner similar to that used previously, indicates escape of cations from the red cell in amounts proportional to the degree of ionization of hemoglobin as a cation.

RESULTS

From an inspection of the data in Figures 1 to 4 it is evident that the 3 dilutions of ACD solution differed in no respect from one another. Moreover there was no significant difference in any of the indices between the present series of experiments and those carried out previously with an acid citrate-glucose solution of double content of glucose (ACD–I).

The data on the regression lines of the individuals indicate that the differences between them were many-fold greater than the differences encountered between different samples of blood of 1 individual. Such data signify clearly, on the one hand, the advantages of comparing preservatives on one blood, and, on the other hand, the variability that may be expected when bloods of different individuals are compared. With *in vitro* testing the variability arises from differences among donors alone; it may be surmised that even greater differences will be found by chance alone, when *in vivo* tests are carried out under conditions where the cumulative variations of donor and recipient exert their influence.

DISCUSSION

The data presented clearly indicate that ACD–15 (ACD–G) solution is in no way inferior to the ACD solution originally described. In addition, the new solution has the advantage of entailing less dilution of blood, with the result that the final plasma protein concentration, calculated on the assumption of an initial concentration of 7 grams per 100 ml., is about 5.8 grams per 100 ml., that is, essentially the same as that in plasma of citrated blood. The explanation for the equality of the plasma protein concentrations in the 2 media lies in differences in the volume assumed by the erythrocytes. While the cells in citrate shrink by about 4 per cent, those in ACD–15 solution swell by about 7 per cent. The water shifts in opposite direction tend to cancel the existing difference of 5 per cent in the dilution of blood. A second advantage of ACD–15 solution as compared with the old acid citrate-glucose mixture is the reduced amount of glucose, which should lessen the difficulties in the preparation of dried plasma.

SUMMARY

In a comparison of 3 dilutions of ACD solution it was found that the extent of dilution of blood may be reduced to 15 ml. per 100 ml. without affecting the preservation of red cells; also, that the amount of glucose may be reduced to ½ of that in

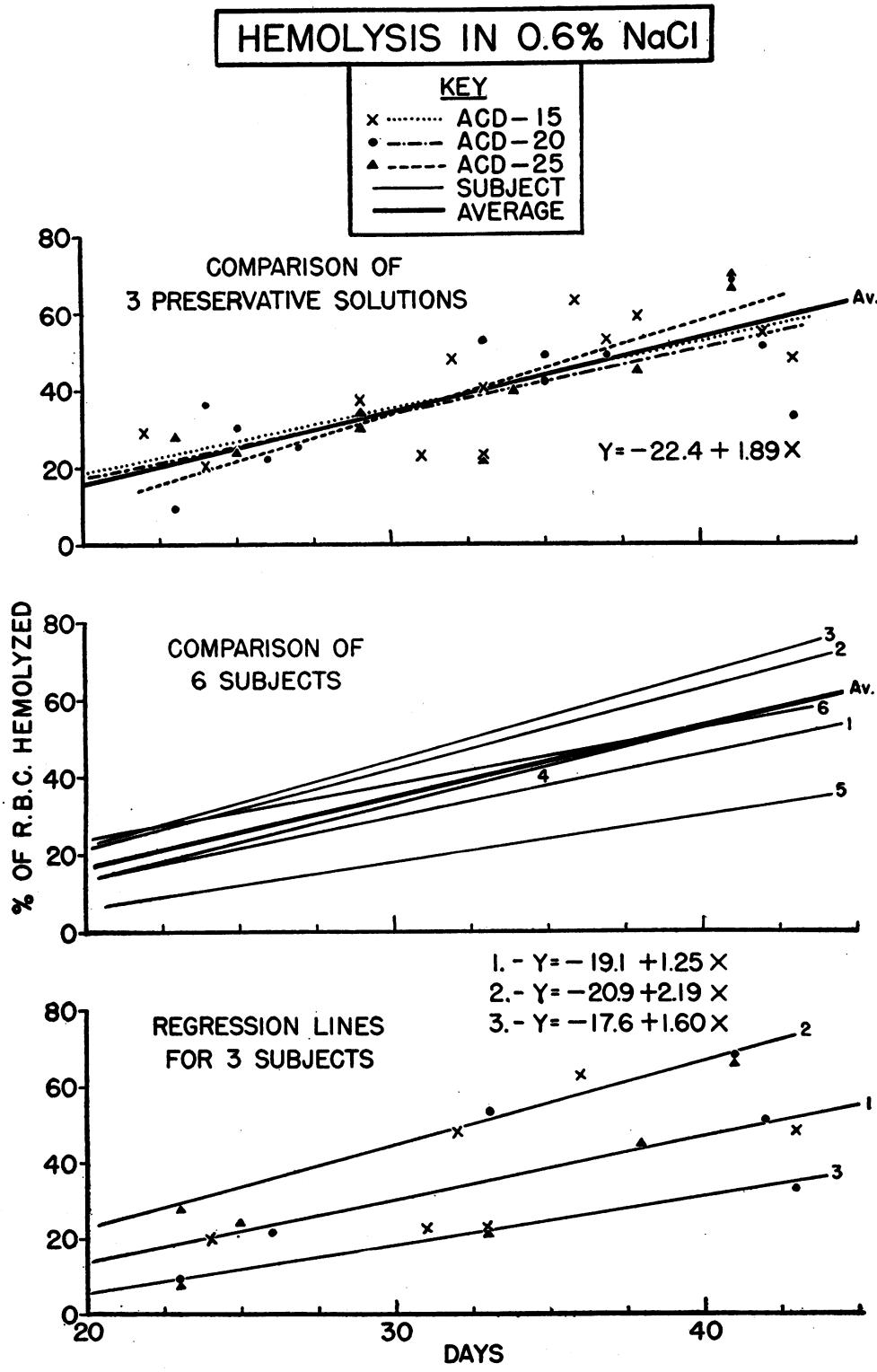

FIG. 1. A COMPARISON OF 3 DILUTIONS OF ACD–I SOLUTION WITH RESPECT TO HEMOLYSIS IN 0.6 PER CENT NaCl

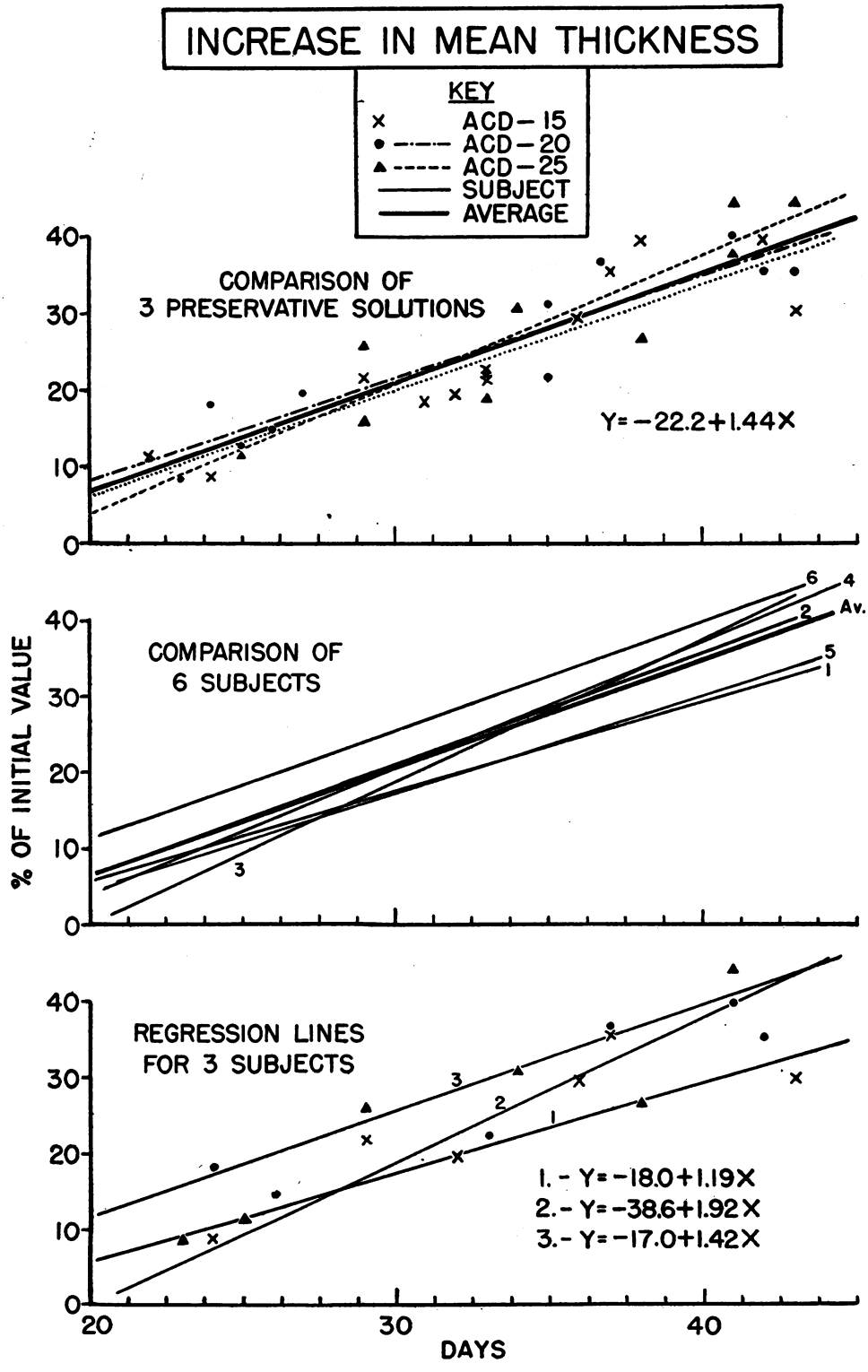

FIG. 2. A COMPARISON OF 3 DILUTIONS OF ACD–I SOLUTION WITH RESPECT TO INCREASE IN MEAN THICKNESS OF CELLS

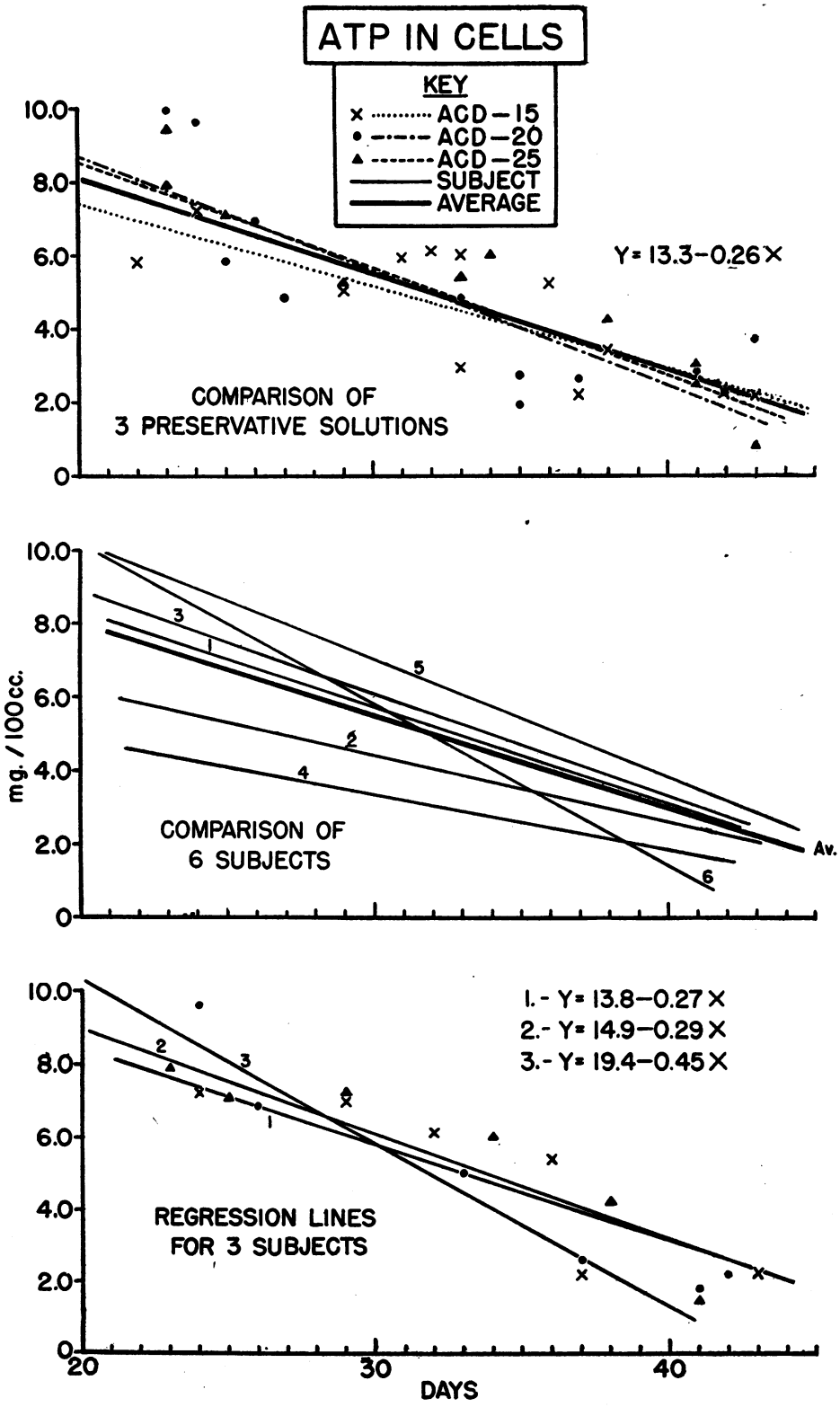

FIG. 3. A COMPARISON OF 3 DILUTIONS OF ACD-I SOLUTION WITH RESPECT TO ADENOSINE TRIPHOSPHATE CONTENT OF CELLS

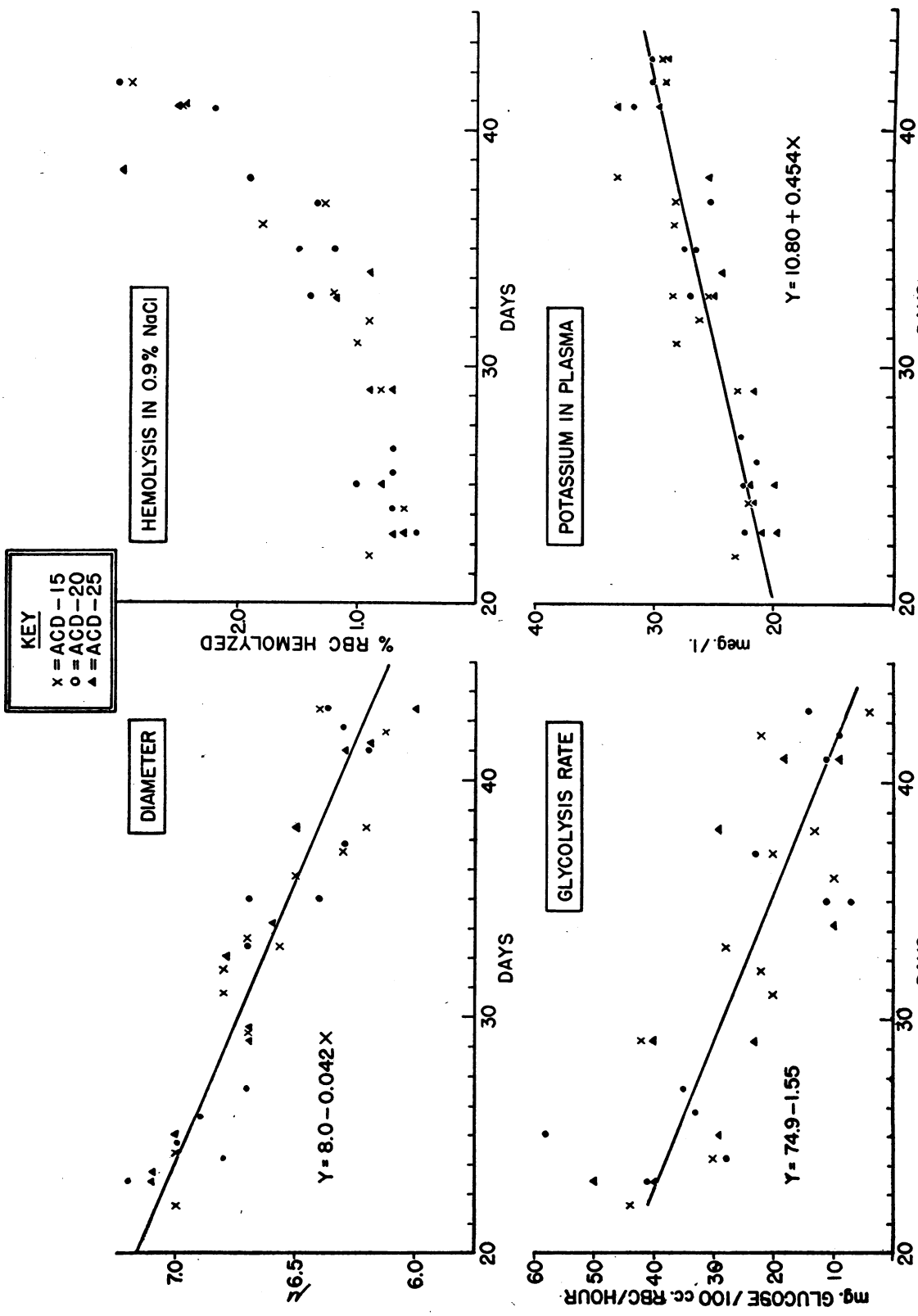

FIG. 4. CHANGES WITH TIME IN (1) DIAMETER, (2) HEMOLYSIS IN 0.9 PER CENT NACL SOLUTION, (3) GLYCOLYSIS RATE OF RED CELLS, AND (4) POTASSIUM IN PLASMA OF BLOOD IN 3 DILUTIONS OF ACD SOLUTION

the original ACD-solution. A greater variability among bloods of different individuals as compared with different blood samples of one individual has been demonstrated.

BIBLIOGRAPHY

1. Rapoport, S., Dimensional, osmotic and chemical changes of erythrocytes in stored blood. I. Blood preserved in sodium citrate, neutral, and acid citrate-glucose (ACD) mixtures. J. Clin. Invest., 1947, **26**, 591.
2. Loutit, J. F., Mollison, P. L., and Young, I. M., Citric acid-sodium citrate-glucose mixtures for blood storage. Quart. J. Exper. Physiol., 1943, **32**, 183.
3. Loutit, J. F., and Mollison, P. L., Advantages of a disodium-citrate-glucose mixture as a blood preservative. Brit. M. J., 1943, **2**, 744.