on the intestinal tract. Obviously, there may be other mechanisms by which vitamin A influences the utilization of carotenoids.

Summary. Forms of vitamin A having alcoholic, aldehydic, or acidic terminal functional groups, when fed at 100,000 IU of vitamin A/kg of diet, depressed carotenoid utilization. The retinoic acid caused the decrease in the carotenoid content of serum, liver, and skin to a greater extent than other forms of vitamin A. In an experiment designed to study the effect of vitamin A in combination with thyroxine or thiouracil on the utilization of carotenoids, a similarity of their effects between high vitamin A and thiouracil was evident.

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Zinc Content of Human Platelets (32993)

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Comparison of the zinc concentration in 16 samples of plasma and serum obtained at the same venipuncture in 14 normal individuals revealed an increase of 16% zinc in serum after clotting (1). The source of this greater zinc content could be from one of the cellular elements which are destroyed during the coagulation process. While zinc in erythrocytes and in leukocytes has been determined (2–6), the presence of zinc in platelets has not been demonstrated (7). Since platelets are largely disintegrated in the process of clotting (8), zinc would be released if it were present. Other factors might include dilution (more volume of plasma than of serum per aliquot of whole blood), and hemolysis. The purposes of these experiments were to explain the difference in the zinc levels between plasma and serum, and to determine whether zinc is present in platelets in a significant amount.

Material and Methods. Nine healthy males aged 25–62 and one female with thrombocytosis each supplied 100 ml of venous blood. This was drawn using a 19-gauge needle[2], through polyethylene tubing into a Fenwal no. 1682[3] polyethylene blood pack unit containing 15 ml of acid citrate dextrose (ACD) as the anticoagulant. Constant, gentle mixing was required. The ACD solution was shown not to be contaminated by zinc. This sample was used for isolating platelets. All samples were collected between 9 and 10 a.m. Preparation of zinc-free glass and plastic ware has been previously described (1).

The 100-ml blood sample was transferred from the plastic donor bag to a 250-ml centrifuge tube and allowed to stand for 1 hour at 4°C prior to differential centrifugation for

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platelet isolation. Slow centrifugation[4] at 800 rpm (175*g*) for 10 min resulted in sedimentation of red and white cells leaving a platelet-rich plasma supernatant. Centrifugation of the pooled plasma was repeated twice to remove any remaining cells, decanting after each centrifugation. The plasma was then centrifuged at 3000 rpm (650*g*) for 15 min. This concentrated the platelets into a button which was washed 3 times in 0.9% saline and suspended in 1.0 ml. Examination of smears by phase microscopy after each centrifugation showed that there were no red or white cells in the final platelet yield. Because of the necessity for purity the yield was low.

The platelet count in both whole blood and the final saline concentrate was performed manually with a phase contrast hemocytometer, using 0.1% ammonium oxalate as diluent, counting 2 chambers. For the platelet concentrate a dilution of 1 part platelets to 9 parts saline was employed. In the case of whole blood the result was compared with that obtained by a Coulter counter. Zinc was determined in serum, plasma, platelet concentrate, and platelet-free plasma by atomic absorption spectrophotometry[5] using a direct dilution method (1).

Plasma and serum volumes were compared in 12 subjects. Ten-ml aliquots taken at the same time from the same donor were added to two graduated test tubes (one containing 0.1 ml of 30% zinc-free sodium citrate as the anticoagulant) and allowed to stand 45 min at room temperature. Both aliquots were then centrifuged at 3000 rpm (1290*g*) for 30 min. The supernatant volume was read from the calibrated scale. The plasma volume was corrected for the volume of anticoagulant.

Hemoglobin, the index of hemolysis, was determined in plasma and serum taken from 10 individuals at the same time by a micromethod described by Hanks *et al.* (9) for plasma. It was necessary to dilute the serum with deionized water (9 parts water, 1 part serum) prior to reading in a Beckman DU spectrophotometer. A hemolyzate containing hemoglobin was prepared from the blood of 1 of these subjects. It was obtained by hemolyzing washed red blood cells (RBC) with deionized water and centrifuging at 2500 rpm (1240*g*) to remove the stroma. The zinc content of the resulting hemolyzate was determined.

TABLE I. Comparison of Zinc Concentration of Serum and Plasma in Normal Subjects.

Subject	μg of Zn/100 ml	
	Plasma	Serum
1	86	104
2	90	92
	90	108
3	76	80
4	88	104
	118	140
5	82	94
6	102	126
7	108	146
8	108	133
9	92	96
10	116	124
11	100	125
12	106	142
13	114	135
14	72	93
Mean	97	115
Difference (%)	16	
	$p < 0.001$	

Results. The mean concentration of serum zinc for the 14 normal individuals in whom serum and plasma zinc was being compared was 115 μg/100 ml. In contrast, the concentration of plasma zinc was 97 μg/100 ml ($p < 0.001$). This represents a 16% difference (Table I).[6]

Platelets were found to contain zinc in amounts ranging from 0.20 to 0.45 μg/10^9 platelets, or 4.6–26.3 μg of platelet zinc per 100 ml of whole blood (Table II). As also shown the final concentration of platelets represented yields of 0.45–26%.

The effect of volume differences between plasma and serum on zinc concentration is

[4] Model PR-2 No. 845 head, International Equipment Co., Boston, Massachusetts.

[5] Model 303, Perkin Elmer, Norwalk, Connecticut.

[6] In addition to these normal subjects the serum and plasma zinc was compared in 44 samples from 32 patients, many of whom had alcoholic cirrhosis where plasma zinc is characteristically low. The mean plasma zinc was 76 μg/100 ml compared to 86 μg/100 ml for serum ($p<0.001$).

TABLE II. Data Regarding Zinc Content of Platelets.

Subject	Number of whole blood platelets (mm^3)	Number of platelets in final concentrate (mm^3)	Platelet yield from 100 ml whole blood (%)	Amount of zinc in 1 ml of platelet concentrate (μg/ml)	Amount of zinc in 10^9 platelets (μg)	Amount of platelet zinc in 100 ml of whole blood (μg)
1	232,000	1,900,000	8.2	0.38	0.20	4.6
2	275,000	2,210,000	8.0	0.66	0.30	8.3
3	231,000	2,670,000	11.6	0.87	0.33	7.6
4	242,000	2,680,000	11.1	1.22	0.45	10.9
5	257,000	280,000	1.1	0.09	0.32	8.2
6	174,000	1,190,000	6.8	0.39	0.33	5.7
7	236,000	6,220,000	26.4	1.24	0.20	4.7
8	255,000	1,820,000	7.1	0.38	0.21	5.4
9	238,000	1,520,000	6.4	0.53	0.35	8.3
10[a]	822,000[a]	370,000[a]	0.45[a]	0.12[a]	0.32[a]	26.3[a]
Mean	238,000	2,277,000	9.6		0.30	7.1

[a] Female with thrombocytosis; not included in the mean.

noted in Table III. The plasma volume averaged 6.2% higher than serum ($p < 0.001$). The amount of hemoglobin found in serum was almost tenfold greater than that found in plasma from 10 subjects (Table IV). The mean value for serum was 21.4 mg/100 ml and that for plasma was 2.24 mg/100 ml. There was 32.7μg of zinc present in an aliquot of RBC hemolyzate containing 1 gm of hemoglobin determined on 1 subject.

Discussion. Many reports on zinc in blood use the terms serum and plasma interchangeably. In 1951, Vikbladh stated that "serum and plasma have the same zinc content" (10). However, until recently methods were not sufficiently sensitive. With the advent of more sophisticated methods such as atomic absorption spectrophotometry the significantly higher value for serum became evident.

Theoretically some of the zinc in serum could come from hemoglobin derived from hemolyzed red blood cells. Shinowara (11) reported that serum, allowed to clot for 30 min averaged nearly 15 mg/100 ml of hemoglobin. Hanks *et al.* (9) using 25 normal subjects found 0.32 mg/100 ml of hemoglobin in plasma. In our laboratory hemoglobin levels determined on serum and plasma from 10 individuals averaged 21.4 mg/100 ml for serum and 2.24 mg/100 ml for plasma (Table

TABLE III. Volume Difference between Serum and Plasma from 10 ml Whole Blood.

Subject no.	Volume serum (ml)	Volume plasma (ml)	Difference (%)
1	4.7	4.9	4.1
2	4.5	4.9	8.9
3	4.6	5.1	9.8
4	4.4	4.9	10.2
5	4.5	5.0	10.0
6	4.6	4.9	6.1
7	4.5	4.6	2.2
8	4.7	4.8	2.1
9	5.0	5.4	7.4
10	5.2	5.4	3.7
11	4.8	5.2	7.7
12	4.7	4.8	2.1
Mean	4.7	5.0	6.2% $p < 0.001$

TABLE IV. Comparison of Zinc in Serum and Plasma Due to Hemolysis.[a]

	Serum	Plasma
Hemoglobin (mg/100 ml)[b]	21.4 ± 9.37	2.24 ± 0.74
Hemolyzate zinc (μg/100 ml)[c]	0.70 ± 0.10	0.07 ± 0.01

[a] Hemoglobin was used as the index of hemolysis.
[b] Mean ± SD of 10 normal individuals.
[c] Values based on 32.7 μg of zinc per aliquot of hemolyzate containing 1 gm of hemoglobin (see text).

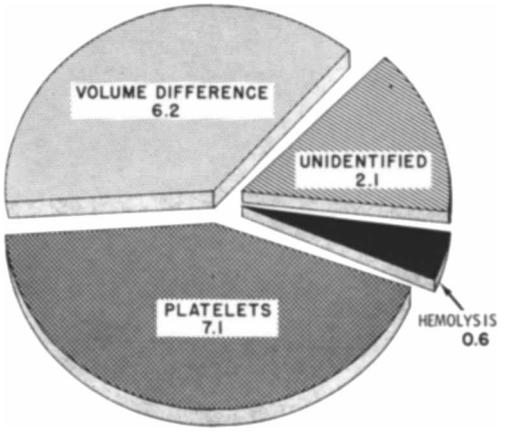

FIG. 1. Factors contributing to the 16% higher zinc concentration in serum when compared to plasma.

IV). In addition 32.7 μg of zinc were found in a single sample of RBC hemolyzate containing 1 gm of hemoglobin. This value is in close agreement with 37.4 μg of zinc/gm of "hemoglobin" reported by Talbot and Ross (5) in 21 normal individuals. Using our value, 32.7 μg of zinc per aliquot of RBC hemolyzate containing 1 gm of hemoglobin, the zinc contributed to serum by hemolysis (Table IV) could be estimated at no more than 0.70 μg/100 ml and thus could not contribute significantly to the 16% higher zinc concentration in serum when compared to plasma (Table I).

A review of the literature did not disclose any studies showing the presence of zinc in platelets. Maupin looked for zinc in platelets by emission spectrography and reported it "absent or negligible in amount" (12,13). Others have referred to this study to support the assumption that there is no zinc in platelets (7).

Although it is generally accepted that platelets disintegrate during clotting (8), little is known regarding the exact percentage. Our observations on a single serum sample revealed approximately 25,000 platelets/mm^3. This subject had 236,000 platelets/mm^3 of whole blood. Thus in this case 9.4% of the platelets survived coagulation after 1 hour.

Of the enzymes which have been reported to be present in platelets several are zinc metalloenzymes (14–19). These include lactic, glutamic, and malic dehydrogenases, protease, aldolase, and carbonic anhydrase. Thus, it is not surprising that this study shows zinc to be present in platelets.

One of the volunteers had a platelet dyscrasia with an unusually high platelet count, bleeding tendency, and familial polycythemia. Nevertheless, the amount of zinc per unit of platelets was similar to that of normal donors (Table II). As also shown, the platelet yield was extremely low for this individual demonstrating that the differential centrifugation method employed isolated platelets of a specific density.

In reference to Tables I–IV it should be noted that the same subjects were not used for each procedure, although there was considerable overlap. All were normal laboratory personnel.

Figure 1 summarizes the conclusions reached in these observations. Of the 16% difference between serum and plasma zinc, 6.2% (or 39% of the total difference), was due to a volume difference, 7.1% (or 44% of the total difference) being zinc contained in platelets, 0.6% (or 4.0% of the total difference) is derived from hemolysis and 2.1% (or 13% of the total difference) is unidentified.

Summary. The zinc content of serum was consistently higher than the zinc concentration in plasma by an average of 16%. Because platelets are largely disintegrated in the clotting process, zinc was determined in platelets concentrated from 10 individuals and was present in a significant amount. It was demonstrated that 39% of the increased concentration of zinc in serum can be attributed to a slightly greater dilution in plasma, 44% being derived from platelets and 4.0% from hemolysis. This is the first known report demonstrating zinc in platelets.

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Postnatal Changes in the Pituitary–Adrenal Axis of the Rat*† (32994)

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Contrary to the findings of Jailer (1) and Schapiro *et al.* (2), recent data from both this laboratory (3–6) and Levine's laboratory (7–8) indicate that the adrenal gland of the neonatal rat responds to certain stressors. The degree of response decreases at around day 7 of age and thereafter gradually increases to the adult pattern. A similar "biphasic" pattern for the growth of the adrenal gland was reported in the rat more than 50 years ago when the adrenal weight data were expressed as a function of body weight (9).

In addition, certain correlations have been found to exist between "early handling" of the neonate and adrenocortical function in the young adult rat (6, 10). Although a causal relationship between the two events has yet to be established, it is assumed that such may exist.

The present study is designed to examine the development of adrenocortical activity in the neonatal rat and to determine whether the decrease in adrenal activity at 7 days of age in the rat is due to changes in the adrenal gland, the pituitary, or both.

Materials and Methods. All rats used in these experiments were of the Purdue-Wistar stain. The litter size was reduced to 6 on the morning the litter was found and the pups were considered to be 1-day old at this time. The lighting schedule consisted of 13 hours light (7 a.m. to 8 p.m.) and 11 hours dark. The rats were born and housed in special rooms and no one except the experimenter was allowed to enter on the day of the experiment. Food and water were supplied *ad libitum*. All rats were killed at 10:30 a.m. Wherever possible, a split-litter technique was used so that control and experimental values were obtained from the same litter.

Results. Expt. 1: Compensatory Hypertrophy. In this experiment animals were used at 2, 5, 7, 10, 15, and 30 days of age. Two rats were unilaterally adrenalectomized (right adrenal gland removed) and 2 others were sham operated. Both groups were killed 72 hours later and the left adrenal removed and weighed to the nearest 0.2 mg. Two control rats were killed at the time of the operation

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