in vivo, participating in formation of the antibody as a template. In other words, the reaction between the degraded protein and the absorbed antiserums might be a kind of cross reaction. These might be the reasons why the reaction was weak and took a long time to appear. The positive PCA reactions with Fraction 1, but not with either Fraction 2 or intact BSA in the guinea pigs sensitized with the absorbed antiserums, also suggest the presence of antibody against fragments of antigen.

The presence of antibody, directed toward the "hidden sites," in the antiserums does not parallel the concentration of anti-BSA (Table I). The antiserums prepared by immunization with soluble antigen reacted with Fraction 1, whereas the antiserums prepared with either the alum-precipitated antigen, or the antigen mixed with Freund's adjuvant did not, indicating that the presence of the antibody relates to the properties of the antigen injected, and/or the schedule of immunization. These findings suggested to us that the rate of destruction of antigen *in vivo* might be delayed by addition of the adjuvants, and that production of antibody against the internal structure of the antigen might have some relation to the process of antigen destruction.

Summary. 1. Positive ring tests and PCA reactions were observed between pepsin-degraded BSA and some of the BSA absorbed anti-BSA rabbit serums, suggesting the presence of the antibody against antigenic determinants which are not on the surface of the native protein molecule. 2. The presence of the antibody to the "hidden sites" did not parallel the concentration of anti-BSA in the serum but rather seemed to be related to the state of the antigen used in the immunization, and to the immunization schedule.

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Early Response of Plasma Volume, Red Cell Mass and Plasma Proteins To Massive Hemorrhage.* (25395)

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The response of blood volume and plasma proteins to massive hemorrhage in the rat has been reported(1). Measurements were made at 12, 24, 48 and 72 hours after bleeding. After hemorrhage of about 50% of measured blood volume, plasma volume was replenished within the first 12 hours. Arterial hematocrit was lowest at 12 hours, and although rising at 72 hours after hemorrhage, had returned to only 67% of control level by that time. Plasma protein concentration was fully restored by 48 hours, and was already 88.5% of control value at 12 hours, which was the earliest post-hemorrhagic measurement made. Since it was apparent that major plasma volume and protein concentration changes occurred prior to 12 hours, the experiments have been repeated with measurements of circulating volumes and plasma proteins at 2, 4, and 8 hours after hemorrhage. Such measurements were not made in the earlier study for 2 reasons. First, the purpose of that study was

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to find a relatively stable experimental preparation to evaluate tolerance to trauma after initial massive bleeding. In pilot studies after massive hemorrhage, maximum hemodilution occurred at 12 hours, and it was believed that 12 hours was suitable for earliest determinations of the measured parameters. Secondly, there was no assurance that radiochromium tagged cells would be suitably mixed for reliable volume studies in animals so recently in hemorrhagic shock. Our purpose here is 2-fold: 1) to report results of mixing time experiments to establish the validity of radiochromium tagged red cell dilution technic in animals in hypovolemic shock and 2) to report results of measurements of plasma volume, red cell mass and plasma proteins 2, 4, and 8 hours after massive, controlled hemorrhage.

Methods. Exp. I. To determine whether chromium 51 tagged red cell mixing was complete in animals in shock, so that measurements of red cell mass in such animals could be considered valid, the following experiment was performed. Rats were subjected to bilateral hind limb tourniquet application(1). Duration of tourniquet application was 4 hours, which produces a 75% mortality by 48 hours after tourniquet release. Rate of fluid accumulation in the hind limbs after tourniquet release is greater during the first 2 hours with a diminishing rate between that time and 4 hours. Three hours after tourniquet release, when animals were in profound hypovolemic shock, tagged red cells were introduced into the circulation and various mixing times were allowed before sampling for red cell mass determinations. The mean red cell masses in groups of animals so treated were compared with the mean red cell mass of a normal control group. *Exp. II.* Details of methods for determination of red cell mass, plasma volume and plasma proteins have been described(1). Male rats of Holtzman Farms weighing 240 to 320 g were used. Light ether anesthesia was used throughout. Controlled hemorhage was performed for 3 to 5 minutes after cannulation of the femoral artery with a polyethylene catheter (PE #10) attached to a Luer-Lok adapter and fitted to 10 ml syringe. In previous experiments the syringe contained approximately 0.1 ml of sodium heparin (10 mg/cc), injected into the artery prior to blood withdrawal. It was observed in those experiments that animals continued to ooze from the site of arterial cannulation after blood withdrawal until volume determinations were made. Since this introduced a slightly variable hemorrhage rather than a completely controlled one, bleeding procedure was modified. The syringes were merely wetted with heparin and any excess heparin was expressed through the cannula prior to cannulation. At end of bleeding period, the syringe was disconnected from the adapter and about 0.1 ml of protamin sulfate (1% solution) was injected intraarterially *via* the cannula. The artery was then ligated and the incision closed with skin clips. The wounds were inspected and were bloodless at time of volume measurements (2, 4, and 8 hours after hemorrhage). The controlled hemorrhage was 2.5 ml/100 g of body weight. Body weight, hematocrit, plasma protein concentration and red cell mass were determined in non-hemorrhaged control group and at 2, 4, and 8 hours after hemorrhage in experimental groups (one group of animals at each time). Blood volume, plasma volume and grams of circulating protein/100 g of body weight were then calculated for each determination.

Results. Exp. I. Reliability of the chromium 51 red cell tagged dilution method for measuring red cell mass in shocked animals is demonstrated by the fact that no difference is evident in the red cell mass as measured in tourniquet shocked animals when 15, 30 and 60 min are allowed for mixing of tagged cells, or between these values and those of control animals (Table I). The hematocrit of shocked animals is greatly elevated over hematocrit readings of non-shocked animals due to plasma loss in the former.

Exp. II. Plasma volume continued to rise following hemorrhage throughout period of observation (Table II). Significantly, it was 93% of control value at 2 hours, 101% of control at 4 hours and 108% at 8 hours after hemorrhage. Concomitant with this finding, the hematocrit reached its lowest value at 8

TABLE I. Measurements of Red Cell Mass in Tourniquet Shocked Rats.

Treatment	Wt	Hematocrit	Red cell mass	p†
Control (6)	216.3 ± 9.94*	46.5 ± 1.05*	2.57 ± .13*	
15 min. mixing (8)	206.2 ± 11.12	66.8 ± 2.28	2.60 ± .13	$p = .20$
30 " " (7)	193.3 ± 9.82	64.4 ± 1.93	2.51 ± .15	$.02 < p < .05$
60 " " (5)	197.9 ± 7.49	66.6 ± 1.34	2.56 ± .09	$.50 < p < .70$

* Stand. dev. † Comparison of means by Student "t" analysis. ( ) = No. of animals.

hours. Red cell mass showed no tendency toward replacement during period of observation.

Plasma protein concentration rose from its lowest level at 2 hours to reach 87% of control level at 8 hours. In spite of super-normal plasma volume at 8 hours total blood volume remained considerably below control value (81%) due to reduced red cell mass.

The calculated amount of plasma proteins/100 g of body weight, although less than the control value throughout the observation period, showed a considerable increase during the first 2 hours and in the last 4 hours of the experiment. Since approximately 50% of plasma protein was removed with the hemorrhage, the 73% of control value at 2 hours represents a sizeable inflow of protein as does the 92% of control value at 8 hours (as compared with the 73% at 4 hours).

Discussion. The present series of measurements at 2, 4 and 8 hours post-hemorrhage fits very well with previously recorded 12, 24, 48, and 72 hour series(1). From the earlier investigation it was not possible precisely to identify the time of greatest plasma dilution or lowest hematocrit level. This occurred between 8 and 12 hours. Value for the hematocrit at 8 hours was 64% of control and that of the 12 hour group 58% of control. The 24 hour hematocrit in the earlier study showed a rising value. Plasma volume is seen to have reached its maximum value by 8 hours and then remained stationary. The low point of hematocrit, therefore, coincides with maximum plasma volume and is uncomplicated by changes in red cell mass since there is little evidence of increase in red cell mass until 24 to 48 hours after hemorrhage.

There is no evidence for or against the possibility that sequestered red cells, not measured by the chromium dilution, are introduced into the circulation during hemorrhage or immediately thereafter. If such an event took place and was reversed sometime after hemorrhage (*i.e.*, when circulating volume was again adequate), a falling red cell mass might be evidenced in measurement sometime after the hemorrhage. The slightly lower red cell mass observed at 4 and 8 hours as compared with the 2 hour observation could be construed to support such a series of events. The theoreti-

TABLE II. Volume, Hematocrit and Plasma Protein Values in the Rat before and at 2, 4, and 8 Hours after Rapid Withdrawal of 2.5 ml Whole Blood per 100 g Body Weight.

Measured values				Calculated values		
Initial body wt, g	Hematocrit	Red cell mass, ml/100 g	Plasma proteins, g/100 ml	Plasma proteins, g/100 g	Total blood vol, ml/100 g	Plasma vol, ml/100 g
Control animals (N = 7)						
274.6 ± 17.3	48.3 ± 1.11 (100%)	2.52 ± .15 (100%)	6.34 ± .346 (100%)	.172 ± .008 (100%)	5.22 ± .21 (100%)	2.70 ± .15 (100%)
2 hr post hemorrhage (N = 6)						
279 ± 19.4	34.7 ± 2.42 (71.8%)	1.35 ± .07 (53.6%)	4.87 ± .15 (76.8%)	.126 ± .009 (73.3%)	3.90 ± .16 (74.7%)	2.55 ± .14 (94.4%)
4 hr post hemorrhage (N = 8)						
277.0 ± 12.8	32.0 ± 1.93 (66.3%)	1.30 ± .07 (51.6%)	4.59 ± .22 (72.4%)	.126 ± .016 (73.3%)	4.07 ± .39 (78.0%)	2.77 ± .22 (102.6%)
8 hr post hemorrhage (N = 9)						
280.1 ± 18.5	31.1 ± 2.50 (64.3%)	1.31 ± .10 (52.0%)	5.51 ± .22 (86.9%)	.158 ± .009 (91.9%)	4.22 ± .12 (80.8%)	2.91 ± .52 (107.8%)

cal remaining red cell mass following hemorrhage should have been 1.35 ml/100 g of body weight. This was the exact value found at 2 hours after hemorrhage. But it was 1.30 and 1.31 ml/100 g of body weight at 4 and 8 hours respectively after hemorrhage.

Plasma protein concentration forms a continuous function through the 2 series. In this series the plasma protein concentration was rising, reaching 87% of control value at 8 hours. Previously, it was 88.5% of control at 12 hours, 95% at 24 hours and equal to control value at 48 hours post-hemorrhage.

Total calculated protein content at 12 hours was 96% of control value. In the present study it was about 92% of control at 8 hours. The pattern of protein replenishment seems to have an early and late component. For example, the theoretical protein content after hemorrhage should have been 92 mg/100 g of body weight (control was 174 mg/100 g of body weight). At 2 hours post-hemorrhage it was 124 mg/100 g, a net gain of 32 mg/100 g or a 20% higher value than expected if no protein replacement had occurred. During the next 2 hours there was no change in total protein content, in spite of plasma volume gain. At 8 hours, however, the protein content had risen to 160 mg/100 g: an inflow of about 40 mg/100 g and a value 22% above that at 4 hours. This would seem to indicate a 2-phase plasma protein replacement, an immediate and a delayed one. The immediate plasma protein replacement is insufficient to restore concentration, while the slower process not only restores but exceeds the control level in terms of absolute amounts of plasma protein. The concentration does not, however, exceed the control value, suggesting a fine regulatory mechanism.

Summary. 1) The chromium 51 red cell tagged dilution method is valid for measuring red cell mass in the presence of hypovolemic shock as no difference was found in red cell mass measured in tourniquet shocked animals when 15, 30 and 60 minutes were allowed for mixing of tagged cells, or between these values and those of control animals. 2) The response of blood volume compartments and plasma proteins to acute withdrawal of 50% of circulating blood volume has been determined at 2, 4, and 8 hours after hemorrhage. Plasma volume is restored to 100% of the control within 4 hours and is 108% at 8 hours post-hemorrhage. Despite the elevated plasma volume, total blood volume remains well below the control, due to persistent red cell mass deficit. Changes in hematocrit inversely reflect plasma volume changes. Plasma protein concentration is restored to 87% of control by 8 hours. Protein replacement seems to occur in 2 stages. An immediate response is evident within the first 2 hours and a delayed response begins at 4 hours after blood withdrawal and extends over a 48 hour period.

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Response of Acutely Starved and Chronically Undernourished Rats to Saline Therapy Following Tourniquet Shock.* (25396)

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We previously demonstrated that a tourniquet trauma of sublethal magnitude in normal rats produced a mortality rate in acutely starved rats which correlated with degree of weight loss(1). Thus, there was 42% mortality in rats acutely starved to 80% of initial body weight and 100% mortality in rats acutely starved to 70% of initial body weight. A method for infusion treatment of tourniquet shock in the normal rat was later presented

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