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.

1. Pareira, M. D., Serkes, K. D., Lang, S., *Am. J. Physiol.*, 1959, v197, 786.

Received October 13, 1959. P.S.E.B.M., 1960, v103.

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

KENNETH D. SERKES, STANLEY LANG AND MORTON D. PAREIRA
Surgical Research Labs., Jewish Hospital, St. Louis, Mo.

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

* Supported by Dept. of Army, Office of Surgeon General.

(2). This method consisted of slow administration of intravenous fluid over period of greatest fluid loss from the circulating volume. This slow uniform rate of infusion allowed achievement of higher survival rates at lower infusion volumes than had previously been reported in the rat. Survival rates were reported in normal rats at different levels of fluid replacement using normal saline, human plasma, 6% dextran in normal saline and 6% dextran in glucose in water. The present study is concerned with survival after saline infusion therapy in acutely starved and chronically undernourished rats with appropriate controls subjected to tourniquet shock.

Methods. Male albino rats of Holtzman Farms were maintained in air-conditioned laboratory on Purina rat chow and water *ad lib.* until start of experiments. Under each experimental condition, animals from each group served as untreated controls. *Exp. 1. Acute starvation.* (Saline infusion of 7 ml/100 g body weight.) Three groups of 16 animals each were used. At 9 a. m. on first day of experiment, 2 groups weighing 270-310 g had food withdrawn and at noon all animals were weighed to nearest ½ g on triple beam balance. This represents initial body weight. Group I (acute starvation group) received no food but water *ad lib.* until conclusion of experiment. Starvation period was 4 days, when their average was 78% of initial body weight. Group II (large control) was returned to cages and allowed food and water *ad lib.* Both groups were weighed daily thereafter. Group III (small control) animals continued food and water *ad lib.*; on day of tourniquet trauma their average weight corresponded to average weight of acutely starved group (*ca* 221 g). This group was intended to control any difference in response to therapy due to weight alone. Approximately 16 hours prior to application of tourniquets, food but not water was removed, (Groups II and III). On morning of experiment the animals were again weighed, anesthetized and bilateral hind limb tourniquets applied for 4½ hours. This duration of tourniquet trauma has been previously shown to be lethal to at least 96% of untreated controls in the 200-250 g weight range (2). At time of tourniquet release, animals were again anesthetized and a polyethylene catheter introduced into right jugular vein for administration of normal saline solution. Infusion was begun 1 hour after release of tourniquets, and rate of infusion so timed that 7 ml of solution was administered/100 g of body weight for 3 hours ± 17 minutes. Then the vein was ligated and the neck closed with skin clips. Animals were then returned to cages where food and water were available. Survival was recorded at 24 and 48 hours after tourniquet release(2). *Exp. 2. Acute starvation.* (Saline infusion of 15 ml/100 g body weight.) The experiment was repeated using this higher level of fluid replacement on each of 2 days. The results for each day and the combined results are indicated in the Table. *Exp. 3. Chronic undernutrition.* (Saline infusion of 7 ml/100 g body weight.) Three groups of 16 animals each were again used. *Group I* started in same weight range as in acute starvation experiments (260-310 g). Over 35 days they were given submaintenance amounts of Purina Lab. rat chow daily in such restricted amounts that at 35 days the animals were at 77.4% of their initial body weight (217 g). *Group II* (large control) also started in same weight range but allowed food and water *ad lib.* and at time of tourniquet trauma weighed 363.5 g. *Group III* (small control) was again a smaller group of rats allowed food and water *ad lib.* so that at trauma their average weight was in the same range as that of chronically undernourished group (215.9 g). All groups were submitted to 4½ hour bilateral hind limb tourniquet trauma. One hour following tourniquet release they received infusion of 7 ml normal saline/100 g body weight for 3 hours. Twenty-four and 48 hour survivals were recorded for all groups. Untreated controls were selected from each group. *Exp. 4. Acute starvation compared to chronic undernutrition.* (Saline infusion of 7 ml/100 g body wt.) A group weighing 260-310 g were placed on submaintenance diet of Purina rat chow so that over 35 days they gradually lost weight to approximately 80% of initial body weight. Four days before anticipated arrival of first group at the selected weight level, another group of equal size and in the same initial weight range was weighed

TABLE I. Survival in Acutely Starved Rats and Fed Controls following 4½ Hr Bilateral Hind Limb Tourniquet Trauma and Normal Saline Infusion of 7 ml/100 g Actual Body Weight (Exp. 1).

		24 hr			48 hr		
	Avg wt (g)	Lived	Died	Survival %	Lived	Died	Survival %
Group I (acute starvation)	%						
Treated	221.2 (77.9)*	3	8	27	0	11	0
Untreated	224.5 (77.8)	0	4	0	0	4	0
Group II (large control)							
Treated	282.9	11	0	100	11	0	100
Untreated	283.8	1	3	25	0	4	0
Group III (small control)							
Treated	241.1	11	1	93	9	3	75
Untreated	235.5	0	4	0	0	4	0

* % of initial body wt.

for initial body weight and thereafter given water but no food. On day of tourniquet trauma this group also weighed approximately 80% of initial body weight. On day of tourniquet trauma both groups were submitted to bilateral hind limb tourniquet application of 4½ hours duration. Beginning 1 hour after tourniquet release all animals were infused *via* right jugular vein with 7 ml normal saline solution/100 g body weight for 3 hours. Twenty-four and 48 hours survivals were recorded for both groups. Untreated controls were included for each group. The experiment was performed on 3 different days. Results for each day and combined results are indicated in Table IV.

Results. Infusions of 7 ml/100 g body weight of saline protected normally fed animals from death produced by tourniquet shock, but not animals acutely starved (Table I). When 15 ml saline/100 g body weight was given, survivors under acute starvation increased to 75% (Table II), while all treated control animals survived (Table II). Chroni-

TABLE II. Survival in Acutely Starved Rats and Fed Controls following 4½ Hr Bilateral Hind Limb Tourniquet Trauma and Normal Saline Infusion of 15 ml/100 g (Exp. 2).

		24 hr			48 hr		
	Avg wt (g)	Lived	Died	Survival %	Lived	Died	Survival %
Group I (acute starvation)	%						
Treated Day 1	209.8 (73.7)*	5	1		4	2	
" 2	231.8 (77.9)	5	1		5	1	
Total		10	2	83	9	3	75
Untreated Day 1	205.5 (73.7)	0	2		0	2	
" 2	226.2 (77.7)	0	2		0	2	
Total		0	4	0	0	4	0
Group II (large control)							
Treated Day 1	291	6	0		6	0	
" 2	289.7	6	0		6	0	
Total		12	0	100	12	0	100
Untreated Day 1	293	1	1		1	1	
" 2	298.5	1	1		1	1	
Total		2	2	50	2	2	50
Group III (small control)							
Treated Day 1	220	6	0		6	0	
" 2	221.2	6	0		6	0	
Total		12	0	100	12	0	100
Untreated Day 1	211	0	4	0	0	4	0
" 2	221.2	0	4	0	0	4	0
Total		0	8	0	0	8	0

* % of initial body wt.

TABLE III. Survival in Chronically Undernourished Rats and Fed Controls following a 4½ Hr Bilateral Hind Limb Tourniquet Trauma and Normal Saline Infusion of 7 ml/100 g Actual Body Weight (Exp. 3).

		24 hr			48 hr		
	Avg wt (g)	Lived	Died	Survival %	Lived	Died	Survival %
Group I (chronic undernutrition)	%						
Treated	217.8 (77.4)*	12	0	100	12	0	100
Untreated	227.1 (82.8)	3	1	75	2	2	50
Group II (large control)							
Treated	363.5	12	0	100	12	0	100
Untreated	369.4	4	0	100	4	0	100
Group III (small control)							
Treated	215.9	12	0	100	11	1	93
Untreated	223.9	1	3	25	1	4	0

* % of initial body wt.

cally undernourished animals under treatment with 7 ml/100 g dose had survival rate comparable to that of treated controls (Table III). Because of previously noted day to day variation in survival rates following tourniquet shock treated by infusion therapy(2), survival rate of animals acutely starved was compared simultaneously with that of chronically undernourished animals of same weight (Table IV). Higher survival rate of the latter was significant ($p<0.001$ by chi square).

Discussion. These experiments established that the response to tourniquet trauma followed by saline infusion therapy in chronically undernourished rats is as good as in normally fed rat in the same weight range. It also demonstrates that both of these groups show considerably better survival than acutely starved rats whose weight was reduced to the same degree.

The reasons for the difference are not clear. It could be suspected that the chronically undernourished group might have a relatively greater plasma or blood volume than the acutely starved group; however, we have previously shown(3) that at this level of body weight loss, total blood volume and plasma volume are higher in acutely starved than in

TABLE IV. Survival in Acutely Starved and Chronically Undernourished Rats following a 4½ Hr Bilateral Hind Limb Tourniquet Trauma and Normal Saline Infusion of 7 ml/100 g Actual Body Weight (Exp. 4).

		24 hr			48 hr		
	Avg wt (g)	Lived	Died	Survival %	Lived	Died	Survival %
Acute starvation	%						
Treated Day 1	231.8 (78.4)*	11	1		8	4	
" 2	242.3 (79.6)	8	5		4	9	
" 3	249.9 (79.8)	9	6		7	8	
Total		28	12	70	19	21	48
Untreated Day 1	240.7 (79.5)	0	4		0	4	
" 2	256.1 (80.4)	1	7		0	8	
" 3	256.3 (81.7)	0	5		0	5	
Total		1	16	6	0	17	0
Chronic undernutrition							
Treated Day 1	234.6 (80.3)	12	0		11	1	
" 2	229.6 (81.3)	13	1		10	4	
" 3	235.2 (83.6)	16	0		14	2	
Total		41	1	98	35	7	83
Untreated Day 1	237.0 (80.8)	4	0		3	1	
" 2	236.2 (83.7)	1	7		0	8	
" 3	233.2 (79.1)	1	5		1	5	
Total		6	12	33	4	14	22

* % of initial body wt.

chronically undernourished animal. Also, values for blood volume as percent of actual body weight were higher in the acute starvation group than the chronic undernutrition group. Serum protein concentration was essentially the same in both groups.

The unexpected survival of some untreated controls in the undernourished group also lends support to the conclusion that in some way chronic undernutrition allows a significantly higher survival rate after tourniquet trauma than does acute starvation of the same degree.

The difference in survival between acutely starved rats of Exp. 1 and 4, points to the variation inherent in such testing situations. We have previously demonstrated(2), in spite of rigid control of all obvious variables, there may be a sizeable day to day variation in survival. This is probably due in some degree to the relatively small numbers of animals which can be tested on any one day and the fact that death of 1 or 2 animals exerts a large effect. Comparison between groups must be made simultaneously on the same experimental day and enough days should be devoted to each experiment to produce significance of results.

Summary. Experiments were performed to ascertain possible differences in survival following tourniquet trauma and saline infusion therapy between rats chronically undernourished to 77.4-80.3%, and those acutely starved to 77-80% of initial body weight. It has been demonstrated that there is a significantly higher percentage of survival when chronically undernourished rats are infused with 7 ml normal saline solution/100 g body weight following an otherwise lethal tourniquet trauma. Acutely starved rats are not protected by this level of replacement, but do show 75% survival when infused at level of 15 ml saline solution/100 g body weight.

1. Pareira, M. D., Sicher, N., Lang, S., *Ann. Surg.*, 1958, v147, 62.

2. Serkes, K. D., Lang, S., Pareira, M. D., *Surgery*, 1959, v45, 623.

3. Pareira, M. D., Sicher, N., Lang, S., *A. M. A. Arch. Surg.*, 1958, v77, 191.

Received June 19, 1959. P.S.E.B.M., 1960, v103.

A Rapid (24 Hour) Bioassay for Detection of Human and Mouse Tumor Factor. (25397)

F. FRIEDMAN, R. KASSEL, L. BURTON, M. L. KAPLAN AND A. ROTTINO
Hodgkin's Disease Research Laboratories, St. Vincent's Hospital, N. Y. City*

A cell free factor has been extracted and refined from human and mouse tumor tissue (1,2). This factor, when injected into C3H (f) mice will induce a wide variety of tumors, *i.e.*, parotid, mammary, and adrenal carcinomas; soft tissue tumors; and leukemia(1,2). For convenience, this factor has been designated as "Tumor Factor" (TF)(2). The method utilized for extraction and refinement of mammalian TF is the BFK (Burton-Friedman-Kassel) procedure(2). This procedure is an adaptation of the method originally developed for purification of the tumor inducing factor (TIF) present in the *tu-e* tumor strain of *Drosophila melanogaster*. Since both methods were closely related, past experience in the study of "*Drosophila* tumors" extending over a period of 10 years, suggested that mammalian TF could produce, in *Drosophila*, the lesion already described in the literature as "*Drosophila* tumor"(3-10,12). Preliminary data concerning the reaction of *Drosophila* to refined mammalian TF indicated that *Drosophila* could be used as an assay animal for rapid detection of mammalian TF. This report is concerned with a description of the technics developed from expansion of the preliminary observations.

* Supported by U.S.P.H.S. and Damon Runyon Memorial Fund for Cancer Research.

Methods and materials. Drosophila hosts. The host line, *wild 51-52*, is a genetically sta-