changed. At this point the value of the neutralization test for diagnostic purposes was further emphasized. After modification had occurred it was possible only by demonstrating a rise in antibody titer to prove that the dogs had been infected. However, diagnosis of ICH has also been made on numerous occasions by direct isolations of the virus in tissue cultures, either from a single drop of blood obtained during the febrile period; or after death from the livers of infected dogs(11). Thus, as has already been established for poliomyelitis, diagnosis can be made either directly by virus isolation or indirectly by demonstrating increase in antibody titer.

Recently, a comparison was made between virus from the 74th tissue culture passage and virulent ICH virus. There were 6 dogs in each group with an equal distribution of litter mates in each group. None of the dogs inoculated with virus from the 74th tissue culture passage developed either a febrile reaction or a leukopenia. Five of the dogs inoculated with virulent ICH virus died and had typical post mortem lesions of ICH, while the 6th dog developed a strong febrile reaction. All of the vaccinates developed a high titer of neutralizing antibody and resisted challenge with the virulent virus.

Summary. 1. Propagation of ICH virus with the production of distinctive cytologic changes has been demonstrated in roller tube cultures of dog kidney. 2. After 51 serial passages the virus apparently had lost its virulence for dogs, although producing a solid immunity. 3. A practical neutralization test for the diagnosis of ICH has been described.

1. Robbins, F. C., Enders, J. F., and Weller, T. H., PROC. SOC. EXP. BIOL. AND MED., 1950, v75, 370.

2. Rubarth, S., *Acta Path. et Microbiol. Scand.*, 1947, Supp. 69, p. 1.

3. Poppensiek, G. C., and Baker, J. A., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 279.

4. Enders, J. F., Weller, T. H., and Robbins, F. C., *Science*, 1949, v109, 85.

5. Weller, T. H., Robbins, F. C., and Enders, J. F., PROC. SOC. EXP. BIOL. AND MED., 1949, v72, 153.

6. Dulbecco, R., and Vogt, M., *J. Exp. Med.*, 1954, v99, 167.

7. Miles, J. A. R., Parry, H. B., Larin, N. M., and Platt, H., *Nature*, 1951, v168, 699.

8. Goret, P., Joubert, L., and Buffet, A., *Bull. Acad. Vet. de France*, 1950, v23, 305.

9. Martin, L. A., and Joubert, L., *ibid.*, 1952, v25, 393.

10. Cabasso, V. J., Stebbins, M. R., Norton, T. W., Cox, H. R., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 239.

11. Fieldsteel, A. H., and Emery, J. B., to be published.

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Effects of Tumbling Trauma, Scalding and Hemorrhage on Rat Tissue Non-Protein Sulfhydryl.* (21243)

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In a preceding publication(1), 2 of us have reported that scalding, tourniquet trauma and exposure to severe cold for 6 hours all induced in the mouse a marked decrease in liver non-protein sulfhydryl (–SH) concentration. The present paper deals with the effects in the rat of tumbling trauma and scalding on non-protein –SH concentration in the blood, liver, and certain other tissues. Data on liver –SH values obtained for rats subjected to severe hemorrhage are also presented.

Materials and methods. Chemical estimations. All distilled water employed in the present experiments was freed of heavy metal ions capable of binding –SH by passing ordinary distilled water through a La Motte Filtrion column. –SH estimations for deprotein-

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TABLE I. Comparative Tissue Non-Protein Sulfhydryl (-SH) Values Obtained for: (A) Control Rats, (B) Rats Traumatized by Tumbling (400 Turns) in a Noble-Collip Drum, or by Scalding (75°C for 15 Seconds).

			Non-protein -SH values, as GSH equivalents, mg %, ± S.E.*				
						Blood	
Exp.	Time of sacrifice after tumbling or scalding	No. of rats in group	Liver	Kidney	Heart	Original	Adjusted to "100% Hb"†
A	Controls	16	194 ± 7	108 ± 4.4	59.3 ± 1.6	39.4 ± 1.6	40.6 ± 1.6
	Tumbled rats:						
	5 min.	10	166	101	64	44.4 ± 1.4	43.0 ± 2.0
	40 "	4	126	96	62	39.4	38.6
	3 hr	4	110	103	43	39.6	36.0
	6 "	4	163	96	55	38.9	39.7
B	Controls	9	214 ± 7	108	64	39.0 ± 1.4	44.0 ± 1.6
	Scalded rats:						
	3 hr	4	170	76	45	48.5	41.9
	6 "	8	148 ± 8	86	67	46.5 ± 1.2	41.4 ± 1.0
	18 "	1‡	137	124	63	47.8	42.3
C	Controls	12	200 ±11	126 ± 2.3			
	Scalded rats:						
	3 hr	8	156 ±12	84 ± 4.9			
	6 "	4	161	92			

P values for control vs. scalded rats of Exp. B: For liver, less than .001.
Idem Exp. C: " " , .01; for kidney, less than .001.

* Stand. error values not available in some cases, due to analytical values having been secured by using extracts from tissues pooled from several rats in the Group.

† See text.

‡ Only 1 of 4 scalded rats survived to 18 hr.

ized tissue or blood extracts secured in the scalding and tumbling experiments were made by the nitroprusside method of Grunert and Phillips(2), adapted to the Fisher Electrophotometer by increasing the volumes of all reagent solutions employed by them by 2.5 times, and the final volume to 25ml. In our experiments this method has given satisfactory and reproducible optical density readings for glutathione and cysteine solutions, tissue and blood extracts, and such extracts to which glutathione had been added. It has the great advantage over the amperometric method(3), which we had previously used(1), of permitting performance of analyses at a much greater rate. Since stability of the color developed by nitroprusside in the presence of –SH is apparently dependent on having a very high salt concentration, and since many proteins are precipitated at such high salt concentrations, it does not appear likely that the nitroprusside method will be readily adapted to estimations of total and protein –SH, which may be made fairly readily by the amperometric method(1). *Deproteinized blood* extracts for –SH estimation by the nitroprusside method were secured as follows: A one ml hypodermic syringe bearing a short 23 gauge needle was filled to the 0.10 mark with heparin in 0.9% NaCl at a concentration of 1000 U. S. P. units per ml. The test rat was etherized, the chest wall opened, and blood drawn from the heart into the syringe to the 1.0 ml mark. This heparinized blood was discharged into a 10 ml glass stoppered volumetric flask. Two hundredths ml of the blood was withdrawn from the flask with a Sahli pipette and added to 5.0 ml of 0.1 N HCl, for estimation of hemoglobin content of the blood by the acid hematin method, using a Fisher Electrohemometer. To the heparinized blood remaining in the 10 ml flask was added 4 ml of distilled water and a pinch of saponin. On completion of hemolysis 2.0 ml of 8% metaphosphoric acid were added. The flask was stoppered and thoroughly shaken. Three grams of crystalline NaCl were added and shaken to solution. Foam in the neck was carefully removed using a wooden applicator dipped in caprylic alcohol. Saturated NaCl solution was added to the 10 ml mark. The contents of the flask were thoroughly mixed and filtered

TABLE II. Effects of Hemorrhage on Rat Liver Sulfhydryl.

Exp.	Avg total bleeding vol as % of body wt	Hr between end of 2nd bleeding & sacrifice	No. of rats in group	Liver -SH as mg %, GSH equivalent, ± S.E.			Protein extracted, as % of liver wt	Protein -SH, as % of extracted protein
				Total -SH	Non-protein -SH	Protein -SH		
A	Control	—	11	518 ± 35	187 ± 5	331 ± 35	17.4 ± 1.8	.20
	3.25	2	2	465	157	309	18.1	.18
	3.25	4	10	419 ± 40	142 ± 9	270 ± 40	16.7 ± 1.4	.17
B	Controls	—	10	615 ± 14	198 ±11	417 ± 15	26.8 ± .4	.17
	2.77	24	10	645 ± 17	212 ± 7	433 ± 15	24.8 ± .6	.19
P values for control vs. bled rats of Exp. A, 4 hr after bleeding:				N.S.	.001	N.S.*	N.S.	
P values for controls of Exp. A (about 150 g body wt) vs. controls of Exp. B (older rats, about 210 g body wt):				.03	N.S.	.05	.001	

* N.S.—Difference not statistically significant.

through Whatman No. 1 paper into a test tube. A 5 ml aliquot of the filtrate, corresponding to 0.44 ml of rat blood, was employed for the nitroprusside optical density estimation.

In Table I, 2 sets of values are given for blood non-protein –SH: a) original values, b) values adjusted to blood containing hemoglobin at 100% of the "normal" value, arbitrarily placed at 15.6 g of hemoglobin per 100 ml of blood. Use of such a "normal" value makes it possible to allow for variations in non-protein –SH content of the blood due to variations in the mass of red cells subjected to analysis. The advisability of having "adjusted" values available for consideration is indicated by the finding of Peterson, Beatty and West(4) that whereas radiation was followed by marked changes in blood glutathione values, parallel changes also occurred in the hematocrit values, so that glutathione expressed as mg per 100 g of red cells was relatively little changed. *Deproteinized tissue* extracts for –SH estimation by the nitroprusside method were secured as follows: Immediately after the blood had been removed from the heart, each tissue for which an analysis was desired was excised, frozen on dry ice, wrapped in tin foil and held in a deep freeze to time of analysis, but not more than 24 hours after sacrifice. The frozen tissue was ground in a large mortar with sand and ice-cold 3% metaphosphoric acid, 4 ml per gram of tissue. Crystalline NaCl, 1.5 g per gram of tissue, was added and ground to solution in tissue brei. Finally, 2% metaphosphoric acid saturated with NaCl was added in the amount of 14.5 ml per gram of tissue. The final mixture was filtered through Whatman No. 1 paper to give a clear filtrate. Concentration of non-protein –SH in the tissue has been estimated by multiplying the concentration found in the tissue extract by 20. In the *hemorrhage experiments* (Table II), the liver extracts were secured essentially as described previously(1), and the –SH estimations were made by the amperometric method(1,3). The values for *protein –SH as per cent of the protein*, shown in the final column of Table II, were secured as follows: Each protein –SH value, expressed as glutathione equivalent, was converted to *–SH per se*. This value was then used in conjunction with the corresponding extractable liver protein value, as estimated by the method of Robinson and Hogden(5), to calculate the term *protein –SH as per cent of protein*.

Animals and treatment. Carworth Farms strain male rats, fed on Rockland rat food pellets, were employed. Approximate average body weights for these rats were as follows: Exp. A of Table I, 240 g; Exp. B of Table I, 320 g; Exp. C of Table I, 270 g; Exp. A of Table II, 150 g; Exp. B of Table II, 210 g. I. *Tumbling trauma:* 400 turns (800 tumbles) were administered, using an apparatus modelled on that of Noble and Collip(6), but having a fixed rotation rate of 41 turns per minute. Preparation of rats for tumbling, and their

appearance and behavior after tumbling, differed in no way from the descriptions of Noble and Collip. For data obtained, see Table I, Exp. A. II. *Scalding:* The method described by Rosenthal(7) was employed. Each test rat was etherized and scalded to the neck in water at 75°C for 15 sec. For data obtained, see Table I, Experiments B and C. III. *Hemorrhage:* Each rat was etherized and bled from the tail by the method described for the mouse by Tabor, Kabat and Rosenthal(8). After completion of the second hemorrhage, the rats were returned to cages containing food and water. The amounts of blood secured by us, expressed as per cent of the body weight, have been appreciably less than the amounts which Tabor *et al* were able to secure from mice. They removed blood in a total amount of 4.5% of the body weight, in 2 equal bleedings an hour apart. While we were successful with each rat in removing blood in the amount of 2.25% of the body weight in the first bleeding, the second bleeding did not in any case yield an amount of blood exceeding 1.25% of the body weight. The amount yielded by rats of over 200 g body weight was appreciably less, in ml per 100 g of body weight, than that yielded by rats of about 150 g body weight. Of 24 rats subjected to hemorrhage, 2 died as a result of the first bleeding. The average total amounts of blood removed are indicated for the various groups of surviving bled rats in Table II. Rats subjected to hemorrhage and sacrificed at 2 or at 4 hours after completion of the second bleeding exhibited dyspnea, prostration and apathy at time of sacrifice. None of this symptomology was exhibited at 24 hours after completion of hemorrhage.

Results. I. *Tumble experiments* (Table I, Exp. A): The liver non-protein –SH values secured for tumbled rats were all less than the average liver non-protein –SH value obtained for control rats. This difference was most pronounced for tumbled rats sacrificed 3 hours after completion of tumbling. Other tissues analyzed (kidney, heart, skeletal muscle, blood) showed relatively little change in non-protein –SH, with the possible exception of heart at 3 hours after tumbling. II. *Scald experiments* (Table I, Exp. B and C): Rats sacrificed at 3 and at 6 hours after scalding exhibited significantly lower non-protein –SH values for both liver and kidney than did control rats. Spleen non-protein –SH values were practically identical for control and scalded rats. Heart non-protein –SH was apparently decreased at 3 hours after severe scalding. At both 3 and 6 hours after scalding a considerable increase in blood hemoglobin had occurred, reflecting no doubt a corresponding hemoconcentration. There was also a considerable increase in the blood non-protein –SH. However, on adjustment of all bloods to a "100% Hb" value, the blood non-protein –SH values of control and scalded rats became statistically indistinguishable. III. *Hemorrhage experiments* (Table II): By 4 hours after the second hemorrhage a significant decrease in the liver non-protein –SH concentration had occurred (Exp. A). At 24 hours after hemorrhage, control and bled rats exhibited indistinguishable liver non-protein –SH values. The data of Table II indicate that no significant alteration in extractable liver protein or protein –SH was induced by the hemorrhage. More protein and protein –SH were extracted from the livers of the larger, older rats of Exp. B than from the livers of the younger rats of Exp. A. It is interesting to note that, within the limits set by the accuracy of the analytical methods employed, a fairly constant value was obtained for the factor *protein –SH as per cent of the extractable liver protein* (last column of Table II).

Discussion. A wide variety of procedures has been developed for producing in experimental animals a condition resembling clinical shock(9). From data presented in this paper and a preceding publication(1) it is apparent that in both the mouse and the rat the use of such procedures has to date invariably resulted within a few hours in a significant decrease in concentration of liver non-protein –SH compounds. Tests made to date have failed to reveal appreciable increases in non-protein –SH of tissues other than the liver, accompanying the significant decreases noted in the liver. Tissues of tumbled or scalded rats examined have included kidney, blood, heart, spleen and skeletal muscle. It would appear, therefore, that decreases in liver non-protein

–SH occurring in severely injured rats (and mice) are not brought about by the transfer to, and storage of, such compounds in unaltered chemical form in tissues other than the liver.

Leaf and Neuberger(10) and Edwards and Westerfeld(11) have reported that by 24 hours after rats were placed on a low protein diet, a considerable decrease had occurred in the concentration of glutathione in the liver. Since animals subjected by us to scalding, trauma, or hemorrhage did not eat for several hours thereafter, it might be postulated that the low liver non-protein –SH values observed by us were due to protein deprivation. However, the changes observed by us have been most pronounced at three and six hours after infliction of injury or hemorrhage, whereas in the experiments of Leaf and Neuberger rats deliberately starved did not exhibit any appreciable decrease in liver glutathione concentration even at 24 hours after the beginning of the starvation.

It is hoped that future experimentation will elucidate some of the metabolic and/or hormonal mechanisms responsible for the decreases in concentration of non-protein –SH compounds which occur in certain tissues, especially the liver, following severe injury.

Summary. 1. Significant decreases in concentration of non-protein –SH were noted in rat liver following tumbling trauma, and in rat liver and kidney following severe scalding. 2. In analyses confined to the liver, a significant decrease in liver non-protein –SH was noted following severe hemorrhage. No appreciable change was noted in either liver protein –SH or extractable liver protein. 3. Blood non-protein –SH concentrations were practically identical for control and tumbled rats. Scalded rats exhibited a moderate increase in concentration of blood non-protein –SH, paralleling the hemoconcentration.

1. Beck, L. V., and Linkenheimer, W., PROC. SOC. EXP. BIOL. AND MED., 1952, v81, 291.

2. Grunert, R. R., and Phillips, P. H., *Arch. Biochem.*, 1951, v30, 217.

3. Weissman, N., Schoenbach, E. B., and Armistead, E. B., *J. Biol. Chem.*, 1950, v187, 153.

4. Peterson, R. D., Beatty, C. H., and West, E. S., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 747.

5. Robinson, H. W., and Hogden, C. C., *J. Biol. Chem.*, 1940, v135, 707.

6. Noble, R. L., and Collip, J. B., *Quart. J. Exp. Physiol.*, 1942, v31, 187.

7. Rosenthal, S. M., *Pub. Health Rep.*, 1942, v57, 1923.

8. Tabor, H., Kabat, H., and Rosenthal, S. M., *ibid.*, 1944, v59, 637.

9. Wiggers, C. J., *Physiology of Shock*, 1950, Harvard Univ. Press, Cambridge, Mass.

10. Leaf, G., and Neuberger, A., *Biochem. J.*, 1947, v41, 280.

11. Edwards, S., and Westerfeld, W. W., PROC. SOC. EXP. BIOL. AND MED., 1952, v79, 57.

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Activation of Bovine Plasminogen by Trypsin.*† (21244)

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Following the early observation that chloroform treatment increased the proteolytic activity of serum(1), chloroform was found to function by reacting with a protease inhibitor, thus allowing an autocatalytic type activation of the protease(2). The inactive zymogen of this protease system has been named plasminogen (profibrinolysin) and the active enzyme, plasmin (fibrinolysin). Plasmin functions in the blood coagulation scheme by splitting fibrinogen and fibrin to smaller fragments. Since chloroform, first used to activate plasminogen, is foreign to a biological system, attempts have been made to find biological

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