was implanted under the skin of the back (about 1 mg). Hormone from the pellet could reach the test organs only provided they still had an adequate blood supply.

Preliminary data on the number of mitoses in transverse sections of the seminal vesicle were obtained by counting the mitotic figures in the last 10 sections preceding junction of seminal vesicle and ductus deferens to produce ejaculatory duct. In Groups F to I the averages were, respectively, 20.5, 8.1, 17.0 and 12.0.

Pending additional data, it would seem that in this species the fetal testes produce a hormone which accelerates the growth of such organs as the seminal vesicles and the bulbourethral glands. If the adrenals of fetal males produce any such hormone, the quantity is not sufficient to prevent the effects of castration upon the accessory reproductive organs.

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Osmotic and Mechanical Fragilities of Erythrocytes of Monkeys Infected with *P. knowlesi* Malaria.*

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The objective of the experiments reported here was (a) to determine the osmotic and mechanical fragilities of the red blood cells of monkeys infected with malaria and (b) to compare the osmotic fragilities of the red blood cells before and after trauma.

Methods. Samples of the freshly drawn oxalated blood of 7 *Macaca mulatta* monkeys experimentally infected with *P. knowlesi* 5 to 9 days previously and of 6 uninfected control monkeys were studied.† For the determination of the osmotic fragility of the red blood cells, 0.1 cc of blood was introduced into 1 cc of a series of progressively hypotonic concentrations of NaCl and into 1 cc of distilled water. Approximately 10 minutes later these samples were centrifugalized at 1500 r.p.m. for 10 minutes. The percentage concentration of hemoglobin in each supernatant was then determined by appropriate use of the Evelyn photoelectric colorimeter, taking that in the tube containing distilled water as a concentration of 100%. A smooth curve was then drawn connecting the points representing the percentages of hemoglobin liberated at each tonicity. From this were read off the respective concentrations of NaCl required to liberate the characteristic percentages of hemoglobin shown in Table I.

The mechanical fragility of the red blood cells was determined according to a recently published method.[1] One-half cc samples of blood with hematocrits adjusted to about 40% were traumatized by glass beads rolling in the greatest internal circumference of 50 cc rubber-stoppered Erlenmeyer flasks, rotated about a horizontal axis perpendicular to their flat base. The percentages of hemoglobin liberated by 1½ hours of such trauma were considered to represent the mechanical fragility of the samples.

Usually an infected and a normal control sample were studied simultaneously. The initial osmotic and mechanical fragilities of a portion of each sample were determined. If there was sufficient blood, the osmotic

* This investigation was aided by a grant from the John and Mary R. Markle Foundation.

† The blood samples were obtained through the kindness of Dr. Quentin M. Geiman from animals being studied under a contract, recommended by the Committee on Medical Research, between the Office of Scientific Research and Development and Harvard University.

[1] Shen, S. C., Castle, W. B., and Fleming, E. M., *Science*, 1944, **100**, 387.

TABLE I.
Characteristics of Blood of Infected Monkeys.

Exp. No.	Time, hr	R.B.C., mils.	Mechanical fragility: Hematocrit, %	Mechanical fragility: Hematocrit (adjusted), %	Mechanical fragility: Hemolysis %	Intracellular parasites: Trophozoites %	Intracellular parasites: Schizonts %	Intracellular parasites: Total, %	% NaCl causing % hemolysis indicated: 1	2.5	5	10	50	75	95	
44-109	0	5.05	40.3	40.3	6.3	2.7	1.8	4.5	.52	.49	.47	.45	.39	.36	.32	
44-112	0	4.22	31.0	39.0	8.6	1.2	5.8	7.2	.68	.59	.48	.43	.38	.35	.28	
44-119	0	2.90	20.0	44.0	8.3	2.7	12.3	15.0	.72	.61	.55	.47	.37	.34	.30	
	9					1.0	4.0	6.2	.72	.66	.58	.45	.34	.31	.26	
	9					0.4	1.8	2.2	.54	.47	.42	.41	.36	.33	.29	Rotated
44-128	0	3.43	27.0	39.0	8.7	0.2	22.4	22.6	.62	.58	.53	.47	.35	.33	.26	
	9					1.0	25.3	26.3	.84	.81	.70	.58	.35	.34	.26	
	9					0.2	8.6	8.8	.78	.70	.60	.43	.34	.31	.25	,,
44-134	0	2.35	18.0	44.4	23.3	0.7	32.3	33.0	.74	.72	.68	.60	.39	.35	.28	
	9					0.2	28.8	29.0	.84	.82	.79	.70	.43	.36	.27	
	9					0.0	3.4	3.4	.59	.57	.54	.52	.42	—	—	,,
45-7	0	2.70	25.1	32.7	12.8	10.0	41.0	51.0	.81	.77	.74	.68	.48	.43	.37	
	9					2.4	15.6	18.0	.89	.88	.85	.80	.49	.44	.37	
	? < 9					9.0	14.2	23.2	.84	.82	.80	.70	.47	.41	.31	,,
45-57	0	2.08	16.0	40.2	15.0	22.7	24.7	47.4	.77	.73	.68	.60	.37	.33	.21	
	9					14.2	13.0	27.2	.87	.82	.73	.62	.37	.32	.21	
	9					14.7	2.8	17.5	.64	.59	.54	.49	.37	.33	.22	,,
						Average Characteristics of Blood of Normal Monkeys.										
6 animals	0		43.5	43.5	3.8	0	0	0	.47	.44	.42	.41	.36	.34	.28	
3 animals	9								.49	.44	.41	.38	.34	.31	.24	,,

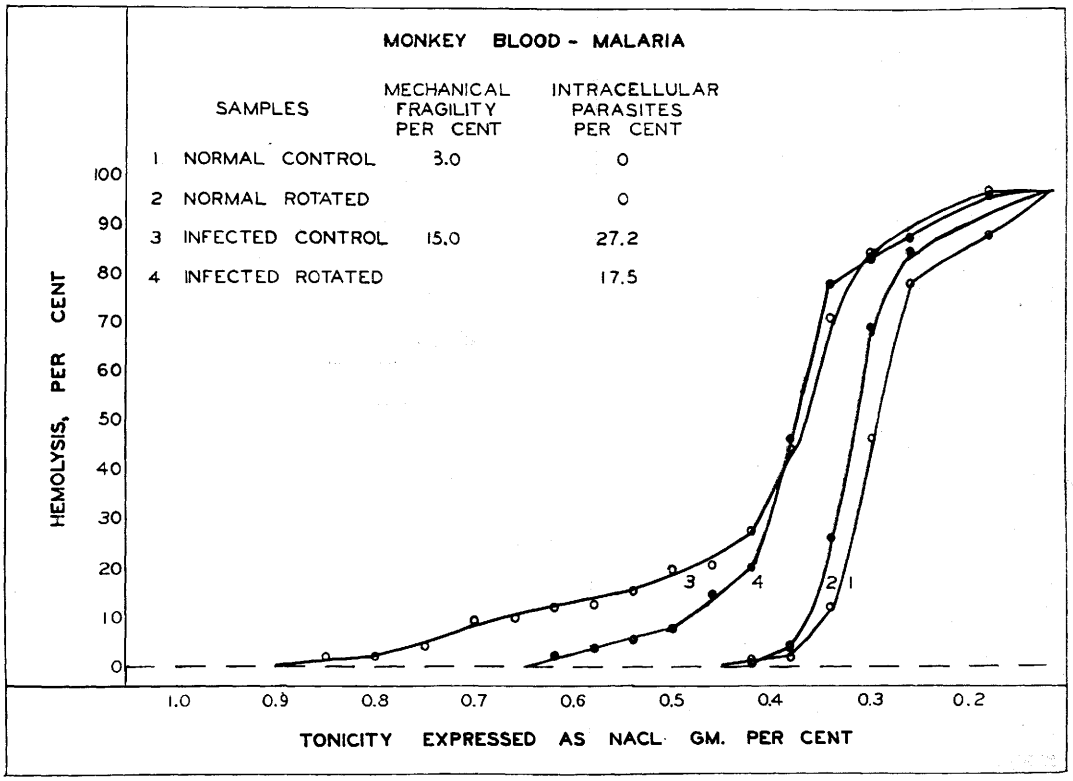

FIG. 1.

Effect of trauma (rotation with glass beads) on the osmotic fragility and percentage of parasitized red blood cells of a monkey with malaria (Exp. 45-57). The osmotic fragility of the red blood cells of this normal control monkey is less than that of the average normal monkey (Table I).

fragility of another portion of each sample was also determined after 9 hours of rotation with glass beads at room temperature. As controls, portions of each sample were also allowed to stand for 9 hours at room temperature and the osmotic fragility again ascertained. In addition, the percentages of red blood cells containing trophozoites and schizonts and their total were determined from blood films stained by Wright's method. Such parasite counts were made from the infected blood samples before and after the 9-hour period of mechanical trauma as well as from the portions of the samples which had stood for 9 hours at room temperature.

Results. Inspection of the data shown in Table I indicates that, as previously observed by Geiman and his associates,[2] the blood of all of the infected monkeys exhibited greater osmotic fragility of a portion of the red blood cells than did that of the uninfected controls. Fig. 1 (Exp. 45-57) exhibits in curve 1 the S-shaped form of the curve of osmotic fragility of the control normal monkey blood although it is to be noted that the red blood cells of this monkey were generally less fragile than those of the average normal monkey (Table I). In contrast to this symmetrical curve is the "trailing" form of the left end of curve 3 representing the osmotic fragility of the infected monkey blood. This asymmetry denotes a red blood cell population containing an increased proportion of osmotically fragile red blood cells. The mechanical fragility of the red blood cells of the infected monkeys was also greater than that of the normal controls. Inspection of the data on percentages of intracellular parasites given in Table I suggests that the

[2] Geiman, Q. M., Anfinsen, C. B., McKee, R. W., Ormsbee, R. A., and Ball, E. G., *J. Exp. Med.*, 1946, **84**, 583.

parasitized erythrocytes, especially those containing schizonts, were largely responsible for both the increased osmotic and mechanical fragilities of the infected samples. If this were so, selective destruction of the infected cells by prolonged mechanical trauma should produce 2 effects: (a) Decrease of the percentage of parasitized cells; (b) some tendency for the osmotic fragility curve of the traumatized sample to return toward normal.

Inspection of the data in Table I shows that effects (a) and (b) occurred in the 5 instances in which complete tests of infected blood were made. An example of effect (b) is shown in curves 3 and 4 in Fig. 1 (Exp. 45-57). It is to be noted that mechanical trauma usually was especially effective in destroying red blood cells containing schizonts. The negligible effect of trauma on the osmotic fragility of normal monkey blood is shown by the similarity of curves 1 and 2 and by the average values in Table I.

Discussion. Because in some instances the plasmas of the infected monkeys contained hemoglobin obvious to the naked eye, and in one animal hemoglobinuria was observed, the increased osmotic and mechanical fragilities of the parasitized red blood cells are considered to have a possible relationship to the mechanism of the production of the anemia in human malaria and possibly to blackwater fever. Theoretically, the parasites could increase the mechanical fragility of the infected red blood cells by damaging the cell membranes, by increasing cohesion between the cells,[3-5] or by increasing their volume and consequently their spheroidicity.[1] Such increased spheroidicity, measurable as increased osmotic fragility, could be brought about by damage to the cell membrane sufficient to affect its permeability, by the expanding growth of the parasites or by increase of the total osmotic pressure of the cell due to the metabolic activities of the parasites within. Tests for the presence of agglutinins for red blood cells at room and ice box temperatures in the sera of 2 infected monkeys were negative.

The motion of the circulation could very well, because of the increased mechanical fragility of the red blood cells,[6,7] play a part in determining the time of release of the mature parasites and in limiting the percentage of parasitized cells in active circulation. Areas of less active vascular flow might thus provide conditions favorable for the maturation of large numbers of parasites. Osmotic lysis there or the sudden reentry of such unusually mechanically fragile cells into active circulation might contribute in man to the hemolytic crisis of blackwater fever.

Summary. Experiments with the blood of control and of *Macaca mulatta* monkeys infected with *Plasmodium knowlesi* indicate that the parasitized red blood cells develop increased osmotic fragility and *in vitro* are selectively destroyed by mechanical trauma. Consequently, it is suggested that because of this increased mechanical fragility of the red blood cells the motion of the circulation *in vivo* plays a part in the production of the hemolytic anemia in malaria of monkeys and, by analogy, of man.

[3] Dameshek, W., and Miller, E. B., *Arch. Int. Med.*, 1943, **72**, 1.

[4] Stats, D., PROC. SOC. EXP. BIOL. AND MED., 1943, **54**, 305.

[5] Lee, J. S., Puh, Y. C., and Tsai, C., *Proc. Chinese Physiol. Soc., Chengtu Branch*, 1944, **2**, 59.

[6] Meltzer, S. J., and Welch, W. H., *J. Physiol.*, 1884, **5**, 255.

[7] Rous, P., and Turner, J. R., *J. Exp. Med.*, 1916, **23**, 219.