(15) and the deleterious effect of isologous whole blood for homologous bone marrow transplantation in mice(16) should be reinterpreted in the light of these findings. Transplantation immunity can be conferred with as few as 50,000 cells of a Peyer's patch(17) and 80,000 isologous bone marrow cells are able to protect against hematopoietic failure induced by X radiation(8). Hence, since the white blood cell count is ~ 8 million per ml of whole blood, the proportion of the circulating cells that are able to divide may be as high as 1%.

Summary. Strains of mice are being produced that are isogenic with inbred stocks except for the hemoglobin locus. With mice of such strains, experiments were carried out to ascertain whether peripheral blood of normal mice contain cells capable of repopulating the marrow of irradiated recipients. In 3 of 5 recipients injected intravenously with ~ 100 million nucleated peripheral blood cells after 600 r, increasing numbers of graft-derived erythrocytes were noted within 60 days after treatment, indicating the presence in peripheral blood of cells that are able to give rise to mature erythrocytes.

1. Bloom, W., in *Handbook of Hematology,* Vol. I, Downey, Ed., Hoeber, New York, 1938, p373.
2. ———, *ibid.,* Vol. II, p1469.
3. Merwin, R. M., PROC. SOC. EXP. BIOL. AND MED., 1959, v101, 9.
4. Smith, L. H., Popp, R. A., St. Amand, W., *ibid.,* 1960, v103, 232.
5. Snell, G. D., *J. Genet.,* 1948, v49, 87.
6. Popp, R. A., Cosgrove, G. E., Jr., Owen, R. D., PROC. SOC. EXP. BIOL. AND MED., 1958, v99, 692.
7. Popp, R. A., Cosgrove, G. E., Jr., *ibid.,* 1959, v101, 754.
8. van Bekkum, D. W., Vos, O., *J. Cell. and Comp. Physiol.,* 1957, v50, Suppl. 1, 139.
9. Smith, L. H., Toha, J., PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 125.
10. Bond, V. P., Fliedner, T. M., Cronkite, E. P., Rubini, J. R., Brecher, G., and Schork, P. K., *Acta haemat.,* 1959, v21, 1.
11. Maximow, H., *Arch. f. exp. Zellforsch.,* 1928, v5, 169.
12. Downey, H., *Folia haemat.,* 1927, v34, 65, 145.
13. Pierce, M., *Arch. Path.,* 1932, v14, 295.
14. Downey, H., Stasney, J., *J.A.M.A.,* 1935, v105, 764.
15. Cole, L. J., Garver, R. M., *Nature, London,* 1959, v184, Suppl. 23, 1815.
16. Goodman, J. W., Congdon, C. C., *Radiation Res.,* 1960, v12, 439 (abstr.).
17. Jacobson, L., Simmons, E., Marks, E., Gaston, E., Kolar, J., *ibid.,* 1960, v12, 444 (abstr.).

Received June 29, 1960. P.S.E.B.M., 1960, v104.

A Study of the So-Called "Cat Effect" of Cruickshank and Whitfield. (25967)

ENID NEIDLE* AND MAGNUS I. GREGERSEN
Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City

Some years ago Cruickshank and Whitfield (1) claimed on rather scant evidence from time-concentration curves on cats that "gobbling" of T-1824 by the reticulo-endothelial system leads to an overestimate of plasma volume upon first injection of this dye. Furthermore, they suggested that "a previous 'saturating' dose of dye should be injected before a determination is made in order to abolish the 'absorption curve' ". Judging from the questions directed to our laboratory from time to time, even as recently as the present year, we have the impression that Cruickshank and Whitfield's report, often referred to as the "cat effect," is still considered unchallenged. To be sure, the experiments of Cruickshank and Whitfield have not, to the best of our knowledge, been exactly repeated but since the question has been raised with us again, we decided to report the results of experiments made by one of us in 1946-47 (E.N.) which were designed to test the "cat effect." Our reasoning was that if the claims regarding this phenomenon were true, then the plasma volume obtained by the second of 2 successive injections of T-1824 should be

* Present address: New York Univ. College of Dentistry, Physiology Dept., New York City.

smaller than the first. In a series of experiments on unanesthetized normal cats and dogs, in which 2 successive determinations of plasma volume were made with T-1824, we found that the outcome was quite the opposite.

Materials and methods. The experiments were carried out on 11 dogs and 9 cats without anesthesia after a preliminary period of training. The T-1824 injections were made into the jugular vein with a calibrated syringe immediately after withdrawing the usual control blood sample. In dogs the average dose of T-1824 for each test was 11.5 mg/kg body weight and in cats, 9 mg/kg. The post injection blood samples were drawn from the contralateral veins at 5, 10, 15, 20, 30, and sometimes also at 45 and 60 minutes after each injection of dye and immediately transferred to hematocrit tubes containing an adequate amount of powdered heparin to prevent clotting. The second injection was made immediately after drawing the last sample from the first series and subsequent dye-tinged samples were read against this "blank," the first series being read against the initial dye-free control sample, either in a Koenig-Martins spectrophotometer at 620 millimicrons (cats) or with a Nickerson decade photometer (dogs). Optical densities were then plotted on semilog paper and extrapolated in the usual manner to determine D_0 for calculating plasma volume. Plasma protein concentrations were measured with a refractometer and from these the dye curves were corrected for fluid shifts to obtain fair estimates of the dye disappearance rates. Hematocrit values obtained by centrifugation at 3000 rpm (radius 19 cm) for 30 minutes were corrected for plasma trapping using the factor 0.96. It should be noted that total blood volumes were calculated using the equation $BV = \frac{PV \times 100}{100 - Hct \times 0.96}$. Hence the total blood volume values have not been adjusted for the F_{cells} factor which in any event is known to be subject to large variations in the unanesthetized dog(2) and perhaps also in the cat.

Results. The relevant data from the experiments on cats and dogs are shown in Tables I and II respectively; mean values and standard deviations are given at the bottom of each table. In all but one experiment on cats (4M where the 2 plasma volumes were the same) the second plasma volume was larger than the first, the mean difference for all 9 experiments being 8.4% (s.d. ± 4.6). In the dogs the second plasma volume was also larger than the first except in 6F where the second volume was nearly 3% less than the first. However, we may note that in this experiment neither hematocrit value nor plasma protein concentration showed the gradual fall characteristic of the other tests and usually observed with repeated sampling. Nevertheless, in the dogs the second plasma volume averaged 6.4% (s.d. ± 7.8) larger than the first, corresponding fairly well with the difference noted in the cat experiments.

Disappearance rates of T-1824 are shown at extreme right of each table. These are higher in cats than in dogs and also higher after the first injection than after the second in both groups of animals. Although heart rates reveal fairly large individual variations in both cats and dogs, it will be seen that in any one experiment the values did not change radically during the period of the test.

Plasma volumes in ml/kg body weight in the cats ranged from about 30 to 56 with a mean value of 41 (± 8). In the dogs corresponding values range from about 45 to 83 with a mean of 63 (± 14). Total blood volumes in cats ranged from about 52 to 97 ml/kg with a mean of 71 (± 16); in dogs the range was from 78 to 145 with a mean of 110 (± 20). As stated above, these total blood volumes cannot be taken as true values since the calculations were made without regard for unequal distribution of red cells in the circulation which in any event is unpredictable in conscious dogs(2) and perhaps also in cats with the spleen intact.

Discussion. The fact that the disappearance rates are higher after the first than after the second injection of dye would seem to lend support to the claims of Cruickshank and Whitfield, *but only if the first plasma volume were larger than the second.* We have seen from Tables I and II that quite the reverse

TABLE I. Normal Unanesthetized Cats.*

Cat No. and sex	Date	Wt	Het × .96, %	Plasma protein, % (refract)	Heart rate/min.	Plasma vol, ml	Plasma vol, ml/kg	Dev. of 2nd plasma vol from 1st plasma vol, %	Blood vol, ml	Blood vol, ml/kg	Disappearance rate, %/hr
1 ♂	11/19/46	4.0	1) 38.5	1) 7.98	1) 208	1) 146	1) 36.5	+ 4.9	1) 243	1) 60.8	1) 15.6
			2) 36.5	2) 7.74	2) 209	2) 153	2) 38.3		2) 254	2) 63.5	2) 11.7
			3) 36.0	3) 7.32	3) 200						
2 ♂	6/20/46	3.3	1) 37.5	1) 7.00	1) 230	1) 100	1) 30.8	+ 9.1	1) 170	1) 52.3	1) 31.0
			2) 35.2	2) 6.45	2) 232	2) 109	2) 33.6		2) 182	2) 56.0	2) 16.1
			3) 32.9	3) 5.98	3) 232						
3 ♀	11/26/46	3.0	1) 42.8	1) 7.32	1) 208	1) 120	1) 40.0	+17.5	1) 218	1) 73.0	1) 30.6
			2) 42.0	2) 7.32	2) 184	2) 142	2) 47.0		2) 252	2) 84.0	2) 15.3
			3) 40.3	3) 7.22	3) 168						
4 ♂	6/12/46	3.6	1) 42.7	1) 7.52	1) 164	1) 202	1) 56.2	0	1) 357	1) 99.1	1) 22.2
			2) 41.1	2) 7.46	2) 148	2) 202	2) 56.2		2) 350	2) 97.3	2) 20.0
			3) 39.2	3) 7.01	3) 142						
5 ♂	6/13/46	3.1	1) 42.6	1) 7.32	1) 210	1) 125	1) 40.4	+ 7.4	1) 227	1) 73.2	1) 13.9
			2) 40.8	2) 6.96	2) 220	2) 135	2) 43.4		2) 246	2) 79.4	2) 14.3
			3) 41.7	3) 6.54	3) 232						
6 ♀ (preg.)	12/12/46	2.5	1) 44.5	1) 7.88	1) 220	1) 89.5	1) 35.8	+ 8.9	1) 166	1) 66.4	1) 26.0
			2) 41.7	2) 7.30	2) 232	2) 97.5	2) 39.0		2) 178	2) 71.1	2) 20.4
			3) 41.0	3) 7.22	3) 248						
7 ♀	1/ 6/47	2.5	1) 36.8	1) 6.60	1) 222	1) 80	1) 32	+ 6.3	1) 135	1) 54.0	1) 39.2
			2) 35.0	2) 6.19	2) 225	2) 85	2) 34		2) 141	2) 56.5	2) 23.8
			3) 34.4	3) 5.62	3) 232						
8 ♀	12/ 5/46	3.3	1) 28.2	1) 7.11	1) 190	1) 149	1) 45	+14.4	1) 218	1) 66.4	1) 34.9
			2) 29.3	2) 7.11	2) 208	2) 170	2) 51.5		2) 250	2) 75.7	2) 19.0
			3) 28.4	3) 6.65	3) 192						
9 ♀	12/ 6/46	2.6	1) 44.8	1) 6.65	1) 152	1) 134	1) 51.5	+12.6	1) 252	1) 97	1) 40.0
			2) 44.7	2) 6.65	2) 152	2) 148	2) 57.0		2) 280	2) 108	2) 18.2
			3) 45.4	3) 6.65	3) 176						
10 ♀	12/ 2/46	1.8	1) 35.2	1) 7.74	1) 240	1) 75.0	1) 41.6	+ 2.6	1) 127	1) 70.5	1) 40.8
			2) 34.2	2) 7.57	2) 220	2) 76.9	2) 42.7		2) 122	2) 67.8	2) 20.8
			3) 32.2	3) 7.00	3) 188						
Mean		2.97	1) 39.4	1) 7.31		1) 123	1) 41.0 ±8.1 s.d.	+ 8.4 ±4.6 s.d.	1) 211.3	1) 71.3 ±15.8 s.d.	1) 29.4
			2) 38.1	2) 7.08		2) 132	2) 44.3 ±8.5 s.d.		2) 225.5	2) 75.9 ±16.9 s.d.	2) 17.9
			3) 37.3	3) 6.72							

* For Het, plasma protein and heart rate: 1) time of first dye inj.; 2) time of second dye inj.; 3) end of second dye curve. For plasma vol, blood vol and disappearance rate: 1) from first dye curve; 2) from second dye curve.

TABLE II. Normal Unanesthetized Dogs.*

Dog No. and sex	Date	Wt, kg	Hct × .96, %	Plasma protein, % (refract)	Heart rate/min.	Plasma vol, ml	Plasma vol, ml/kg	Dev. of 2nd plasma vol from 1st plasma vol, %	Blood vol, ml	Blood vol, ml/kg	Disappearance rate, %/hr
1 ♂	4/29/46	11.0	1) 43.5 2) 41.6 3) 41.3	1) 6.01 2) 5.86 3) 5.50	1) 60 2) 64 3) 63	1) 553 2) 560	1) 50.3 2) 50.7	+ .80	1) 993 2) 990	1) 90.2 2) 90.0	1) 6.1 2) 8.9
2 ♀	6/ 4/46	8.9	1) 32.2 2) 31.5 3) 29.0	1) 6.16 2) 6.16 3) 5.66	1) 96 2) 88 3) 92	1) 604 2) 652	1) 67.8 2) 73.2	+8.00	1) 909 2) 967	1) 102 2) 109	1) 11.1 2) 9.0
3 ♂	4/14/46	9.5	1) 42.5 2) 40.5 3) 39.6	1) 6.06 2) 5.71 3) 5.50	1) 68 2) 64 3) 64	1) 785 2) 810	1) 82.5 2) 85.2	+3.3	1) 1376 2) 1380	1) 145 2) 145	1) 13.3 2) 5.2
4 ♀	2/ 4/46	9.6	1) 40.4 2) 42.0 3) 40.4	1) 6.62 2) 6.26 3) 5.61	1) 108 2) 116 3) 108	1) 572 2) 595	1) 59.5 2) 62.0	+4.20	1) 1058 2) 1011	1) 110 2) 105	1) 9.9 2) 12.5
5 ♀	4/10/46	6.7	1) 34.7 2) 32.6 3) 32.5	1) 6.21 2) 5.86 3) 5.45	1) 92 2) 96 3) 88	1) 527 2) 566	1) 78.6 2) 84.5	+7.50	1) 824 2) 866	1) 123 2) 129	1) 11.2 2) 6.8
6 ♀	5/ 2/46	7.0	1) 42.1 2) 42.7 3) 41.7	1) 7.77 2) 7.77 3) 7.67	1) 72 2) 72 3) 76	1) 471 2) 457	1) 67.4 2) 65.5	−2.8	1) 855 2) 792	1) 122 2) 113	1) 8.3 2) 6.0
7 ♀	4/29/46	13.2	1) 39.8 2) 38.6 3) 38.0	1) 6.62 2) 6.52 3) 6.42	1) 55 2) 60 3) 64	1) 602 2) 626	1) 45.6 2) 47.5	+4.2	1) 1031 2) 1044	1) 78.4 2) 79.0	1) 6.4 2) 4.1
8 ♀	6/10/46	9.0	1) 41.2 2) 40.8 3) 40.1	1) 6.36 2) 6.11 3) 5.91	1) 112 2) 100 3) 92	1) 640 2) 652	1) 71.0 2) 72.4	+2.0	1) 1107 2) 1113	1) 123 2) 124	1) 13.8 2) 10.2
9 ♀	3/25/46	7.7	1) 44.9 2) 43.1 3) 42.5	1) 6.62 2) 6.36 3) 6.01	1) 120 2) 112 3) 112	1) 452 2) 498	1) 59.7 2) 64.6	+8.2	1) 834 2) 900	1) 108 2) 117	1) 15.0 2) 7.0
10 ♀	6/ 4/46	8	1) 44.2 2) 44.6 3) 42.4	1) 6.47 2) 6.16 3) 6.01	1) 96 2) 96 3) 88	1) 354 2) 453	1) 44.4 2) 56.6	+27.4	1) 660 2) 827	1) 82.5 2) 103	1) 21.6 2) 4.5
11 ♀	3/27/46	8.4	1) 34.6 2) 33.1 3) 32.9	1) 7.62 2) 7.42 3) 7.32	1) 60 2) 56 3) 56	1) 720 2) 725	1) 85.5 2) 86.3	+ .94	1) 1119 2) 1097	2) 134 2) 130	1) 11.9 2) 14.4

(Continued on following page)

TABLE II (continued).

Dog No. and sex	Date	Wt, kg	Het × .96, %	Plasma protein, % (refract)	Heart rate/min.	Plasma vol, ml	Plasma vol, ml/kg	Dev. of 2nd plasma vol from 1st plasma vol, %	Blood vol, ml	Blood vol, ml/kg	Disappearance rate, %/hr
12 ♀	6/10/46	9.2	1) 46.5	1) 7.17	1) 140	1) 464	1) 50.5	+12.8	1) 920	1) 100	1) 17.9
			2) 43.5	2) 6.87	2) 136	2) 524	2) 57.0		2) 940	2) 102	2) 6.4
			3) 42.0	3) 6.47	3) 144						
	Mean	9.01	1) 40.6	1) 6.64		1) 562	1) 63.6 ±14.2 s.d.	+ 6.4 ±7.8 s.d.	1) 973.8	1) 109.8 ±20.4 s.d.	1) 12.2
			2) 39.6	2) 6.42		2) 593	2) 67.1 ±13.4 s.d.		2) 993.9	2) 112.2 ±18.3 s.d.	2) 7.9
			3) 38.5	3) 6.12							

* See footnote Table I.

obtains. Loss rates can obviously be influenced by regional distribution of blood and rates of flow as well as by "gobbling" mechanisms; and on distribution and flow we have no more information in our experiments than did Cruickshank and Whitfield. Cruickshank and Whitfield also show one test (how many others were done is not reported) with small and large injections of dye purporting to indicate that "the amount of dye taken up by the absorbing system is practically independent of the concentration of dye in plasma" (1). This question was investigated extensively in dogs by Allen and Gregersen(3) who found satisfactory agreement in volumes determined in succession first with small and then with excessively large amounts of T-1824 giving plasma concentrations in excess of 1 mg/ml.

While no one denies that the reticulo-endothelial system and other tissues take up dye, the crux of the matter is whether or not this absorption is sufficient during the "mixing period" to invalidate the extrapolation method for determining the theoretical value C_0 used in calculating plasma volume. The strongest kind of circumstantial evidence supports extrapolation as a valid method of correcting for the loss of dye (and other test substances) during mixing. Briefly this evidence in part consists of a) the experiments of Allen and Gregersen(3) and b) the repeated demonstration that in those species in which T-1824 is firmly bound to albumin this dye has the same volume distribution as various other test substances including bovine albumin, bovine globulin, the polysaccharide S III, hemoglobin, I^{131} tagged albumin(4) and dextran (5,6,7).

The decreases in hematocrit values and plasma protein concentrations shown in Tables I and II are of interest here chiefly because they accord both in direction and magnitude with the changes in plasma volume measured by successive injections of T-1824.

Conclusion. In animal species in which T-1824 is firmly bound to the plasma proteins, absorption of dye by the reticulo-endothelial system and other tissues during the "mixing period" is not a significant source of error in

measurement of plasma volume with this dye. With T-1824, as well as with other suitable test substances, back-extrapolation of the time concentration curve on a semi-log plot provides satisfactory correction for loss during the mixing period.

1. Cruickshank, E. W. H., Whitfield, I. C., *J. Physiol.*, 1945, v104, 52.

2. Gregersen, M. I., *Am. J. Med.*, 1953, v15, 785.

3. Allen, T. H., Gregersen, M. I., *Am. J. Physiol.*, 1953, v172, 377.

4. Gregersen, M. I., Rawson, R., *Physiol. Rev.*, 1959, v39, 307.

5. Semple, R. E., Sauderson, B., *Proc. Canad. Physiol. Soc. in Rev. Canada. Biol.*, 1955, v14, 285.

6. Semple, R. E., Thomsen, A. E. T., Ball, A. J., Excell, B. J., *Amer. J. Physiol.*, 1956, v187, 631.

7. Semple, R. E., Thomsen, A. E. T., Ball, A. J., *Clin. Sci.*, 1958, v17, 511.

Received July 6, 1960. P.S.E.B.M., 1960, v104.

F_{cells} Values in the Normal and Splenectomized Cat: Relation of F_{cells} to Body Size. (25968)

PATRICIA N. FARNSWORTH, CORAZON M. PAULINO-GONZALEZ* AND MAGNUS I. GREGERSEN

Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City

The purpose of the present investigation was to carry out simultaneous measurements of plasma and cell volume in normal and splenectomized cats with T-1824 and Cr^{51} to determine the F_{cells}† value in this species and to study the effect of filling and emptying of the spleen on the F_{cells} factor.

Materials and methods. Fourteen healthy male cats were selected for the studies which covered the period from Sept., 1958 to May, 1959. All cats were given anti-feline distemper serum and acromycin and kept for one month under uniform laboratory conditions before making any observations. Nine cats were then splenectomized and allowed to recuperate for 4 to 5 weeks before attempting measurements on blood volume. With one exception, 2 determinations were done on each cat and these were made not less than 10 days apart to avoid the effect of previous blood sampling on the results. It should be noted that the procedures were designed to limit blood sampling to 5 to 10 ml for a complete determination of both red cell volume and plasma volume.

Since the spectral absorption curve of T-1824 is different in the plasma of various animals(1), the dye was standardized in cat plasma. Values for optical density of a 1:500 dilution of 0.5% T-1824 (Warner, lot No. 046033) in plasma were obtained on a Beckman B spectrophotometer using 10 mm absorption cells. The peak of spectral absorption on our instrument was at 630 mu. At this wave length average optical density was 0.80 (s.d. ± 0.035). Hematocrit determinations were done by centrifugation at 3000 rpm (radius 19 cm) for 30 minutes. The trapping factor was determined by the method described by Wang(2). In 12 experiments, average value for the trapping factor was found to be 0.96 with a standard deviation of ± 0.01.

Utilizing the methods described below, a series of beaker experiments were performed on known amounts of cat blood. The overall error in these *in vitro* experiments was well under ± 2%.

On the day before each experiment, a 2 ml sample of blood was withdrawn from the cat and incubated at 37°C for 45 minutes with approximately 20 μc of Abbott's rachromate. The cells were then separated from the plasma by centrifugation and carefully washed 2 or 3 times with cold isotonic saline to remove un-

* Public Health Service Fellowship, Division of International Health, present address: University of the Philippines College of Medicine, Manila.

† $F_{cells} = \frac{\text{overall cell \%}}{\text{venous cell \%}}$.