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.

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

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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On the day before each experiment, a 2 ml sample of blood was withdrawn from the cat and incubated at 37°C for 45 minutes with approximately 20 μc of Abbott's rachromate.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-

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

bound Cr^{51} and stored in the icebox for subsequent use.

All animals were given water *ad libitum* but no food for at least 12 hours before experiment to avoid the interference of lipemia in the optical density readings of T-1824. For experiments under anesthesia, 30 mg of Nembutal per kilo of body weight were given intraperitoneally. In experiments without anesthesia the cat was placed in a supine position on a cat board and tied down as lightly as possible to prevent obstruction of the circulation as described by Hamlin and Gregersen (3). After the cats became accustomed to this routine, blood sampling generally proceeded smoothly.

The amount of T-1824 injected was measured by weighing a tuberculin syringe before and after filling with about 0.20 ml of dye. The radioactive cells which had been prepared on the preceding day were washed once again and resuspended in cold isotonic saline. Just prior to injection a calibrated 2 ml syringe was used to withdraw an aliquot sample of the labeled cells. At the same time, 3 standards were prepared by placing 1 ml aliquot samples of the cell suspension in each of 3 calibrated 25 ml volumetric flasks which were then filled to the mark with distilled water to produce hemolysis. One ml aliquots of the standards were later counted in an Atomic Associates multiscaler counter, model 1071. From the average result, number of counts per ml of cell suspension and total number of counts injected were calculated.

Using a heparinized syringe, 1.5 ml of blood was withdrawn from the femoral vein to serve as a "blank" for the cell and plasma volume determinations. The needle was left in the vein and the blank sample promptly transferred to a 4 ml hematocrit tube. The tagged cells were injected first, the dye injection following immediately thereafter and the times noted. The syringe used for radioactive cells was rinsed in 20 ml of distilled water and a 1 ml aliquot sample was counted to calculate the residual activity in the syringe. This value was subtracted from total number of counts originally in the syringe to determine the actual number of counts injected into the cat. Likewise, the dye syringe (and the needle) were withdrawn and rinsed in 10 ml of saline. The rinse was then centrifuged and the clear, blue-tinged supernatant fluid was read against saline in the Beckman B spectrophotometer at a wave length of 620 mμ to calculate amount of residual dye in the syringe. This was subtracted from the original weight of dye in the syringe to determine actual amount of dye injected.

Following the injections of labeled cells and dye, five 1.5 ml samples were drawn from a femoral vein with heparinized syringes at about 10 minute intervals and transferred to 4 ml hematocrit tubes. These were centrifuged and hematocrit values recorded and corrected, using the trapping factor of 0.96 to obtain the cell percentage for each sample. The plasma was pipetted off and centrifuged to insure a clear solution for the optical density readings which were made in micro cells against the "blank" plasma. Plasma protein concentrations in all samples were calculated from refractive index readings on the Abbe refractometer. The time concentration curve of the dye was plotted using appropriate corrections for fluid shifts as revealed by the plasma protein readings and plasma volume calculated by back extrapolation in the usual manner(4).

A very simple procedure was devised for preparation of cell samples for counting. The plasma that had been removed from the hematocrit tube was *exactly* replaced by a 1% saponin solution using a fine pipette. One ml from each sample of the hemolyzed cells was then counted and from this and from the cell percentage the counts per ml of cells can be accurately calculated. Total cell volume was determined by dividing this value into total number of counts injected.

From the determined values of plasma volume, cell volume and venous cell percentage, the following calculations were made:

$$\text{Total blood volume} = \text{Cell volume} + \text{plasma volume}$$

$$\text{Overall cell \%} = \frac{\text{Total cell volume}}{\text{Total blood volume}}$$

$$F_{cells} = \frac{\text{Overall cell \%}}{\text{Venous cell \%}}$$

Results. Table I shows the results of 2 plasma volume determinations on 9 splenec-

TABLE I. Plasma Volumes on Splenectomized Cats under Nembutal.

Cat No.	N_1*	Body wt (kg)	PV (ml)
1	2	2.93	37.8
2	2	3.23	40.5
3	2	3.25	34.8
4	2	3.38	42.1
6	2	4.19	37.7
7	2	3.30	38.4
8	2	3.06	42.4
9	2	2.84	43.9
10	2	3.66	41.4
Mean of 9 cats and S.D.			39.9 ± 2.8

* N_1, No. of observations on each animal.

tomized cats anesthetized with Nembutal. The average plasma volume per kilo body weight is 39.9 (± 3.0). In Table II, we have tabulated the results of simultaneous measurements with T-1824 and Cr^{51} on 7 of the same cats when unanesthetized. (Two of the animals had died before these tests could be completed.) In this series mean plasma volume is 36.65 ml/kg, cell volume (RCV) 11.8 ml/kg, total blood volume 50.3 ml/kg. The calculated F_{cells} values fall within a narrow range from 0.76 to 0.80 with a mean of 0.78 ± .01. It should be noted that the average disappearance rates of T-1824 in anesthetized and unanesthetized animals were 14.8 and 15.4% per hour respectively.

The upper part of Table III shows the results of simultaneous cell and plasma volume determinations from 5 normal cats (spleen intact) under Nembutal anesthesia before and after injections of adrenaline. The lower part of Table III gives results of the same tests on 3 splenectomized cats, also under Nembutal. In the former group before injection of adrenaline, F_{cells} values ranged from 0.88 to 1.06. In each instance the F_{cells} value falls markedly after adrenaline is given, while venous cell percentage is seen to rise. In the 3 anesthetized splenectomized cats, F_{cells} value ranged from 0.79 to 0.83 and these values hardly change after injection of adrenaline, nor is there any marked change in venous cell percentage.

The contrast in the effects of adrenaline on F_{cells} value and venous cell percentage in the normal and splenectomized cats is shown graphically by the typical experiments plotted in Fig. 1 and 2.

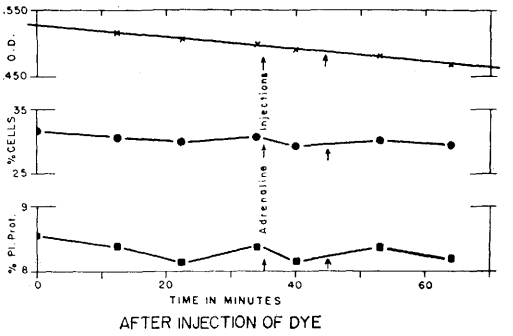

FIG. 1. Results from a typical experiment on an unanesthetized splenectomized cat showing the characteristic time-concentration curve of T-1824, and the minor variations in both venous cell percentage and plasma protein concentration. Cr^{51} counts not included in the diagram were constant within ±1.0% throughout. Note that the 2 adrenaline inj. (indicated by the arrows) had little if any effect on the variables shown.

Discussion. When using either the plasma volume-hematocrit or the cell volume-hematocrit method for "estimating" total blood volume, the F_{cells} factor $\left(\frac{\text{overall cell \%}}{\text{venous cell \%}}\right)$ must be known and included in the calculation

TABLE II. Simultaneous Determinations of Plasma Volume and Cell Volume on Splenectomized, Unanesthetized Cats.

Cat No.	N_1*	Body wt, kg	PV	RCV	TBV	Venous cell	Overall cell	F_{cells}
			ml/kg			%		
2	2	3.10	41.5	11.0	52.4	27.2	20.8	.77
3	2	3.20	32.5	11.5	44.7	32.7	25.8	.79
6	1	3.60	33.7	11.8	46.2	33.1	25.5	.77
7	2	3.09	33.6	11.5	50.6	29.8	22.8	.76
8	2	2.70	35.6	12.5	48.5	32.1	25.7	.80
9	2	2.78	43.8	12.6	57.1	28.4	22.0	.77
10	2	3.80	35.9	11.7	52.8	28.5	22.2	.78
	Mean		36.7	11.8	50.3	30.3	23.5	.78 ± .01†

* N_1, No. of observations on each cat. † Stand. dev.

TABLE III. The Effect of Adrenaline* on F_{cells} Value and Venous Cell Percentage.

Cat No.	Date	Body wt, kg	PV (ml/kg)	RCV (ml/kg)	TBV (ml/kg)	Venous cell %	Overall cell %	F_{cells}
				Normal, anesthetized† cats				
5	2/19/59	2.79	34.6	17.7	52.3	35.5	33.6	.95
						45.7	*33.6*	*.74**
11	2/18/59	3.48	44.9	16.8	61.7	25.4	26.8	1.06
						36.7	*26.8*	*.73*
13	3/ 5/59	3.23	36.9	13.7	50.6	30.7	26.9	.88
						40.3	*26.9*	*.67*
15	3/ 3/59	3.33	52.0	13.7	65.7	20.7	20.8	1.00
						33.9	*20.8*	*.61*
16	3/25/59	3.57	35.1	12.2	47.3	27.0	25.7	.95
						38.5	*25.7*	*.67*
				Splenectomized, anesthetized† cats				
3	1/16/59	3.3	33.6	12.7	46.3	31.1	25.8	.83
						32.2	*25.8*	*.80*
3	2/10/59	3.48	33.0	12.8	45.8	33.5	26.4	.79
						31.9	*26.4*	*.83*
2	2/ 4/59	3.3	39.6	13.7	53.3	31.3	25.6	.82
						32.3	*25.6*	*.79*

* Values obtained after injections of adrenaline are shown in italics. For typical spacing of injections, see Fig. 1 and 2.

† Nembutal, 30 mg/kg intraper.

to arrive at a true estimate for total blood volume(4). The present experiments show that in the splenectomized cat this F_{cells} factor is 0.78 (s.d. ± .01).

From the experimental results given in Table III it is obvious that in cats with spleen intact, as in normal dogs(5), "estimates" of blood volume from measurement of either cell volume or plasma volume alone are unreliable since the F_{cells} factor is highly variable depending on the state of the spleen.

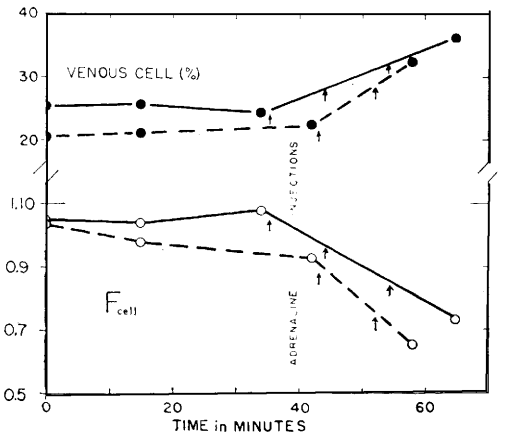

FIG. 2. Graphic presentation of effects of adrenaline inj. (arrows) on venous cell percentage and F_{cells} value in cat #11 (solid line) and cat #15 (broken line) from Table III.

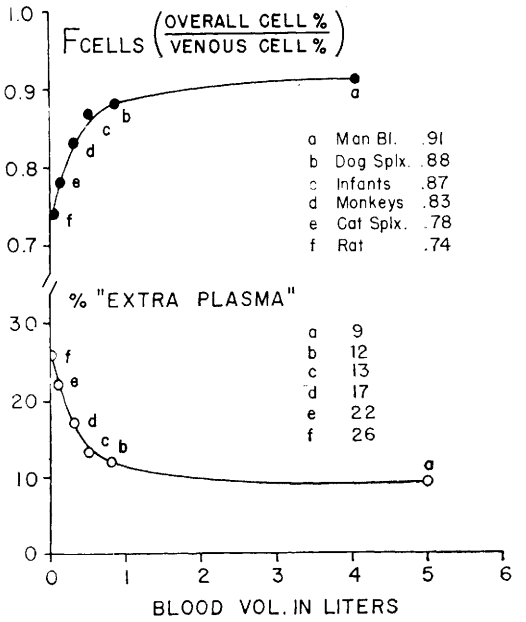

FIG. 3. Showing that the observed differences in F_{cells} values (upper curve) and "extra plasma" (lower curve) in various species are related to body size. Revised from figure published previously (Gregersen, M. I., *Chinese Med. J.*, 1959, v6, 171).

The mean F_{cells} value of 0.78 in splenectomized cats agrees with other evidence that species differences in the F_{cells} value may be fundamentally related to body size. This is

shown in Fig. 3 originally constructed from F_{cells} values from the literature tabulated by Gregersen and Rawson(4) and here revised to include the final value obtained in the splenectomized cat. It is apparent from Fig. 3 that the smaller the animal, and hence the smaller the F_{cells} value, the greater is the deviation in uncorrected "estimates" of total blood volume from the "true" or "measured" blood volume. This fact largely explains the differences between the "measured" total blood volumes on cats reported here and uncorrected "estimates" reported by Neidle and Gregersen(6) because the mean of the measured plasma volume (in ml/kg) in their study is in close agreement with ours, the two values being 41.0 ml/kg and 39.9 ml/kg respectively.

Summary. The F_{cells} factor in the splenectomized cat is 0.78 (s.d. ± .01), but with spleen intact the F_{cells} factor is highly variable as shown by the striking effects of adrenaline on the F_{cells} value in the normal anesthetized cat. Evidence is presented indicating that species differences in the F_{cells} value may be fundamentally related to body size.

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Blood Volume in Sympathectomized-Splenectomized Dogs.* (25969)

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When both plasma and cell volumes are determined simultaneously, one obtains a true measure of total blood volume and can calculate the overall cell percentage(1). The ratio of overall cell percentage/large vessel cell percentage, or F_{cells} factor, is quite constant in normal splenectomized dogs and averages 0.87 or 0.88(2-4). The most reasonable explanation for an F_{cells} factor lower than unity is that the cell percentage of the blood in small vessels is lower than that in the large arteries and veins(1). Thus, the F_{cells} value would depend upon the relative volume, as well as relative cell percentage, of blood in the 2 compartments, namely the small vessels and the large vessels. Since peripheral circulation is under the control of sympathetic nervous system, it is interesting to see if the removal of sympathetic activity would alter distribution of cells and plasma, which is reflected in the F_{cells} value. A few measurements of cell and plasma volumes in sympathectomized-splenectomized dogs were briefly reported in a previous paper dealing with the circulatory adjustment to hemorrhage(5). In the present study, 33 measurements of blood volume were made on 20 sympathectomized-splenectomized dogs. The F_{cells} factor, as well as cell volume, plasma volume, arterial cell percentage, and plasma protein concentration, are reported and compared with those in splenectomized dogs with sympathetics intact.

Material and methods. Nine male and 11 female mongrel dogs were used. Total thoracolumbar sympathectomy and splenectomy were performed as described earlier(5), at least 4 weeks before the experiments. Blood volume was measured without general anesthesia. Preparation of the animal, measurement of cell volume with Cr^{51} and plasma volume with T-1824, and determination of hematocrit and plasma protein concentration were carried out in the manner reported previously(4).

Results. The results are listed in Table I. Arterial cell percentage is calculated from the

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