

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 splenec-

tomized cat is 0.78 (s.d. $\pm .01$), 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.

1. Allen, T. H., Ochoa, M., Roth, R. F., Gregersen, M. I., *Am. J. Physiol.*, 1953, v175, 243.
2. Wang, L., *Am. J. Physiol.*, 1959, v196, 188.
3. Hamlin, E., Gregersen, M. I., *ibid.*, 1939, v125, 713.
4. Gregersen, M. I., Rawson, R. A., *Physiol. Rev.*, 1959, v39, 307.
5. Gregersen, M. I., *Am. J. Med.*, 1953, v15, 785.
6. Neidle, E., Gregersen, M. I., *Proc. Soc. Exp. Biol. and Med.*, 1960,

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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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TABLE I. Blood Volume in Sympathectomized-Splenectomized Dogs.

Dog No.	Date of last op.	Exp. date	B.W. (kg)	Art. cell %	P.P. (g %)	PV	CV	BV	F _{cells} factor	PV	CV	BV
A. Male dogs												
1	5/17/56	6/19/56	9.8	42.5	6.62	531	298	829	.838	54.2	30.4	84.6
		9/22/56	10.5	41.4	6.01	535	342	877	.915	51.0	32.6	83.5
		11/16/56	10.6	40.9	6.21	575	345	920	.917	54.2	32.5	86.8
2	7/10/56	8/ 7/56	7.6	46.6	7.32	428	277	707	.845	56.3	36.6	92.9
		9/15/56	8.2	36.0	6.47	522	245	767	.862	63.7	29.9	93.5
		12/ 3/56	8.6	38.7	6.87	538	284	822	.871	62.6	33.0	95.6
4	10/ 8/56	11/16/56	9.9	43.6	6.21	484	296	780	.871	48.9	29.9	78.8
		1/ 3/57	10.7	45.8	6.47	508	352	860	.893	47.5	32.9	80.4
7	6/11/58	7/ 9/58	11.6	45.4	6.26	559	373	932	.881	48.2	32.2	80.3
		8/ 4/58	11.7	43.0	6.31	647	391	1038	.877	55.3	33.4	88.7
8	6/17/58	7/18/58	9.7	42.1	5.86	480	292	772	.898	49.5	30.1	79.7
		3/24/59	10.0	43.8	5.66	447	277*	724	—	44.7	27.7	72.4
9	6/30/58	7/31/58	9.6	37.5	6.56	583	278	861	.861	62.3	30.6	93.0
		8/25/58	9.7	40.0	6.42	604	297	901	.825	60.8	29.0	89.8
12	7/29/58	8/27/58	10.7	41.2	6.62	682	378	1060	.867	63.7	35.3	99.1
		12/29/59	12.9	49.8	6.47	607†	469	1076	—	47.2	36.3	83.5
13	2/10/59	4/22/59	10.0	45.7	6.31	545	341	886	.848	54.5	34.1	88.6
16	3/12/59	5/29/59	10.8	41.6	6.42	673	357	1030	.832	62.3	33.0	95.4
Mean			10.1	42.5	6.39				.869	54.8	32.2	87.0
Stand. dev.			1.26	3.41	.37				.028	6.44	2.47	7.15
B. Female dogs												
3	7/26/56	8/23/56	8.1	36.8	5.61	418	196	614	.870	51.6	24.3	75.9
		11/ 4/56	7.9	43.9	7.02	423	261	689	.863	54.2	33.0	87.2
5	11/16/56	2/ 5/57	9.1	34.4	6.31	582	257	839	.892	64.0	28.2	92.2
6	1/22/57	2/24/57	8.6	41.0	6.26	482	277	759	.890	56.0	32.2	88.2
10	7/17/58	8/13/58	10.7	41.4	5.96	547	302	849	.860	51.1	28.2	79.3
		8/29/58	10.9	42.7	5.81	636	360	996	.846	58.3	33.0	91.3
		4/17/59	11.5	41.7	6.36	556	320	876	.875	48.3	27.8	76.1
11	7/15/58	8/15/58	7.2	45.5	5.35	377	223	600	.819	52.3	31.0	83.3
		11/10/58	8.5	47.4	5.76	386	260	646	.848	45.5	30.6	76.0
14	2/17/59	4/24/59	7.5	45.4	6.16	391	253	644	.866	52.1	33.8	85.9
15	3/ 3/59	5/15/59	11.5	41.3	6.21	608	328	936	.850	52.9	28.5	81.4
17	6/25/59	8/ 5/59	7.6	39.4	5.15	363	179	542	.838	47.7	23.6	71.3
18	6/29/59	8/13/59	9.4	47.1	5.30	538	375	914	.870	57.2	39.9	97.1
19	7/13/59	9/ 3/59	9.3	44.7	6.26	432†	276	708	—	46.5	29.7	76.2
20	7/21/59	11/19/59	8.1	41.9	6.26	407†	234	641	—	50.2	28.9	79.1
Mean			9.1	42.3	5.99				.861	52.5	30.2	82.7
Stand. dev.			1.46	3.61	.49				.020	4.91	4.00	7.40

* CV calculated from PV, art. cell %, and an assumed F_{cells} factor of .87(4).

† PV " " CV,

Idem

ratio of height of red cell column plus buffy coat to total sample height in the hematocrit tube, with correction for the plasma trapping factor 0.96(6). Plasma protein concentration is computed from the refractive index(7). Cell volume (CV) is calculated from the red cell volume (RCV) with the formula:

$$CV = RCV \times \frac{\text{arterial cell \%}}{\text{arterial red cell \%}}.$$

Blood volume (BV) is obtained by summing plasma volume and cell volume. The F_{cells}

factor is computed from the formula:

$$F_{\text{cells}} = \frac{CV/BV}{\text{arterial cell \%}} \times 100.$$

Discussion. 1. Comparison of male and female dogs. Average PV/kg, CV/kg, and BV/kg are all higher in the male animals (Table I). This is what one would expect from the findings in man(8). These sex differences in the present study, however, are not statistically significant ($P > 0.05$ with t test for all 3 volumes). The arterial cell percentage is al-

TABLE II. Analysis of Variance for Male Sympathectomized-Splenectomized Dogs.

Nature of measurement	Source of variation	Sum of squares	Degrees of freedom	Mean square	F-ratio	s	s _A	$\sqrt{s^2 + s_A^2}$
CV, ml/kg	Among dogs	68.67	8	8.58	2.18			
	Composite error	35.46	9	3.94				
	Total	104.13	17					
	Stand. dev.					1.98	1.53	2.51
PV, "	Among dogs	491.33	8	61.42	2.59			
	Composite error	213.69	9	23.74				
	Total	705.02	17					
	Stand. dev.					4.88	4.39	6.55
BV, "	Among dogs	662.42	8	82.80	3.60*			
	Composite error	207.28	9	23.03				
	Total	869.70	17					
	Stand. dev.					4.80	5.50	7.30
F _{cells} factor	Among dogs	.00615	8	.000781	1.03			
	Composite error	.00530	7	.000758				
	Total	.01145	15					
	Stand. dev.					.0275	.00364	.0278

* Significant at .05 level.

most identical in the 2 groups, whereas plasma protein concentration is higher ($P < 0.02$) in the male. The F_{cells} factor, 0.869 for the male and 0.861 for the female, is almost the same in the 2 groups ($P > 0.3$).

2. Analysis of variance(9). Since repeated measurements were made on several male dogs, one can analyze and study the components of variance in this group (Table II). Two possible components of variation can be isolated from these experiments. One component, which is estimated as s^2 (mean square for 'composite error' in Table II), is that resulting from repeat observations made on the same dog. It may include measurement error, dietary effects, seasonal and age changes, etc. Another component, estimated as s_A^2 , is that resulting from observations made on different dogs and reflects biological variability. If there are true differences among these male dogs as far as can be detected here, then the mean square for 'among dogs' should contain the second component of variance estimated by s_A^2 , in addition to the first component estimated by s^2 (mean square for 'composite error'). The F-ratio (mean square for 'among dogs'/mean square for 'composite error') thus tests the extent to which differences among dogs exceed the error in the same dog. The magnitude of the F-ratio for various measurements is in the order: BV/kg, PV/kg, CV/kg, and F_{cells} factor (Table II). From the F-ratio and a comparison of s and s_A (Table II), it would appear that the individual differences

for F_{cells} factor are no greater than would be expected from chance alone. For blood volume, on the other hand, these male sympathectomized-splenectomized dogs appear to exhibit true biological differences. These findings are similar to those in the rhesus monkey(10).

3. Comparison of sympathectomized with non-sympathectomized dogs. In Table III, the results for the male sympathectomized-splenectomized dogs in the present study are compared with those of 22 male splenectomized dogs subjected to the same experimental procedures(4). The sympathectomized dogs have higher averages for PV/kg, CV/kg, and BV/kg. The differences are significant at the 5% level for cell volume and blood volume, but not for plasma volume. In an earlier report on a smaller number of animals, sympathectomized-splenectomized dogs(5) were also found to have a higher blood volume per unit body weight (85.9 ml/kg) than splenectomized dogs (81.4 ml/kg). The difference in blood volume in that study, however, was entirely attributable to plasma volume (sympathectomized - splenectomized, 56.1 ml/kg; splenectomized, 50.8 ml/kg). The discrepancy between the 2 series in this aspect is difficult to explain. For one thing, the comparison in the earlier report(5) was made without regard to sex. Difference in nutritional status, seasonal variations and age may also be contributory factors. Comparison of data on the same animal before and after sympathectomy would probably be more conclusive than the

TABLE III. Comparison of Sympathectomized-Splenectomized and Splenectomized Dogs.

		B.W. (kg)	Art. cell %	P.P. (g %)	PV (ml/kg)	CV (ml/kg)	BV	F _{cells} factor
A. Splenectomized*	Mean	10.6	41.5	6.18	52.2	30.2	82.4	.88
	S.D.		2.72	.30	6.02	3.12	7.48	.020
B. Sympathectomized-splenectomized	Mean	10.1	42.5	6.39	54.8	32.2	87.0	.87
	S.D.		3.41	.37	6.44	2.47	7.15	.028
	Mean B - Mean A		+1.0	+1.18	+2.6	+2.0	+4.6	-.01
	t		1.03	1.69	1.32	2.21	1.97	1.10
	P		.3	.1	.2	.04	.05	.3

* Data taken from 22 male splenectomized dogs reported by Rawson, Chien, Peng and Dellenback(4).

comparison of population means.

Arterial cell percentage, plasma protein concentration and F_{cells} factor for the 2 groups of animals show no significant difference ($P > 0.05$, Table III). An unchanged F_{cells} value after sympathectomy would indicate that the removal of sympathetic system does not cause marked changes in distribution of cells and plasma in the large and small vessels. Although sympathectomy causes some dilatation of blood vessels and tends to increase the volume of blood in the small vessels, the result on F_{cells} factor suggests that this increase is either not very large or is accompanied by a concomitant decrease in cell percentage in these vessels.

Summary. With the use of Cr⁵¹ and T-1824, cell and plasma volumes are measured in sympathectomized-splenectomized dogs. Average cell volume, plasma volume, and blood volume are 32.2 ml/kg, 54.8 ml/kg, and 87.0 ml/kg respectively for the male dogs; and 30.2 ml/kg, 52.5 ml/kg, and 82.7 ml/kg respectively for the females. These sex differences are not statistically significant. A comparison between sympathectomized-splenectomized dogs and splenectomized dogs shows higher cell and blood volumes (significant at

the 5% level) for the former group. Analysis of variance demonstrates significant individual differences in blood volume among the male sympathectomized-splenectomized dogs. The F_{cells} factor shows neither significant individual difference nor sex difference, and is unchanged by sympathectomy.

1. Gregersen, M. I., Rawson, R. A., *Physiol. Rev.*, 1959, v39, 307.
2. Reeve, E. B., Gregersen, M. I., Allen, T. H., Sear, H., *Am. J. Physiol.*, 1953, v175, 195.
3. Reeve, E. B., Gregersen, M. I., Allen, T. H., Sear, H., Walcott, W. W., *ibid.*, 1953, v175, 204.
4. Rawson, R. A., Chien, S., Peng, M. T., Dellenback, R. J., *ibid.*, 1959, v196, 179.
5. Chien, S., *ibid.*, 1958, v193, 605.
6. Gregersen, M. I., Schiro, H., *ibid.*, 1938, v121, 284.
7. Neuhausen, B. S., Rioch, D. M., *J. Biol. Chem.*, 1923, v55, 353.
8. Allen, T. H., Peng, M. T., Chen, K. P., Huang, T. F., Chang, C., Fang, H. S., *Metabolism*, 1956, v5, 328.
9. Snedecor, G. W., *Statistical Methods*, 5th ed., Iowa State Coll. Press, Ames, Iowa, 1956.
10. Gregersen, M. I., Sear, H., Rawson, R. A., Chien, S., Saiger, G. L., *Am. J. Physiol.*, 1959, v196, 184.

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