BLOOD DESTRUCTION DURING EXERCISE.

II. Demonstration of Blood Destruction in Animals Exercised after Prolonged Confinement.

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In a previous communication[1] it was shown that a slight decrease in the total red cell volume and hemoglobin content of the organism frequently occurs during a single day of exercise in dogs previously kept under sedentary conditions. The decrease, however, is of very small proportions and not invariable, so it scarcely yields convincing proof of an increase in blood destruction under the circumstances mentioned. Observations on the effects of several consecutive days of exercise are obviously called for.

Nothing is more certain than that under the varied conditions of normal life blood formation keeps exact pace with blood destruction. If blood destruction is relatively greater during exercise, then assuredly the marrow must put forth more cells in animals habituated to exercise. Otherwise anemia would develop, and this is not observed. But, as is well known, stimulation of the marrow, even from considerable blood losses by hemorrhage, is not effective at once. Only after several days does the replacement of lost corpuscles go on actively. In the experiments to be described this circumstance has been utilized. Animals long accustomed to a sedentary life, and in which presumably little blood was being destroyed or made, were exercised vigorously with the idea that the blood-forming tissue might be "caught napping" and not at once make up such unusual corpuscle losses as exercise would entail.

Experimental Procedures.

Both the carbon monoxide and the dye methods for blood volume determination were employed.

[1] Broun, G. O., *J. Exp. Med.*, 1922, xxxvi, 481.

The technique of the dye method has already been described.[1] In that with carbon monoxide the procedure of Van Slyke and Salvesen[2] was used in the blood gas analysis. The CO readings were checked by absorbing the gas with cuprous chloride solution. The arrangement of the respiratory chamber resembled that described by Arnold and his coworkers.[3] The CO, however, was introduced directly into the base of the metal cone that closely covered the nose and mouth of the animal. The determination of residual CO in the respiratory chamber was likewise made by the method which Arnold employed.

Dogs were used, as in the preceding study, and only such as had been kept caged for several months. All were healthy adults and, with one exception, males. They were fed upon a generous mixed diet containing considerable meat. No change was made in the amount or quality of the food during the period of observation. On the days on which the animals were exercised, they were fed after its conclusion, since when food was given before or during exercise vomiting was frequent. Treadmills were employed with the tread at an angle of 20° to the horizontal. The average day of exercise consisted of two 2 hour periods of treadmill work separated by a rest period of 1 hour. The dogs worked willingly and, especially in the first few days, were very tired when released. Water was allowed in the rest interval. Each dog was given 3 to 6 consecutive days of exercise. All ate well and remained in excellent health and spirits, towards the end of the 6 days appearing to take pleasure in the exercise.

The number of preliminary blood volume determinations and the intervals between them were intentionally varied in order to rule out the possibility that the determinations themselves were responsible for such changes as occurred. The control observations vary, therefore, in number from one to five, and the preliminary observation periods from a single day to 3 weeks. The control periods of some animals overlap the exercise periods of others of the same series and hence one can be certain that no common factor of intercurrent nature was active in depressing the cell volume. It did not seem wise to use the dye and the carbon monoxide methods together, on the same individual, since the double procedure is taxing. A separate series of animals was therefore used for each type of determination. The observations with the CO method were made during the winter months, those with the dye method during the spring and summer, so no seasonal factor can be invoked to explain the parallelism in result.

In some instances a determination of blood volume was made during the days of exercise. In all cases a determination was made on the day on which exercise was discontinued. Five out of six of the animals of Series A (Table I) were kept under observation until the cell volume returned to normal levels, or went above, as sometimes happened. Only occasionally was this done with the animals of the other two series (Tables II and III).

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[2] Van Slyke, D. D., and Salvesen, H. A., *J. Biol. Chem.*, 1919, xl, 103.

[3] Arnold, H. R., Carrier, E. B., Smith, H. P., and Whipple, G. H., *Am. J. Physiol.*, 1921, lvi, 313.

The average of such cell and plasma volume determinations as were made prior to exercise furnished the "normal" in each instance. In the tables all determinations are given both as the actual number of cubic centimeters of cells or plasma and as percentages of this normal, taking the latter as 100 per cent. The percentage figures are made the basis of the graphic representation in the charts.

Results by the Carbon Monoxide Method.

The findings in the six animals of Series A are given in Table I and Text-fig. 1. In each instance the blood volume was determined by the CO method. It will be seen that all the animals showed a decrease in cell volume during the exercise period. The average decrease for the series was 19 per cent. The changes in plasma quantity were very irregular; but the CO method is known to be ill suited for plasma volume determinations,[4] so no significance pertains to the variations observed.

Results by the Dye Method.

Two series of animals were exercised, and the changes in blood volume followed by the dye method.

In Series B (Table II, Text-fig. 2) the blood volume determinations during the preliminary or control period were made in the absence of any exercise, so far as this could be avoided in animals as lively as dogs; while, with one exception (Dog 6), 18 hours of rest were allowed to elapse after exercise before the later determinations, in order that the distribution of cells, natural during rest, might reestablish itself. In Series C (Table III, Text-fig. 3) every determination was immediately preceded by exercise of at least 10 minutes duration, to secure the altered distribution of cells which brief activity brings about. As has already been shown[1] a false increase in cell volume consequent on changed cell distribution occurs during brief exercise when the determinations are made by the dye method.

In all of the animals a distinct fall in cell volume was noted. For Series B the average decrease amounted to 25 per cent; for Series C 18 per cent. The agreement with the findings by the CO method is

[4] Smith, H. P., Arnold, H. R., and Whipple, G. H., *Am. J. Physiol.*, 1921, lvi, 336.

TABLE I.

Series A. Blood Destruction during Exercise as Shown by the CO Method.

Animal.	Time.	Cell volume.		Plasma volume.		Remarks.
		Cc.	Per cent of normal average.	Cc.	Per cent of normal average.	
						Animal caged 5 mos. at the time observations were begun.
No. 0. Male mongrel; weight 16¾ kilos.	4 days before beginning exercise.	670	96	960	101	No exercise.
	1 day " " "	723	104	939	99	" " Exercise begun the next day.
						Animal exercised 4 hrs. daily for 4 days.
	After 4 days of exercise.	591	85	919	97	Blood volume determination at end of exercise period.
	19 days later.	677	97	825	87	No exercise since preceding determination.
	5 " after preceding determination.	710	102	672	71	No exercise since preceding determination.
						Animal caged 3 mos. at the time observations were begun.
No. 1. Male collie; weight 22 kilos.	6 " before beginning exercise.	1,165	94	1,090	104	No exercise.
	4 " " " "	1,270	103	975	93	" "
	The day exercise was begun.	1,276	103	1,074	103	" " before blood volume determination. Exercised 2 hrs. on this day after blood volume determination and 4 hrs. daily for the next 5 days.

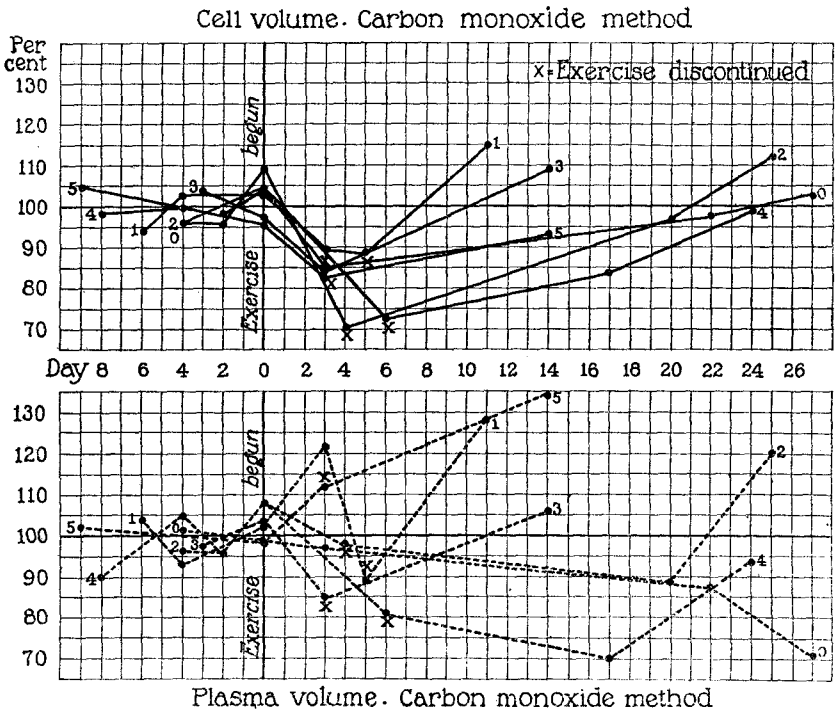
	After 4 days of exercise.	1,096	89	1,272	122	Blood volume determination at the end of exercise period.
	" 6 " " "	1,089	88	936	89	Blood volume determination at the end of exercise period.
	6 days after preceding determination.	1,425	115	1,335	128	No exercise since preceding determination.
						Animal caged 5 mos. at the time observations were begun.
No. 2. Male mongrel; weight 14½ kilos.	4 " before beginning exercise.	686	96	714	96	No exercise.
	2 " " " "	682	95	710	96	" "
	The day exercise was begun.	778	109	802	108	" " before blood volume determination. Exercised 4 hrs. on this day and 4 hrs. daily for the next 4 days.
	After 5 days of exercise.	504	70	726	98	Blood volume determination at end of exercise period.
	16 days after preceding determination.	694	97	658	89	No exercise since preceding determination.
	5 " " " "	800	112	888	120	No exercise since preceding determination.
						Animal caged 5 mos. at the time observations were begun.
No. 3. Male pointer; weight 20¾ kilos.	3 " before beginning exercise.	825	104	1,110	97	No exercise.
	The day exercise was begun.	767	96	1,180	103	" " before blood volume determination. Exercised 4 hrs. after blood volume determination and 4 hrs. daily for the next 3 days.
	After 4 days of exercise.	669	84	968	85	Blood volume determination at end of exercise period.
	11 days after preceding determination.	866	109	1,206	105	No exercise since preceding determination.

TABLE I—*Concluded.*

Animal.	Time.	Cell volume.		Plasma volume.		Remarks.
		Cc.	Per cent of normal average.	Cc.	Per cent of normal average.	
						Animal caged 5 mos. at the time observations were begun.
No. 4. Female collie; weight 23½ kilos.	10 days before beginning exercise.	1,128	98	1,084	90	No exercise.
	6 " " " "	1,148	100	1,267	105	" "
	4 " " " "	1,130	98	1,160	96	" "
	2 " " " "	1,195	104	1,305	108	" " 2 days later exercise was begun and the animal exercised 4 hrs. daily for 5 days.
	After 5 days of exercise.	834	73	978	81	Blood volume determination at end of exercise period.
	11 days after preceding determination.	960	83	840	70	No exercise since preceding determination.
	7 " " " "	1,143	99	1,127	94	No exercise since preceding determination.
						Animal caged 2 mos. at the time observations were begun.
No. 5. Male collie; weight 20 kilos.	9 " before beginning exercise.	1,081	103	1,043	102	No exercise.
	The day exercise was begun.	1,010	97	1,010	98	" " before blood volume determination. Animal exercised 4 hrs. on this day after blood volume determination and 4 hrs. daily for the next 3 days.
	After 4 days of exercise.	867	83	1,145	112	Blood volume determination at end of exercise period.
	11 days after preceding determination.	972	93	1,373	134	No exercise since preceding determination.

therefore striking. Since the factor of cell distribution was controlled as already indicated, the findings point to an actual decrease in the volume of circulating cells.

As one would expect, the plasma volume curves obtained by the dye method are much more regular than those from the CO determinations. In the majority of instances, an increase in plasma was



TEXT-FIG. 1. Series A. Blood destruction during exercise as shown by the CO method. Exercise was begun 2 days after the point indicated in Dog 4.

found at the end of the days of exercise. Such was the case in eight of the eleven experiments recorded (Tables II and III). In two animals (Nos. 8 and 9, Table II, Text-fig. 2), although a slight decrease occurred from the plasma volume noted at the beginning of exercise, the figures are still above the normal average. A distinct decrease below the normal took place only during the first experiment in Dog 7 (Table II, Text-fig. 2). This increase in plasma, occurring as it

TABLE II.

Series B. Blood Destruction during Exercise as Shown by the Dye Method (Preliminary Observations Carried Out with the Animal Quiet).

Animal.	Time.	Cell volume.		Plasma volume.		Remarks.
		Cc.	Per cent of normal average.	Cc.	Per cent of normal average.	
						Animal caged 3 mos. at the time observations were begun.
No. 7. Male pointer; weight 18 kilos.	2 days before beginning exercise.	1,003	101	1,212	95	No exercise.
	The day exercise was begun.	984	99	1,346	105	" " before blood volume determination. Exercised 4 hrs. on this day after blood volume determination and 4 hrs. daily for the next 2 days.
	After 3 days of exercise.	620	62	1,160	91	Animal rested 18 hrs. before blood volume determination.
	7 days after preceding determination.	760	77	1,300	102	No exercise since preceding determination.
						Animal caged 3 mos. at the time observations were begun.
No. 8. Male pointer; weight 14¾ kilos.	14 " before beginning exercise.	532	98	706	96	No exercise.
	4 " " " "	548	101	698	95	" "
	3 " " " "	511	94	759	103	" "
	The day exercise was begun.	577	106	795	108	" " before blood volume determination. Exercised 4 hrs. on this day after blood volume determination and 4 hrs. daily for the next 3 days.
	After 4 days of exercise.	451	83	763	103	Animal rested 18 hrs. before blood volume determination.

						Animal caged 3 mos. at the time observations were begun.
No. 9. Male collie; weight 14 kilos.	21 days before beginning exercise.	415	99	878	100	No exercise.
	18 " " " "	390	93	818	93	" "
	14 " " " "	406	97	839	96	" "
	3 " " " "	420	100	900	103	" "
	The day exercise was begun.	469	112	957	109	" " before blood volume determination. Exercised 4 hrs. on this day after blood volume determination and 4 hrs. daily for the next 4 days.
	After 5 days of exercise.	312	74	916	104	Animal rested 18 hrs. before this determination.
	3 days after preceding determination.	352	84	918	105	No exercise since preceding determination.
						Animal caged 4 mos. at the time observations were begun.
No. 6. Male pointer; weight 20½ kilos.	9 " before beginning exercise.	923	103	1,062	91	No exercise.
	6 " " " "	902	101	1,150	99	" "
	4 " " " "	849	95	1,136	98	" "
	2 " " " "	873	97	1,212	104	" "
	The day exercise was begun.	936	104	1,244	107	" " before blood volume determination. Exercised 4 hrs. on this day after blood volume determination and 4 hrs. daily for the next 4 days.
	After 5 days of exercise.	737	82	1,295	112	Blood volume determination at end of exercise period. Exercised 2 hrs. the next day. Exercise then discontinued.
	3 days after preceding determination.	911	102	1,164	100	No exercise.

TABLE III.

Series C. Blood Destruction during Exercise as Shown by the Dye Method (Preliminary Determinations Preceded by Brief Activity).

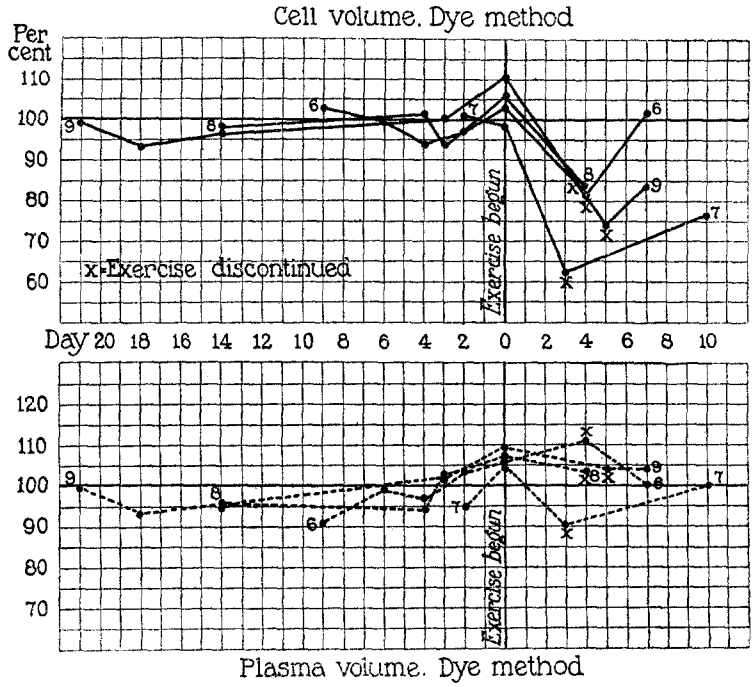
Animal.	Time.	Cell volume.		Plasma volume.		Remarks.
		Cc.	Per cent of normal average.	Cc.	Per cent of normal average.	
						Animal caged 1 mo. without exercise since observations recorded in Text-fig. 2 were made. Cell volume has returned to normal level.
No. 7. Male pointer; weight 18 kilos.	The day exercise was begun.	990	100	1,133	100	10 min. exercise before blood volume determination. Animal exercised 4 hrs. on this day after blood volume determination and 4 hrs. daily for the next 5 days.
	After 2 days of exercise.	960	97	1,330	117	Blood volume determination at end of exercise period.
	" 6 " " "	811	82	1,177	104	Blood volume determination at end of exercise period. Animal rested 1 day. Exercised 1 hr. daily on the 2 following days.
	3 days later.	820	83	1,290	114	1 hr. of exercise before blood volume determination. Animal rested 1 day. Exercised 1 hr. daily for 3 days and again rested 1 day.
	6 days after last determination.	926	94	1,279	113	10 min. exercise before blood volume determination.

						Animal caged 4 mos. at the time observations were begun.
No. 10. Male mongrel; weight 14 kilos.	The day exercise was begun.	618	100	762	100	10 min. exercise before blood volume determination. Animal exercised 4 hrs. on this day after blood volume determination and 4 hrs. daily for the next 2 days.
	After 1 day of exercise.	589	95	785	103	No exercise before this blood volume determination.
	" 2 days " "	490	79	795	104	4 hrs. exercise before this blood volume determination.
	" 3 " " "	483	78	793	104	15 min. exercise before this blood volume determination.
						Animal caged 2 mos. at the time observations were begun.
No. 11. Male setter; weight 19½ kilos.	6 days before beginning exercise.	1,025	105	990	100	10 min. exercise before blood volume determination. Otherwise no exercise.
	4 " " " "	940	96	1,032	105	10 min. exercise before blood volume determination. Otherwise no exercise.
	The day exercise was begun.	970	99	935	95	10 min. exercise before blood volume determination. Animal exercised 4 hrs. on this day after blood volume determination and 4 hrs. daily for the next 5 days.
	After 4 days of exercise.	754	77	998	101	4 hrs. of exercise before blood volume determination.
	" 6 " " "	755	77	992	101	4 hrs. of exercise before blood volume determination.
						Animal rested 1 day and then exercised 1 hr. daily for 3 days.
	4 days after preceding determination.	804	82	1,038	105	10 min. exercise before blood volume determination. Otherwise no exercise.

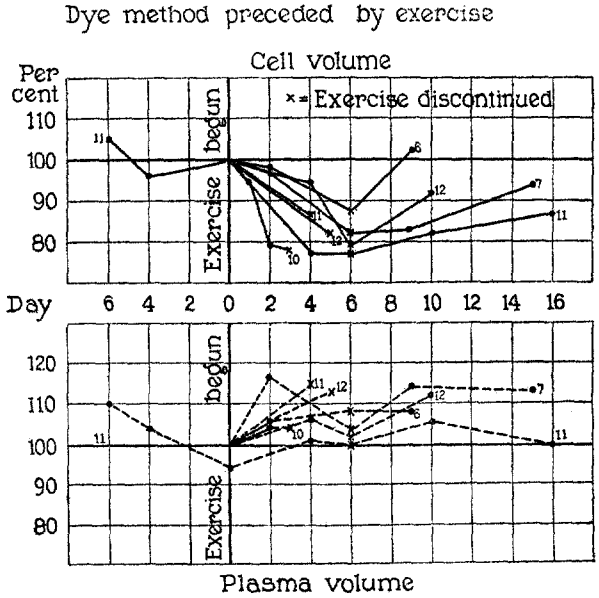
TABLE III—*Continued.*

Animal.	Time.	Cell volume.		Plasma volume.		Remarks.
		Cc.	Per cent of normal average.	Cc.	Per cent of normal average	
No. 11—*continued.*	6 days after preceding determination.	848	87	990	100	10 min. exercise before blood volume determination. Otherwise no exercise.
						Animal caged 40 days without exercise since observations noted above.
No. 11. Male setter; weight 19½ kilos.	The day exercise was begun.	863	100	918	100	10 min. exercise before blood volume determination. Animal exercised 4 hrs. on this day after blood volume determination and 4 hrs. daily for the next 3 days.
	After 4 days of exercise.	740	86	1,052	115	10 min. exercise before blood volume determination.
						Animal caged 5 mos. at the time observations were begun.
No. 12. Male mongrel; weight 18½ kilos.	The day exercise was begun.	940	100	912	100	10 min. exercise before blood volume determination. Animal exercised 4 hrs. on this day after blood volume determination and 4 hrs. daily for the next 5 days.
	After 4 days of exercise.	887	94	968	106	4 hrs. exercise before blood volume determination.
	" 6 " " "	740	79	935	102	4 hrs. exercise before blood volume determination. Animal rested 1 day and then exercised 1 hr. daily for 2 days.

	4 days after preceding determination.	866	92	1,024	112	15 min. exercise before blood volume determination.
						Animal caged 45 days without exercise since observations noted above. Animal has lost weight and has smaller blood volume.
No. 12. Male mongrel; weight 16½ kilos.	The day exercise was begun.	760	100	848	100	10 min. exercise before blood volume determination. Animal exercised 4 hrs. on this day after blood volume determination and 4 hrs. daily for the next 4 days.
	After 5 days of exercise.	624	82	948	113	Blood volume determination made after 4 hrs. of exercise.
						Animal exercised 6 days during preceding week. Rested a day and a half. Cell volume has returned to normal level (see No. 6, Text-fig. 2 and Table II).
No. 6. Male pointer; weight 20¼ kilos.	The day exercise was begun.	911	100	1,164	100	10 min. exercise before blood volume determination. Animal exercised 4 hrs. on this day after blood volume determination and 4 hrs. daily for the next 5 days.
	After 2 days of exercise.	891	98	1,233	106	Blood volume determination at end of exercise period.
	" 6 " " "	797	87	1,253	108	Blood volume determination at end of exercise period.
						Exercise discontinued.
	3 days later.	930	102	1,262	108	1 hr. of exercise before blood volume determination.



TEXT-FIG. 2. Series B. Blood destruction during exercise as shown by the dye method. The preliminary observations were carried out with the animal quiet.



TEXT-FIG. 3. Series C. Blood destruction during exercise as shown by the dye method. The preliminary determinations were preceded by brief activity.

did simultaneously with a decrease in cell volume, may represent an effort to maintain a constant total blood volume. On the other hand, evidence has already been given[1] that as little as several hours of exercise on a single day cause an increase in plasma. It is significant that the three determinations that show a decrease in plasma to have been present at the end of the exercise were made, not immediately thereafter, but when 18 hours of rest had elapsed.

The Period of Recovery.

The time required for the cell volume to return to normal levels after the depression due to exercise is subject to wide variations. All but one of the animals of Series A (Table I, Text-fig. 1) were kept in their cages until recovery was complete. Dog 1 recovered completely during the 1st week after exercise was discontinued; No. 3 in the course of the 2nd week; while Nos. 0, 2, and 4 did not reach normal levels until nearly 3 weeks had elapsed.

Nos. 1, 2, and 3 later developed a well marked plethora; No. 1 yielding the highest cell volume per kilo that the writer has encountered in supposedly normal dogs. No less than three blood volume determinations were made on the animal at this time, two by the CO method and one by the dye method. All gave practically the same results. Only the first of these three determinations is recorded in Text-fig. 1 and Table I. The others find place in a subsequent paper.[5] Dog 5 of the series was utilized for other purposes after the 11th day following exercise. At this time his cell volume was 93 per cent of the normal.

In Series B (Table II, Text-fig. 2) and Series C (Table III, Text-fig. 3) the course of recovery was seldom followed to its close. Dog 6 recovered within 3 days after the initial period of exercise (Table II, Text-fig. 2). Further exercise during the following week (Table III, Text-fig. 3) caused a second decrease in cell volume, repaired by the animal with equal rapidity. No. 7 (Table II, Text-fig. 2) showed but slight tendency to recovery 1 week after the first exercise period, but 1 month later the cell volume was normal (Table III, Text-fig. 3). A second period of exercise by this animal was followed

[5] Broun, G. O., *J. Exp. Med.*, 1923, xxxvii (in press).

by a more rapid recovery,—the cell volume coming up to 94 per cent in the 1st week. The cell volume of Dog 11 (Table III, Text-fig. 3) reached 87 per cent of the normal within 10 days after the 1st week of exercise. 1 hour of exercise was given on 3 days of this interval. 40 days later the cell bulk was still far below the normal, as is seen from the preliminary determinations for the second experiment. No. 12 (Table III, Text-fig. 3) under similar conditions had, within 4 days after the first exercise period, a cell volume that amounted to 92 per cent of the normal. A month and a half later a very small cell volume was found, one practically of the same proportions noted at the end of the first exercise period. The body weight had decreased by 2 kilos but the animal seemed in excellent health and spirits and remained in good condition for several months thereafter. A second period of exercise caused the cell volume of both Dogs 11 and 12 to fall below the new level that had been established (Table III, Text-fig. 3).

Control of the Nutritive Factor.

As has already been stated, food in plenty was provided and the amount taken was frequently weighed. The appetite of the animals

TABLE IV.

Control of the Nutritive Factor.

Animal.	Time.	Cell volume.		Plasma volume.		Remarks.
		Cc.	Per cent of normal average.	Cc.	Per cent of normal average.	
No. 25. Female collie; weight 22½ kilos.	The day observation was begun.	1,148	100	1,026	100	
						Food withheld 4 days.
	4 days later.	1,144	100	1,028	100	
No. 26. Male pointer; weight 17 kilos.	The day observation was begun.	855	100	1,038	100	
						Food withheld 4 days.
	4 days later.	872	102	1,061	102	

Determinations were made by the CO method.

in no instance suffered from the exercise, yet as a control to possible underfeeding during the exercise period, the following experiment was performed.

Food was entirely withheld from two animals during a period of 4 days. Blood volume determinations were made at the beginning and end of this period.

The results are given in Table IV. It will be seen that no decrease in cell volume occurred.

DISCUSSION.

The fact cannot be too clearly emphasized that the decreases in circulating hemoglobin and total cell volume which were caused by the exercise employed in the present work occurred in animals previously kept in confinement for several months. Experiments, which will be described in detail in a later communication,[5] show that in dogs allowed liberty or previously exercised during a considerable period of time, the cell volume either fails to decrease when exercise is given according to the method described or shows but slight decrease.

Most of the literature on the effect of exercise on blood has to do with the changes occurring in the course of a single day of exercise. It has already been considered.[1] Schneider and Havens[6] followed the red count and hemoglobin on three athletes at intervals during a period of training, and noted slight increases in both at the end of the period. The blood volume was followed on a single subject, several observations being made, before the training period and after training had been in progress for more than 3 weeks. No marked changes occurred. These findings are not contradictory to the ones here reported. The athlete studied had doubtless led an active life prior to training. Moreover, in the space of 3 weeks of training the hematopoietic system may completely adapt itself to the demands of exercise. Feigl[7] has shown that after strenuous marches many individuals show traces of hemoglobin and hematin in the blood serum and urine—evidence of injury to the blood.

Whipple[8] has reported that carbon monoxide is to some extent taken up by the myohematin of the muscles. It may be asked whether

[6] Schneider, E. C., and Havens, L. C., *Am. J. Physiol.*, 1914–15, xxxvi, 239.
[7] Feigl, J., *Biochem. Z.*, 1916, lxxvi, 88.
[8] Whipple, G. H., *The Harvey Lectures*, 1922 (in press).

the changes in cell volume shown by the CO method are referable in any part to this factor. For this to be so a marked decrease in myohematin must occur during exercise. But similar changes in cell volume are demonstrable with the dye method and the dye employed in the work, vital red, is not taken up by the respiratory pigments. It follows that the changes in cell volume are dependent on actual changes in the amount of circulating cells. It is possible that the high volume figures found by the CO method during the period of recovery may be due in some small part to an increase in myohematin consequent on hypertrophy of the muscles, though there is abundant precedent for these figures in the plethora that ordinarily follows recovery from the experimental anemias.

SUMMARY.

A great decrease in total circulating hemoglobin and red cell volume occurs in dogs long maintained under sedentary conditions when they are exercised vigorously during several consecutive days. This would appear to be consequent on increased blood destruction, unrepaired for the time being.