SOME BLOOD CHANGES IN THE DOG DURING COMPLETE BLOOD STAGNATION:

A STUDY IN CAPILLARY AND ERYTHROCYTE PERMEABILITY

by

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INTRODUCTION

The blood as the main media of transfer of ionic substances to and from the tissues is normally in constant movement through the vessels. Any interruption of this flow may be followed by a change of concentration of these ions in the blood. Thus the ionic concentration of the blood substances, normally very carefully regulated, may be altered considerably; however, there is a limit beyond which life cannot exist. These changes appear to be closely related to the length of time of the flow interruption. The official first aid treatment for an injured limb allows the application of a complete tourniquet for 20 to 30 minutes (1). In surgery, such as aortic grafts, the blood flow to the lower half of the body may be stopped for 20 to 60 minutes without lethal effects (2). During wars limbs have been lost simply because a tourniquet had been applied too long (3). There are numerous diseases that produce a slow or limited circulation and others that allow pooling of blood in various areas of the body. The blood cells move sluggishly and pool in some of the larger blood vessels (4,5). In the standing position there is a tendency for the blood to pool in the legs and at the same time filter into intercellular spaces with the result that the volume of blood returning to the heart is decreased below that of the reclining position. In the case of greatly impaired venous return, the blood may pool in large quantities in the overdistended and weak varicose veins. This

condition may be produced by sitting on ill-fitting chairs when the edge of the chair acts as a partial tourniquet. Crossing the legs may also produce the same effect.

The literature citations of blood studies during blood stagnation are very few. For this reason they are included in this introductory section. Isolated studies have been made of the effect of various periods of blood flow stoppage on a few of the blood constituents. One of the first changes that may take place is an increased capillary filtration of fluid from the plasma (6 - 11). This results in a hemoconcentration of the solid elements and the dissolved molecules of large size (7, 9, 12). In a more complete or longer period of stagnation a small amount of plasma protein may also move through the capillary into the tissue (8). This is primarily albumin (13).

The sodium and potassium content of the blood changes, depending on the type of blood stagnation - arterial, venous or complete. Baetjer (14) by reducing the volume of blood in the limb of cats 80 per cent by hemorrhage found no increase in the potassium of the remaining blood but after a 90 per cent reduction, a 60 per cent increase in the potassium concentration was observed. Dennis and Moore (15) reported an increase of 13.3 mg. % (50 per cent increase) during 5 - 9 minutes of myocardial ischemia of the cat and dog following ligation of both the coronary arteries and veins but no change after ligation of the coronary veins and not the arteries. Farber et al.(19) similarly reported that there was no change in venous plasma potassium during venous stasis of two minutes duration in the

human. Laufman et al. (16) reported that during a 50 per cent restriction of the inferior vena cava of dogs there was an increase of sodium and potassium in the serum. In a special type of partial venous stagnation in humans, congestive heart failure, the values of both sodium and potassium in the plasma increased (17, 18).

Berry et al. (7) are joined by other workers in reporting an increase in serum protein, hemoglobin (20), specific gravity (21, 22) and packed cell volume (20, 23) in the blood of congested vessels. Landis et al. (8) had previously reported that a 80 mm. Hg. compression will produce a 1.5 per cent loss of proteins from the plasma.

The acidity of stagnant blood in the cat and dog increased during a combination of arterial and venous ligation. During only venous stagnation for 5-9 minutes no change occurred as reported by Moore and Greenberg (24). Similarly, during asphyxia, the blood decreased (23).

The blood sugar increased in man during the beginning of venous stagnation but then later decreased, whereas, during the use of an arterial tourniquet for five minutes, it changed very slightly (25).

Reports on the carbon dioxide and oxygen changes in the blood during stagnation could not be found. In the case of asphyxia the oxygen saturation decreases and carbon dioxide saturation increases (23).

Water and salt move from the blood plasma into the tissues in venous stasis thus reducing the chloride concentration (6,26,27).

At the same time there is a gain of chloride ion in the erythrocyte.

Many of the above reports deal with only one or two factors. Thus sodium and potassium, which are generally inversely related, should not be studied singly. "It is important to consider the pathology of the blood as a whole " (28). In many cases pathology of the cell may influence the plasma and vice versa. In some cases, the alteration of two components may be concomitant, while in other cases the alteration of one may produce an alteration of the other (28). In most cases either the total blood or plasma was studied individually so that only the general movement in or out of the blood stream was detected. Little is known about the exchange of ions between the blood cells and the plasma during stagnation.

There has been much variation in the types of blood stasis used in previous work, which could be either partial or complete, of either the arteries, or the veins, or both. The type of stasis employed makes a considerable difference in the final blood composition (7, 8, 24, 29).

The purpose of this work was to show a more complete picture of the changes that take place between the blood cells and the plasma and between the blood and the tissue during complete stagnation of blood for about one-half hour in the hind leg of the dog.

The terms stagnation, stasis, stoppage, ligation, compression, and ischemia have all been used by previous authors to indicate a partial or complete interference with the blood flow and will be used frequently in this paper.

METHODS

A total of 14 mongrel dogs weighing between 30-60 pounds each, in a post-absorptive state, were subjected to 20-30 minutes of tourniquet application on the upper part of the thigh close to the hips. Most of these animals were used the second time, 2-4 weeks after their first experimental run thus giving a total of 26 experimental tests. The contralateral leg was used for the second test.

The TQ (abbreviation for tourniquet to be used hereafter in this report) was a simple strand or two of flexible 8 mm. rubber tubing stretched and wrapped very tightly around the thigh and held together by a strong hemostatic forceps. The TQ was supported on the leg by an attendant who kept constant watch that it did not loosen or slip down the tapered leg. There were cases where the animals were especially quiet so that a specially built table platform could be used which supported and maintained the TQ in the upper thigh area. Even in these cases someone was in the immediate vicinity to keep close watch on the TQ. In two cases (animals 620 and 79) the TQ broke in the middle of the experimental period but was re-adjusted within 30 seconds. This was followed by 20 minutes of complete stagnation.

Some dogs rarely moved their legs while others moved them quite frequently; some appeared annoyed and others in definite discomfort. In two or three cases the TQ was removed after 20

minutes because of extreme annoyance. In general, the animals
were quiet and calm. They had been cared for daily by the
experimenter for a period of 2-6 months previous to the experi-
ment and had become very responsive to his voice so that a few
gentle words from him would reassure them. This is the factor,
in many cases, that made it possible to perform this experiment
without anesthesia.

Constant checks were made for the signs of complete stag-
nation -- considerable cooling of the skin and absence of a
pulse. There is a possibility that when the dogs moved their
legs a very small quantity of blood was forced through the
arteries.

At the end of the period just prior to TQ removal, 20 ml.
of blood were withdrawn, distal to the TQ, from a superficial
vein which was quite distended and tender by that time. A few
times the TQ was removed for one second and with one swift
stroke of the hand, the vein was massaged in the direction of
the heart. After the TQ was reapplied the sample was withdrawn.
This procedure was tried in an attempt to get pooled capillary
blood which has been subjected to a longer period of ionic
exchange in the tissues.

A control sample was drawn from the contralateral leg
30-60 minutes before the TQ was established. This was the
length of time necessary to begin the analysis of this blood.

In some of the runs a sample was drawn from the contra-
lateral leg immediately before the TQ release.

The samples were withdrawn into a 20 ml. syringe sealed

with grease. The syringe contained 3-4 drops of heparin which
was the equivalent of 5 mgm. One part of the sample was im-
mediately injected under oil through a long needle into a test
tube, and another part of the sample was placed into a centri-
fuge tube and centrifuged for 30 minutes at approximately
3500 rpm.

Twelve factors were studied during each of the first few
experiments. Since considerable time was required to make
these tests even after very careful planning it was feared that
blood changes may take place which would alter the in vivo
picture. For this reason the factors were divided into two
groups, and only one group of factors was tested on each
experimental run.

One group consisted of:

hematocrit	total leucocyte count
sugar	differential count
chloride	pH
erythrocyte count	specific gravity

The second group included:

hematocrit	carbon dioxide content
erythrocyte count	sodium
oxygen content	potassium

The hematocrit was determined by two methods: Wintrobe
and Van Allen. (30). The values from the Wintrobe method were
used as the packed cell volume, except in a few cases where
there was insufficient blood, at which time the Van Allen method
was used.

The erythrocyte and leucocyte counts were made on the
hemocytometer using the standard methods (30). For differential
leucocyte studies Wrights stain was used. The lymphocytes and

monocytes were counted as mononuclear cells.

The sodium and potassium were analyzed by the Barclay flame spectrophotometer using the internal standard (31). The amounts of these ions in both whole blood and plasma were determined. The blood and plasma were diluted by lithium sulfate and water as soon as possible after withdrawal from the dog to prevent erroneous results from shifts of ions between the plasma and cells. Sometimes the diluted sample was refrigerated for a day prior to analysis.

The amount of sodium and potassium in the erythrocyte was calculated from the plasma and blood values on the basis of the hematocrit reading.

The chloride ion in plasma and whole blood was analyzed by the method of Schales and Schales as outlined by Hawk, Oser and Summerson (32) and Simmons and Gentzkow (33), respectively. The amount of chloride ion in the erythrocyte was calculated from the measured values in the whole blood and plasma on the basis of the hematocrit. Duplicate samples which were run frequently gave similar results.

The sugar content of whole blood was analyzed by the method of Somogyi-Shaffer-Hartmann, as outlined by Hawk, Oser and Summerson (32). Frequent duplicate samples revealed similar values. Generally two standards were determined and the average value used.

The carbon dioxide and oxygen contents of the blood were analyzed with Van Slyke manometric gas analysis apparatus by the method reported by Hawk, Oser and Summerson (32). The oxygen

absorbent used was the special oxygen reagent containing potassium ferricyanide.

The pH was determined almost immediately after withdrawal from the vein. Sometimes the sample was first placed under oil and other times it was immediately placed into the analysis chamber from the needle of the syringe. The Coleman electrometer was used.

The specific gravities of the whole blood and the plasma was determined by the copper sulfate method of Phillips et al. (34).

Numerous preliminary analyses were made for each factor studied on known solutions as well as duplicate blood samples to assure accuracy.

All animals were generally observed during and after the experiment for edema and any signs of shock or impaired functions.

Hematocrit. The packed cell volume was determined on
every animal. The mean value for 26 animals before TQ applica-
tion was 40.1 compared with 43.2 after TQ application. (Table 1).
This is a mean increase of 20.2 per cent. Only one of the
animals, a pregnant female, showed a decrease in hematocrit.

Blood was drawn from the contralateral leg before the TQ
was removed. In five cases the hematocrit had not changed or
it changed only slightly from the normal control value. In
two cases the hematocrit value was about half-way between the
control and experimental value.

The Wintrobe and Van Allen micro methods were used for the
hematocrit determination. The Van Allen method gave slightly
less packed cell values but there was not a consistent decrease.
Since any inaccuracies are amplified when working with micro-
methods, the readings from the Wintrobe tubes were used generally,
with a few exceptions, where only small quantities of blood were
available.

Erythrocytes. The number of erythrocytes per cu. mm. of
blood increased in every case but one during TQ application. This
one case was a post-parturition dog (Table 2, animal 626). It is
suspected that if a count would have been made on this same
animal when it was used the first time for an experimental run
while pregnant, a decrease in the erythrocyte count would also
have been revealed since there was a decrease in the hematocrit

Table 1.   Hematocrit   Changes

Animal[1]	Before TQ.	After TQ.	Change	Per Cent Change
59	33.5	44	10.5	37.3
515	38.6	39.2	0.6	1.5
520	43.7	46.5	2.8	6.4
523	43.9	49.7	5.8	13.2
54	43.3	55.4	12.1	27.9
55[2]	23.5	19.0	-4.5	-19.1
56	42.5	58.0	15.5	35.5
511	42.5	48.0	5.5	12.9
512	44.0	51.2	7.2	16.4
611	44.6	49	4.4	9.9
617	39.5	43.5	4.0	10.1
618	39.5	46.5	7.0	17.7
620	45.5	54.5	9.0	19.8
623	49	50	1.0	2.0
624	36	55.5	19.5	54.2
626	28	31.0	3.0	10.7
627	39.5	54.4	14.9	37.7
79	40	48	8.0	20.0
710	46.5	52.5	6.0	12.9
715	47.5	58.5	11.0	23.2
716	41.5	53.5	12.0	28.9
718	44	49.5	5.5	12.5
722	37.5	43.5	6.0	16.0
723	45.2	57.7	12.5	27.7
729	32.3	39.3	7.0	21.7
730	32.2	54.4	19.2	54.5
SX	10438	12523		505.5
x̄	40.1	48.2		19.4

N=26

[1] The animals had been numbered originally by the date that they were used and the same numbers were maintained here for ready reference to these data sheets.

[2] This is a pregnant dog about 11 days before parturition.

(Table 1, animal 65). In general, however, the erythrocyte count rose from a mean 5.71 million (range 4.65-7.2) in normal blood to 7.14 million (range 3.95-10.04) after TQ application (Table 2). The mean per cent of increase was 24.8.

The size of the erythrocyte seems to diminish during stagnation as suggested by the difference between the per cent of increase of the erythrocyte count of 24.8 and the hematocrit increase of 19.4 per cent. In order that the cell volume remain the same these values should increase in equal amounts. Also the mean corpuscular volume reveals a decrease in 12 out of 16 cases. The mean normal value obtained here was 74.7 cu. microns while during stagnation it dropped to 67.1 cu. microns. This does not include animal 626, the post-parturition animal, and animal 624 both of whom had a large increase of the M. C. V. Statistical analysis of the difference reveals a t-value of 3.60 which indicates significance at the 1 per cent level (35).

There was no uniformity of total cell count on samples taken from the contralateral leg. In three cases there was a definite increase; in another case, an increase equal to the experimental value; and in another case, no change from the normal control value.

Leucocytes. The total number of leucocytes in one cu. mm. of stagnant blood generally decreased during tourniquet application. This decrease has a mean value of 5.8 per cent (calculated from columns 1 and 2 of Table 3). The t-test, however, indicated that this is a non-significant difference (35). There was an increase in 6 out of 18 animals. Since an increased

ERYTHROCYTE COUNT CHANGES

Animal	Before TQ Million Per cu. mm.	After TQ Million Per cu. mm.	Change Million Per cu. mm.	Percent Change
59	4.84	6.76	1.92	39.7
520	5.73	6.19	0.46	8.0
523	6.54	8.25	1.71	26.1
66	6.18	9.23	3.05	49.4
612	6.71	8.24	1.53	22.8
617	5.10	6.01	0.91	17.8
618	4.82	5.97	1.15	23.9
624	6.00	7.04	1.04	17.3
626*1	4.60	3.95	-0.65	-14.1
79	5.55	6.54	0.99	17.8
710	7.06	8.16	1.10	15.6
718	5.87	6.82	0.95	16.2
722	5.06	6.31	1.25	24.7
723	7.23	10.04	2.81	38.9
729	5.30	6.67	1.37	25.8
730	4.78	7.98	3.20	56.9
SX	91.37	114.16	22.78	396.9
X̄	5.71	7.135	1.42	24.8

N= 16   *1   11 days post-parturition

hematocrit and erythrocyte count revealed a hemoconcentration
the leucocyte count was expected to increase in proportion.
Expected leucocyte counts based on both the hematocrit and the
erythrocyte counts were calculated and the difference between
these expected counts and the actual counts after tourniquet
application have been determined (Table 3).

The difference between the expected count based on the
hematocrit increase and the actual count after tourniquet applica-
tion revealed a mean loss of 4182 leucocytes per cu. mm. of
stagnant blood. This is a mean loss of 23.9 per cent of the
expected value. When the increase of erythrocyte count was
used to indicate hemoconcentration instead of the hematocrit
there was a mean loss of 3359 leucocytes per cu. mm. This is
20.1 per cent of the expected leucocytes.

The type of leucocytes lost was predominantly neutrophilic,
as revealed by a mean differential count decrease of 6.1 cells
per hundred (Table 4). This, however, does not account for the
total loss of leucocytes, therefore, there must have been also
a loss of some of the remaining types. The monocytes decreased
in number the least of the cells. The mean number of neutrophils,
eosinophils and mononuclear cells found in normal control blood
was 67.5, 7.6 and 24.8, respectively.

Sodium and Potassium. Sodium and potassium tests were made
on both whole blood and plasma. On the basis of the packed
cell volume the amount of these electrolytes was calculated in
the erythrocyte. Thus a change of concentration of these ions
would indicate exchange between the erythrocyte and the plasma,

LEUCOCYTE COUNT CHANGES

Animal	Before TQ	After TQ	Expected Count Based On Change of Hematocrit	Difference Between Expected and Actual Count	Expected Count Based on Change of Erythrocytes	Difference Between Expected and Actual Count
59	8700	9200	11,423	− 2223	12,154	− 2954
520	17225	14325	18327	− 4002	18,603	− 4278
66	11750	7600	15921	− 8321	17,554	− 9954
612	18375	17150	21389	− 4239	22,565	− 5415
617	13775	13400	15166	− 1766	16,227	− 2827
613	11475	8525	13506	− 4981	14,218	− 5693
624	13775	17475	21241	− 3766	14,883	+ 2592
626*1	11925	15525	13200	+ 2325	13,606	+ 1919
627	19850	15137	27333	−12196		
79	10300	12950	12360	+ 590	12,133	+ 817
710	11350	11662	12814	− 1152	13,121	− 1459
715	25900	19100	31909	−12809		
716	8625	5650	11123	− 5473		
718	20550	19850	23119	− 4269	23,880	− 5030
722	12330	18266	14303	+ 3963	15,376	+ 2890
723	13500	12825	17240	− 4415	18,752	− 5927
729	11900	9600			14,970	− 5370
730	13325	12560	20587	−20587	22,239	− 9689
SX	25463	23979	300961	79648	250261	53780
X̄	14146	13326	17703.5	− 4685	16,685	− 3359
Range	17275	13450				
S.D.	4356	3934				

t.=0.932 for column 2 and 3 ═ This is non significant at the 5 percent level.

*1 post parturition animal

Animal	Neutrophil Change Before	After	Eosinophil Change Before	After	Mononuclear Cell Before	After Change		Total Leucocyte Before	After
7 -16	57	61 +4	17	6 - 11	25	33 + 8		3,625	5,650
7 -15	31	30 - 1	2	3 + 1	17	17 0		25,900	19,100
7 -10	64	54 -10	4	3 - 1	32	43 +10		11,350	11,662
7 -23	64	47 - 17	15	17 + 2	21	36 +15		13,500	12,825
7 -22	53	49 - 4	8	11 + 3	39	40 + 1		12,330	18,266
7 -13	77	67 - 10	5	3 - 2	13	30 +12		20,550	18,850
7 -29	66	62 - 4	6	11 + 5	28	27 - 1		11,900	9,600
7 -30	78	71 - 7	4	1 - 3	13	23 +10		13,325	12,550
SX	540	491 - 49	61	55 - 6	198	254 55		117479	108503
X̄	67.5	61.4- 6.1	7.6	6.9-0.75	24.8	31.8+6.9		14685	13,562
range	24	31							
S.D.	10.2	12.1							

t = 2.687 for the neutrophil count changes. This is significant at the 5 per cent level.

and also the plasma and the extravascular area. By determining
the concentration of the erythrocyte and plasma separately on
the basis of hematocrit changes it was possible to determine
whether any shift of ions was due simply to the movement of
fluid from the blood taking with it its normal concentration of
sodium or potassium, or whether the actual concentration of the
remaining blood had changed. The measured values were sodium
and potassium in whole blood and in plasma and the hematocrit
reading. The remaining values are calculated (Table 5).

There was a decrease of both sodium and potassium concen-
tration in the total blood and in the plasma. The values for
sodium in the blood dropped 3.0 per cent from the control value
of 128.4 to 124.6 mEq.; and in the plasma, 3.0 per cent from the
control value of 147.5 to 143.2 mEq. (Tables 5 and 6). The
value for potassium in the blood dropped 33 per cent from 7.3
to 4.9 mEq.; in the plasma, 10 per cent, from 6.7 to 6.0 mEq.;
in the blood cells, 80 per cent from an expected 6.2 to 3.4
mEq. In the normal dog the quantity of potassium found in the
erythrocytes is almost one and one half times that found in the
plasma (Table 7). This ratio decreased during stagnation of
blood. The quantity of sodium in the cell is normally about
nine times the quantity of potassium in the cell. This value
increased during stagnation of blood. The quantity of sodium
in the plasma is normally about 24 times greater than the potassium
in the plasma. This increased slightly during stagnation. The
quantity of sodium in the plasma is normally about two times
greater than the sodium in the erythrocyte. This decreased

Table 5 Sodium Changes in the Blood

Animal	Blood Sodium		Plasma Sodium		Sodium in Plasma of 1000 cc. of Blood		Expected Sodium in Plasma of 1000 cc. of blood after TQ.
	before	after	before	after	before	after	
	MEQ.	MEQ.	MEQ.	MEQ.	MEQ.	MEQ.	MEQ.
55	121.9	125.0	143.7	150			
	126.5	125.0	134.4	132.8			
520	128.1	123	140.6	140.6	79.2	75.2	75.2
523	125.0	121	133.1	143.7	74.7	72.3	66.9
64	129.1	120.3	141.7	139.1	80.3	62.0	63.2
613	137.5	125.0	146.9	145.3	181.4	74.0	74.9
620	182.8	178.2	192.8	190.6	105.1	86.7	37.7
710	125.0	114.1	151.6	127.7	81.1	60.7	72.0
624	120.3	115.6	142.1	146.9	90.9	65.4	63.2
626*	103.0	120.3	137.5	134.4	99.0	92.7	94.9
627	120.3	111.0	137.5	125.0	83.2	57.0	62.7
715	139.1	118.0	165.6	146.9	86.9	61.0	68.7
716	137.5	126.6	159.4	146.9	93.2	68.0	74.1
x̄	128.4	124.6	147.5	143.2	85.7	68.5	70.9

*[1] post parturition animal – has not been averaged in with the other results.

Change of Na. conc.in Plasma of 1000 cc. of Stagnant Blood	Na. in Cells in 1000cc. of Whole Blood before after		Expected Na. in Cells of 1000 cc. of Blood	Change of Na.conc.in Cells of 1000cc. of Blood	Total Change of Na. from 1000 cc. of Blood Remaining at the end of TQ application
MEQ.	MEQ.	MEQ.	MEQ.	MEQ.	MEQ.
0	48.9	47.8	52.0	-4.2	-1.2
5.4	50.3	48.7	56.9	- 8.2	- 2.8
- 1.2	47.8	77.1	61.1	18.0	14.8
- 0.9	56.1	51.0	61.7	-10.7	-11.6
- 1.0	77.7	91.5	93.1	- 1.6	- 2.6
-11.3	43.9	53.5	49.5	4.0	- 7.3
2.2	29.4	50.2	45.3	4.9	7.1
- 2.2	4.0	27.6	4.4	23.2	21.0
- 5.7	37.1	54.0	51.1	2.9	- 2.8
- 7.7	52.2	57.0	64.3	- 7.3	-15.0
- 6.1	44.3	58.6	56.7	1.9	- 4.2
———	———	———	———	———	———
- 2.54	48.0	56.6	59.2	- 0.2	-2.86

Table 6. Potassium Changes in the Blood

Animal	Blood Potassium		Plasma Potassium		Potassium in plasma of 1000 cc. of blood		Expected Potassium in plasma of 1000 cc. of blood after TQ
	Before	After	Before	After	Before	After	
	mEq.	mEq.	mEq.	mEq.	mEq.	mEq.	mEq.
55	9	6.5	4.1	4.7			
	9	4.0	12.0	8.0			
520	5	5.5	8.9	5.4	5.0	2.9	4.8
523	5	3.5	6.1	4.2	3.4	2.1	3.1
64	5	3.3	6.0	4.5	3.4	2.0	2.7
613	8.5	3.2	7.5	2.4	4.2	1.2	3.8
620	13	11.0	8.9	8.3	4.9	3.8	4.1
710	7	3.5	6.8	4.6	3.6	2.2	3.2
624	6.5	4.5	5.4	5.5	3.5	2.4	2.4
626[1]	6.5	6.0	5.3	6.7	3.7	4.6	3.7
627	7.5	4.0	5.8	7.2	3.5	3.3	2.6
715	5.8	4.7	4.7	7.4	2.5	3.1	2.0
716	6.5	3.7	6.0	6.5	3.5	3.0	2.8
X	7.3	4.9	6.7	6.0	3.7	2.8	3.2

[1] Post-parturition animal

Change of K conc. in plasma of 1000 cc. of stagnant blood	K in cells in 1000 cc. of whole blood		Expected K in cells of 1000 cc. of blood	Change of K conc.in cells of 1000 cc. of blood	Total loss of K from 1000 cc.of blood remaining at the end of TQ application
	Before	After			
mEq.	mEq.	mEq.	mEq.	mEq.	mEq.
- 1.9	2.5	4.0	2.7	+ 1.3	- 0.6
- 1.0	3.3	2.4	3.7	-1.3	- 2.3
- 0.7	3.3	2.3	4.2	- 2.0	- 2.7
- 2.6	6.4	2.6	7.0	- 4.4	- 7.0
- 0.3	10.6	9.1	12.7	- 3.6	- 3.9
- 1.0	5.2	2.4	5.8	- 3.4	- 4.4
0	4.7	3.3	7.3	- 4.0	- 4.0
+ 0.9	4.6	3.7	5.1	- 1.4	- 0.5
+ 0.7	5.7	2.4	7.9	- 5.5	- 4.8
+ 1.1	4.5	3.2	5.6	- 2.4	- 1.3
+ 0.2	4.8	2.3	6.1	- 3.8	- 3.6
- 0.4	5.0	3.4	6.2	- 2.8	- 3.2

during stagnation. The amount of sodium contained in the cells found in 1000 ml. of blood increased from 44.4 mEq. in normal blood to 56.1 mEq in stagnant blood. At the same time, there was an increase in the concentration of cells in the blood during stagnation. Therefore the total amount of sodium contained in the cells of a unit volume of blood was expected to increase in proportion. In order to determine whether there was actually a change in concentration of the sodium, the per cent increase of the cells must be multiplied by their original sodium quantity to determine the expected sodium content of the cells. The difference between this value and the actual value will reveal the amount gained or lost by the cells. In six animals there was a gain; in five, a loss of sodium from the cells. (Table 5). These individual cases revealed considerable variation indicating that sodium could move either in or out of the cell. There was a slight statistically non-significant overall increase of sodium in the cell.

In the same manner that the actual loss of sodium from the cell was determined, the potassium change was also calculated. In ten animals there was a loss and in one animal a gain in the potassium content of the blood cells during the stagnation of blood by a complete tourniquet (Table 5, 6).

The plasma volume, being inversely proportional to the packed cell volume, likewise changed during stagnation (Table 1) thus requiring calculations similar to the ones above to determine any change in the actual concentration of sodium and potassium in the plasma of a unit volume of blood. The sodium and potassium

contained 1000 ml. of normal plasma were each multiplied by
the per cent of plasma contained in the stagnant blood to deter-
mine the expected amounts of sodium and potassium on the basis
that the actual concentration does not change. This expected
value is compared with the actual amount of these ions measured
and a gain or loss determined. In eight animals there was a
loss, in two animals a gain, and in one animal no change of the
plasma sodium. For plasma potassium there were six animals
with a loss, four with a gain and one animal with no change during
a half hour period of complete stagnation. When these separate
changes of electrolytes in the plasma and the cells were averaged
the mean loss of sodium from stagnant blood was 5.9 mEq. per
1000 ml. of blood.

Table 7. Changes in the Ratio of Sodium and Potassium in the
          Blood

			Before	After
Ratio	K	in plasma to	0.730	.723
	K	in erythrocyte		
contained in 1 cc. of whole blood				
Ratio	K	in erythrocyte to	0.112	0.060
	Na	" "		
Ratio	K	in plasma to	0.042	0.040
	Na	" "		
Ratio	Na in plasma		1.955	1.256
	Na in erythrocyte			

Chloride Content. The chloride content of the blood was
measured as sodium chloride. The blood remaining in the vessels

after TQ application contained 16.6 mg.% less sodium chloride than the normal blood. This was largely at the expense of the plasma. At the same time the sodium chloride contained in the cells found in 100 ml. of blood increased 64.8 per cent from 57.9 to 98.4 mg.% (Table 8). To determine whether this increase was due to a change in packed cell volume alone or also to a change in the concentration of sodium chloride in the cells, the expected amount of sodium chloride based on the hematocrit increase was calculated. This was compared with the existing value which showed an increase of 29.9 mg. of sodium chloride in the erythrocytes contained in 100 ml. of blood (Table 8). Likewise, the expected plasma sodium chloride value was calculated on the basis of the plasma volume change during stagnation. A slight loss in concentration in the plasma of 4.8 mg. per 100 cc. of blood was revealed. When these original concentrations of sodium chloride in the blood cells and in the plasma were compared with the expected value based on a change in the amounts of blood cells and plasma found per unit volume of blood, an overall increase of 25.1 mg. of sodium chloride per 100 ml. of blood was found. This was due to a 43 per cent increase of sodium chloride in the blood cells.

Sugar. The sugar contents of the blood decreased during one-half hour of complete stagnation by a mean 15.2 mg.%. This was a drop from the mean for normal blood of 56.7 mg.% to the mean of 41.5 mg.% for stagnant blood. This was a decrease of 24.2 % from the normal value. The range in normal blood was 42.8-65. During stagnation it was 21.6-58 mg.%.

Table 8 Sodium Chloride Changes in the Blood

Animal	Plasma NaCl before	after	Whole Blood before	after	NaCl in plasma contained in 100 ml. of BEFORE	AFTER	NaCl in cells in 100 ml of blood before	after
	mg.%	mg.%	mg.%	mg.%	mg.%	mg.%	mg.%	mg.%
59	597.6	572.0	420.2	366.4	397.4	320.3	22.8	46.1
515	562.3	623.0	408.9	407.2	349.6	384.4	59.3	22.8
520	595.1	579.8	434.0	408.9	335.0	310.2	99.0	98.7
66	620.5	628.0	439.4	385.5	356.8	263.8	82.6	121.8
612	635.8	597.6	439.4	426.3	356.0	289.8	83.4	136.5
617	640.8	600.1	430.7	428.0	394.1	339.1	36.6	88.9
619	623.0	607.8	414.0	395.9	376.9	325.2	37.2	70.7
624	615.4	597.6	415.9	399.3	393.9	265.9	22.0	133.4
626*1	549.3	532.3	443.7	435.0	395.5	401.8	48.2	33.2
79	590.0	599.0	403.7	405.4	354.0	311.5	49.7	94.0
710	628.1	584.9	426.3	413.3	336.0	277.8	90.3	135.4
713	590.0	574.7	406.3	394.1	330.4	290.2	75.9	103.9
722	633.2	656.1	436.7	445.4	395.7	370.7	41.0	74.7
723	602.7	610.3	415.9	399.3	330.3	258.2	85.6	141.1
730	577.3	595.1	398.5	381.9	373.1	271.4	25.4	110.5
X̄	607.8	601.5	420.8	404.2	362.8	306.0	57.9	98.4

N=15
*1 post parturition dog- has not been averaged in with other figures

Expected NaCl in cells in 100ml. of bl.	Change in NaCl in total cells not accounted for by increase in no.of cells	Expected NaCl in plasma of 100ml. bl. after TR.	Change in NaCl in plasma of 100ml.of remaining blood	Total change from expected value of NaCl in 100ml.of stagnant bl.
MG.%	MG.%	MG.%	MG.%	MG.%
29.9	+ 16.2	334.6	− 14.3	+ 1.9
60.2	− 37.4	346.1	+ 38.3	+ 0.9
99.6	− 0.9	323.4	− 5.7	− 6.6
111.9	+ 9.9	279.4	− 15.6	− 5.7
97.1	+ 39.4	310.1	− 20.3	+ 19.1
40.3	+ 48.6	368.1	− 29.0	+ 19.6
43.8	+ 26.9	333.2	− 8.0	+ 18.9
33.9	+ 99.5	273.8	− 7.9	+ 91.6
53.4	− 20.2	373.9	+ 22.9	+ 2.7
59.6	+ 34.4	306.9	+ 4.6	+ 39.0
101.0	+ 34.4	304.1	− 26.3	+ 8.1
85.4	+ 18.5	298.0	− 7.8	+ 10.7
47.6	+ 27.1	357.7	+ 13.0	+ 40.1
109.3	+ 31.8	255.0	+ 3.2	+ 34.0
39.2	+ 71.3	262.7	+ 8.7	+ 80.0
68.5	+ 29.9	310.9	− 4.8	+ 25.1

"Because of the free diffusibility of glucose between the erythrocyte and plasma, distinction between the analysis of whole blood and plasma is relatively unimportant" (32). For this reason only the whole blood sugar has been determined. However, even though the free diffusion theory seems to be generally accepted (36) LeFevre (37) reported that some sugars, primarily trioses will inhibit the free movement of others.

Carbon Dioxide and Oxygen Content. The carbon dioxide content of the stagnant blood increased by a mean value of 2.6 volumes per cent. However, there were 2 cases out of 16 with a very slight decrease of the carbon dioxide content. The range of change was -0.9 to $5.4 volumes per cent.

The oxygen content fell from a control mean of 14.1 volumes per cent to 10.4 volumes per cent. At the same time the packed cell volume increased, thus increasing the oxygen carrying capacity (Table 10). The expected amount of oxygen on the basis of hematocrit increase was 17.1 volumes per cent. This was 6.7 volumes per cent above the actual value after stagnation. Therefore, the actual value fell 64 per cent below the expected value. The range of change in oxygen concentration was from -0.4 to -12.8 volumes per cent.

In four cases blood was drawn from the contralateral leg before the IQ was released. The oxygen and carbon dioxide contents were found to vary only slightly from the original control sample.

The carbon dioxide combining capacity was determined in a few cases merely to get a general idea of any change. There was

Table 9.   Sugar Changes in Stagnation

Animal	Before mg.%	After mg.%	Difference mg.%
515	44.6	40.8	- 3.8
520	49.0	32.1	-16.9
66	63.2	50.6	-12.6
612	55.1	40.6	-14.5
617	42.5	33.4	- 9.1
618	62.0	35.2	-26.8
624	66.8	28.1	-38.7
626	64.6	44.2	-20.4
79	62	58	- 4.0
710	51.2	50.2	- 1.0
718	46.3	37.1	- 9.8
722	61.5	46.8	-14.7
723	56.2	53.3	- 2.9
729	65.0	51.2	-13.8
730	61.0	21.6	-39.4
$\bar{x}$	56.7	41.5	-15.2

Table 10 Carbon Dioxide and Oxygen Changes in the Blood

Animal	CO_2 Content		O_2 Content		CO_2 Change
	before	after	before	after	
	VOL.%	VOL.%	VOL.%	VOL.%	VOL.%
520	23.4	27.6	14.7	11.5	+ 4.2
64	20.7	23.9	15.1	12.9	+ 3.2
611	25.2	27.0	15.4	12.6	+ 1.8
613	24.2	26.6	15.8	14.1	+ 2.4
620	28.3	27.4	9.1	10.5	− 0.9
623	21.8	21.5	18.5	17.9	− 0.6
626	26.7	31.6	9.3	3.5	+ 4.9
627	27.6	31.4	13.0	5.1	+ 3.8
710	21.1	22.2	18.2	17.8	+ 1.1
715	28.8	30.4	7.6	1.7	+ 1.6
716	28.2	31.9	16.5	8.9	+ 3.7
718	28.1	29.4	15.8	11.0	+ 1.3
722	27.0	32.4	12.2	7.8	+ 5.4
723	26.0	27.7	16.2	15.0	+ 1.7
729	30.0	33.8	13.2		+ 3.8
730	30.8	34.7	14.2	5.3	+ 3.9
$\bar{x}$	26.1	28.7	14.1	10.4	+ 2.6
S. D.	3.1	3.9	3.2	4.9	

t = 2.985   t = 5.061

O_2 Change	Percent that new packed cell volume is of the old	Expected O_2 Content	Change in O_2 conc. in RBC. in 100 cc. of blood
VOL.%	per cent	VOL.%	VOL.%
- 3.2	106.4	15.6	- 4.1
- 3.2	127.9	20.6	- 7.7
- 2.8	112.9	17.4	- 4.8
- 1.7	109.9	17.4	- 3.3
+ 1.4	120.0	10.9	- 0.4
- 0.6	102.0	18.9	- 1.0
- 5.8	110.7	10.3	- 6.8
- 8.1	137.7	17.9	-12.8
- 1.2	112.9	20.5	- 2.7
- 5.9	123.2	9.4	- 7.7
-7.6	128.9	21.3	-12.4
- 3.6	112.5	16.9	- 5.9
- 4.4	116.0	14.2	- 6.4
- 1.2	127.7	20.7	- 5.7
—	—	—	—
- 8.9	168.9	24.0	-18.7
= 3.7		17.1	- 6.7

a definite decrease during stagnation. Insufficient tests
were made to give statistical validity.

Acidity. The pH of the blood changed from a mean pH 7.34
to 7.19 during stagnation. This was a mean decrease of pH 0.16
(range -0.11 to -0.57). There was only one animal with an
increase of pH (Table 11).

Specific Gravity. The relative specific gravity of blood
increased from a mean of 1.050 to 1.0543 (Table 12). Every
animal but one showed increase. In this one case the specific
gravity remained the same after as before stagnation.

The mean specific gravity of plasma increased from 1.023
to 1.028 during stagnation. The major factor causing plasma
specific gravity changes is considered to be proteins. Accord-
ing to the work of Phillips et al. (34) the protein concentration
can be calculated from the blood and plasma specific gravities.
Using their method the plasma protein concentration for 10
control samples was 5.9 gm.% which changes to 7.6 gm.% after
stagnation. This was a 29 per cent increase of the plasma
protein. The hemoglobin content was calculated on the same
basis which was 12.3 gm.% both before and after stagnation.
Since there was no change in the hemoglobin concentration of the
erythrocyte the specific gravity changes of the blood must have
been due solely to the plasma protein increase and erythrocyte
count increase.

There is some question as to the accuracy of these cal-
culated figures since the hematocrit value calculated by this
method varied considerably from the actual experimental value.

Table 11 pH Change of Blood

Animal	Before	After	Difference
59	7.34	7.31	− 0.03
65	7.34		
66	7.34	7.22	− 0.12
612	7.38	7.20	− 0.18
617	7.30	7.20	− 0.10
618	7.37	7.18	− 0.19
624	7.42	7.53	+ 0.11
626	7.45	7.25	− 0.20
79	7.25	7.15	− 0.10
710	7.40	7.30	− 0.10
718	7.32	7.28	− 0.14
722	7.17	6.84	− 0.33
723	7.3	7.26	− 0.04
729	7.38	7.25	− 0.13
730	7.31	6.74	− 0.57
$\overline{X}$	7.34	7.19	− 0.15

N = 15

Changes in the Specific Gravity of Whole Blood and Plasma with
Calculated Protein and Hemoglobin values[1]

Animal	Blood		Plasma		Protein in gm. %		Hemoglobin in gm. %	
	before	after	before	after	before	after	before	after
59	1.052	1.0565						
520	1.054	1.054						
66	1.0515	1.0555						
612	1.056	1.063						
617	1.050	1.0515						
618	1.051	1.054						
624	1.047	1.0545						
626	1.0465	1.050	1.023	1.033	7.6	9.7	9.1	9.0
627	1.0515	1.054	1.0245	1.0335	6.5	9.9	12.5	11.9
79	1.051	1.053	1.0235	1.027	6.1	7.4	12.7	12.6
710	1.0515	1.054	1.022	1.024	5.5	6.3	13.3	14.0
715	1.0545	1.0575						
716	1.0515	1.0545	1.0235	1.0275	6.1	7.6	12.9	13.2
718	1.049	1.0515	1.0235	1.027	6.1	7.4	11.8	11.8
722	1.048	1.0515	1.020	1.023	4.7	5.9	12.3	13.0
723	1.0515	1.054	1.020	1.025	4.7	6.7	13.9	13.7
729	1.044	1.045						
730	1.048	1.0545	1.024	1.035				
$\bar{X}$	1.050	1.0543	1.023	1.028	5.8	7.6	12.3	12.3

*1 for method see reference (32)

In four dogs, blood was drawn from the contralateral leg just before the TQ was released at the end of the experimental period. The blood and plasma specific gravities were determined and found to vary only slightly from the normal control sample taken before the experimental period.

General. There was an increased respiration shortly after TQ application which continued throughout the experimental period.

Toward the end of this period and for some time thereafter, a few of the animals developed noticeable edema which resulted from a blockage of the lymph channels (38). A general hyperemia (39, 40, 41, 42, 43) with enlarging of the capillaries up to five times their resting size (44) may be partly responsible.

Analysis of the sample of blood from the experimental leg taken at the end of 30 minutes after a release of the TQ for one second revealed less change than in the sample taken just before TQ release (See Methods). This was an attempt to get capillary blood rather than venous blood but apparently the sample contained arterial blood freshly supplied to the capillaries. For this reason all the experimental samples designated as "after stagnation" were taken from the distended vein before the TQ removal.

A few pressure tests by the cannulation-and-mercury-manometer method were made on the femoral artery upon the application of a TQ. The pressure immediately began a steady decrease from a normal of 90 mm. until after about one and one-half to two minutes when it reached a steady state at a pressure of about 40 mm. of Hg. A few times it dropped lower to about 26 mm.

Once this steady state was reached there seemed to be little alteration up to about eight minutes, beyond which time no tests were made.

DISCUSSION

<u>Hematocrit</u>. Numerous calculated results reported in
this study are based on the change of the hematocrit read-
ings during stagnation as an index of hemoconcentration.
Ashworth and Tigertt (21) have found this to be an accurate
measure of the hemoconcentration. Swingle <u>et al.</u> (20) also
used the hematocrit as a gauge of circulating plasma volume
changes and found it compared favorably with the dye-injection
method. However, Fine <u>et al.</u> (12) believe that the hematocrit
change cannot be used to calculate the change in plasma volume.
Landis and Jonas (8) reported that various degrees of venous
compression produced fluid loss in proportion to the duration
and degree of compression. Perlow (9) reported that a defin-
ite increase in the hemoconcentration existed in the blood
distal to the TQ during venous occlusion but later when the
animal was in TQ shock small hemorrhages developed allowing
the escape of erythrocytes through the capillary wall.

The hemoconcentration that develops during general
asphyxia may be due largely to a release of blood cells from
the spleen, and to a small extent, to the increase of erythro-
cyte size from the entrance of plasma fluid, and not from a
loss of fluid from the blood stream (45). However, this
suggested change in the size of the erythrocyte is contrary
to results of this present work reported under <u>Erythrocytes</u>
(also see Figure 1).

In this present study the hematocrit increase from
40.1 to 48.2 is very similar to the report of Swingle et al.
(20) of an increase from 38.3 to 48.8 in 5 hours of TQ ap-
plication. Similarly Scott and Robbins (46) reported an
average increase from 39.3 to 56.2 during an average of 5
hours of TQ application to the leg of dogs. The greatest
hemoconcentration results in the first 1-2 hours of TQ appli-
cation. However, 6, 8, and 11 hours after the release of the
TQ the hematocrit rose still higher, up to 71. Damage had
been inflicted on the capillaries during the ischemia; at
the same time, the acid metabolites in the capillary had
increased causing increased permeability to fluids, which
passed through the wall very rapidly when the full force of
the arterial blood pressure was released into them (47).
The capillaries forming the kidney glomeruli also showed in-
creased permeability to fluid during the application of a
limb TQ (48). Berry et al. (7) reported that a partial venous
TQ for 5 minutes to the human arm will produce a 6 per cent
increase of the hematocrit.

Herber (23) reported a very rapid increase from 39.4
to 50.5 in 10 minutes of partial asphyxia of the trachea of
the dog. Here, of course, the picture is complicated by re-
leased cells from the spleen.

The mean normal hematocrit value of 40.1 is within the
range of mean values for dogs reported by other workers. Some
of these values are 38.3 from Swingle et al. (20), 39.3 from
Scott and Robbins (46), 39.4 from Herber (23), 44.28 from

Coffin (49), and 44.5 from Albritton (50).

The pregnant dog had a low hematocrit before the TQ application which decreased slightly during the experimental procedure. This was the only animal which showed a decrease which suggests that there is some tie-up with pregnancy.

However, for this there is no clear explanation. Numerous hypotheses may be suggested to explain the decrease: First, the capillary permeability may have been increased allowing the erythrocyte to pass into the intercellular spaces; second, the erythrocyte fragility may have been increased allowing excessive destruction of the cells; and third, the fluid balance may have been reversed by the movement of proteins or other substances so that fluid was drawn into the blood from the intercellular spaces.

In most cases as determined in the contralateral limb before TQ release there was no general change of packed cell volume in the general body blood. There were, however, a few that did change for which there is no clear explanation. Numerous possibilities include: (1) a reflex constriction of the vascular tree of the contralateral leg, (2) a general loss of fluid from the blood, and (3) a leakage of blood past the tourniquet limb into general circulation.

Erythrocytes. The erythrocyte count revealed a general increase slightly greater than the hematocrit increase. (Figure 1). This suggests a definite decrease in the cell size. In addition the mean corpuscular volume decrease also indicates a general diminution of the erythrocyte size. This decrease

might be accomplished by the movement of fluid from the cell into the plasma. If, at the same time, the sodium and chloride ion did nøt move freely with the fluid across the cell membrane, the concentration of each in the cell would increase. This may partly explain the increase of sodium and chloride ion observed in this present study. However, the increase of these ions in the cell may be accomplished by movement from the plasma as during the normal "chloride shift". Since sugar possibly leaves the cell when the plasma sugar concentration decreases (see section on Sugar under Results) a decrease of cell size might be expected. A reduction of cell size has been reported with a decrease of glucose in the cell (37). A reduction of oxygen may also decrease the erythrocyte size (51).

The hemoglobin may also be expected to increase in concentration but no such change was demonstrated. One investigator, Herber (23), suggested that an increase in the hematocrit of the dog during asphyxia was partly, two per cent, due to the shifting of water from the plasma to the cell. This is contrary to the findings during ꝙ stagnation in the present study.

Generally speaking among the animals there is an inverse relationship between the size of the circulating erythrocyte in the peripheral blood and their number per unit volume of blood (52).

In the one case of an erythrocyte count decrease, the post-parturition animal, there was an increase in hematocrit

which indicated an increase in the cell size. At the same
time there was a considerable increase in the plasma protein
concentration. It is difficult to suggest the mechanism of
this shift. It may be possible that some of the endocrine
changes associated with pregnancy altered the erythrocyte
permeability. Estrone, for example, increased the amount
of extracellular fluid in the tissues around the genitalia
of the monkey according to the report of Eric Ponder (53).
In toxemia of pregnancy the erythrocyte M.C.V. increased (54).
It is commonly observed that pregnancy and also anemia (55)
sped up sedimentation of these cells. Some maintain that it
is due to the alteration of the plasma chemistry (56). Others
suggest that this is produced by a change in the cell sur-
face or membrane of the erythrocyte. The cells in this
post-parturition dog did settle out very rapidly - in about
10 minutes. This change of the cell surface may also effect
permeability. Thus an increase in the erythrocyte perme-
ability may have allowed the fluid to move into the cell as
well as through the capillary wall. At the same time some
chloride ion^s moved into the cell along with the fluid but
not enough to maintain normal concentration, so the concen-
tration of the chloride ion in the cell decreased slightly.
The plasma chloride increased some through an influx from
the intercellular space. Sodium content was very low in the
red blood cells but increased greatly. However, it still was
much less in this post-parturition dog than in other animals
of this series. The sodium in the plasma also was low but

increased. This means that the sodium comes from the inter-cellular fluid. At the same time the number of red blood cells decreased. This could not have been from a dilution of the blood because the plasma proteins increase consider-ably in concentration; therefore, the red blood cells must have been lost from the capillary while sodium, chloride ions, plasma proteins and white blood cells were retained since in this case there was an increase in the number of leuco-cytes per cu. mm. of blood.

The mean normal erythrocyte count of 5.7 million in this present study is very similar to the report of 5.5 million by Malkmus (57) and falls within the range of mean values (4.5 - 8.0 million) reported by other investigators (50, 52, 57-60).

No definite significance is drawn from the irregular erythrocyte counts found in the contralateral sample.

Leucocytes. The leucocyte has been studied infrequent-ly as a part of the blood picture change during stagnation Massey (61) reported an increase of leucocytes in disturb-ances of coronary circulation. So, in general, the loss of leucocytes from the blood stream reported in this study may be unexpected (Figure 2). It is well known, however, that the leucocyte possesses the ability to "crawl" (53) and is ameboid (62) in its ability to change its shape so that it may pass through the small openings between individual cells in the wall of the capillary. There is a substance called leukotaxine, a dialyzable polypeptid crystallized from

inflammatory exudates by Menkin (65) which appears to stimulate chemotaxis of the leucocytes, drawing it from the capillaries at the site of bacterial infection. At such areas there may be a great accumulation of leucocytes outside of the blood stream. In the present study numerous factors reported such as carbon dioxide increase, oxygen decrease and pH decrease (Table 10, 11) are the very factors mainly responsible for the dilatation of the capillary, at which time the pores between the cells would be the largest (53, 64). The ameboid leucocytes were the types that showed the greatest decrease in number. This was mainly the neutrophils and eosinophils with the greatest decrease among the neutrophils.

Since no attempt has been made here to demonstrate the presence of increased numbers of leucocytes in the lymph and intercellular fluid the possibility may not be overlooked, that some of the cells were destroyed in the blood stream, which may account for the increase in the plasma protein. Gamma globulin is known to be released from lymphocytes upon their dissolution (65, 66). This is a normal process stimulated by the adrenal cortical hormone. No leucocytic fragments were observed during the differential study.

The mean total leucocyte count of 14,146 for normal dogs reported in this present work is very similar to the report of 14,180 by Coffin (49). The values found by three other investigators range from 9,000 to 12,600 (50, 60, 67).

The normal mean per cent of neutrophils, eosinophils and mononuclear cells of 67.5, 7.6 and 24.8 is very similar

to the averages reported by Albritton (30) of 68, 5.1 and
26.2 respectively. These mean values lie also within the
range reported by other investigators (58, 60, 67).

The only detected correlation between the total and the
differential pattern was the high beginning neutrophil count
in the cases of high total leucocyte count.

Sodium and Potassium. The metabolism of sodium and
potassium has been given much attention recently. Normally
both cations move through the cell wall of the erythrocyte[1]
in such amounts that a concentration gradient exists with the
surrounding plasma under normal conditions. However, the
dog erythrocyte seems to be the least permeable of the com-
mon animals tested (68). Rates of transfer were measured
for numerous animal and human erythrocytes (68,-72). These
rates vary from one animal to another. In the rat erythro-
cyte the potassium exchange is fairly rapid amounting to
about 5 per cent of the total amount contained in the cells
per hour which is about $3\frac{1}{2}$ times the rate for the human
erythrocyte (68, 72). The cat erythrocyte exchanges 45 and
the frog 1.4 per cent of potassium per hour (101). The move-
ment of sodium into the human erythrocyte in vitro is almost
double the potassium exchange (70). This movement may be

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[1]The leucocyte is also permeable to cations (73) but
since the total leucocyte volume is so relatively small any
ionic change that may occur in it is included in with the
erythrocyte in this discussion.

FIGURE 1. PACKED CELL PLASMA
AND PROTEIN CHANGES

PC–PACKED CELL VOLUME
E –EXPECTED VALUE BASED ON PC. INCREASE
EE–EXPECTED VALUE BASED ON R.B.C.
        COUNT INCREASE
A –AFTER 30 MIN. OF TQ.
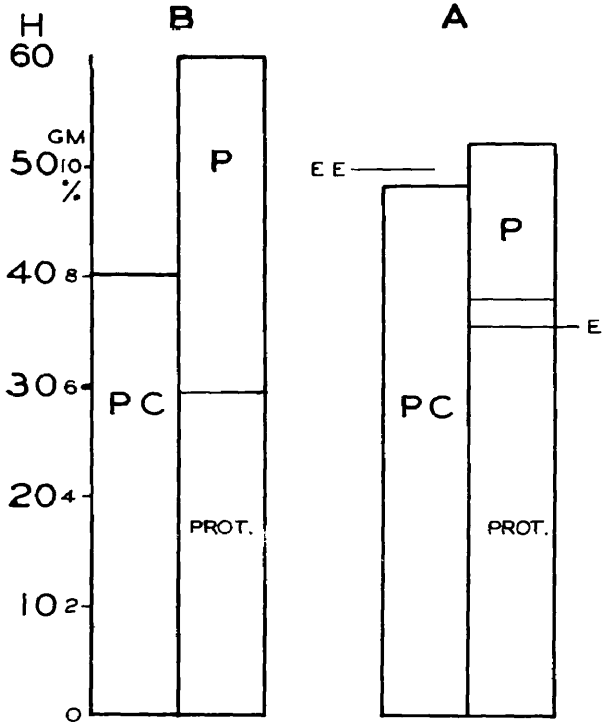B –BEFORE STAGNATION
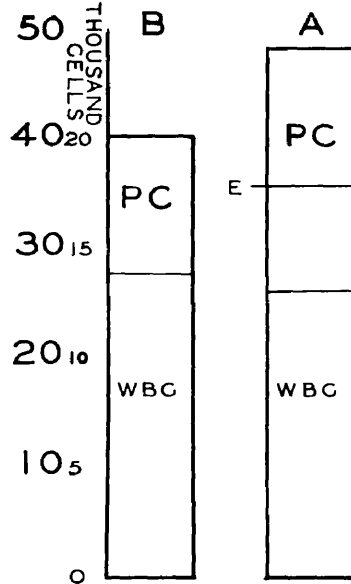P –PLASMA
PROT.– PROTEIN
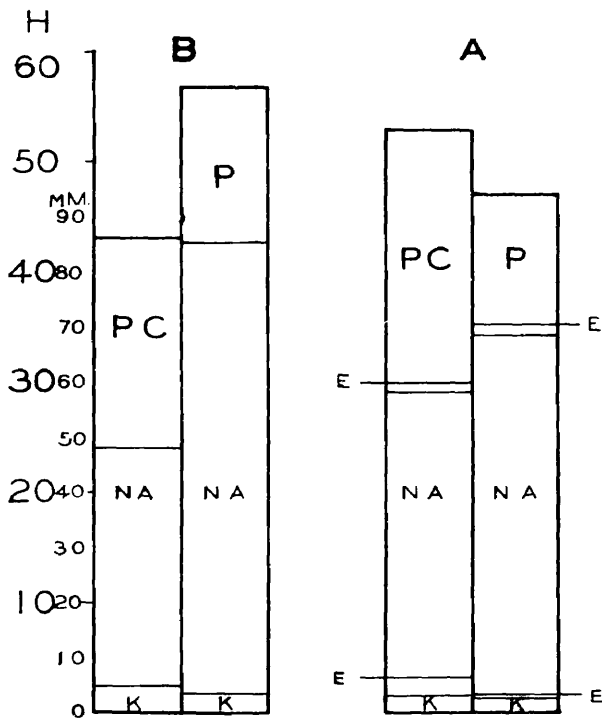H –HEMATOCRIT

FIGURE 2. PACKED CELL AND
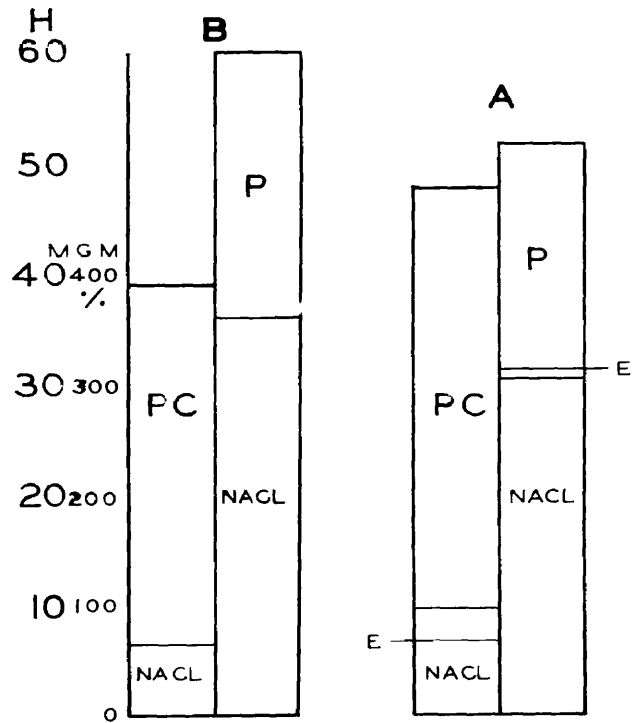LEUCOCYTE CHANGES

FIGURE 3. SODIUM AND POTASSIUM
CHANGES

FIGURE 4. SODIUM CHLORIDE CHANGES

explained by simple diffusion in the case of the dog
erythrocyte (75) but not in numerous other animal erythrocytes
(74).

The transfer of potassium into the cell and the main-
tenance of a concentration gradient was closely associated
with carbohydrate metabolism (69, 76). If glucose is present
the absorption of potassium is greatly increased. Increasing
glycogenesis by injecting glucose or insulin may lower the
serum potassium very considerably (77). On the other hand,
when various inhibitors of glycolysis such as a fluoride or
iodoacetate are present, absorption of the potassium ion by
the human erythrocyte _in vitro_ is prevented (69). The energy
released by the carbohydrate metabolism, it is believed,
supplies the energy for the cation transfer. Solomon (70)
and Weller and Taylor (72) have measured this energy and
reported that the human utilizes nearly a millimole of glu-
cose for every millimole of potassium that enters the cell;
in the rat this ratio is $\frac{1}{2}$ to 1.

Both the cold (69) and barbiturates (78) which slow up
metabolism decrease the potassium absorption by the erythro-
cyte. At the same time acetylcholine and cholinesterase
are intimately concerned with maintenance of the ion perme-
ability of the cell surface (69).

It has been suggested also that the erythrocyte potas-
sium content is directly related to the B.M.R. of the body (18).

The erythrocyte, however, is not nearly so permeable to
potassium as are many other tissues of the body; consequently,

excessive potassium introduced into the plasma will be quick-
ly removed by the viscera to be slowly released to the
muscles later (74).

A concentration gradient of sodium and potassium also
exists between the internal and the external cellular com-
ponents of muscle tissue. Twenty-five times more potassium
by weight was found in dog's muscle than in the serum accord-
ing to Eichelberger (79). But, this gradient remained only
so long as the cell membrane remained undamaged as reported
by Baetjer (14, 80) on ischemia studies.

There is limited information available on the movement
of electrolytes across the capillary wall. The capillaries
are the main source of supply for the muscles so it is
expected that they along with the lymphatics, facilitate
any total change of cellular and interstitial fluid electro-
lyte that may occur. Fenn et al. (74) as mentioned previous-
ly suggested that the viscera takes up any excess potassium
very rapidly from the blood. Then, too, during TQ shock
after the tissues had been injured large quantities of sodium
moved through the capillary into the tissue cells (109).
The electrolyte concentration of the blood was altered by
partial and complete asphyxia of the dog which produced an
increase of serum potassium (83, 84, 85). The source of
this increase, however, was probably the liver rather than
the muscle tissue as suggested by the fact that upon extir-
pation of the liver no increase of serum potassium could be
observed. Brewster et al. (76) suggested that this release

from the liver is accomplished by the sympathetic nerve
fibers.

The work of Baetjer (14) with hemorrhage of the cat
and Dennis and Moore (15) with coronary artery and vein liga-
tion of the cat suggested a movement of potassium from the
muscle into the capillary blood. Gellhorn et al. (32) re-
ported, however, that the sodium movement through capil-
laries is reduced as a result of traumatic shock. However,
in the first case, the volume and pressure of the blood were
reduced considerably by the experimental conditions which
makes it unlike the present experiment. This work of Dennis
and Moore approached the experimental conditions of complete
stagnation as closely as any. The discrepancy of the results
cannot be explained. In another series Dennis and Moore
during venous ligation found no evidence of sodium and potas-
sium movement across the capillary wall. The increase of
serum potassium during a partial venous stagnation reported
by Lauffman et al. (16) was probably also from the liver and
not the muscle.

In the present study (Figure 3) the possibility of the
release of potassium from the liver was eliminated by a com-
plete TQ of all of the leg vessels at the upper thigh, so
that the loss of sodium and potassium from the blood means a
transfer through the capillary into the interstitial space.
This movement was contrary to the reports of the few workers
who have demonstrated a transfer of cation during stagnation
(14, 15). The fact that in these cases the blood was under

a very reduced volume and pressure may account for the difference.

The decrease of the sodium concentration of the blood in this present study may be expected since this is the general direction of change following the release of the TQ. Since the movement of sodium from the erythrocyte was by simple diffusion (75), a slight loss from the erythrocyte might have been expected. The lack of any consistent change may be due to the small concentration gradient between cell and plasma.

Also following the TQ release there is a loss of potassium from the rat (86, 87) and rabbit (88) muscle cells. Thus, in this present study an anticipated increase in the blood potassium might be expected rather than the considerable decrease observed. The greatest shift of the cations studied was the potassium movement from the erythrocyte. Scheer (69) reported that when the metabolism of the erythrocyte was decreased by a decrease in available sugar, the outward flux of potassium was increased. Sugar was definitely reduced during stagnation which may explain the potassium movement.

The one factor that makes a comparison of these electrolyte values in the dog with the human, the rat and the rabbit electrolytes extremely difficult is that the main base of the erythrocytes of these animals is potassium while for the dog it is sodium (89, 90).

The pattern of these cations was clearer after the TQ release. The concentration gradient became changed as a result of the damage during ischemia. In the animals in which

potassium is the chief intracellular cation there was a
loss of potassium from the tissue cells, and an increase
of sodium in these cells (86, 88). The potassium content
of the serum increased (17, 86, 91, 92, 93). It has not
yet been determined whether this increase was a direct re-
sult of the lost intracellular potassium moving into the
blood, as would seem probable, or from a release of potas-
sium from the liver stimulated by a substance in turn re-
leased by the ischemic tissues (40, 91).

The increase of sodium in the tissue cell was greater
than could be expected on the basis of simple movement of
interstitial fluids moving into the cell; therefore, it
must have escaped from the capillaries (86, 88, 1-5 of
literature reviewed by Fuhrman and Crismon (86)).

In the contralateral leg the changes were reversed.
There was an increase of potassium concentration and a de-
crease of the sodium concentration in the muscle cells of
mice as reported by Fox and Baer (13). This may suggest
that the changes take place in the uninjured leg at the ex-
pense of the contralateral leg.

The mean sodium content in the present study of the
normal blood, plasma and erythrocytes of 128.4 mEq.and
114.2 mEq.[1] respectively is very similar to the reported

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[1]This value was determined on the basis of milliequiv-
alents (mEq.) per 1000 cc.of erythrocytes.

values of Albritton (50), of 127, 150 and 97 mEq. Reports
of others range from 83 to 159.8 mEq. for blood, from 74.4
to 185.8 mEq. for plasma and from 120.7 to 153.9 mEq. for
erythrocytes (94, 95).

The mean potassium content of 7.3, 6.7 and 12.0 mEq.[1]
in whole blood plasma and erythrocytes respectively, lies at
the upper border of the range of means reported by other in-
vestigators (50, 94, 95). This range of mean values is: for
blood, 4.5-10.3 mEq.; for plasma, 4.3-6.6 mEq.; and for
erythrocytes, 7.0-11.5 mEq.

Chloride. There was an overall decrease of sodium
chloride from the blood along with the fluid which escaped
into the tissue, but the loss was not as great as that ex-
pected based on the actual fluid loss from the blood. In
other words, the NaCl concentration in the blood increased
above the expected amount. This was due to the large in-
crease in NaCl concentration of the erythrocyte. There was
a slight decrease in plasma NaCl concentration (Figure 4).
Peters and Van Slyke (96) and others (26, 27) reported that
during a venous stasis the plasma chloride concentration fell
while the erythrocyte chloride concentration rose. They
suggested that this movement of the chloride anions into the
cells of both the blood and tissues was a result of the ac-
cumulation of carbonic and organic acids in the ligated limb
on the Donnan distribution. Fuhrman et al. (97) reported an
increase in muscle chloride concentration in rabbit muscle

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[1]Ibid.

during injury by partial blood flow stoppage by a micro-
sphere injection. After the release of the RL, however,
Fuhrman and Crismon (86) in another experiment on rats found
an increase in plasma chloride concentration.

It is generally believed that the movement of the chloride
ion is closely associated with the movement of the cations.
There was no quantitative relationship demonstrated here in
this present study. The chloride ion in the intracellular
spaces was generally considered in combination with a cation.
Calculations on the quantity of cation in the cell revealed
a sufficient amount to combine with even the large increase
of erythrocyte chloride ions.

The relationship of the chloride ion to the sugar changes
will be discussed in the following section under Sugar.

The sodium chloride values of normal blood found in this
present study vary somewhat from the reports of other in-
vestigators. The values obtained here are 607.8, 420.8, and
147.5[1] mg.% for plasma, whole blood and erythrocytes, re-
spectively. The values given by Albritton (50) are 579-
643.5 mg.% for plasma, 479.7-532 mg.% for blood, and 356-
403.6 mg.% for erythrocytes. The plasma value is within
range but the erythrocyte value is considerably lower. There
is no reasonable explanation for this at the moment. Other
investigators (60, 94, 98) concur with Albritton but suggest

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[1]The value for the erythrocyte was obtained by adjusting
the mean value of columns 8, Table 8 by the hematocrit value
to give the NaCl for 100 cc. of erythrocytes.

a wider range of normal values.

Sugar. The sugar decrease can be accounted for by three methods: (1) loss across the capillary wall to supply that lost by constant metabolism, (2) utilization by the cations in their flux with the blood cell, and (3) glucolysis into lactic acid by the leucocytes (99) and erythrocyte (37). Evidence that the erythrocyte does metabolize sugar has been suggested, as reported at a very recent symposium (37), by the appearance of lactic acid in the erythrocyte. Another sugar biproduct, diphosphoglycerate, appears to be in equilibrium with glucose in the cell.

Loughlin et al. (25) reported a decrease of 20 to 25 mg.% during 6 minutes of venous compression in man which is higher than the values found in this work on dogs. However, caution must be exercised in making such a comparison between man and dog. The initial increase of blood sugar in the venous compression which Loughlin et al. reported is probably a result of the hyperemia in the affected capillaries and veins allowing fresh sugar-ladened arterial blood into the veins (43, 100, 101, 102).

No relationship could be demonstrated between the sugar and chloride changes of the whole blood or plasma during stagnation. An inverse relationship has been reported by other investigators (103, 104, 105) during various conditions of sugar deprivation or inhibition. There appears to be no correlation between the sugar decrease and any other factors studied here.

The normal mean blood sugar found in this present work is 56.7 mg.%. This value is at the lower end of the range of 50-100 mg.% reported by Dukes (60).

It is interesting to note that according to Schneider and Karpovich (4) if the blood sugar level in dogs falls below 40 mg.% death results, since in the present study the mean value at the end of stagnation was 41.5 mg.% and in some individual cases it dropped to 21 mg.%. However, it is necessary to keep in mind that these values are a measurement of the blood in the stagnant limb and not of the total body blood. There was no noticeable general effect of this low blood sugar value on the animal after the release of the TQ. Supposedly, it was immediately mixed with the general body blood and the sugar quantity increased.

Carbon Dioxide and Oxygen. A carbon dioxide increase and an oxygen decrease were expected. Perhaps because these changes are so easily anticipated very little literature can be found reporting these values during blood stagnation. The reports of asphyxia may indicate the trend. In ten minutes of asphyxia the carbon dioxide increased about 25 per cent and the oxygen decreased from 18 vol. % to 0.33 vol. % as reported by Herber (23).

Reports of the normal carbon dioxide and oxygen values of dog venous blood are very few. Dukes (107) reported for normal dog venous blood a CO_2 value of 45.3 and for O_2 11.9 vol. %. Austin et al. (99) reported about 41 vol. % of CO_2 for venous blood of dog.

No correlation could be seen between the carbon dioxide
and oxygen shifts or the carbon dioxide and chloride shifts.

Acidity. An increase in the acidity of the blood was
expected on the basis of continued influx of the acid metabo-
lites from the involved tissues.

Numerous tests were made on the carbon dioxide combining
power of the blood which revealed a decrease in every case
during stagnation thus supporting the observation of a pH
decrease. These values were not recorded in this study because
of the possibility of inaccuracy from exposure to the atmos-
pheric gases. Moore and Greenberg (24) reported a much more
drastic reduction of the cardiac blood in shorter time (pH
drop of 0.22-0.81 in 3-9) during coronary artery and vein
occlusion than the mean pH 0.15 reduction found in the present
study. During asphyxia of the dog for 10 minutes there was
a drop in pH of 0.26 (23).

The pH value of normal blood found in this present
study, pH 7.34, is within the range of means, pH 7.1-7.68,
reported by other investigators (23, 59, 60, 98). There
appears to be a relationship between the pH and the carbon
dioxide change but the samples were too few to make a statisti-
cal analysis.

Specific Gravity. The increase in the specific gravity
of the blood is normally produced by an increase in both the
packed cell volume and the plasma protein concentration. This
plasma protein concentration almost solely determines the
specific gravity of the plasma. The specific gravity increase

is a result of the loss of fluid from the blood. Plasma proteins in general appear to be retained within the capillaries during light vascular compression while the fluid moves through the wall. The increase of the plasma specific gravity reported by numerous investigators (7, 21, 22) along with this present work would support this view. The 29 per cent increase of plasma protein is greater than the per cent increase of both the hematocrit and the erythrocyte during stagnation. This fact is difficult to reconcile. It was previously suggested that the Van Slyke et al. method of hemoglobin and protein determination from the specific gravity appeared somewhat irregular when applied to dogs. Perhaps this would account for the difference between the hematocrit and protein increases. If not, then in searching for a possible explanation it may be suggested that: (1) the leucocytes which decreased in numbers disintegrated releasing their proteins into the plasma, (2) small numbers of erythrocytes also released proteins into the plasma which would account for the decrease in erythrocyte size, and, (3), protein entered the capillary from the lymph spaces. This latter is very unlikely on the basis of previous evidence.

Then, in more severe compression some plasma protein may escape from the capillaries. This view is supported by the work of Landis et al. (8). It is rather evident from the previous paragraph that up to 30 minutes of complete stagnation there was no protein lost from the capillaries of the dog's hind limb.

Berry et al. (7) during 5 minutes of venous compression in the human obtained a plasma specific gravity increase of 0.0012 in comparison with 0.005 of the present study. At the same time he reports a plasma protein increase of 0.29 mg.% in comparison with 1.7 mg.% reported in the present work which is also considerably more than the 0.9 mg.% increase reported by Swingle et al. (20) during 2.5 hours of complete stagnation in the dog. A total of 5 hours did not increase the protein concentration above the previous level. This latter fact demonstrates along with the hematocrit changes, previously discussed, that the greatest hemoconcentration resulted in the first 1-2 hours of TQ application.

The reports of plasma protein changes, following the release of the TQ, are contradictory. The plasma protein, 5 to 6 hours following the release of the TQ, have decreased in the rabbit (22) but increased in the dog (20). Moore (13) reported that after release of the TQ from the hindlimb of the mouse the serum albumin decreased while the globulin increased.

The hemoglobin content of the cell remained unchanged during stagnation according to the method of Phillips et al. (34). This method gave very irregular results with this dog blood when it was used to calculate the hematocrit from the specific gravity of blood and plasma which could be compared with the measured hematocrit values. The calculated hemoglobin value of 12.3 gm.% of the present study (Table 12) compares favorably with the 13.0 gm. reported by Dukes (60).

No correlation could be demonstrated between the specific gravity of blood or plasma to other factors studied.

The normal mean blood specific gravity observed here was 1.050 which is at the lower border of the range of means (1.052-1.060) reported by numerous workers (50, 57, 60, 98, 107). The normal mean plasma specific gravity observed here was 1.023 which is within the range (1.0205-1.0256) reported by numerous workers (7, 60, 98). Likewise the normal mean plasma protein value of 5.9 mg.% (Table 12) is within the range of means (5.7-7.08) previously reported (7, 20, 50).

SUMMARY AND CONCLUSIONS

1. Blood studies were made on 26 samples from the hind limb of dogs comparing blood before and after 30 minutes of a tight tourniquet application to the limb and the following observations were made.

2. The capillary and erythrocyte permeability were altered during the stagnation of blood.

3. A hemoconcentration developed as a result of excessive fluid leakage from the capillaries during stagnation as revealed by:

    (a) A 20.2 per cent increase of the hematocrit value

    (b) A 24.8 per cent increase in the erythrocyte count

    (c) An increase of the specific gravity of the whole blood from a value of 1.050 to 1.0543 and of the plasma from a value of 1.023 to 1.028.

4. The erythrocyte diminished in size from a mean corpuscular volume (M.C.V.) of 74.7 to 67.1 cu. microns. This loss of size was accomplished partly by the loss of sugar, potassium and fluid from the cell.

5. The number of leucocytes found in the stagnant blood had decreased 35 per cent from the expected value which was based on the hematocrit increase.

    (a) The neutrophilic leucocytes were lost in largest numbers followed by the eosinophilic

and mononuclear leucocytes.

(b) The loss of leucocytes from the stagnant blood is largely the result of diapedesis and may be partly due to the destruction of the cells in the blood.

6. The concentration of the sodium in the blood decreased largely as a result of a decrease of the sodium in the plasma. This suggests that the fluid that escaped from the capillaries contained a higher sodium concentration than did the normal plasma.

7. The potassium ions were lost from the blood stream largely at the expense of the blood cells during the stagnation of the blood. There was an 80 per cent reduction of potassium concentration from its expected value in the blood cell.

8. The chloride concentration of the blood increased during stagnation largely through a 39 per cent increase of the blood cell chloride content.

9. The sugar content of the blood decreased 15.2 per cent during stagnation.

10. The plasma carbon dioxide content increased 10 per cent during stagnation of blood.

11. The total blood oxygen content decreased 30.5 per cent from normal to stagnant blood. However, there was a 64 per cent decrease from the expected value which was based on the erythrocyte increase in the stagnant blood.

12. A slight uncompensated acidosis or acidemia developed

in the stagnant blood which is revealed by a pH dropped from a mean 7.34 to 7.19 during 30 minutes of stagnation.

13. The calculated plasma protein concentration increased 24.8 per cent during stagnation of blood. This is slightly greater than erythrocyte count and hematocrit increase.

14. There was little demonstrable relationship between the changes of any of these factors during stagnation.

15. One dog studied during TQ application one and one half weeks before and then after parturition revealed considerably different results from the other animals. These were: a low hematocrit, a decrease of the erthrocyte count, a considerable increase of the M.C.V., an increase in the leucocyte count, an increase in the sodium content of the blood cells, a large increase of the plasma chloride concentration, a large decrease of the oxygen content, and an increase above average of the plasma specific gravity. More studies are needed on pregnant and post-parturition animals before any conclusion may be made.

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