Influence of Blood Volume on Cardiovascular Response to Anemia in the Dog.* (29662)

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If oxygen uptake in the tissues of the body is to remain unaltered when the concentration of hemoglobin in the circulating blood is reduced, either cardiac output must increase to maintain the amount of oxyhemoglobin delivered to the tissues per minute or the extraction of oxygen from the available hemoglobin must be increased, or both mechanisms must operate.

Studies in animals and man have shown that cardiac output is increased in anemia (1-6), but the relative importance of all the factors which may influence the output has not been established. With the exception of the studies by Fowler and associates(1) and of Murray and co-workers(4), few measurements have been made on the relation of changes in total blood volume to changes in cardiac output in anemia. The present studies were therefore designed to assess in dogs the cardiovascular changes which accompany acute anemia and hypervolemia when these conditions are imposed singly or in combination.

Methods. Experiments were carried out in 21 dogs anesthetized with sodium pentobarbital (25 mg/kg) and in 3 conscious dogs. The dogs weighed from 9.4 to 19 kg.

Six dogs were made hypervolemic and anemic by transfusions at body temperature of 4 aliquots of 250 ml of 6% dextran (75,000 molecular weight) in normal saline. Each aliquot was transfused over a 5-minute period. Four more dogs had similar treatment, one after cardiac denervation(7), two after cardiac denervation and bilateral thoracoabdominal sympathectomy, and one after receiving atropine and pentolinium.

In 6 dogs normovolemic anemia was achieved by establishing a temporary extracorporeal bypass connected to a reservoir which was initially filled with dextran. The volume of the reservoir was kept constant. The bypass allowed a progressive equilibrium between the animal's blood and the dextran, resulting in anemia with little or no change in blood volume. By substituting fresh reservoirs of dextran, step-wise reductions in hematocrit were achieved.

Six other dogs were made hypervolemic by intra-arterial or intravenous transfusions over 5 minutes of 4 aliquots of 250 ml of fresh blood obtained from a donor dog. All bleedings and transfusions were made through wide-bore tubing to minimize trauma to the blood. An equal amount of blood from a second donor was given to the original donor dog after each bleeding in order to reduce the amount of vasoactive substances which might be liberated by the decrease in arterial pressure.

Observations were made before any transfusion and 15 minutes after completion of the transfusion of each 250-ml aliquot or each exchange transfusion. Cardiac output was measured by the indicator-dilution technic. Indocyanine green dye was injected into the pulmonary artery, and changes in dye concentration were recorded continuously at the root of the aorta by a densitometer attached to a catheter. The densitometer was calibrated by drawing through it known concentrations of dye in aliquots of the dog's own blood. Calibrations were made after each transfusion of blood or of dextran. Stroke volume was calculated from the average heart rate recorded during the measurement of cardiac output.

Red cell delivery to the tissues per minute was obtained by multiplying cardiac output by the arterial hematocrit.

Total blood volume was measured during the control period and 15 minutes after completion of the last transfusion. Total blood volume was derived from plasma volume

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TABLE I. Dextran-Induced Hypervolemic Anemia in 6 Anesthetized Dogs (12 to 15 kg).

Measurement		Total dextran infused: Control	250 ml	500 ml	750 ml	1000 ml
Cardiac output (ml/min/kg)	Mean	153	227	271	338	392
	Range	(125–189)	(127–320)	(173–379)	(235–447)	(274–540)
	S.D. ±	27	71	72	100	116
Arterial hematocrit (%)	Mean	48	37.2	29.2	23.7	20.2
	Range	(40.7–52)	(30.5–42.5)	(19.4–34.3)	(20.7–27.2)	(19–22.3)
	S.D. ±	4.3	5.4	6	3.5	1.5
Red cell delivery (ml/min/kg)	Mean	73.6	83.5	74.2	81.2	71.1
	Range	(61.6–98.2)	(52.7–108.1)	(58.3–108.7)	(58.3–121.5)	(52.2–100.9)
	S.D. ±	15.3	29.0	21	29.5	21.2
Heart rate (beats/min)	Mean	156	171	169	152	155
	Range	(119–207)	(132–240)	(130–240)	(130–168)	(144–174)
	S.D. ±	33	48	40	17	11
Stroke volume (ml/kg)	Mean	1.03	1.47	1.73	2.26	2.49
	Range	(.62–1.34)	(.53–2.22)	(.72–2.75)	(1.71–3.39)	(1.86–3.10)
	S.D. ±	.3	.6	.7	.7	.6
Mean femoral artery pressure (mm Hg)	Mean	125	125	122	122	125
	Range	(111–136)	(100–144)	(93–143)	(84–149)	(108–137)
	S.D. ±	7	18	18	25	13
Mean right atrial pressure (mm Hg)	Mean	0	1.2	2.7	4	4.4
	Range	(–3 to +1)	(0 to +4)	(0 to +6)	(1 to +10.5)	(1 to +13)
Total blood vol (ml/kg) (measured in 3 dogs)	Mean	105.5				160
	Range	(105–110)				(141.6–180.5)

measured by radioiodinated serum albumin (8) and the hematocrit.

The volume of blood in the lungs and the left side of the heart was calculated as the product of cardiac output and mean transit time of the indicator between sites of injection and sampling (pulmonary valves to aortic valves). Mean transit time was determined from the recorded dilution curve and was corrected for delay due to the sampling system(9).

For measurement of pressures, catheters were inserted into the right atrium and the femoral artery. The pressures were measured by strain-gauge transducers, and the zero reference point was sited at the approximate level of the right atrium. The percentage oxygen saturation of the arterial blood was obtained from measurements of oxygen content and capacity by the Van Slyke manometric technic.

One normal dog and one with chronic cardiac denervation were trained to run on a treadmill. These dogs performed graded exercise before and after transfusions of dextran. A tracheostomy was performed one week before the experiment to permit measurement of oxygen uptake(10).

Results. Dextran-induced hypervolemic anemia. The response in 6 anesthetized dogs to each 250-ml infusion of dextran is given in Table I. Cardiac output, stroke volume and right atrial pressure measured 15 minutes after the completion of each 250-ml transfusion of dextran showed a progressive increase; hematocrit showed a progressive decrease. Arterial blood pressure, heart rate and red cell delivery per minute remained close to the control values.

After 1000 ml had been transfused, cardiac output was increased by an average of 156%, this increase being due almost wholly to an increase in stroke volume. Arterial oxygen saturation remained at the control values of between 93 and 95%.

Normovolemic anemia induced by exchange transfusion. The response in 6 dogs to 4 successive exchange transfusions with dextran is given in Table II. Average cardiac output, which increased with the first transfusion, remained about this value with the subsequent exchanges as the hematocrit progressively decreased. Average heart rate showed a modest increase and arterial blood

TABLE II. Normovolemic Anemia in 6 Dogs (9.4-13.8 kg) (Exchange Transfusion with Dextran).

Measurement		Control	1st Exchange	2nd Exchange	3rd Exchange	4th Exchange
Cardiac output (ml/min/kg)	Mean	184	238	227	238	229
	Range	(76–282)	(219–262)	(132–297)	(166–299)	(187–344)
	S.D. ±	68	18	65	55	77
Arterial hematocrit (%)	Mean	40.2	28.5	21.3	16.0	11.8
	Range	(31.0–47.8)	(27.9–29.1)	(20.5–22.5)	(14.4–17.9)	(9.4–14.6)
	S.D. ±	5.9	.50	.80	1.3	2.2
Red cell delivery (ml/min/kg)	Mean	76.1	68.0	48.7	38.4	28.0
	Range	(23.6–125.5)	(61.2–74.9)	(27.1–66.8)	(26.2–53.5)	(18.4–50.2)
	S.D. ±	32.6	5.8	15.3	11.5	14.9
Heart rate (beats/min)	Mean	164	172	189	200	191
	Range	(94–203)	(126–216)	(168–213)	(181–228)	(151–217)
	S.D. ±	44	42	15	19	29
Stroke volume (ml/kg)	Mean	1.22	1.43	1.20	1.20	1.20
	Range	(.41–2.15)	(1.21–1.76)	(.73–1.64)	(.91–1.46)	(.91–1.59)
	S.D. ±	.63	.27	.35	.25	.31
Mean femoral artery pressure (mm Hg)	Mean	132	134	132	125	117
	Range	(105–154)	(124–154)	(115–149)	(109–140)	(78–139)
	S.D. ±	19	13	13	12	29
Mean right atrial pressure (mm Hg)	Mean	1.16	+.76	+1.84	+1.12	+2.67
	Range	(−2.7 to 7.5)	(−1.0 to 4.1)	(−.8 to +6.0)	(−1.5 to +4.0)	(−.1 to +4.1)

pressure a modest decrease as the amount of blood exchanged with dextran increased. The stroke volume, apart from a minor increase after the first exchange, remained at control value. The red cell delivery per minute showed a progressive decrease. Two of the dogs differed from the values reflected by the averages in that the cardiac output increased progressively with each exchange transfusion, in one from 188 ml/min/kg (hematocrit 38%) to 341 ml (hematocrit 14.6%) and the other from 78 ml/min/kg (hematocrit 31.5%) to 187 ml (hematocrit 9.6%). The increased output was due both to an increase in heart rate and in stroke volume. The arterial blood pressure increased (mean 127 to 138 mm Hg and 106 to 126 mm Hg respectively). The mean right atrial pressure did not show any progressive rise as cardiac output increased.

In the 4 dogs which did not show a progressive rise in cardiac output as the hematocrit decreased, the dextran-blood mixture was centrifuged after the hematocrit had been reduced to the desired level, and the packed red blood cells were returned to the dog. This increased the hematocrit toward the initial value. Hypervolemic anemia was then induced by dextran transfusions. Unlike the situation in normovolemic anemia, there was an increase in cardiac output with each 250 ml transfusion. After 1000 ml had been transfused, the cardiac output was from 180 to 300% of control value. Data from the 2 animals showing the greatest response are shown in Fig. 1.

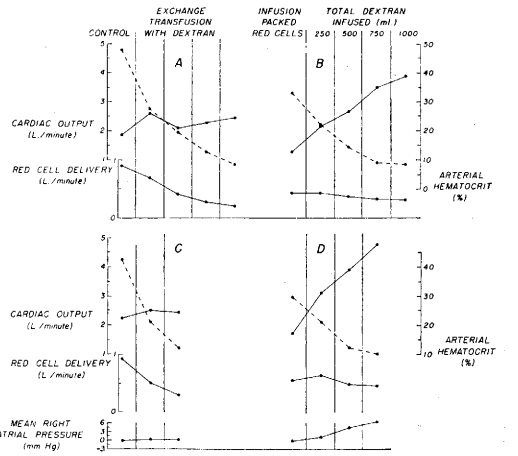

FIG. 1. Effects of anemia with normovolemia and of anemia with hypervolemia on cardiac output, arterial hematocrit and red cell delivery. Top panel: Conscious dog (11.3 kg) trained to lie quietly; catheters introduced under local anesthesia; A, normovolemia and B, hypervolemia. Lower panel: Dog (13 kg) anesthetized with pentobarbital; C, normovolemia and D, hypervolemia. Arterial hematocrit is indicated by the dotted line. For details see text.

TABLE III. Hypervolemia Induced by Transfusions of Fresh Blood in 6 Anesthetized Dogs (11.5-19 kg).

Measurement		Control	Total blood given 250 ml	500 ml	750 ml	1000 ml
Cardiac output (ml/min/kg)	Mean	137	144	132	129	121
	Range	(104–170)	(130–159)	(114–143)	(94–180)	(98–178)
	S.D. ±	28	19	24	33	33
Arterial hematocrit (%)	Mean	41.5	42.6	46	50.7	53
	Range	(35.8–47.2)	(38–50.5)	(43–50)	(47.9–55)	(46.3–62)
	S.D. ±	4.3	6.6	3.6	3.1	3.0
Red cell delivery (ml/min/kg)	Mean	57.7	62.1	60.8	67.2	65.6
	Range	(42.1–80)	(49.4–80)	(52–81)	(51.7–92)	(45–96)
	S.D. ±	15.5	14.4	12.0	20.5	23
Heart rate (beats/min)	Mean	186	180	140	146	127
	Range	(138–242)	(120–228)	(96–168)	(90–192)	(69–198)
	S.D. ±	44	50	27	22	49
Stroke volume (ml/kg)	Mean	.77	.85	.99	.90	1.01
	Range	(.51–.98)	(.60–1.32)	(.68–1.46)	(.56–1.14)	(.76–1.42)
	S.D. ±	.19	.30	.26	.22	.27
Mean femoral artery pressure (mm Hg)	Mean	124	116	123	133	140
	Range	(76–173)	(73–167)	(79–176)	(85–198)	(98–151)
	S.D. ±	38	38	37	46	33
Mean right atrial pressure (mm Hg)	Mean	−.5	0	+1.7	+3.2	+4
	Range	(−2 to +7.5)	(+1.5 to +2)	(−2 to +9)	(+2 to +7)	(+2 to +6)
Total blood vol (ml/kg) (3 dogs)	Mean	81				138
	Range	(73.5–89.5)				(119.5–166)

Hypervolemia induced by transfusion of blood. The response in 6 anesthetized dogs to each 250-ml infusion of blood is given in Table III. With each transfusion there was a progressive increase in hematocrit and atrial pressure and a progressive decrease in heart rate. Cardiac output showed a modest decrease, red cell delivery a modest increase and femoral arterial pressure an increase.

In an attempt to keep the hematocrit constant, a mixture of red blood cells in dextran was given to a dog in four 250-ml aliquots. The hematocrit of each aliquot was similar to the hematocrit of the dog before the transfusion. The results were similar to those in which fresh whole blood was used. The control cardiac output was 2.1 l/min, and after each transfusion it was 2.5, 1.9, 1.8, and 1.8 l/min, respectively. The hematocrit was maintained within 3% of the control value of 40%. After 1000 ml had been infused, right atrial pressure increased by 5 mm Hg and systemic pressure by 14 mm Hg.

Hypervolemic anemia in absence of cardiac nerve activity. When a dog with complete cardiac denervation was given dextran, the response was similar to that of a normal animal. The cardiac output increased progressively because of increased stroke volume (Fig. 2). Two dogs with cardiac denervation and bilateral thoracolumbar sympathectomy behaved similarly.

Another dog was atropinized (4 mg atropine sulfate intravenously followed by 2 mg every 2 hours) and given pentolinium (2 mg/kg intravenously) to cause a sympathetic blockade. The results with dextran infusions were in all respects similar to those in normal dogs (Fig. 3).

Exercise and hypervolemic anemia. A normal dog trained to run on a treadmill was made hypervolemic and anemic by transfusions of dextran. After 1000 ml had been given, the resting cardiac output increased by 77% and the arterial hematocrit decreased by 58%; the dog was able to exercise and to achieve an oxygen uptake of approximately two-thirds of its maximal uptake before the dextran. For any given oxygen consumption during exercise, cardiac output was much higher when the animal was hypervolemic and anemic than when normal; the red cell

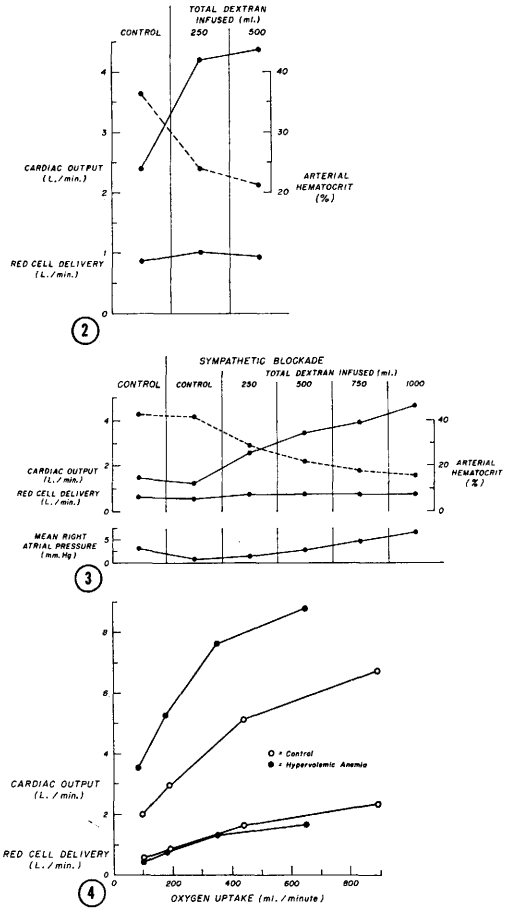

FIG. 2. Effect of hypervolemic anemia on cardiac output and red cell delivery to the tissues in a dog (13.2 kg) with chronic cardiac denervation.

FIG. 3. Effect of hypervolemia and anemia on cardiac output and red cell delivery to the tissues in an anesthetized dog (13 kg) after atropinization and sympathetic blockade by pentolinium.

FIG. 4. Effect of hypervolemia in augmenting cardiac output in the anemic dog to maintain red cell delivery at control values at rest and during graded exercise (dog, 12 kg).

delivery per minute was approximately the same at any given exercise level before and after the transfusions (Fig. 4). For comparison, a dog (13.2 kg) with cardiac denervation also was exercised before and after receiving 1000 ml of dextran. The resting cardiac output increased from 2.2 to 4.4 l/min; as a consequence, the red cell delivery to the tissues was unaltered. With the maximal exercise that the dog could perform after the transfusion, the oxygen consumption increased from 150 to 630 ml (S.T.P.D.); the cardiac output reached 6.0 l/min, and the red cell delivery at this time (1.6 l/min) was similar to the value obtained during exercise with the same oxygen consumption before the transfusion was given.

Lung and left heart blood volume. This was measured in 5 dogs made anemic and hypervolemic with dextran and in 6 made hypervolemic by transfusions of blood. Both groups showed a progressive increase in lung and left heart blood volume with each 250-ml aliquot of fluid given. After 1000 ml of blood had been given, the volume increased by 55% and the cardiac output decreased by 12%. After the 1000 ml of dextran, the increase in volume was 74 and in cardiac output 180%.

Discussion. In these studies the combination of anemia and hypervolemia resulted in augmentation of cardiac output of sufficient magnitude to maintain a control delivery of red blood cells to the periphery even when the arterial hematocrit had been reduced to about 20%. This constancy of red cell delivery was seen in the normal dog and in the dog with chronic cardiac denervation or with autonomic blockade induced by drugs. Since the oxygen saturation of the arterial blood showed no significant change from control values, the amount of oxygen delivered to the tissues was maintained in spite of the anemia. This was true also during exercise; indeed, with graded exercise the animal continued to maintain his red cell delivery at values comparable to those when blood volume and hematocrit were normal. Failure to achieve the maximal work levels reached in the control state was due to inability of the heart to pump out more blood since measurement of the mixed venous oxygen content at the peak work which the dog performed showed that nearly all the oxygen had been extracted.

Gowdey and associates(11) and Fowler and co-workers(2) have also reported a constant red cell delivery to the periphery in anesthetized dogs with hypervolemic anemia. In the present experiments, increase in cardiac output was linearly related to degree of hypervolemic anemia. The associated pro-

gressive rise in right atrial pressure and in lung and left heart volumes and the fact that the increase in flow was almost wholly due to an increase in stroke volume suggest that hypervolemia played the dominant role in augmenting cardiac output through an increase in ventricular filling pressure. In 4 of the 6 dogs studied the combination of anemia and normovolemia did not result in any substantial increase in cardiac output; since in these 4 dogs the arterial hematocrit was reduced to the same level in normovolemic anemia as in hypervolemic anemia, the increase in cardiac output with the hypervolemic anemia cannot be attributed to a stimulant action of the dextran itself. The decreased viscosity must account in part for the absence of an increase in blood pressure despite the increased blood volume and cardiac output. The finding of a reduced arterial pressure without a substantial change in cardiac output in 4 of the dogs with normovolemic anemia lends support to this possibility.

Fowler and associates(2) induced normovolemic anemia by bleeding dogs and replacing the volume of blood removed by equal volumes of dextran. The cardiac output increased in a comparable manner to that seen for a similar degree of anemia when dogs were made hypervolemic; this result suggests that the hematocrit level is the major factor determining the cardiac response. Results similar to this were seen in 2 of the 6 dogs in the present series in which exchange transfusions were made. Since in these 2 dogs the arterial blood pressure and the stroke volume increased, the possibility exists that total blood volume may have increased. If this occurred it was not reflected by an increase in right atrial pressure and the explanation for the increased cardiac output remains obscure. Murray and associates(4) also found that cardiac output had an inverse correlation to hematocrit at any given blood volume. However, the cardiac output was higher in the hypervolemic compared to the normovolemic state at any comparable hematocrit.

In the present studies, like those of Fowler and associates(1), normal dogs made hypervolemic with no change or an increased hematocrit had a modest increase, no change or, as the hematocrit increased, a modest decrease in cardiac output. In both circumstances the increase in right atrial pressure and in lung and left heart volumes was comparable to those seen in hypervolemic anemia. Systemic arterial pressure increased progressively with increase in blood volume while there was a stepwise decrease in heart rate. The increased peripheral vascular resistance resulting from the increased viscosity due to the elevated hematocrit must contribute to this rise in pressure(12). The failure of cardiac output to increase in these situations might be ascribed to sino-aortic reflexes arising from the elevation in arterial pressure. Some substance to this viewpoint was given by the results of an experiment in which a dog with chronic cardiac denervation was made hypervolemic with fresh blood. In this case the infusion of 500 ml of blood resulted in a 60% increase in cardiac output accompanied by a rise in right atrial pressure and in femoral arterial blood pressure. Frye and Braunwald(13) studied the cardiovascular responses to whole blood transfusions in normal humans; only when the autonomic nervous system was chemically blocked did the cardiac output increase.

Difficulty was experienced in interpreting these experiments with blood loading because in most cases some arterial hypotension accompanied the infusion of the first 250 ml of blood. Though the fall in arterial pressure was from 5 to 10 mm Hg mean pressure, the possibility that the subsequent course of the experiment was modified by a reaction to the transfused blood could not be entirely ruled out.

No analysis is yet possible of the interaction of all the factors which may govern the cardiac output in anemia. Justus and his colleagues(3) have evidence suggesting that a humoral factor is involved. Sharpey-Schafer (14) claimed that there is increased venous tone which could aid in increasing cardiac output. When anemia is acutely induced in the presence of a constant blood volume,

there is an increase in cardiac output proportional to the reduction in hematocrit(1,5, 15). Nevertheless, the circulatory adjustments are insufficient to maintain a normal oxygen delivery to the body tissues. In the anesthetized dog with constant blood volume the amount of oxygen arriving at the tissues is at its maximum only when the hematocrit is normal; it is reduced both in polycythemia and in anemia(4,15). In the present study the increase in output due to the anemia alone was insufficient to maintain the delivery of oxygen to the tissues at the control value; only when blood volume was increased as the hematocrit decreased did the output increase sufficiently to deliver the same number of red cells to the periphery. Thus, in spite of the similar changes in blood viscosity, it was only when the filling pressure of the ventricles was augmented that the heart was enabled to increase its output sufficiently to maintain oxygen transport to the tissues.

1. Fowler, N. O., Bloom, W. L., Ward, J. A., *Circulation Res.*, 1958, v6, 163.

2. Fowler, N. O., Franch, R. H., Bloom, W. L., *ibid.*, 1956, v4, 319.

3. Justus, D. W., Cornett, R. W., Hatcher, J. D., *ibid.*, 1957, v5, 207.

4. Murray, J. F., Gold, Philip, Johnson, B. L., Jr., *J. Clin. Invest.*, 1963, v42, 1150.

5. Richardson, T. Q., Guyton, A. C., *Am. J. Physiol.*, 1959, v197, 1167.

6. Roy, S. B., Bhatia, M. L., Mathur, V. S., Virmani, Shyama, *Circulation*, 1963, v28, 346.

7. Cooper, Theodore, Gilbert, J. W., Jr., Bloodwell, R. D., Crout, J. R., *Circulation Res.*, 1961, v9, 275.

8. Beierwaltes, W. H., Johnson, P. C., Solari, A. J., *Clinical Use of Radioisotopes*, W. B. Saunders Co., Philadelphia, 1957, 456 pp.

9. Fox, I. J., Sutterer, W. F., Wood, E. H., *J. Appl. Physiol.*, 1957, v11, 390.

10. Donald, D. E., Shepherd, J. T., *Am. J. Physiol.*, 1963, v205, 393.

11. Gowdey, C. W., Hatcher, J. D., Sunahara, F. A., *Canad. J. Biochem. & Physiol.*, 1954, v32, 282.

12. Levy, M. N., Share, Leonard, *Circulation Res.*, 1953, v1, 247.

13. Frye, R. L., Braunwald, Eugene, *J. Clin. Invest.*, 1960, v39, 1043.

14. Sharpey-Schafer, E. P., *Brit. M. Bull.*, 1963, v19, 145.

15. Murray, J. F., Gold, Philip, Johnson, B. L., Jr., *Am. J. Physiol.*, 1962, v203, 720.

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Effect of Sodium Citrate on Milk Yield in Rats.* (29663)

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The existence of an inhibitory factor in muscle tissues was reported(1,2). The presence of this factor prevented muscular contraction even in the presence of adenosine triphosphate (ATP). By adding a calcium solution, however, the inhibition was prevented and contraction occurred. It was suggested that the calcium released by nerves during their stimulation in some way negated the inhibitory factor thus making possible the contraction of the innervated muscle(3). In the mammary gland, myoepithelial cells have been observed lying in close contact with the epithelium of the alveoli and ducts. It has been suggested that these cells have contractile properties which respond to the stimulus of oxytocin causing the milk in the alveoli to be discharged into the larger ducts and thus becoming available to the nursing young (4).

Evidence that myoepithelial cells are contractile comparable to smooth muscle cells is based on visual examination of the exposed

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