MUSCLE HEMOGLOBIN IN HUMAN AUTOPSY MATERIAL *

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Experiments in this laboratory have shown that for the dog, muscle hemoglobin is a variable factor.[1] It is in highest concentration in adult active dogs and lowest in young pups. It is probable that exercise is more important than the level of the blood hemoglobin in determining the hemoglobin concentration in muscle fibers. Severe long-continued anemia in dogs may reduce the muscle hemoglobin level somewhat, but in healthy anemic adult dogs it rarely falls below 400 to 500 mg. muscle hemoglobin per 100 gm. We may observe lower normal values in quiet, confined, non-anemic, adult house dogs.

A method satisfactory for dogs was described in detail and we thought it would be possible to modify this muscle hemoglobin method sufficiently to give worth-while determinations in human necropsy material. In dogs it is possible to render the muscles practically blood hemoglobin-free by simultaneous bleeding and perfusion, massage and careful washing.[2] There are many difficulties in the necropsy procedure and these results of necessity are not so satisfactory as were the experiments in dogs. Access to muscles is limited in the usual postmortem examination and most of our readings concern the rectus abdominis and adductor longus muscles.

We have shown that in dogs it is necessary to free the muscle of contained blood hemoglobin in order to obtain accurate values, and it must be admitted that only occasionally will necropsy material be found which can be prepared free from blood hemoglobin. Moreover, it is not difficult to show that under certain conditions muscle hemoglobin can be washed out of the muscles by an excess of gravity perfusion. For example a dog perfused on one side immediately after death may be placed in the cadaver ice-box forty-eight hours. The perfusion of the other side with pressure, using the standard glucose salt solution, will give distinctly lower readings in the late

* Received for publication November 20, 1927.

perfused side in spite of the fact that some blood clots still persist in the smaller vessels of these muscles. This indicates obviously that muscle hemoglobin has been washed out of these muscles perfused forty-eight hours after death. It is possible that physical changes in the muscle hemoglobin and muscle fibers due to rigor mortis, accumulation of lactic acid or other factors are in part responsible for this escape of muscle hemoglobin in the perfusion fluids.

In spite of these many difficulties we decided to proceed with a study of the human material. Care must be used in the interpretation of results and no emphasis can be placed upon minor variations in the muscle hemoglobin readings, but we believe the larger variations in muscle hemoglobin values do have some significance. It is significant that these human values and abnormalities coincide with the more easily controlled observations in dogs. It appears that the level of muscle hemoglobin in man as in the dog is very largely determined by exercise or work done. Probably low blood hemoglobin values and diet factors have some little influence on the level of muscle hemoglobin.

Method

The results reported herein were compiled using the adductor longus and the rectus abdominis muscles. A few minor modifications were made in the method during the study, but essentially the method employed at all times was to wash through the right external iliac artery 4000 cc. to 7000 cc. of 1 per cent sodium chloride plus 5 per cent glucose solution. This washed out the blood in the vessels in the right leg and the right rectus abdominis. The muscles were not massaged either *in situ* or after removal as it invariably caused an increase in the amount of edema. It was also found that the washing if done slowly with a moderate amount of pressure was more satisfactory than if done quickly with high pressure. A four-liter bottle was used and the pressure supplied by a hand pump. It was not unusual to take from twenty minutes to one half-hour for the actual washing. Of the utmost importance was the length of time which had elapsed since death. In those cases in which the blood had clotted in the vessels it was impossible to remove any great amount of blood, and even small amounts of perfusing fluid

gave edema, while larger amounts frequently seemed to wash out a certain amount of muscle hemoglobin.

The right side having been perfused, approximately 30 gm. samples were removed from both adductor longus and rectus abdominis muscles, the left being kept as controls. In taking these sections due care was exercised to avoid contamination of the muscle with blood, all vessels being ligated before cutting. At first, samples were taken from each of the four muscles for microscopic examinations as to the blood content. Later sections were taken only from the muscles which had been perfused. Microscopic sections have shown that in nearly every case there is much less blood in the capillaries of the perfused side than in the unperfused side, and in some cases the perfused capillaries are entirely clear of red blood cells.

The remaining portions of the muscle were trimmed free from fat, fascia and any large blood vessels and were then chopped moderately fine with a razor blade, using this method to cut the fibers without crushing them. Duplicate 10 gm. samples from each muscle were taken and 40 cc. of 0.4 per cent ammonium hydrate added to each. The remainder of the chopped muscle was set aside temporarily. The ammonia extract was thoroughly stirred and placed in the ice-box at 4° to 7° C for sixteen hours. During this time the extract was thoroughly stirred two to three times. It was now either filtered or centrifugalized. If filtered it was first put through a wire gauze strainer, then several thicknesses of cheesecloth and then through double thickness of filter paper giving a clear filtrate. Centrifugalization at high speed for twenty minutes and removal of the supernatant fluid by pipette gave equally clear solutions and was especially advantageous in those muscles particularly rich in fat. In cases in which the centrifugalized fluid was not absolutely clear it was put through filter paper. The filtrate was a 20 per cent extract of the muscle, and because of the dilution was unsuitable for analysis by the oxygen capacity method of Van Slyke, the percentage of error due to the physically dissolved oxygen being too high.

Three different quantitative colorimetric estimations of pigment were utilized. The clear extract was examined in the spectrophotometer and the concentration estimated from the density. Kennedy,[3] and Kennedy and Whipple [4] have discussed the relation between the concentration of hemoglobin and its optical density and the identity

of blood hemoglobin and muscle hemoglobin. Small portions of the extract were taken and the oxyhemoglobin changed to carbon monoxide hemoglobin by bubbling illuminating gas through the solution. This was then read in a Duboscq colorimeter against a 1 per cent solution of dog's blood, the hemoglobin content of which had been determined by the Van Slyke method. There was frequently some difficulty in matching the solutions, the muscle hemoglobin seeming to have a slightly more yellow tint than the blood standard. To obviate this difficulty a Wratten light filter No. 74 was

TABLE I

Comparison of Hemoglobin Values on Identical Muscle Samples Estimated by Different Methods. Muscle Hemoglobin Expressed in Mg. per 100 Gm. Muscle.

	Acid hematin	CO hemoglobin		Spectrophotometer	Average
		No filter	With filter		
Left adductor not perfused.....	1190	1425	1035	1270	1220
	1190	1400	1010	....	....
Right adductor perfused.....	870	810	730	835	815
	880	790	790	....	....
Left rectus not perfused.....	810	1000	710	780	825
Right rectus perfused.....	600	710	530	570	600
	620	680	470	....	....
Right pectoral not perfused.....	1130	1290	1025	1110	1160
	1210	1380	1000	....	....

placed in the eye piece of the colorimeter. Under these conditions the colors of the solutions were more nearly comparable. Kennedy [5] has discussed the value of these light filters in colorimetry. The remaining portions of the original muscle extract were now changed into acid hematin and read against a similar standard prepared from dog's blood. The solution is prepared as follows: Add 4 drops of 5N hydrochloric acid to 10 cc. of the extract, shake until the precipitate is redissolved, and then place in the ice-box over night.

The pigment content of the muscle of ten cases was determined by using three colorimetric methods; namely the *spectrophotometric*, the *acid hematin*, and the *carbon monoxide hemoglobin* both with and without the aid of light filters. Table I shows the relative agreement of these four methods on one case (A–209). In our hands the determination by the carbon monoxide method was the most troublesome

and it was thereafter dispensed with. All other figures appearing in this paper are averages of determinations by the acid hematin and the spectrophotometric methods, each of these usually made by a separate observer * and all of them averages of duplicate examinations.

Since even with the greatest care edema occurred in certain cases it was thought wise to make a correction for this in so far as was possible. From the original chopped muscle, duplicate 1 gm. samples were weighed into weighing bottles and dried to constant weight in a vacuum oven with a temperature of 72° to 76° C and a vacuum of from 23 to 27 inches of mercury. As a rule, at the end of ten hours the specimens were so dehydrated that another six to ten hours drying did not change the weight more than 1 to 2 mg. Correction for edema may be readily calculated from these figures. As there was no assurance that the rectus and the adductor muscles were equally perfused and equally edematous, it was necessary to determine each of them separately on each side and each in duplicate. This method is faulty in many respects because the perfusing fluid itself contained 6 per cent of solids, and there is no assurance that during the perfusion a certain amount of protein might not have been washed out of the muscles. So in addition to estimating the relative dry weights of the different samples the amount of nitrogen was determined on the dried samples by the Kjeldahl method. On this basis the protein content was estimated and a correction established for the edema. In practice, for any pair of muscles, we usually averaged the factor obtained by comparing the dry weights and the factor obtained by comparing the protein content, and used this average factor to correct for edema of the muscles. Table II (page 80) illustrates the points mentioned.

Usually the correction factor is greater than 1. It is always greater than 1 where there is edema due to perfusion. However, in a certain percentage of our cases the correction factor is less than 1. For example, in a case such as A-296 where death was due to myocardial failure with marked anasarca, it is probable that the hypertonic perfusing solution caused absorption of fluid from the tissues.

It may be recalled that the spectrophotometric curve of the absorption bands of muscle hemoglobin is just slightly but definitely

* We wish to acknowledge the assistance of Miss Beatrice Moshier, who made most of the acid hematin readings.

different from that of blood hemoglobin, as pointed out by Kühne,[6] Günther[7] and others. The difference is that the points of maximum absorption of each of the two main bands in the muscle hemoglobin are moved about 5 microns toward the longer wave end of

TABLE II

Method of Calculating Correction for Edema in Perfused Muscle (A-258)

1. COMPARISON OF DRY WEIGHTS

		Wt. of muscle in gm.	Dry wt. in gm.	Per cent dry weight	Average		
Right rectus perfused	A ...	0.824	0.172	20.9			
	B ...	0.976	0.202	20.7	20.8	25.0 / 20.8	1.2
Left rectus not perfused	A ...	0.702	0.174	24.8			
	B ...	0.641	0.161	25.1	25.0		

2. COMPARISON OF PROTEIN CONTENTS

		Wt. of muscle	Protein in gm.	Per cent of protein	Average		
Right rectus perfused	A ...	0.824	0.139	16.9			
	B ...	0.976	0.160	16.3	16.6		
Left retcus not perfused	A ...	0.702	0.155	22.1		21.7	1.31
	B ...	0.641	0.137	21.3	21.7	16.6	1.2
							1.25 Av.

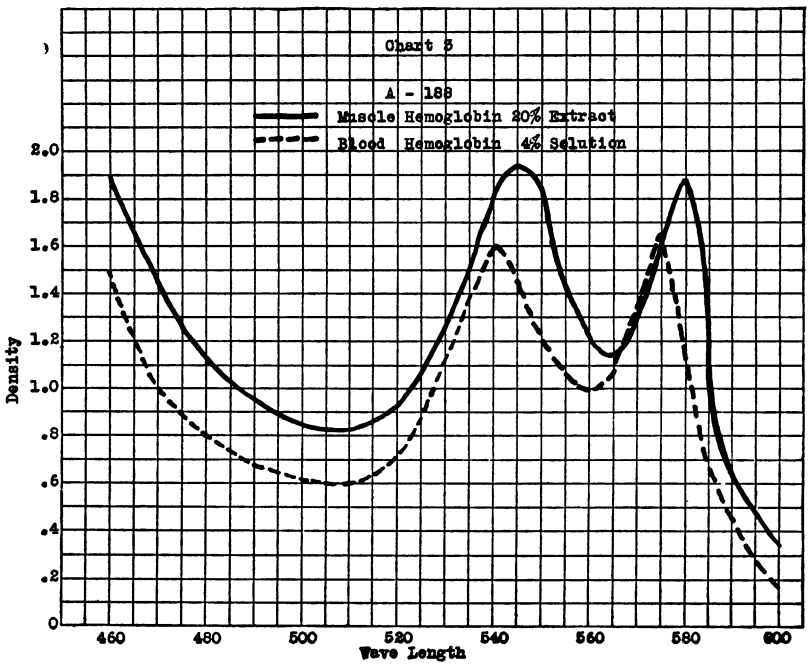
3. CALCULATION OF HEMOGLOBIN CONTENT IN MG. PER 100 GM. OF MUSCLE

	Duplicate samples	Right rectus perfused	Average
Spectrophotometer.....	A	770	
	B	760	765
Acid hematin.....	A	730	
	B	720	725
			745 × 1.25 = 930 mg. of hemoglobin per 100 gm. muscle corrected for edema.

the spectrum. This fact gives us an opportunity to test in a gross way the importance of the blood hemoglobin in the capillaries in any given muscle sample. Chart 3 presents the curves of the blood

hemoglobin and muscle hemoglobin from the same case (A–188). These curves are presented as they are entirely typical of the hemoglobin from the two sources. In this particular case the blood hemoglobin was estimated one month before death. The two readings were made by separate observers. The muscle hemoglobin was estimated from an unperfused muscle which still contained blood in the

CHART 3



The Curves of the Blood Hemoglobin and Muscle Hemoglobin (A–188)

capillaries. It will be seen, however, that this particular case was one of very severe anemia and the hemoglobin content of the blood was much lower than normal. However, we have repeatedly observed this same type of muscle hemoglobin curve in both the perfused and unperfused muscle.

That there is a noticeable difference between the perfused and unperfused side is shown by Table IV which gives the values of the perfused side for the adductor longus as illustrated above. Except in three instances the corrected value for the perfused muscle is lower than that of the unperfused side. The average of these is

around 10 to 15 per cent below the value of the unperfused muscle. It must be admitted that perfusion rarely removes all of the contained red cells so that this correction is an inaccurate variable. In three instances the corrected value of the perfused muscle is greater than that of the unperfused side. This point is discussed above and is probably largely due to the hypertonic perfusate in the presence of tissue edema of disease.

TABLE IV

Muscle Hemoglobin before and after Perfusion. Mg. per 100 gm. of Muscle

No.	Leg unperfused	Leg perfused	Leg perfused; corrected for edema	No.	Leg unperfused	Leg perfused	Leg perfused; corrected for edema
X-670.....	1010	805	....	A-268.....	975	860	840
A-172.....	1000	630	....	A-274.....	1050	900	900
A-188.....	1140	940	1000	A-280.....	1225	670	840
A-198.....	960	930	970	A-281.....	600	650	590
A-204.....	820	615	690	A-282.....	540	580	580
A-209.....	1230	860	1120	A-283.....	970	990	1030
A-253.....	750	790	....	X-835.....	570	550	550
A-254.....	1095	945	1040	A-285.....	1170	1100	1050
A-258.....	1160	910	1090	A-295.....	800	510	550
A-260.....	1015	855	....	A-296.....	670	660	590
A-262.....	1180	740	930	A-297.....	1050	1000	990
A-267.....	795	370	550				

Necropsy Material. In Table V we present muscle hemoglobin values together with the cause of death, age of patient and blood hemoglobin. The values for the blood hemoglobin and all other data pertaining to the patient we have obtained from the clinical history. The blood hemoglobins are recorded on the basis of the Sahli standards. It will be noted that the values range all the way from 550 mg. to 1120 mg. of hemoglobin per 100 gm. of muscle. In comparing the relationship between blood hemoglobin and muscle hemoglobin it is interesting to note that seven of sixteen cases have blood hemoglobin of 75 per cent or below. Of these seven only three have muscle values in the lower half of the group and the remaining four have muscle values in the high upper part of the group. In fact the highest muscle value obtained had a blood hemoglobin of 70 per cent while the two lowest blood hemoglobins, 30 per cent and 43

per cent, have very high muscle hemoglobin values, 1090 and 1000 mg. respectively. These two cases are quite remarkable; the lower value of 30 per cent hemoglobin was found in a case of rapidly

TABLE V

Muscle Hemoglobin as Related to Blood Hemoglobin. Cause of Death, and Age of Patient

Case No.	Muscle hemoglobin *		Age	Blood hemoglobin per cent	Red cell count	Cause of death
	Adductor	Rectus				
A–267 ...	550	490	72	80	4,250	Combined sclerosis. Bronchopneumonia.
A–295 ...	550	475	58	60	2,400	Colitis. Starvation.
A–282 ...	580	550	58	77	4,910	Carcinoma of breast.
A–296 ...	590	...	53	90	....	Myocardial failure.
A–281 ...	590	...	77	84	....	Arteriosclerosis. Chronic nephritis.
A–204 ...	690	...	56	65	....	Perinephritic abscess. Starvation.
A–253 ...	790	710	57	90	....	Arteriosclerosis. Lobar pneumonia.
A–268 ...	840	...	41	70	2,800	Alcoholism. Pneumonia.
A–274 ...	900	670	55	84	....	Carcinoma of trachea.
A–262 ...	930	950	13	92	5,570	Scarlet fever. Bronchopneumonia.
A–198 ...	970	870	10	75	4,568	Pneumococcus meningitis.
A–188 ...	1000	1030	4.5	43	2,600	Aplastic anemia.
A–283 ...	1030	750	15	78	4,800	Post diphtheritic paralysis and bronchopneumonia.
A–254 ...	1040	...	32	95	4,650	Lobar pneumonia.
A–258 ...	1090	970	25	30	1,740	Aplastic anemia.
A–260 ...	1100	820	38	70	3,810	Post scarlatinal nephritis.
Additional cases in which blood values are not available						
X–835 ..	550	...	60	..	....	Cirrhosis.
A–205 ...	620	...	43	..	....	Cirrhosis. Lobar pneumonia.
X–670 ..	805	625	45	..	....	Fractured skull.
A–280 ...	840	...	51	..	....	Strangulated hernia. Peritonitis.
A–297 ...	990	...	66	..	....	Carbuncle. Septicemia.
A–285 ...	1050	790	27	..	....	Syphilitic menigitis. Basilar hemorrhage.
A–209 ...	1120	770	40	..	....	Diffuse bronchopneumonia.

* Hemoglobin in mg. per 100 gm. of muscle. Values of perfused muscle corrected for edema.

developing aplastic anemia (A–258). The entire duration of the illness was three to four weeks in an active young woman, 25 years of age. The other case in this group is an unusual one of a young child

4½ years old who had been confined to his bed during most of a severe months illness. We should not expect such a high reading here for two reasons, first, age, and second, the long stay in bed. However, there is an interesting factor present; this patient had twenty-one transfusions of 50 to 150 cc. of blood within five weeks. There was much blood pigment in the endothelial phagocytes of the lymph nodes and spleen. It is probable that this greatly increased blood destruction and storage of pigment have been in some part responsible for this increase in muscle hemoglobin.

We hope to measure the muscle hemoglobin in cases of pernicious anemia as it is generally observed that the skeletal muscles are often very deep red in these cases. It is interesting to note that the case with the lowest muscle hemoglobin had a blood hemoglobin of 80 per cent. There are several cases with low muscle hemoglobin values which also have moderately low blood hemoglobin, 70 to 85 per cent, but these are usually in inactive individuals. Although this series of sixteen cases in which the blood findings are known is entirely too small to allow us to draw general conclusions, nevertheless the figures agree with those recorded in dogs and indicate that severe anemia causes only a slowly progressing slight decrease in the muscle hemoglobin.

Table VI is a redistribution of Table V showing the muscle hemoglobin values both in the acute and chronic diseases. In the thirteen necropsies in which we have listed an acute illness as the cause of death the average muscle value is 980 mg. of hemoglobin per 100 gm. of muscle. The lower values in this group are from, (1) an obese alcoholic, 45 years old, no occupation, who sustained a fractured skull; (2) an obese woman, 51 years old, who was also a diabetic and who died following an operation for a strangulated hernia; and (3) an emaciated woman of 41 "who looks much older," who was a drug addict and had suffered from pain and stiffness in the joints for several years. With the exception of these three patients, this group was fairly active, most of them young or middle-aged. In this group we have included a patient having carcinoma of the trachea; who died from obstruction and who had been fairly active until shortly before death.

Of the ten chronic diseases listed, the average hemoglobin value is 651. Not including the aplastic anemia of four weeks duration noted above it would be 620. The three lowest values listed were,

(1) a case of combined sclerosis who had been bedridden for seven months; (2) an obese habitual drunkard of 66 who had been doing no active work; and (3) an extremely emaciated woman of 54 who died of colitis and starvation. The difference of the muscle hemoglobin values in the acute and chronic diseases is quite striking.

TABLE VI

Muscle Hemoglobin as Related to Cause of Death

	Adductor longus Muscle Hemoglobin *	Average
Acute Diseases		
Pneumococcus meningitis	970	
Lobar pneumonia	1040	
Aplastic anemia	1090	
Post scarlatinal nephritis	1100	
Scarlet fever. Bronchopneumonia	930	
Alcoholism. Pneumonia	840	
Carcinoma of trachea and obstruction	900	
Post diphtheritic paralysis. Bronchopneumonia	1030	
Diffuse bronchopneumonia	1120	
Strangulated hernia, peritonitis	840	
Syphilitic meningitis	1050	
Carbuncle and septicemia	990	
Fractured skull	805	
		980
Chronic Diseases		
Aplastic anemia	1000	
Perinephritic abscess with starvation	690	
Combined sclerosis. Bronchopneumonia	550	
Arteriosclerosis. Chronic nephritis	590	
Carcinoma of breast	580	
Colitis. Starvation	550	
Myocardial failure	590	
Arteriosclerosis. Lobar pneumonia	790	
Cirrhosis. Lobar pneumonia	620	
Cirrhosis	550	
		651

* Muscle hemoglobin values expressed in mg. per 100 gm. of muscle.

Every case with an extremely low value is one in which there is a chronic wasting disease necessitating inactivity.

Muscle Hemoglobin Related to Age. There are four cases in this series below 20 years of age (see Table V). They show high values in the neighborhood of 1000 mg. There are only three cases over 60 years of age. Two of these show low values but both had chronic diseases. The highest values occur in a 40 year-old laborer who had

been working hard until six days before death, and in a 38 year-old well developed man. Other high values occur in patients whose ages are 25, 27, and 32. We believe that here again we observe the result of activity rather than of age itself, for the previously healthy young adults show the highest values.

In conclusion it may be pointed out that the behavior of muscle hemoglobin is very like that of blood hemoglobin particularly as regards the end products formed within the body. It is known [8] that muscle hemoglobin introduced intravenously, intraperitoneally or intramuscularly is promptly excreted in part as bile pigment in the urine. Therefore it will be of much interest to study clinical cases of myositis or other related diseases to obtain evidence as to the quantitative contribution of bile pigment arising in the striated muscles.

Summary

A method is described for the quantitative analysis of muscle hemoglobin in autopsy material.

In twenty-three cases studied, values of 550 mg. to 1120 mg. per 100 gm. of muscle are found.

The muscle hemoglobin is apparently only slightly influenced by fluctuations in the blood hemoglobin.

The highest values are found in active young adults who have died after a short illness.

Low values are found in the chronic diseases and wasting illnesses. In every case in which there is an exceptionally low value there has been prolonged inactivity.

We conclude that in man as in the dog the concentration of the muscle hemoglobin within the muscle fibers is determined more by muscular activity than by the level of blood hemoglobin.

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