V. THE IRON CONTENT OF BLOOD FREE TISSUES AND VISCERA

Variations Due to Diet, Anemia and Hemoglobin Injections

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(Received for publication, January 4, 1932)

When one studies the conservation of hemoglobin in the body, a knowledge of the storage of iron in various tissues is much to be desired. This information could not be obtained from the literature on this subject extensive as it is. It will be found that the distribution of iron corresponds roughly with the deposit of iron containing pigment (Paper IV) but there are many discrepancies which are obviously explained by iron deposits which give no iron stain.

When one reviews the published experiments of many investigators dealing with the iron content of various organs, it becomes apparent that two serious difficulties must be faced. Iron analyses are difficult[1] when applied to viscera and method errors may actually mask the true iron content. Analysis of viscera containing blood within the capillaries gives no accurate measure of the tissue iron as the contained blood may contain more iron than the tissue. We believe we have overcome both of these difficulties.

A glance at the tables below shows that the *kidney* as well as the spleen, bone marrow and liver is concerned with the conservation of iron after frequent hemoglobin injections. In Paper IV we have discussed the iron staining pigment found in the epithelium of the convoluted tubules of kidney. When the minimal renal threshold for hemoglobin is exceeded, we note a deposit of iron staining pigment in the tubular epithelium but there are no pigment deposits of this

[1] We acknowledge with much appreciation the invaluable assistance of Dr. S. H. Bassett of the Department of Medicine in working out the method of iron analysis as applied to body tissues.

nature if the hemoglobin injections are kept below the minimal renal threshold. The iron analyses of the kidneys are in harmony with these observations. With subthreshold hemoglobin dosage the kidney shows a normal or control iron content but following many superthreshold hemoglobin injections the iron content of the kidney may increase to 5 or 6 times the control level (Table 53).

Under a variety of dietary conditions we observe that the iron content of spleen, liver and bone marrow fluctuates widely but the kidney is relatively constant. We believe this is in part due to the fact that our dogs maintain an unusually high hemoglobin level (120 to 150 per cent) and presumably have a high storage of iron and iron staining pigment.

Anemia reduces the storage of iron staining pigment (Paper IV) as well as the tissue iron (Table 52) which indicates that this stored iron is used at least in part for the elaboration of new hemoglobin. To attain the most accurate base line in the *study of iron storage* due to diet or other factors we believe an initial anemia period of 2 months or more is highly desirable.

Methods

Healthy adult dogs were used in all experiments with the few exceptions specifically mentioned. These normal dogs presented a red cell hematocrit of 50 to 55 per cent which corresponds to 140 to 150 per cent hemoglobin. Some method procedures have been described in the other papers of this series. The pigment studies of these same dogs are given in Paper IV.

The body tissues and viscera were freed from red cells by a method previously described (4). In brief, the method is as follows: Under ether anaesthesia the jugular vein and carotid artery are canalized. A Locke's solution containing 5 per cent glucose flows by gravity into the jugular while simultaneously equal amounts of blood are removed from the carotid. By the use of graduated containers the inflow of Locke's solution and the outflow of blood is kept equivalent and there are no large fluctuations in the blood volume. Under such conditions with ether anaesthesia the heart continues to beat with its usual force until the end of this viviperfusion and the tissues are washed free of red cells as in no other way. If the bleeding is not too rapid the procedure occupies about 30 minutes and the red cell hematocrit falls from 50 to 55 per cent or normal to a fraction of 1 per cent which we consider representative of a successful type of perfusion. There is no gross edema of tissues to confuse the picture as is true even with an unsatisfactory gravity perfusion. At the end of the perfusion the heart beats begin to fail and at this time a little adrenalin given through

the jugular will raise the blood pressure for a time and facilitate the washing out of red cells within the viscera. When the heart stops the viscera are removed promptly, permitted to drain, placed in a towel moistened with Ringer's solution to prevent drying, then cut up and weighed for analysis. The ribs used for analysis while fresh are scraped free of all muscle and periosteum and no cartilage is included. Small bits of tissue are saved for histological study.

In a satisfactory experiment the tissues are bloodless in gross and show no red cells in histological section with the exception of the spleen and bone marrow. The lungs, gastrointestinal tract and pancreas are a pale grayish white. The heart and striated muscle present the usual color due to muscle hemoglobin which is not washed out. The spleen is small and a pale pinkish red. The liver is a deep buff or maple sugar color. The kidneys are a pale buff often modified by pigment of a brown color. The rib marrow appears as usual and is a pale pink color. The lymph glands are often a bit pigmented. The weights of the specimens taken for iron analysis depend upon the mass of tissue available. The weights run from a few grams in the case of lymph glands up to 80 to 90 gm. in the case of the liver.

Muscle hemoglobin solutions used in Table 53 were prepared by method previously described in detail (4).

The method for *iron analysis* is accurate and dependable but somewhat time consuming. It is described in detail in a recent publication by Bassett, Elden and McCann (1). The dry method of ashing by means of the electric furnace was found to be unsatisfactory. In all analyses given below the wet method as described was used. In the ashing of tissue a preliminary digestion with fuming nitric acid was carried out. Usually 50 cc. of acid was added and allowed to stand some hours. Subsequently digestion over a water bath gives complete solution. The solution while warm is transferred to 800 cc. pyrex Kjeldahl flasks, care being taken to secure a complete rinsing of the beaker. Sulphuric acid (20 cc.) is added and the flasks heated to drive off the water and nitric acid. Perchloric acid (8 to 10 cc. of 60 per cent solution) is added and heat applied until the solution is clear. This final solution while still hot is transferred quantitatively to a 200 cc. volumetric flask. After cooling to room temperature the flask is made up to the mark with distilled water. This solution is stored in tightly stoppered flasks and represents the original sample of tissue dissolved in 200 cc. of fluid.

25 to 50 cc. portions of these solutions are now pipetted into 100 cc. pyrex Florence flasks and heated over the open flame until the dense white fumes of sulphuric acid are evolved. This procedure insures the removal of all traces of nitric and perchloric acid or any other oxydizing agent which prevent accurate titration. The solution is now diluted with 20 per cent sulphuric acid which has previously been boiled 2 hours, cooled in presence of carbon dioxide and stored in a flask protected from the air by a blanket of pure carbon dioxide. These specimens are now ready to be titrated electrometrically with dilute titanous sulphate solution protected from the air by means of carbon dioxide gas. The

apparatus and method as described by King and Washburne (2) was used. We did not use the mercury seals at the top of the burette and on the flask containing the titanous sulphate solution as described by these authors; tight fitting rubber stoppers were satisfactory for our purposes. In place of the ferrous ammonium sulphate standard, as used by the authors, a solution of iron wire in hydrochloric acid was prepared. One-tenth of a gram of iron wire C.P. is accurately weighed and dissolved in dilute hydrochloric acid. A small amount of 30 per cent hydrogen peroxide is added to insure complete oxidation. The solution is now diluted to 2000 cc. Twenty cc. portions are now pipetted into 100 cc. beakers and allowed to concentrate slowly on the steam bath. When the volume approaches 5 cc. the samples are transferred to 100 cc. pyrex Florence flasks with the specially prepared sulphuric acid and titrated with titanous sulphate using the apparatus referred to above. One troublesome source of error, resulting in failure to get checks on different iron standard specimens, often occurs if the standard is allowed to concentrate below a volume of about 5 cc., due to the loss of iron by volatilization. Five cc. of concentrated sulphuric acid may be added and the standard heated down over the open flame in the same manner as the tissue specimens are heated. Two determinations were made upon each specimen and in some cases two separate digestions of the same organ were checked. The method although cumbersome proved to be quite accurate and dependable even for very small amounts of iron.

EXPERIMENTAL OBSERVATIONS

We believe that in our hands the method employed in these dogs prepares tissues and viscera which are practically free from blood—let us say 1 per cent of normal or less. The spleen and bone marrow are exceptions but even here the tissues contain relatively few red cells and those cells are largely in the intersinusoidal stroma. With anemia we note that the spleen and bone marrow show very low iron figures as compared with normal controls (Table 52) and this gives support to our belief that even in these organs the method of viviperfusion gives a pretty complete washing out of mature red cells.

The iron content of colon, jejunum, stomach and urinary bladder was determined on seventeen of the dogs and is not included in the tables. The fresh specimens include the mucosa. The content of the *stomach* varied from 0.9 mg. to 2.1 mg. per 100 gm. of fresh tissue averaging about 1.5 mg. The content of the *jejunum* varied from 0.9 mg. to 3.3 mg. with an average content of 1.7 mg. The lowest value for the colon was 0.8 mg. per 100 gm. of fresh tissue in Dog 30-174 which was fed on a diet of anemia bread and milk for

31 days (Table 51). The highest values for the colon were 4.5 mg. in Dog 29-288 which presented an unexplained hemoglobinuria (Table 52) and 3.7 mg. in Dog 30-125 which received 500 mg. of iron daily as iron citrate added to the kennel diet. The average for the *colon* was 2.2 mg. per 100 gm. of fresh tissue. The iron content of the urinary bladder averaged 2.2 mg. per 100 gm. of fresh tissue. These figures indicate that the *gastrointestinal tract* although concerned with the absorption and excretion of iron in its varied combinations, is not concerned with storage of any iron compound. These low values also give an admirable control of the viviperfusion method as it is not possible to free the intestinal villi of red blood cells by any type of gravity perfusion. These analyses serve also as controls for the method of iron analysis.

When the experiments in Table 51 were planned we did not realize that our stock dogs with high blood hemoglobin (140 to 150 per cent) presented a very considerable iron storage as compared with anemic dogs (Table 52). In the face of this conspicuous iron storage in the dogs taken out of stock (Dogs X-2806 and X-2884, Table 51) we hesitate to stress the findings observed in other widely different diets. For a comparison with the iron staining pigment refer to Table 43, Paper IV. It is apparent that the ideal diet experiment would be preceded by a 2 to 3 months' anemia period due to bleeding, at a hemoglobin level of 1/3 normal (40 to 50 per cent hemoglobin).

The first two experiments in Table 51 (Dogs 30-174 and 30-250) were continued on a diet of anemia bread and milk for 31 and 21 days respectively. The make up of this bread has been described elsewhere (3). This diet permits of a minimal regeneration of new hemoglobin in anemia due to bleeding. The iron analysis of various organs is close to the average values.

The third and fourth experiments in Table 51 (Dogs 30-125 and 30-103) were continued 31 and 30 days on a liver diet and diet rich in iron citrate. Both these diets are very favorable to rapid regeneration of new hemoglobin in anemia due to bleeding. The liver diet dog shows average values for all tissues except the liver and spleen. The spleen is above the average if we exclude from the average the dog fed iron citrate. The liver iron analysis is only slightly above the average. We do not attach any significance to these figures.

The dog fed large amounts of iron citrate (Dog 30-125) does show an unusually high iron content for the spleen but the liver content is below normal. The lung also shows a high iron analysis. It is possible that the iron feeding did effect this iron storage.

The dog fed carrot and mush (30-270, Table 51) for 25 days received a diet unfavorable for new hemoglobin regeneration in anemia due to bleeding but rich in carotin. This healthy airedale shows such high iron values for his liver, spleen and bone marrow that we suspect some unknown condition leading to blood destruction in the weeks or months preceding this experiment. This experiment is entirely out of accord with the other diet dogs and we do not feel that these changes are in any way related to the diet.

TABLE 51

Diet Factors and Iron Content of Perfused Tissues

Dog No.	Type of diet	Duration	Iron in mg. per 100 gm. of fresh tissue								
			Kidneys	Liver	Spleen	Mes. lymph glands	Ribs	Heart	Psoas muscle	Lung	Pancreas
		days									
30-174	Anemia Bread and milk	31	4.7	20.4	38.0	—	22.8	6.5	6.3	10.2	2.4
30-250	Anemia Bread and milk	21	6.9	22.7	40.1	8.3	20.5	3.7	2.8	6.7	2.5
30-125	Fe (500 mg.) + kennel	31	4.0	17.1	86.8	9.7	17.5	3.3	4.3	13.4	1.6
30-103	Liver (300–450 gm.) + meat + bread	30	5.4	30.3	46.1	5.5	14.4	4.0	2.4	6.1	2.0
30-270	Carrot + mush	25	5.0	42.2	53.6	—	14.9	—	3.9	5.5	3.2
X-2806	Kennel	30+	2.2	13.9	23.3	4.8	7.9	3.0	3.4	3.7	1.6
X-2884	Kennel	30+	4.0	25.7	37.8	3.7	7.4	2.9	3.1	2.7	1.3
Average			4.6	24.6	46.5	6.4	15.0	3.9	3.7	6.9	2.1

The last two dogs (X-2806 and X-2884, Table 51) are healthy stock dogs which had been on the kennel diet of mixed hospital food scraps for more than 1 month. This diet is moderately favorable for new hemoglobin regeneration in anemia due to hemorrhage. The iron analyses for the tissues are slightly below the general average for Table 51.

Table 52 shows that in anemia the iron content of all tissues and viscera is reduced to minimal levels. The low values for iron are conspicuous in the pancreas and this again serves as an admirable check on the methods of perfusion and iron analysis. For the study of the iron staining pigment in these dogs (Table 52) refer to Table 44, Paper IV. It is of interest that the muscle tissue of the heart

and psoas show no significant change from the control samples. It is probable that much of this iron is a part of the muscle hemoglobin.

The first two experiments (Table 52) are examples of a pretty severe anemia due to bleeding continued for 1 and 1.5 months respectively. Even this short anemia period removes practically all the iron reserve storage in spleen, liver and bone marrow. The kidney iron level is about 1/2 normal for the controls. The liver is reduced to 5 and 4 mg. iron level which is about 1/5 normal for the controls. The spleen and bone marrow are reduced to about 1/4 and 1/3 normal iron content but part of this iron is surely contained in red blood cells not removed by the viviperfusion.

The third experiment in Table 52 deals with an unusual case of spontaneous hematuria in a dog otherwise normal and healthy. This dog was under observa-

TABLE 52

Hemorrhage, Anemia and Iron Content of Perfused Tissues

Dog No.	Anemia		Iron in mg. per 100 gm. of fresh tissue								
	Hb. level	Duration	Kidneys	Liver	Spleen	Mes. lymph glands	Ribs	Heart	Psoas muscle	Lung	Pancreas
	per cent	*mos.*									
30-271	50–60	1.0	1.4	5.0	17.5		4.1	2.9	4.0	3.3	0.7
30-257	50–60	1.5	2.9	4.0	6.6		3.5	2.5	3.3	2.1	1.1
29-288	95–100	*3.0±	3.0	13.8	15.3	9.4	6.4	2.8	7.8	4.1	1.0
29-8	95–100	**1.0±	3.9	11.4	52.0	8.3		2.9	4.3	5.2	1.3
Average.....			2.8	8.6	22.8	8.8	4.7	2.8	4.8	3.7	1.0

* Spontaneous hematuria.

** Closed sterile bile fistula for 9 months.

tion for over 3 months and his blood hemoglobin level was about 2/3 normal (95 to 100 per cent hemoglobin). Autopsy did not reveal the bleeding spot and all tissues were normal. Under these conditions the iron reserve storage is not reduced to minimal levels but is distinctly subnormal.

The last experiment in Table 52 concerns an animal with a closed sterile bile fistula which during the last month of life developed an obstruction in the tube and a mild grade of secondary anemia. The high values for iron in the spleen are probably related to icterus and blood destruction.

Table 53 shows the result of hemoglobin injections of various sorts and in varying amounts both superthreshold and subthreshold, with and without hemoglobinuria. The organs involved in the reaction

to hemoglobin injections with consequent iron storage are the kidney, spleen, liver and bone marrow.

For the clinical histories, data related to the hemoglobin injections and pigment study in these same dogs, refer to Table 41, Paper IV, and accompanying short histories.

The first two dogs in Table 53 (29-252 and 30-135) were given superthreshold hemoglobin doses of dog hemoglobin. The first dog received a few very large

TABLE 53

Hemoglobin Injections and Iron Analyses of Perfused Body Tissues

Dog No.	Hemoglobin injected				Iron in mg. per 100 gm. of fresh tissue								
	Type	Mg. per kilo daily	Total	Days inj.	Kidneys	Liver	Spleen	Mes. lymph glands	Ribs	Heart	Psoas muscle	Lung	Pancreas
			gm.										
29-252	Dog	625	31	7	44.8	37.5	177.1	13.8	24.7	3.9	2.5	4.7	3.2
30-135	Dog	125	56	25	17.4	36.8	122.7	22.6	11.9	3.4	4.0	8.4	1.4
29-213	Dog	73	32	29	11.0	27.2	40.2	15.4	11.0	5.1	4.9	3.6	2.3
30-84	Dog	35	48	85	6.2	45.3	101.9	—	41.5	3.7	4.5	7.0	3.1
29-219	Sheep	147	32	15	32.7	30.7	144.6	30.6	64.4	3.5	3.8	5.5	6.3
29-228	Sheep	85	42	17	25.7	34.3	58.7	—	35.6	4.3	3.6	5.6	2.5
30-108	Goose	124	10	15	35.2	20.9	111.8	—	28.6	4.1	2.2	4.5	1.6
30-231	Goose	80	15	15	17.4	21.1	43.8	10.0	—	4.3	2.5	—	1.6
30-149	Muscle	17	3.6	15	14.1	31.4	107.6	—	29.5	4.2	5.9	10.1	3.5
30-228	Muscle	12	2.5	16	9.1	26.6	143.5	—	25.5	4.1	4.8	4.9	1.9
Average					21.4	31.2	105.2	18.5	30.3	4.1	3.9	6.0	2.7

doses with conspicuous hemoglobinuria. The second dog received only a few superthreshold doses at the end of the series and this accounts for the great difference in the renal iron content. The values for spleen and liver are maximal.

The third experiment (Dog 29-213, Table 53) shows a long series of hemoglobin injections very close to the glomerular hemoglobin threshold. There was no iron staining pigment in the renal tubules but the iron analysis is above the control kidney figures so that it is probable that a little hemoglobin seeped through occasionally. The iron figures for the spleen and liver are somewhat below the average. Considering the large amount of hemoglobin injected and the low spleen values one suspects the possibility that the iron reserve storage had been depleted because of unknown factors before this period of observation.

The fourth experiment (Dog 30-84, Table 53) is of especial interest because

this dog received many hemoglobin injections during 3 months to a considerable total but each injection was well below the minimal renal or glomerular threshold for hemoglobin. The kidneys show a control level for iron content while the spleen, liver and bone marrow show the expected large reserve store of iron.

The fifth and sixth experiments (Dogs 29-219 and 29-228, Table 53) show the effect of injections of sheep hemoglobin in considerable total amounts. Both dogs were given superthreshold doses with consequent hemoglobinuria. There is abundant reserve storage of iron in the kidney, liver, spleen and bone marrow. The unusually high figures for the spleen and bone marrow in Dog 29-219 might suggest a less satisfactory viviperfusion than the average.

The seventh and eighth experiments (Dogs 30-108 and 30-231, Table 53) show the reaction to injections of goose hemoglobin, one a superthreshold and the other very close to the minimal renal threshold. The latter (Dog 30-231) shows a low iron figure for the kidney yet above the control average which may indicate an occasional seepage of hemoglobin into the tubules.

The last two experiments in Table 53 deal with muscle hemoglobin injections which caused definite hemoglobinuria. As has been pointed out in Paper IV, muscle hemoglobin is different in several respects from blood hemoglobin. The preparation of muscle hemoglobin has been described elsewhere (4). The final product obtained by passage through a medium Berkefeld filter is crystal clear, having the usual hemoglobin color, and quite sterile. It may be preserved a few days in the ice box but the sooner it is used the better. As muscle hemoglobin ages it does not change in its appearance but does change in some of its physical aspects. When used fresh this solution of muscle hemoglobin is non-toxic to most dogs but with aging in the ice box it develops toxicity. Because the preparation of muscle hemoglobin is quite time consuming there is always a tendency to use the solution for several days rather than prepare new solutions. Sometimes fatal shock is produced. When non-lethal shock is produced one may suspect that hemolysis or injury of red cells may follow. We are inclined to explain the high figures for iron analysis in the spleen on this basis as it is obvious that the iron contained in the few grams of muscle hemoglobin injected cannot account for the large surplus iron storage in the spleen.

DISCUSSION

The importance of the *kidney* in relation to iron conservation comes out clearly in the experiments tabulated above. Under normal conditions in a healthy dog the blood free kidney contains about 4.6 mg. iron per 100 gm. fresh tissue. This normal level is reduced to 2.8 or less by short anemia periods. When there is escape of hemoglobin in the blood stream the kidney plays its most important rôle. The kidney glomeruli establish the minimal renal threshold for hemoglobin and prevent its escape so that various phagocytic cells may

completely conserve and remove it from the circulation. Above this minimal threshold the tubular renal epithelium picks up this hemoglobin from the tubular lumen and saves it for future use. When the hemoglobin escape is very large or the renal epithelium is stuffed to repletion, we observe the escape of hemoglobin in the urine. This conservation of iron following superthreshold doses of hemoglobin may raise the iron content of the kidney from normal or 4.6 mg. to 35 to 45 mg. per 100 gm. fresh tissue.

The *liver* is without doubt the most important organ in the conservation and utilization of iron in the body. One may look upon the liver as a warehouse in which the turn over is active and the surplus storage considerable but held within fairly narrow limits. Because of its size the liver contains the largest amount of stored iron although the spleen contains more per 100 gm. fresh tissue.

The blood free liver normally contains about 25 mg. iron per 100 gm. fresh tissue with maximal figures of 42 and minimal of 14. Short periods of anemia will reduce this surplus store to 4 or 5 mg. iron per 100 gm. fresh tissue. Long periods of hemoglobin injection do not increase the liver storage of iron to any remarkable extent and we observe average values of but 31 mg. iron per 100 gm. fresh tissue. This is in striking contrast to the spleen (Table 53).

The *spleen* in contrast to the liver we look upon as a favored storehouse for iron but the turn over here is probably much slower than in the liver. Much of the iron in the spleen is in the form of iron staining pigment (Paper IV). The iron storage in the spleen can be notably depleted by anemia and in certain cases (Table 52) may even approximate the liver values. In fact during periods of continued anemia in the dog all the stores of iron are drawn upon and conspicuous depletion is obvious in spleen, liver and bone marrow.

The *red marrow* of the ribs is of considerable interest when compared with the spleen and runs a remarkably close parallel to it. Ribs in the adult dog consist of bone and red marrow, the latter making up 1/3 to 1/2 the total volume. If we figure the marrow as 1/3 by weight and compare rib marrow and spleen, the parallelism is remarkably close. In three anemia dogs, excluding the bile fistula (Table 52) the iron reads 4.7 mg. for marrow and 13 mg. for spleen. In the normal group (Table 51) the iron analyses read 15 mg. for

the marrow and 39.8 mg. for the spleen, excluding the high spleen value in the iron feeding experiment (Dog 30–125). In the group injected with hemoglobin (Table 53) the red marrow reads 30.3 mg. and the spleen 105 mg. iron per 100 gm. fresh tissue.

In histological study of the spleen and marrow (Paper IV) it was found that the iron staining pigment appeared to be identical in general distribution in both tissues so that the spleen tabulation was considered representative of both spleen and marrow. The mesenteric and retroperitoneal lymph glands show relatively little of interest. The histological study (Paper IV) shows the presence of iron staining pigment when there is evidence that pigment was being deposited in related viscera whose lymph drainage is carried through the lymph glands studied. During anemia periods this iron store is drawn upon but probably more slowly even than the spleen. The small amount of tissue makes analysis relatively inaccurate. In normal dogs this gland tissue assays about 7 mg. iron per 100 gm. fresh tissue as compared with 18 mg. iron in the hemoglobin injected dogs. The amount of iron concerned in lymph gland tissue therefore is trivial.

Heart muscle and *skeletal muscle* (psoas) show great uniformity of iron content. The figures for iron in heart or skeletal muscle run close to 4 mg. per 100 gm. fresh tissue whether the dog is normal or has received a series of hemoglobin injections. In anemia the heart muscle may show a slight decrease. It is probable that a large part of this iron is within the muscle hemoglobin molecule which is an essential part of these muscle fibres.

The *lungs* show a low iron content which fluctuates somewhat from a minimum of 2.7 to a maximum of 10 mg. per 100 gm. fresh tissue with average values of 6 to 7 mg. in dogs which are normal whether they have been injected with hemoglobin or not. During anemia the iron content falls somewhat to an average of 3.7 mg.

The *pancreas, stomach, jejunum, colon* and *urinary bladder* all present minimal values for iron. The low normal values for the pancreas (2.1 mg. iron) are increased very slightly by hemoglobin injections (2.7 mg. iron) and depleted by anemia to minimal tissue levels (1.0 mg. iron). The stomach shows average values of 1.5 mg. iron, the jejunum 1.7 mg. iron and the colon 2.2 mg. iron. The iron content of the urinary bladder is 2.2 mg. iron per 100 gm. fresh tissue.

These last figures indicate that *smooth muscle* contains very little iron. The same statement applies to the *mucosa* of the stomach, jejunum and colon which was a part of the tissue used for analysis.

SUMMARY

When hemoglobin is set free in the circulation the *kidney* plays an important part in the *conservation of iron.* When the renal threshold is not exceeded by the hemoglobin in the blood there is little or no excess iron deposited in the kidney but when superthreshold doses of blood hemoglobin are given the epithelium of the convoluted tubules accumulates much iron and the iron analyses may show 5 times normal values.

The normal dog (140 to 150 per cent hemoglobin) has a large reserve store of iron in the liver, spleen and marrow. Diets may modify this storage of iron in these tissues. To bring conclusive proof relating to the individual diet factors, the reserve store of iron should be depleted by an anemia period of 2 to 3 months.

Complete removal of red cells from tissue capillaries is essential for accurate iron assays of fresh tissue. The method described accomplishes this without causing gross tissue edema.

The lowest iron content is observed in the pancreas, stomach, jejunum, colon and urinary bladder. These figures average from 1 to 2 mg. iron per 100 gm. fresh tissue. This shows that smooth muscle and mucous membranes contain little iron.

Striated muscle (heart, psoas) shows a relatively low iron content but uniform values close to 4 mg. per 100 gm. tissue.

Lungs show a considerable fluctuation with low iron values in anemia (3.7 mg.) and higher values in health (6 to 7 mg.).

The *spleen* shows maximal fluctuations and the highest reserve storage of iron per 100 gm. fresh tissue. The spleen iron analyses show low values in anemia (7 to 15 mg.) and wide differences in controls (25 to 50 mg.). With hemoglobin injections the iron storage is conspicuous and iron analyses may run as high as 150 to 175 mg. iron per 100 gm. fresh tissue.

Bone marrow of the rib runs in parallel with the spleen as regards iron storage following hemoglobin injections and depletion following anemia periods.

The *liver* because of its weight always contains the main bulk of the iron stored in the blood free tissues of the body. Its store is depleted by anemia even to levels of 4 to 5 mg. iron per 100 gm. fresh tissue. In the normal dog the iron store in the liver averages 25 mg. per 100 gm. tissue. Frequent hemoglobin injections may increase this level to 31 mg. iron per 100 gm. The liver is considered the most active clearing house for iron storage and utilization.

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