METABOLISM STUDIES IN HEMOPHILIA*

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Since the year 1820 when Nasse of the University of Bonn formulated the law that males alone are the bleeders, and the disease, hemophilia, is transmitted by normal females through their marriage with normal males, much has been written on the subject of hemophilia. This disease, which Grandidier termed "The most hereditary of hereditary diseases," has been discussed from all angles and points of view, and the only conclusion that has been reached is that the coagulation of the blood was defective (Wright, Addis, et al.).

What the cause of this delayed coagulation is has been explained in various ways. Sahli and Morawitz have stated that the calcium content of the blood is normal in this disease; that the thrombogen content of the blood is normal; and they ascribe the delayed coagulation to a deficiency in the thrombokinase (the activator of the thrombogen) of the blood. This theory has been contradicted by Addis, who believes that the fault lies in the composition of the thrombogen, which (though normal in quantity) is so changed in character that it liberates the thrombin with difficulty.

According to Weil, the delayed clotting is due to an excess of anti-thrombin, whereas, according to Howell the deficiency of the blood in thrombogen is to be blamed for the defect. It was Wright who ascribed the delayed coagulation of the blood in hemophilic subjects to a lack of calcium.

In examining the literature on this subject I have failed to find any records of metabolism studies in this condition. Since I have had occasion to make such studies of two cases of hemophilia, I think it will be interesting to report my results.¹

The two cases studied were of different types of hemophilia. They were both in boys. Julius was about 9 years old, and gave a typical history of hereditary hemophilia. The boy Alter, was about 6 years old and was not a hereditary "bleeder." Dr. Hess thus presents the clinical history of this boy (Alter):

The child has been under our observation for the past three years. . . . Attention was first directed to him on account of his tendency to bleed from

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1. The clinical studies of these cases were reported by Dr. A. F. Hess, Johns Hopkins Hosp. Bull., 1915, xxvi, 372. A preliminary communication was made by A. F. Hess and Max Kahn, Biochem. Bull., 1915, iv, 213.

minor cuts, to manifest frequent "black-and-blue marks" and to have severe hemorrhages from the nose. As far as can be ascertained, he is the sole member of his family who is a bleeder, so that we must consider him as affording an instance of the sporadic type of hemophilia. He is fairly well nourished, weighs about 50 pounds and shows no other abnormality except a moderate degree of ichthyosis of the skin.

In both of the cases it was found that there was delayed coagulation of the blood. In the case of Julius the blood clotted in about eighteen minutes, and in the case of Alter it clotted in thirty-four minutes. On analyzing the blood of these patients for calcium and comparing the results with normal cases we obtained the results shown in Table 1:

TABLE 1.—CALCIUM OXID IN BLOOD

Normal Controls, Gm.	Alter, Gm.	Julius, Gm.
0.072.....	0.047	0.065
0.068.....	0.056	0.069
0.074.....	0.058	
0.066.....	0.058	
0.071.....	0.062*	
0.073.....	0.064*	
0.062.....		
0.068.....		
0.075.....		
0.069.....		
0.075.....		
0.065.....		

* Calcium lactate (2 gm.) had been given for the previous three days by mouth.

It will be seen that in the case of Julius, atypical hemophilic, the lime in the blood was normal, whereas in the case of Alter the calcium content of the blood was decreased.

We reasoned that if the calcium deficiency was the sole cause of the failure of the blood to clot in the case of the boy Alter, this defect could be remedied by adding to the blood the missing lime salt. We, therefore, prepared a solution of calcium chlorid in physiologic saline (0.04 gm. Ca calculated from CaCl_2 in 1,000 gm. saline) of such strength that the addition of a drop of this solution to ten drops of blood will approximately supply the deficiency in calcium. (Normal CaO in blood calculated to be 0.08 parts per thousand). Table 2² shows the effect of the addition of the calcium solution on the clotting

2. Hess, A. F.: Johns Hopkins Hosp. Bull., 1915, xxvi, 373.

TABLE 2.—EFFECT ON CLOTTING TIME OF BLOOD OF ADDITION OF CALCIUM CHLORID SOLUTION

A. NORMAL CASE

Clotting Time	Control	Drops of Ca Solution added			
		1	2	3	4
4 min.	+		+	+	+
5 min.	++		++	++	+
6 min.	+++		+++	++	++
7 min.				++	++
9 min.				++	++
11 min.				++	++
13 min.				++	++
15 min.				+++	++
17 min.					++

B. HEMOPHILIA CALCIPRIVA (ALTER).

5 min.	—	—	—	—	—
9 min.	—	—	—	—	—
11 min.	—	+	++	—	—
13 min.	—	+	+++	+	—
17 min.	—	+++		++	++
21 min.	—			++	+++
26 min.	+			+++	+++
34 min.	+++				

C. TYPICAL HEMOPHILIA

4 min.	—	—	—	—	—
8 min.	—	—	—	—	—
10 min.	+	++	+	++	+
14 min.	++	++	++	++	++
18 min.	+++	++	++	++	++
22 min.		++	++	++	++
26 min.		++	+++	++	++
30 min.				+++	++

+++ denotes complete clotting time.

time of the blood. The determinations of the coagulation time of the blood were made by Dr. A. F. Hess. This table is taken from Dr. Hess' report.

It will be observed that in the normal case the addition of two drops of the lime solution had no effect, and the addition of three and four drops delayed the coagulation. In the case of Alter the addition of one drop quickened coagulation from thirty-four minutes to seventeen minutes; the addition of two drops caused clotting in thirteen minutes; the addition of three and four drops again slowed coagulation to twenty-six minutes. In the case of Julius the addition of calcium chlorid slowed the coagulation of the blood.

Having observed these effects we thought it advisable to study the general metabolism of the two hemophilic children, and compare them with normal children. The following procedure was adhered to in the study of the metabolism.

Both patients were fed on a diet of milk, eggs, butter and bread. Duplicate samples of each substance given the patients were sent daily to the laboratory where they were weighed and analyzed. The urine and the feces were collected daily for examination.

The experiment may be divided into three periods: 1. A fore-period, of a duration of three days, during which time the patients were kept in bed and fed the diet above mentioned, without being given any medication. 2. A calcium-feeding period also lasting for three days, from August 19 to 22, 1914 (during this time the patients received daily 2 gm. of calcium chlorid and 1 gm. of magnesium oxid). 3. An after-period, lasting three days, during which time there was given no medication.

EXPERIMENTAL METHODS

In the analysis of the food the following methods were employed: Nitrogen by the Kjeldahl method; total sulphur by the Wolff and Osterberg method, a modification of the Benedict method; calcium and magnesium by the McCrudden method, after ashing and extracting the ash with hydrochloric acid; phosphorus by the Neumann method.

In the urinary analyses, in addition, ammonia was determined by the Folin method, urea by the Benedict method, and total and ethereal sulphates by the Folin method. The inorganic sulphates were computed by subtracting the ethereal sulphates from the total sulphates, and the neutral sulphur by subtracting the total sulphate sulphur from the total sulphur. Total phosphorus was determined by the Neumann method, the phosphorus being weighed as magnesium pyrophosphate; creatin and creatinin by the Folin method, and uric acid by the Krieger-Schmidt method.

For the feces the same methods were used. In the case of the fat the Kumagawa and Suto method was employed. The results of the examinations are summarized in the four accompanying tables (Tables 3, 4, 5 and 6). These show that the disturbances of nutrition are not at all similar in the two children. In order to appreciate the results we have obtained it is wise to compare them with the metabolism of normal children, and we take the case of Herman S., a boy 5 years of age, as a standard (Tables 7 and 8).

TABLE 3.—SUMMARY OF METABOLISM DURING THE THREE PERIODS
A. TYPICAL HEMOPHILIA (JULIUS)

Substance	Fore Period (Average per Diem)				Feeding Period (Average per Diem)				After Period (Average per Diem)			
	Food	Urine	Feces	Bal.	Food	Urine	Feces	Bal.	Food	Urine	Feces	Bal.
Nitrogen.....	16.14	10.55	3.75	+1.84	16.10	10.13	2.28	+3.69	14.61	10.33	1.93	+2.30
Sulphur.....	1.90	0.98	0.87	+0.05	1.93	0.91	0.95	+0.07	2.06	0.91	0.95	+0.20
Phosphorus..	3.78	2.13		+1.65	3.26	3.03		+0.23	3.79	2.51		+1.28
Chlorin.....	7.94	6.74		+1.20	9.19	8.17		+1.02	8.58	6.78		+1.80
Calcium.....	1.59	0.206	0.824	+0.56	3.39	0.466	2.06	+0.85	1.47	0.17	0.71	+0.59
Magnesium...	0.51	0.177	0.133	+0.20	1.64	0.84	0.61	+0.19	1.45	0.70	0.53	+0.22

B. HEMOPHILIA CALCIPRIVA (ALTER)

Nitrogen.....	11.53	6.75	2.07	+2.71	11.50	6.99	2.17	+2.54	11.30	7.26	2.78	+1.26
Sulphur.....	1.31	0.88	0.30	+0.13	1.94	1.02	0.33	+0.59	1.84	0.95	0.29	+0.60
Phosphorus..	3.49	2.98		+0.51	3.36	3.09		+0.27	3.91	3.55		+0.36
Chlorin.....	4.60	4.09		+0.51	6.00	5.06		+0.94	5.03	3.44		+1.59
Calcium.....	1.37	0.39	1.80	—0.82	3.60	0.73	2.24	+0.63	1.33	0.21	0.81	+0.31
Magnesium...	0.42	0.19	0.11	+0.12	1.56	0.67	0.32	+0.57	0.67	0.14	0.07	+0.46

TABLE 4.—CALCIUM AND MAGNESIUM METABOLISM (ALTER)

Day	Intake	Urine	Feces	Balance	Intake	Urine	Feces	Balance
1.....	1.35	0.37	1.73	—0.75	0.35	0.14	0.09	+0.12
2.....	1.40	0.43	1.94	—0.97	0.47	0.21	0.12	+0.14
3.....	1.37	0.39	1.71	—0.72	0.45	0.21	0.14	+0.10
4.....	3.55	0.72	2.31	+0.52	1.92	1.21	0.56	+0.15
5.....	3.62	0.73	2.14	+0.75	1.35	0.42	0.21	+0.72
6.....	3.65	0.75	2.28	+0.62	1.41	0.37	0.20	+0.84
7.....	1.32	0.12	0.46	+0.74	0.87	0.16	0.09	+0.62
8.....	0.27	0.24	0.88	+0.15	0.62	0.10	0.05	+0.47
9.....	1.42	0.27	1.11	+0.04	0.54	0.17	0.09	+0.28

During Period 2, calcium and magnesium were added to the food.

TABLE 5.—URINARY NITROGEN PARTITION
A. HEMOPHILIA CALCIPRIVA (ALTER)

Day	Amount, c.c.	Total N, gm.	Urea N, Per Cent. of Total N.	Ammonia N, Per Cent. of Total N.	Creatinin N, Per Cent. of Total N.	Uric Acid N, Per Cent. of Total N.
1	750	10.56	82.7	4.2	3.1	2.25
2	720	9.87	83.4	4.4	3.4	1.98
3	810	11.23	82.5	3.9	2.9	2.72
4	690	10.07	82.9	3.9	3.2	2.15
5	760	10.11	82.7	4.1	3.1	2.22
6	770	10.27	83.2	4.3	3.2	2.14
7	840	9.87	83.5	3.8	3.4	2.07
8	720	10.56	81.9	4.4	3.3	2.24
9	730	10.72	82.8	4.2	3.4	2.05

B. TYPICAL HEMOPHILIA (JULIUS)

1	450	6.78	82.7	4.2	3.5	1.2
2	690	7.18	83.8	4.4	3.4	1.4
3	470	6.27	83.6	3.9	3.7	1.4
4	490	6.86	85.2	4.3	3.4	1.3
5	520	7.02	82.9	4.4	3.1	1.5
6	580	7.10	84.4	4.1	3.6	1.2
7	620			...	...	...
8	490			...	...	...
9	510	7.26	82.8	4.2	3.5	1.2

TABLE 6.—SULPHUR OUTPUT IN URINE AND FECES
A. HEMOPHILIA CALCIPRIVA (ALTER)

Day	Urine				Feces, Total S, Gm.
	Total S, Gm.	Ethereal SO ₄ Per Cent. of Total S	Inorganic SO ₄ Per Cent. of Total S	Neutral S Per Cent. of Total S	
1.....	0.82	12.6	72.9	14.5	0.27
2.....	0.97	14.2	70.1	15.7	0.32
3.....	0.85	13.7	72.0	14.3	0.31
4.....	0.89	12.9	69.9	17.2	0.38
5.....	1.02	12.4	75.0	12.6	0.29
6.....	1.15	12.2	74.7	13.1	0.33
7.....					0.27
8.....					0.28
9.....	0.95	13.4	71.1	15.5	0.31
Average.....	0.95	13.0	72.2	14.7	0.31

B. TYPICAL HEMOPHILIA (JULIUS)

1.....	0.87	14.3	68.1	17.6	
2.....	0.92	14.5	69.2	16.3	
3.....	1.05	16.2	65.7	18.1	0.87
4.....	0.97	12.9	67.9	19.2	0.98
5.....	0.86	16.1	68.4	15.5	0.85
6.....	0.89	14.4	68.0	17.6	1.04
7.....	0.87	14.7	69.0	16.3	0.95
8.....	0.91	13.8	70.6	18.6	0.90
9.....	0.94	13.2	69.2	17.6	1.02
Average.....	0.93	14.3	68.3	17.3	0.94

TABLE 7.—NITROGEN METABOLISM—NORMAL BOY—5 YEARS OLD
(AFTER SCHWARZ)³

Nitrogen in food.....	9.567 gm.
Nitrogen in urine.....	7.485 gm.
Nitrogen in feces.....	0.696 gm.
Retention	14.5 per cent.
Absorption	92.7 per cent.

3. Schwarz, H.: Jahrb. f. Kinderh., 1910, lxxii, 549.

TABLE 8.—SULPHUR METABOLISM—NORMAL BOY—5 YEARS OLD
(AFTER SCHWARZ)³

Total sulphur in food.....	0.968 gm.
Sulphur in urine.....	0.592 gm.
Sulphur in feces.....	0.075 gm.
Retention	31.1 per cent.
Absorption	92.2 per cent.

TABLE 9.—(MCCRUDDEN AND FALES.)⁴ NORMAL BOY, W. McC.
Weight at beginning of experiment, 27 kg.; weight at end, 27.2 kg.

URINE

Day	Nitrogen	Sulphur	Oxid	Magnesium Oxid	Phosphorus Pentoxid
1.....	10.43	0.729	0.374	0.153	2.34
2.....	10.65	0.744	0.406	0.139	2.22
3.....	10.35	0.717	0.373	0.154	2.23
4.....	11.07	0.832	0.348	0.152	2.06
5.....	11.35	0.808	0.332	0.157	2.58
6.....	11.12	0.810	0.390	0.154	2.68
Total.....	64.97	4.640	2.223	0.909	14.11

FECES

	Dry Feces					
1.....	2.28	1.232	0.133	1.518	0.267	0.686
2.....	19.0	0.997	0.085	1.846	0.263	0.718
3.....	20.9	1.165	0.115	1.784	0.288	0.768
4.....	23.5	1.173	0.113	2.064	0.301	0.839
5.....	15.3	0.611	0.063	1.318	0.220	0.526
6.....	21.1	1.110	0.073	1.643	0.279	0.856
Total...	122.6	6.488	0.582	10.193	1.618	4.393

Total excretion.....	71.46	5.222	12.42	2.53	18.50
Total in food.....	86.10	6.138	14.988	3.263	24.709
Retention.....	14.64	0.916	2.57	0.73	6.20
Per cent. retained.....	17.0	14.9	17.1	22.4	25.0

4. McCrudden, F. H., and Fales, H. L.: Jour. Exper. Med., 1912, xv, 453.

The protein metabolism as measured by the nitrogen and sulphur output, in the case of the two hemophilic children, is similar to the nitrogen and sulphur metabolism in the 5-year-old boy studied by Schwartz. The absorption, retention and excretion are about the same, and the partition of the nitrogen and the sulphur into the various fractions gave normal average results.

The results of the study of the mineral metabolism should be compared with the records of the study by McCrudden and Fales⁴ of the metabolism of a boy weighing 27.3 kg.

Compared with the normal boy, the excretion of calcium in the urine as compared with the excretion of calcium in the feces was about the same in both the hemophilic patients. In the case of Alter—the case of hemophilia calcipriva—there was a negative calcium balance in the fore-period, a positive calcium balance in the line-feeding period, and a decreasingly positive calcium balance in the after-period. The mineral metabolism in the true hemophilic, Julius, appears normal.

From these observations it would appear that not all hemophilia patients present similar pathologic-chemical disturbances. There seems to be no derangement in the metabolism as measured by the intake and output of nitrogen, sulphur, calcium, etc., in the case of hemophilia vera. There are, however, certain bleeders in whom the disturbing factor seems to be a lack of calcium content of the blood, and an inability on the part of their organisms to assimilate properly the lime from the food. In these cases the remedy indicated would be to administer the lacking mineral constituent in the form of the chlorid or the lactate of calcium.⁵

5. In addition to the previous numbered references, the following may be consulted:

Pratt, J. H.: Osler and McCrae, *Modern Medicine*, 1915, iv, 717.

Bulloch and Fildes: *Treasury of Human Inheritance*, London, 1911, xiva, 169.