EXPERIMENTAL LIPEMIA IN RABBITS.[1]

By THOMAS R. BOGGS, M.D., and ROGER S. MORRIS, M.D.

(From the Clinical Laboratory of the Johns Hopkins Hospital and University.)

PLATE XXV.

INTRODUCTION.

The phenomenon here briefly discussed was observed by the writers during some comparative studies of experimental anemias undertaken for class demonstrations. It was our intention to study with a group of students the changes in the blood of rabbits induced by (*a*) repeated daily hemorrhage and (*b*) poisoning with pyrodin.

In conducting the experiments rabbits were kept in metabolism cages and fed on a mixed diet of bread and cabbage, with water unrestricted. The rabbits which received pyrodin were given graduated doses of the drug, 0.015 to 0.06 gm., by stomach tube, and the blood changes observed by cell counts and hemoglobin determinations daily; smears were made with each count for morphological study.

The rabbits with hemorrhage were bled from the ear veins in amounts varying from 15 c.c. to 45 c.c. each day, averaging about 25 c.c., over long periods. The bleeding was done at the same time each day and in constant relation to feeding. It was observed after bleeding during a number of days that the serum was acquiring an increasing opacity, eventually taking on an appearance resembling rich milk or cream. It was then determined to study this phenomenon in more detail.

APPARENT RELATION OF MILKY SERUM TO HEMORRHAGE AND PHYSICAL CONDITION OF THE RABBITS.

The rabbits under constant conditions of diet, as above stated, were bled from the ear veins six days a week, an average of 25 c.c.

[1] Received for publication April 25, 1909.

per day. And in this way it was noted that, when the count was reduced to about 2,000,000 red cells per cubic millimeter and so maintained, there was a marked loss of body weight and the gradual development of the lipemia to an intense degree. With a day's respite there was constantly a gain of 500,000 to 1,000,000 cells, and increase in weight with a marked diminution in, or disappearance of opacity. Bleeding during eight to sixteen days was required to produce the milky serum, as rabbits showed a considerable individual variation in this respect.

These rabbits killed after prolonged bleeding, showed emaciation with entire disappearance of gross body fat, even about kidneys and heart. Except for a transient weakness immediately following the bleeding, the rabbits appeared well and active. They showed a very marked thirst, increased appetite and active excretion of urine. The feces were normal in appearance. Lastly, when the bleeding was kept up for many weeks the animal became a mere skeleton and the serum gradually lost its milky character. One animal lost 1,468 c.c. of blood in seventy bleedings.

After a short period of rest and gain in weight, the phenomenon was easily reproduced. In one rabbit which was apparently normal after a rest of eight months, during which time it was fed on green vegetables, the serum became intensely milky after a few hemorrhages of 30 to 45 c.c. on successive days.

It was interesting to note that the rabbits which received pyrodin with the same or lower cell counts did not at any time show lipemia In one of our animals, which died during the experiments from excessive bleeding, we tested the tissues for lipase. Loevenhart's ethyl butyrate method was used in the tests. We found lipase present in the liver, spleen and muscles, the amounts being greatest in the order named.

CHARACTERISTICS OF THE MILKY SERUM.

It was constantly observed, as in other anemic blood, that the relative volume of clot was small (see Plate XXV) and from 50 to 65 per cent. of serum was spontaneously separated from the blood on standing. When the opacity was at its height the uncoagulated blood had a definitely whitish tinge as it flowed from the

ear. The serum was quite opaque, and the fresh blood laked with hydrochloric acid for determination of hemoglobin, by Sahli method, showed a cloudy serum, and a consequently higher reading than that of the correspondingly anemic rabbits which received pyrodin.

This milky serum was little affected by strong centrifugation (3,000 revolutions). Only occasionally a thin line of denser "cream" separated in this way or on standing in the ice-box. Microscopically, under a magnification of 1,000 diameters, fine dust-like, slightly refractile droplets could be made out. These did not stain with osmic acid or with Sudan III, even on prolonged contact; thus marking a difference from the fat droplets seen in lipemia after ether narcosis, ingestion of fat, or in diabetes mellitus. A few coarser and more refractile fat droplets, such as are seen in normal serum, stained characteristically with these stains.

On treating the serum with ether it was but slightly altered, even after repeated agitation in a separator funnel for days. The same negative result was obtained with carbon disulphide, carbon tetrachloride and chloroform. In none of these substances was fat extracted to any appreciable extent. With the three last mentioned a permanent emulsion could be obtained by shaking. Here again was a striking contrast to the forms of lipemia previously recorded, as in these the fat is readily removed from the serum by simply washing with ether.

It was not until the close of the experiments, when our material was almost exhausted, that, through a suggestion of Dr. Loevenhart, we found a method for the direct washing of the serum by the previous precipitation of calcium. To effect this change ammonium oxalate crystals were added to the serum in excess and allowed to stand twelve hours, when a fine crystalline precipitate of calcium oxalate was thrown down. Now, after washing with ether, the serum became clear and normal in appearance, and, on evaporating the solvent, a large residue of fat was obtained (see Table III at end of the article).

Before this method was tried, however, Soxhlet extractions had already revealed the fatty nature of the serum and in fact, the chemical study of the fats appended in the table below is based largely on material obtained in this way. For this purpose the

serum was treated with five volumes of absolute alcohol for twelve hours. The protein precipitate was collected in a filter, washed, and dried in a dessicator. The filtrate and washings were united, distilled under reduced pressure, and the fat taken up in pure ether. This ether was put in the receiver of a Soxhlet apparatus and the dry precipitate in the capsule and the extraction continued for from eighteen to twenty-four hours. The ether solution of fat was then evaporated in a weighing dish and the resultant fat weighed and used for further chemical tests.

This ethereal extract consisted of a pale yellow oily substance, of low melting point, and numerous white particles about 1 mm. in diameter which, on microscopic examination, showed the acicular needles and characteristic arrangement of fatty acid crystals. The melting point of the white crystalline masses was definitely higher than that of the non-crystalline oil. On exposure to light and air for several days the fats became darker and slightly rancid.

The fat obtained from different lots of serum by this process varied from 2 to 4.5 per cent. by weight of the serum (see Table III). Only a few direct extractions after decalcification could be made with the material at our disposal and these showed from 2.4 to 2.5 per cent. fat. No attempts were made to crystallize fractionally or separate the individual fats as all the described methods required larger amounts of fat than we had for study. The variation in the percentage of fat obtained is due to the fact that the serum from individual bleedings was used for extraction, and the opacity of the serum varied greatly from day to day.

In order to get some idea of the general character of the fats we determined some of the "constants" used in commercial fat analysis (see Tables I and II).

The *saponification coefficient* is the potassium hydroxide neutralized, expressed as percentage per gram of fat.

Iodine absorption is represented by the actual percentage of iodine absorbed by the fat when digested with a standard alcoholic solution of iodine (Hubl's solution) the excess being titrated with standard thiosulphate solution. This factor is accepted as bearing a constant relation to the unsaturated fatty acids or their glycerides,

and is utilized for the identification of specific fats, as butter, lard, beef fat, etc.

The *acid value* is determined by the number of milligrams of potassium hydroxide required to saturate the free fatty acids in one gram of fat.

In addition, lecithin was determined indirectly as phosphorus pentoxide and calculated as dioleyllecithin, and tests were made for cholesterin and volatile fatty acids. A determination of specific gravity was made. The melting point was roughly estimated for the mixed fats. Parallel extractions were made of normal serum, but no constants determined as the amounts were too small to permit analysis.

In the direct extractions after decalcification, the calcium oxalate with the clear serum, was ashed. The calcium was separated from phosphoric acid, reprecipitated as calcium oxalate, and titrated with standard permanganate.[2] In this way the calcium content of the serum was found to be from 0.165 to 0.176 gm. per 1,000 (normal 0.116),[3] an increase of about 46 per cent.

While certain of the physical and chemical properties of these fats are empirically measured, we have, as yet, no definite knowledge of their actual structure. The iodine absorption indicates a high percentage of unsaturated fatty acids.

CONCLUSION.

In reviewing the literature, no description of a lipemia occurring in relation to simple hemorrhage was found, so that the observation of the phenomenon here recorded would seem to be new.

Very high percentages of fat have been found in the blood of diabetics. Fischer's[4] case showed 18.1 per cent total ether extract. Of this very little was free fat (0.0018 gm. potassium hydroxide per gram of fat); iodine absorption was 60.6 per cent.; cholesterin, 2.6 per cent. Chatin's case, cited by Fischer, showed 1.2 per cent. cholesterin, 66.5 per cent. olein, 32.2 per cent. margarin in the

[2] For method see Boggs, *Johns Hopkins Hospital Bull.*, 1908, xix, 201.
[3] Abderhalden, *Ztschr. f. physiol. Chem.*, 1898, xxv, 65.
[4] Fischer, *Virchows Arch.*, 1903, clxxii, 30, 218.

fat. Neisser and Derlin[5] in the ether extract of blood from a patient with diabetic coma found 19.7 per cent. fat, with melting point of from 39° to 41° C.; iodine absorption was 53.6 per cent. Javal[6] in a similar case found 25.4 per cent. of fat in ether extract of dry serum (perhaps by Soxhlet method); 21 per cent. of the fat was lecithin.

Bleibtreu[7] produced alimentary lipemia in geese by feeding barley and butter. Ether extract of serum showed 6 per cent. of fat. The serum was milky with invisible droplets. Iodine absorption was 57 to 58 per cent. The fat was quite different, chemically, from the fat in the food. Lipemia disappeared a few days after discontinuing the forced feeding.

Our experiments suggest, by analogy, the possible occurrence of lipemia in human anemias. In this connection it is of interest to note that we have recently demonstrated a moderate lipemia in a case of marked secondary anemia from hemorrhoids. The emaciation in such cases, as contrasted with the well-recognized conservation of the fat in pernicious anemia, suggests in human pathology a still further analogy which we now have under investigation.

The fat in our lipemic rabbits differs from fats described above in its insolubility, as well as in its "constants." The change after precipitation of calcium from the serum suggests that the fat may be present in the serum as a protein-calcium-lecithin combination which is decomposed by decalcifying.

While we are not prepared to offer an explanation of the mechanism of this lipemia, it is possible that the great loss of tissue proteins may have some influence on the abnormal fat metabolism. That the fat is derived from the tissues is a fair inference when its occurrence in connection with the loss of weight and the previous disappearance of the body fat are taken into consideration. A more careful study of the lipase in the blood and tissues is desirable. It may be that lowered oxidation following great loss of red cells plays a part.

[5] Neisser and Derlin, *Ztschr. für klin. Med.*, 1904, li, 428.
[6] Javal, *Comp. rend. Soc. de Biol.*, 1908, lxiv, 137.
[7] Bleibtreu, *Deutsch. med. Wchnschr.*, 1907, xxxiii, 446.

TABLE I.

*Constants and other Data Obtained from the Analysis of Fat of Rabbits' Serum.**

	Sp. Gr. at 15.5°C.	Iodine absorption.	Saponification value (KOH)	Free acid.	Volatile acids.	Lecithin.	Cholesterin.
Rabbit I Lipemic	934.5	*a*) 105.6% *b*) 119.8%	*a*) 17% *b*) 22% *c*) 21%	0.84% 0.85%	Traces	10%	None
Rabbit II Lipemic		134.1%	*a*) 19% *b*) 19%		Traces	11.5%	None

TABLE II.

Constants Obtained in the Analysis of Commercial Oils.† For Comparison with Table I.

	Sp. Gr. at 15.5° C.	Iodine absorption.	Saponification value (KOH).	Free acid.
Lard oil	917.0	76.2 per cent.	—	—
Cod liver oil	926.5	166.6 per cent.	18.51 per cent.	0.36 per cent.
Marrow fat (ox)	858.5	45.1 per cent.	19.70 per cent.	—
Linseed oil	934.5	178.8 per cent.	19.28 per cent.	—

TABLE III.

Table of Fat Percentages in Serum.

Rabbit.	Date.	R. b. c.	Hb.	Character of serum	Sohxlet extraction.	Extraction direct from decalcified serum	Calcium content of serum. (In parts per thousand.)
Normal 1	6-V-1908	6,800,000	70%	Clear	0.3%		
Normal 2	7-V	6,200,000	65%	Clear	0.5%		
Pyrodin 7	14-V	2,037,000	33%	Clear	0.49%		
Lipemic I	4-V	2,024,000	29%	Very milky	4.4%		
"	8-V	1,664,000	26%	" "	4.53%		
"	14-V	—	27%	Slightly milky	2.08%		
"	19-V	2,300,000	27%	Milky	3.0%		
"	26-V	1,400,000	20%	Slightly milky	2.3%		
"	4-VI	1,400,000	19%	Clear			
"	9-VI	—	14%	"			
"	‡13-III-1909	—	74%	"			
"	9-IV	—	28%	Milky		2.4%	0.165
"	16-IV	—	25%	"		2.5%	0.176
Lipemic II	16-V-1908	1,700,000	24%	"	2.8%		
"	17-V	1,500,000	23%	"	2.3%		
"	19-V	—	22%	Very milky	3.9%		

* Lipemia has been produced in other rabbits, but analyses of the fats have not been undertaken.

† From Sutton, Volumetric Analysis, London 1904, p. 391.

‡ Experiments resumed on Lipemic Rabbit I, March 13, 1909.

EXPLANATION OF PLATE XXV.

Tubes photographed against black background.

A. Normal rabbit serum. Chloroform at bottom of tube.

B. Fatty serum.

E. Fatty serum washed with ether (*R*) fourteen hours. (1) Slight protein emulsion.

O. Fatty serum treated with ammonium oxalate twelve hours. (*X*) Washed with ether one-half hour. (*L*) Unseparated emulsion of protein. (*Y*) Clear serum with excess ammonium oxalate at bottom of tube. (*R*) Ether containing the fat. (*M*) Fatty serum as separated on standing; (*T*) (*T*) clot showing small bulk of coagulum.

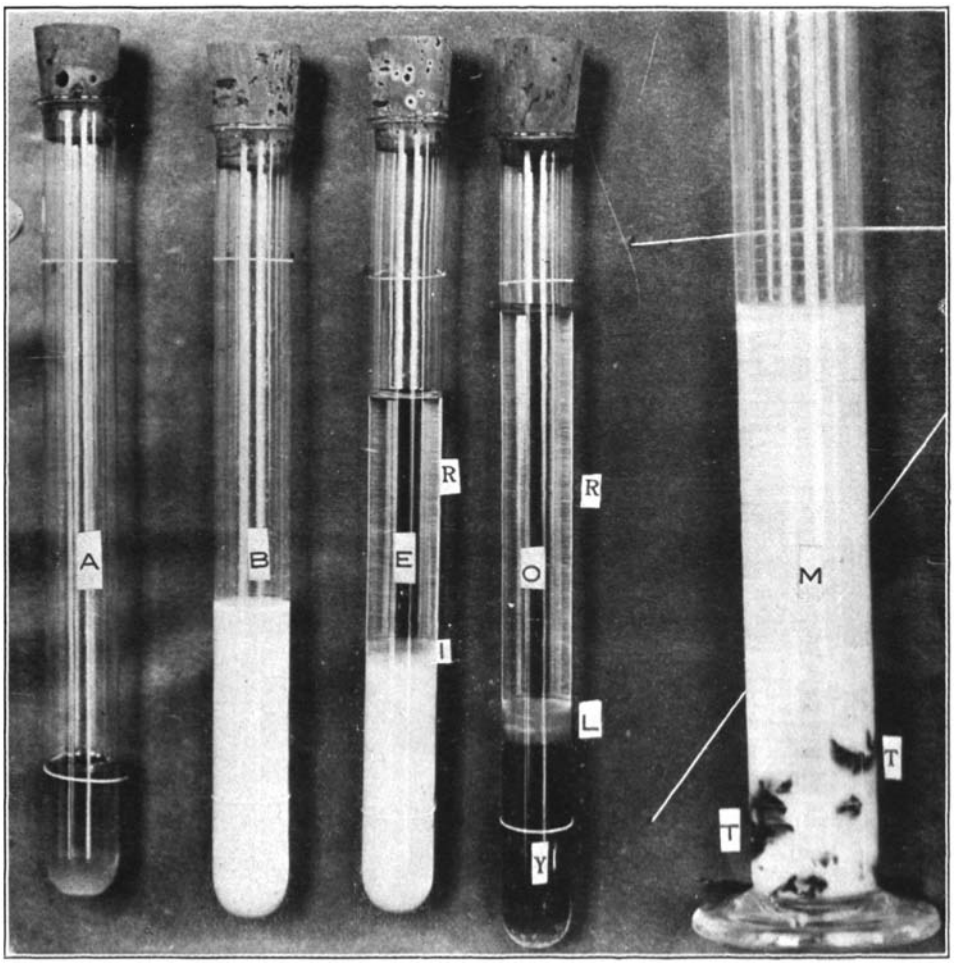

FIG. 1.