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ANALYSIS OF A CHYLOUS FLUID.

**A Thesis Submitted to the Faculty of the
College of Arts and Sciences
of the
UNIVERSITY OF LOUISVILLE
Through Dr. Charles J. Robinson,
in Partial Fulfillment for the Degree
"Master of Science,"
by
Elizabeth C. Franke.**

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Louisville, Kentucky.

May, 1916.

ANALYSIS OF A CHYLOUS FLUID.

Not many analyses of chylous fluids have been published. The analyses reported here were accordingly undertaken when a somewhat unusual opportunity for obtaining chylous fluid presented itself, with the object of adding to the data already published, confirmation or correction of the usually accepted ideas regarding the composition of chyle.

The samples of the fluid analysed were drawn from the thorax of a woman fifty-eight years old and were collected over a period of about three months. From the second of November until the eighth of April (date of the patient's death) fifteen extractions, with a total volume of forty-eight and one-fourth pints, were made. At first the fluid was present only in the left side of the thorax, then for a short time in both the right and left sides, and during the last month and a half, in the right side only. Eight analyses were made, five of the fluid taken from the left side, and three from the right.

In appearance the first three samples were creamy and opaque; the others varied from a dirty white to a straw-yellow, with, in two instances, a distinct pink coloration, due to blood. A bacteriological examination of the fluid by Dr. Stuart Graves showed it to be sterile and free of cells. The fluid clotted within a few hours after drawing and once the clot began to form, coagulated with great rapidity, forming at first a solid gel, which contracted on slight agitation to a small volume. In

the fluid left by the contraction of the clot, minute emulsoid particles could be seen, under the microscope, in active Brownian movement. Rapid agglutination of the particles took place upon the addition of minute quantities of a saturated calcium chloride solution. Addition of one-fourth cubic centimeter, or more, of saturated calcium chloride to five cubic centimeters of the fluid caused coagulation to such an extent that a thick creamy mass of coagulated material rose to the top of the fluid in a few minutes.

In a preliminary examination of the first sample the following facts were determined, which were characteristic of the other samples:

Reaction;	Alkaline to litmus.
Fehling's test;	Strongly positive.
Clotting;	The fluid clotted spontaneously in a few hours after drawing. The clot contracted rapidly, leaving a creamy fluid.
Heat clotting;	The fluid left by the contraction of the clot, upon heating, with or without the addition of acid, did not coagulate.
Nucleoprotein;	Dilution and acidification with acetic (cold) of the fluid left by the contraction of the clot, gave no precipitate. Considering the emulsoid character of the fluid, this cannot be considered as proof of the absence of nucleoprotein.

Protein; Saturation with sodium chloride (cold) gave a precipitate, presumably globulin. Acidification (acetic) of filtrate gave a second precipitate, presumably albumin. Negative ninhydrin reaction indicates the absence of protein and amino acids in the filtrate from this second precipitate.

Fat: Shaking with ether caused only partial clearing of the fluid.

The absence of detectable amounts of protein splitting products and the apparent absence of nucleoprotein which are always present in pus; with the presence of sugar, fibrinogen (as shown by the clotting), and of globulin and albumin, all indicate that the fluid was chyleous in origin. .

The usual methods were employed in the analysis of the fluid.

The total nitrogen (exclusive of fibrinogen nitrogen) was determined by the Kjeldahl method, using 5 cc. of fluid with about 10 g. potassium sulphate, and 25cc. of concentrated sulphuric acid.

The amount of non-protein nitrogen was ascertained by treating 10cc. of the fluid with enough 2.5% trichlor-acetic acid solution to make a volume of 100cc; filtering after thirty minutes; digesting 50cc. of the filtrate with concentrated sulphuric acid; and determining the nitrogen in the usual way.

The difference between the total nitrogen and the

non-protein nitrogen was taken as the total protein nitrogen, exclusive of the fibrinogen nitrogen.

To determine the amount of globulin and albumin present, 10cc. of the fluid was treated with enough saturated sodium chloride solution to make a volume of 100cc; the globulin filtered out, and a nitrogen determination made on 50cc. of the filtrate for the albumin nitrogen present. Since serum albumin contains 15.93% nitrogen* the factor used for converting the albumin nitrogen to albumin was 6.38. The amount of globulin nitrogen was obtained by difference and converted into globulin by using the factor 6.31, as serum globulin contains 15.83% nitrogen.** In one case the globulin was determined directly by washing the precipitate with saturated salt solution; drying; weighing, and dissolving it in enough water to make a volume of 25cc; titrating 5cc. of the resulting solution with N/10 silver nitrate solution to determine the amount of sodium chloride present; and determining the nitrogen present in the remaining 20cc. of the solution. The result thus obtained (.1191% globulin) agreed so closely with that obtained by difference (.1207% globulin) that, considering the fact that complete filtration was extremely slow and difficult, the method by difference was followed.

* Matthews' Physiological Chemistry (first edition) page 110 -
Analysis made by Michel.

** Matthews' Physiological Chemistry (first edition) page 110 -
Analysis made by Chittenden and Gies.

To determine the amount of sugar in the fluid, Soxhlett's method, as applied to milk, was used with a 25cc. sample; the proteins, etc. were precipitated by copper sulphate; and the calculation based upon the weight of the copper resulting from the reduction of Fehling's solution, which was carried out in the customary way. The sugar was assumed to be glucose.

For the lipin substances 20cc. of the fluid were dried with a known weight of plaster of paris, ground up and extracted with ether for fifteen hours in a Soxhlett apparatus; the ether evaporated; and the residue, after drying at 100cc. weighed. In two instances the ether soluble material was analysed for phosphorus and nitrogen in order to estimate the amount of phospholipins present. The Pemberton-Neumann method, volumetric, was used for phosphorus.

(1) .237 g. ether soluble material yielded .00088 g. phosphorus.

(2) .267 g. ether soluble material yielded .00055 g. phosphorus.

(1) .237 g. ether soluble material yielded .00154 g. nitrogen.

(2) .267 g. ether soluble material yielded .00154 g. nitrogen.

Calculating as $C_{42}H_{48}NPO_4$, the percentage of lecithin in the first sample is 9.38; in the second 5.17, based on the phosphorus content. Calculations based upon the nitrogen give respectively 36.07% and 32.01% lecithin. Such small quantities of phosphorus and nitrogen doubtless make the error in the determinations very large.

The results of the analyses follow in tabulated form:

T h o r a c i c F l u i d .

Sample from	Left side	Left side	Left side
Received	November 24th	December 2nd	December 6th
Examined	November 26th	December 3rd	December 6th
Specific gravity	1.0145 at 20°C	1.0145 at 20°C	1.013 at 22°C
Total Solids	7.7%	7.35%	7.64%
Ash	0.79%	0.76%	0.91%
Total nitrogen	0.548%		0.539%
Non-protein nitrogen	0.0409%		0.0409%
Total protein nitrogen (Excluding fibrinogen)	0.506%		0.498%
Albumin nitrogen	0.487%		0.4705%
Albumin (factor 6.28)	3.059%		2.954%
Globulin Nitrogen	0.0191%		0.0276%
Globulin (factor 6.31)	0.1208%		0.174%
Total protein (Exclusive of fibrinogen)	3.18%	3.21%	3.128%
Fibrinogen (clot)			.09%
Total Protein			3.218%
Sugar	0.49%		0.40%
Ether-soluble material	2.33%		2.63%
Total Volume	2000cc	740cc exam. 2000cc drawn	2225cc

Left side	Right side	Left side	Right side	Right side
Feb. 6th	Feb. 6th	Feb. 15th	Feb. 15th	Feb. 20
Feb. 7th	Feb. 7th	Feb. 16th	Feb. 16th	Feb. 21
1.0158at 19°C	1.0178at 19°C	1.016at 20°C	1.016 at 20°C	1.015at 19°C
5.26%	5.26%	5.035%	5.205%	5.209%
0.78%	0.80%	0.715%	0.717%	0.813%
0.612%	0.633%	0.507%	0.570%	0.503%
0.0695%	0.0655%	0.0313%	0.0502%	0.0375%
0.542%	0.567%	0.476%	0.520%	0.466%
0.516%	0.554%	0.440%	0.513%	0.451%
3.24%	3.474%	2.761%	3.22%	2.830%
0.0265%	0.0138%	0.0359%	0.007%	0.015%
0.167%	0.0866%	0.226%	0.045%	0.095%
3.407%	3.56%	2.987%	3.26%	2.925%
				0.04%
				2.964%
0.295%	0.257%	0.188%	0.181%	0.220%
0.567%	0.307%	0.637%	0.426%	0.891%
890cc exam	840cc exam	603cc exam.	805cc exam.	860cc exam
1000cc drawn	2000cc drawn	1500cc drawn	2000cc drawn	1000cc drawn

The first samples drawn while the effusion was confined entirely to the left side show a marked similarity to published analyses of chylo-thorax fluid.

Thoracic fluid under examination (Average of first 3 samples)	Chylo-thorax Buchtala-Salkowski***	Chyle Panzer****
Water	92.44%	91.34%
Total Solids	7.55%	90.29 - 94.53
Proteins	3.22%	8.66%
Fats	2.48%	4.86%
Cholesterol		2.16
Ash	0.82%	2.50%
Sugar	0.445%	6.59
		0.26%
		traces
		0.80 - 1.04
		traces

In chyle the proteins and fats vary greatly with the diet; thus Sollman ***** found variations in protein from 1.85 - 6.5%.

***Buchtala Zeit. Physiol. Chem. 1910. (67), 42.

Salkowski Virchow's Arch. 1909 (198), 189. Cited from Wells' Chemical Pathology (second edition) page 331.

****Panzer Zeit. Physiol. Chem. 1900., (30), 113, cited from Wells' Chemical Pathology (second edition) page 330.

*****Sollmann, Amer. Jour. Physiol. 1907 (17), 487, cited from Wells' Chemical Pathology, (second edition) page 330.

After the last of the three samples referred to in the above tables was drawn, there was no more fluid analyzed for two months. The marked decrease in fat content, the noticeable diminution in sugar, and the very slight increase in protein in the composition of these later samples, are very probably due to a decrease in the absorption of nutrients in the weakened state of the patient. The general composition of the fluid, especially that drawn from the right side, is approaching more and more to that of a sample of lymph analyzed by Guebler and Quevenne as shown in the following table, Column II.

	I. Fluid from right side. Drawn Feb. 6th.	II. Lymph (Thigh of a woman) Guebler & Quevenne*****
Water	94.74%	93.99%
Total Solids	5.26%	6.00%
Protein	3.56%	4.32%
Fat.	0.307%	0.38%
Ash	0.80%	0.73% - 0.82%

In the post-mortem examination, by Dr. Graves and Dr. Moore, of the patient from whom this fluid was drawn, no rupture of the thoracic duct was found; the presence, however, of a tumor, involving the head of the pancreas and some of the lymph nodes, was demonstrated. All of the lymph nodes from the neck to the pelvis were enlarged. Hence the fluid had its origin in a partial obstruction of the lymphatic circulation and an escape of the chyle by transudation.

*****Guebler & Quevenne "Gaz. Med. de Paris," #24, 27, 30, 34; 1854, cited from Physiologic and Pathologic Chemistry, Bunge, page 224.

In order to test out the hypothesis that bile salts enter into a loose physical or chemical union with the fatty acids before the latter are absorbed from the intestine, tests for bile salts were made. Two hundred cubic centimeters of the fluid were mixed with animal charcoal and dried over a water bath. The residue was ground up and extracted over a water bath for twenty minutes with 95% alcohol, and filtered. The lipin substances, precipitating out of the alcohol extract upon cooling, were filtered off and the filtrate treated with ether until a permanent precipitate formed. This precipitate gave negative results with the Pettenkofer, Udransky and Hayes tests for bile salts. Nitrogen and phosphorus were both absent in this precipitate. Hence we are unable to get from this fluid any confirmation of the hypothesis mentioned.