(2) A quinine-aspirin mixture, which has been kept until it has changed to a brown-red mass, is no more toxic to several of the lower animals than the freshly made mixture.

LABORATORY OF JOHN T. MILLIKEN
AND COMPANY.

HYDROLYSIS OF THE CRUDE PROTEIN FROM LIQUOR FOLLICULI.*[1]

BY BRYANT FULLERTON AND FREDERICK W. HEYL.

In a recent publication[2] it was noted that the precipitation of liquor folliculi with alcohol yielded crude protein to the extent of 6.34%. This crude protein, which may of course consist of a mixture has been subjected to the Van Slyke process and the nitrogen distribution determined. The tyrosine and tryptophane content has also been determined colorimetrically. The results of our analysis are tabulated below; and also those showing the composition of the albumin of ovarian residue, of mammary gland and casein for purposes of comparison.

	Follicular fluid.	Albumin ovarian residue.	Mammary[3] giand.	Casein.
Total N	100.0	100.00	100.00	100.00
Amide N	8.3	7.80	5.50	10.27
Humin N	1.9	1.44	2.50	1.28
Arginine N	12.6	11.60	11.10	7.41
Cystine N	0.95	0.70	....	0.20
Histidine N	0.00	0.20	4.66	6.21
Lysine N	14.80	14.69	12.47	10.30
Mono Amino N	61.4	60.55	57.17	55.81
Non Amino N	1.5	3.08	6.40	7.13

	Follicular fluid.	Albumin ovarian residue.
Tyrosine	7.1%	8.5
Tryptophane	2.5%	2.1
Molisch test	−	+

In the first place, it is very difficult (except in sulphur content) to decide whether the protein of the follicular fluid is actually different from that of the ovary itself. While the former does not give the Molisch test, the positive test in ovarian protein may be due to absorption of positively testing material. It does not appear that any great alterations of structure, such as are noted in comparing casein and mammary gland, have been produced by the secretory process.

The analysis of mammary gland protein is really not comparable because in this hydrolysis no effort was made to separate the soluble proteins. It appears, however, that the arginine (12.6% N) is very similar to that found in various other organs; the absence of histidine is a conspicuous difference; lysine is somewhat higher than for any other organ protein except placenta.[4]

* From the Chemical Research Laboratory, The Upjohn Company.

[1] Received for publication December 1, 1925.

[2] JOUR. A. PH. A., XIV, 210 (1925).

[3] See Harding and Fort, *J. Biol. Chem.*, 35, 37 (1918).

[4] *J. Biol. Chem.*, 35, 37 (1918).

EXPERIMENTAL.

The crude protein was analyzed with the following results:

0.6247 Gm. lost at 110°, 0.0511 Gm. or 8.18% moisture.

0.2032 Gm. gave 0.0027 Gm. or 1.32% ash.

A quantity equivalent to 0.2049 Gm. ash and moisture free protein gave 0.0169 Gm. $BaSO_4$ or 1.13% S.

A quantity equivalent to 0.1936 Gm. ash and moisture free protein gave 0.0373 Gm. $PbMoO_4$ or 0.13% P.

Hydrolysis.—A quantity of this material equivalent to 3.1544 Gm. ash and moisture free protein was hydrolyzed by boiling with 100 cc. of 20 per cent hydrochloric acid for about 26 hours. Nitrogen estimations showed 14.97, 14.70, 14.51 and 14.44% N taking 14.65% N as an average. (Amino N = 13.31 and 13.34%.) For the check analysis a quantity equivalent to 3.5758 Gm. ash- and moisture-free protein was used. In calculating, the results tabulated below have been corrected for the solubilities of all the phosphotungstates.

	Analysis Crude Protein. Found.		Parts per 100.[1]
Total Nitrogen	14.65	15.28	100.00
Amide N	1.22	1.61	8.3
Humin N	0.28	0.26	1.9
Arginine N	1.85	1.81	12.6
Cystine N	0.14	0.14	0.95
Histidine N	0.00	0.00	0.00
Lysine N	2.17	2.07	14.8
Mono Amino N	8.99	8.95	61.4
Total Filtrate N	9.21	9.45	....
Non Amino N	0.22	0.50	1.5
			101.45

Although the histidine N, calculated according to the Van Slyke method is zero, the base solution responded to the Pauly diazo test. In comparing the colors thus produced with a standard histidine dichloride solution, this base appeared to be present to the extent of about 0.1%.

The analysis of the decomposed phosphotungstates gave the following figures:

	I.	II.
Total Base N	0.1057 = 4.02	0.1262 = 4.16
Arginine N	0.0476 = 1.81	0.05605 = 1.85
Amino N	0.0759 = 2.88	0.0858 = 2.84
Non Amino N	0.0298 = 1.14	0.0404 = 1.32
Cystine N	0.0038 = 0.14	0.0042 = 0.14

From these figures we calculate the percentage of hexone bases as follows:

	I.	II.
Arginine	5.62	5.75
Histidine	0.00	0.00
Lysine	10.79	11.32

It is observed that the calculation for histidine in both analyses yields negative quantities although from the Pauly test, its presence may be suspected. It is not impossible that the phosphorus present in the crude protein might be present

[1] Calculated on first analysis.

as nucleic acid and that a small quantity of purines have been precipitated with the hexone bases.

Determination of Tyrosine and Tryptophane.—These were determined colorimetrically, tyrosine by the method of Folin and Looney[1] was present to the extent of 7.1%. By the method of May and Rose[2] we found tryptophane = 2.5%.

The Molisch test gave negative results.

SUMMARY.

Hydrolysis of the crude total protein of follicular fluid showed the presence of Arginine, 5.7%; histidine, trace; lysine, 11.0%; tryptophane, 2.5%; tyrosine, 7.1%. All of these values very closely approximate those of the albumin ovarian residue. The latter contains appreciably more sulphur (1.5%), only 1.1% being found in the protein of follicular fluid.

KALAMAZOO, MICHIGAN,
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CHEMICAL EXAMINATION OF OVARIAN RESIDUE.*[1]

I. THE PROTEIN FRACTION.

BY BRYANT FULLERTON[2] AND FREDERICK W. HEYL.

When, in general, any glandular material is extracted in the cold with water or dilute saline solutions, a precipitate is usually produced upon acidification (acetic acid). Preparations of this class have been classified by Samuely[3] in two groups (*a*) "cell globulins" and (*b*) tissue or cell nucleoproteins. Under (*a*) it is noted that from liver tissue of horse or cat, from kidney, spleen, lymph cells, testicles and thymus, such preparations have upon analysis shown from 0 to 1.3% phosphorus. Approximately the same substances are also listed under (*b*). Perhaps one should infer that if, on treating such extracts with methods suitable for the precipitation of globulins, a nonphosphorus compound results, we have a "cell globulin." For instance, from thyroid gland extracts, such a substance can be prepared.

Such substances, when containing phosphorus are best classified according to Jones[4] as α nucleoproteins, and they are probably variable colloidal mixtures or salt like combinations of cell globulins or albumins with nucleic acid.[5] In Osborne's work on wheat embryo, it was shown that such extracts contain both albumin (leucosin) and globulin associated with nucleic acid.

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[1] *J. Biol. Chem.*, 51, 421 (1922).

[2] *J. Biol. Chem.*, 54, 213 (1922).

* From the Chemical Research Laboratory of the Upjohn Company.

[1] Received for Publication December 1, 1925.

[2] This paper is based upon the thesis presented by Mr. Fullerton to the Faculty of Kalamazoo College, in partial fulfillment of the requirements for the degree of Master of Science.

[3] "Abderhalden's Hand-lexikon IV," p. 93 and 130.

[4] "The Nucleic Acids."

[5] "Pohl, Abder. Handbuch, V," 665 reports positive pentose reaction for liver nucleoproteins. See Fleugel, 1919.