Chemical Studies on Sex Differences of Proteins in Animals and Plants (Third Report) Sex Differences of Muscle Proteins

By

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This report is an extension of the first report[1] and the second report[2] with the same authors. Some of the experiments described in the present report were undertaken using the same method as before but different samples. Additional new experiments were also added. Therefore, the same conclusion as before was arrived at; but new premises, and conclusions were added and also new summaries, were formulated from this as well as the first report. The references already stated in the first and the second report have been omitted this time and here only new ones are given.

Short review of the experiments described in the first and second report

(A) Short review of the experiments done with muscle of ox, hen and rabbit in the first report.

(1) The ash and phosphorus contents of female myosin and myogen are always greater in quantity than those of the male by about 20–35%.

(2) The specific rotatory power of the male myosin and myogen is always greater than that of the female, the latter corresponding to about 88–97% of the former.

(3) The content of free amino nitrogen of the male myosin and myogen is always superior to that of the female. The quantity of the latter is about 80–90% that of the former.

(4) In the separation and determination of amino acids of myosin and myogen, the content of histidin nitrogen of the female is always found to be superior to that of the male. The quantity of the former is about 127–240% that of the fatter.

(5) In the experiments with serum albumin and globulin, the same tendencies of ash, phosphorus, specific rotatory power, and free

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amino nitrogen contents were observed between the two sexes.

(B) Short review of the experiments done with muscle fibers of mammals, fowls and fish in the second report.

(6) The male muscle fibers always developed greater acidity in the neutral salt solution than did the female muscle fibers.

(7) The content of histidin form nitrogen of female muscle fibers is always far superior to that of the male.

I. Water, ash and phosphorus content of myosin and myogen

Beside myosin and myogen of bull, cow, cock, hen, male and female rabbit, samples of the same protein from boar, sow, male and female wild duck, male and female codfish and both sexes of hatahata (Arctoscopus japonicus) were newly prepared by the same method as described in the first report. These new samples were analysed by the ordinary methods and their ash and phosphorus contents estimated in percent of dry matter in the following table:

TABLE I.

The water, ash and phosphorus contents of myosin and myogen.

Proteins	Animal	Sex	Water %	Ash %	Ratio	Phosphorus %	Ratio
Myosin	Boar		7.104	0.7177	100	0.1670	100
	Sow		9.061	0.8109	113	0.2073	124
	Male codfish		7.516	0.7765	100	0.1599	100
	Female codfish		6.923	0.8073	104	0.1849	116
	Male hatahata		6.474	0.4250	100	0.1262	100
	Female hatahata		7.467	0.8268	195	0.1659	131
Myogen	Boar		9.263	0.5538	100	0.1256	100
	Sow		9.132	0.8410	152	0.1817	145
	Male wild duck		4.689	0.5575	100	0.1234	100
	Female wild duck		6.168	0.7479	134	0.1313	106
	Male codfish		8.210	0.4256	100	0.1243	100
	Female codfish		7.992	0.5200	122	0.1349	109
	Male hatahata		7.130	0.4712	100	0.0976	100
	Female hatahata		8.101	0.6065	129	0.1638	168

From the above table the ash and phosphorus content of female myosin and myogen are seen to be greater always than those of

male. This seems to explain the greater retention of phosphoric acid in the female body.

II. Iso-electric point of myosin and myogen

The iso-electric point of myosin and myogen in the two sexes was estimated by following Michaelis and Nakayama's method. The principle of the method is the estimation of the optimum precipitation in a certain pH value which is situated between the different iso-electric points of two colloids. If a greater quantity of one colloid was used than the other, the optimum precipitation point approaches the iso-electric point of the former. In conducting the experiment, the authors used the following mastix sol and protein solution:—(1) To prepare mastix sol, 5 g. of mastix were dissolved in 100 ccm of 96% alcohol and filtered. 10 ccm of the filtrate were poured in a large beaker and 200 ccm of distilled water added. The milky solution was filtered and the filtrate preserved for the experiment. (2) 0.05 g. of protein were dissolved in 50 ccm of 1/200 normal NaOH solution. (3) Mixtures were made in different propertions of the two solutions with the ratios of mastix to protein, 5:1, 5:1,2 etc. The buffer solution was prepared according the following formula.

No.	1	2	3	4	5	6	7	8	9
N/10 Na-acetate solution ccm.	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
N/10 Acetic acid solution ccm.	0.1	0.25	0.5	1.0	2.0	4.0	—	—	—
N/1 Acetic acid solution ccm.	—	—	—	—	—	—	0.8	1.6	3.2
Distilled water ccm.	3.9	3.75	3.5	3.0	2.0	0	3.2	2.4	0.8
Colloid solution ccm.	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0

The pH value of the maximum precipitation point was estimated with a Cambridge potentiometer using a quinhydron electrode. In the following table, (−)=no precipitate, (*)=turbid, (**)=precipitation and (***)=maximum precipitation.

TABLE II.

Estimation of iso-electric point of myosin and myogen.

Protein Ratio of mixture		Hours	Female						Male					
			No. 1	2	3	4	5	6	1	2	2	4	5	6
Pig myogen	5.2	0.5	—	—	*	*	*	—	—	—	*	*	*	—
		1.0	—	*	**	**	**	*	—	*	**	**	**	*
		2.4	—	**	***	***	***	**	—	**	***	***	***	**
	5.4	0.5	—	—	—	—	—	—	—	—	—	—	—	—
		1.0	—	*	**	**	—	—	—	*	**	*	—	—
		2.4	—	**	***	***	—	—	—	**	***	***	—	—
	5.8	0.5	—	—	—	—	—	—	—	—	—	—	—	—
		1.0	—	*	**	—	—	—	—	*	**	—	—	—
		2.4	—	**	***	—	—	—	—	**	***	—	—	—
	pH				5.393						5.427			
Codfish Myogen	5.4	10 min.	—	—	—	—	—	—	—	—	—	—	—	—
		30	—	—	—	*	*	—	—	—	*	*	*	—
		1 hr.	—	—	*	**	**	—	—	—	**	**	**	—
		24 hrs.	—	*	**	***	***	—	—	**	***	***	***	—
	5.8	10 min.	—	—	—	—	—	—	—	—	—	—	—	—
		30	—	—	*	—	—	—	—	—	*	*	—	—
		1 hr.	—	—	**	*	—	—	—	*	**	**	—	—
		24 hrs.	—	*	***	**	—	—	—	**	***	***	—	—
	5.12	10 min.	—	—	—	—	—	—	—	—	—	—	—	—
		30	—	—	*	—	—	—	—	—	*	—	—	—
		1 hr.	—	—	**	*	—	—	—	—	**	—	—	—
		24 hrs.	—	—	***	**	—	—	—	**	***	—	—	—
	pH				5.381						5.464			
Wild Duck Myosin	5.4	10 min.	—	—	—	—	—	—	—	—	—	—	—	—
		30	—	—	*	*	*	—	—	—	*	*	*	—
		1 hr.	—	—	**	**	**	—	—	—	**	**	**	*
		24 nrs.	—	*	***	***	***	—	—	**	***	***	***	*
	5.8	10 min.	—	—	—	—	—	—	—	—	—	—	—	—
		30	—	—	*	*	—	—	—	—	*	*	—	—
		1 hr.	—	*	**	**	—	—	—	—	**	**	—	—
		24 hrs.	—	**	***	**	—	—	—	**	***	***	—	—
	5.12	10 min.	—	—	—	—	—	—	—	—	—	—	—	—

		30	—	—	*	—	—	—	—	—	*	—	—	—
		1 hr.	—	—	**	—	—	—	—	—	**	—	—	—
		24 hrs.	—	**	***	—	—	—	—	**	***	—	—	—
	pH				5.581						5.656			
Codfish Myogen	5.4	10 min.	—	—	—	—	—	—	—	—	—	—	—	—
		30	—	—	*	*	*	*	—	—	*	*	*	*
		1 hr.	—	—	**	**	**	**	—	—	**	**	**	**
		24 hrs.	—	—	***	***	***	***	—	*	***	***	***	***
	5.8	10 min.	—	—	—	—	—	—	—	—	—	—	—	—
		30	—	—	*	*	*	—	—	—	*	*	*	—
		1 hr.	—	—	*	**	*	—	—	—	**	**	**	—
		24 hrs.	—	—	**	***	*	—	—	*	***	***	***	—
	5.12	10 min.	—	—	—	—	—	—	—	—	—	—	—	—
		30	—	—	—	*	—	—	—	—	—	*	—	—
		1 hr.	—	—	*	**	*	—	—	—	*	**	*	—
		24 hrs.	—	—	**	***	**	—	—	—	***	***	*	—
	pH					5.144					5.178			

In the experiment on muscle protein of cock and hen, a buffer solution after the following formula was used.

No.	1	2	3	4	5	6	7	8	9	10
N/10 Na-acetate solution ccm.	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0
N/10 Acetic acid solution ccm.	0.1	0.2	0.3	0.4	0.5	0.6	0.7	0.8	1.0	1.5
Distilled water ccm.	3.9	3.8	3.7	3.6	3.5	3.4	3.3	3.2	3.0	2.5
Colloid solution ccm.	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0	2.0

From the above experiment, we summarized the results in the following table.

TABLE III.

pH value of the iso-electric points of myosin and myogen.

Animals Sexes	Myosin		Animals Sexes	Myogen	
	Female	Male		Female	Male
Cock & hen	5.440	5.525	Pig	5.393	5.427
Wild duck	5.581	5.656	Codfish	5.381	5.464
Codfish	5.144	5.178	Cock & hen	6.183	6.270

The iso-electric point of the female body proteins is always more acidic i.e. the pH value is lower than that of the male.

III. The specific rotatory power of myosin and myogen

The specific rotatory power of myosin and myogen alkaline solution was estimated with a Haensch-Schmidt half shadow polariscope by the same treatment as described in the first report.

TABLE IV.

Kinds of animals (20°C)	After 24 hours		Ratio	After 48 hours		Ratio
	Reading	Spec. rotat.		Reading	Spec. rotat.	
Myosin Boar	−1.3	−89.96	100	−1.4	−96.83	100
Sow	−1.2	−83.04	92	−1.3	−89.96	92
Male wild duck	−1.6	−110.72	100	−1.7	−117.64	100
Female wild duck	−1.4	−96.88	87	−1.55	−107.26	91
Male codfish	−1.3	−89.96	100	−1.5	−103.80	100
Female codfish	−1.2	−83.04	92	−1.4	−96.88	93
Male hatahata	—	—	—	−1.6	−110.72	100
Female hatahata	—	—	—	−1.5	−103.80	93
Myogen Boar	−1.4	−96.88	100	−1.5	−103.8	100
Sow	−1.5	?−103.80	?	−1.5	?−103.80	?100
Male wild duck	−1.4	−96.88	100	−1.5	−103.80	100
Female wild duck	−1.0	−69.20	71	−1.2	−83.04	80
Male codfish	−1.1	−76.12	100	−1.2	−83.04	100
Female codfish	−1.0	−69.20	92	−1.1	−76.12	91
Male hatahata	−1.3	−89.96	100	−1.4	−96.88	100
Female hatahata	−1.1	−76.12	84	−1.2	−83.04	85

From the above table, the specific rotatory power of male myosin and myogen is seen to be always greater than that of female. This condition constitutes one of the remarkable differences between the proteins of the two sexes.

IV. The determination of the quantities of HCl combined with muscle-myosin and myogen

The simplest case of combination of an inorganic acid with a protein occurs according to the free amino group of the protein.

This is due to the abundance of amino group which combines with HCl more easily in the common than in the glutinous protein. In our supposition, the quantity of free amino nitrogen of serum and muscle protein would vary according to the sex of the animals. As the following experiment was undertaken with the same manipulation as reported with oryzenin of rice the explanation of the principle of Cohnheim's method is omitted here.

Twenty mg. of protein were disolved in 5 ccm of 1/10 normal HCl solution and the whole was stood overnight. To the protein was added 25 ccm of ca. 5% sucrose solution. The mixture was inverted for exactly 5 minutes in a boiling water bath. At the end of the time, the solution was cooled to room temperature and the quantity of inverted sugar was estimated by Bertland's method. The quantity of sucrose was estimated by Bertland's method. The quantity of HCl in free state is calculated as follows, where 1/10 normal HCl solution contains 18.35 mg (C) of HCl in 5 ccm and the concentration of used sucrose was 1423.966 mg in 25 ccm. This calculation was made with the formula $\frac{C \log A - \log(A-X)}{C' \log A - \log(A-X')}$, where C represents the hydrogen in concentration of pure HCl, C' the same of a solution of protein in HCl, A the quantity of sucrose used, X and X' the quantities of sucrose inverted by pure acid and protein acid solution respectively. C and C' are figures proportional to the hydrogen ion concentration.

TABLE V.

(Combining power of the proteins with HCl)

Kinds of protein	$KMnO_4$ (ccm)	Cu (mg)	Invert sugar (mg)	Sucrose (mg)	Sucrose in 25 ccm (mg)
HCl only	35.00	362.558	198.409	118.482	1130.895
Serum-albumin of ox	34.00	352.199	191.849	182.251	1093.505
,, of cow	34.15	353.753	193.052	183.394	1100.362
Serum-globulin of ox	34.30	355.307	193.704	184.013	1104.078
,, of cow	34.40	256.343	194.550	184.817	1108.900
Muscle-myogen of cock	34.40	356.343	194.550	184.817	1108.900
,, of hen	34.60	358.414	195.810	186.014	1116.082

Kinds of protein	$KMnO_4$ (ccm)	Cu (mg)	Invert sugar (mg)	Sucrose (mg)	Sucrose in 25 ccm (mg)
Muscle-myosin of cock	34.50	357.379	195.076	185.316	1111.898
,, of hen	34.60	358.414	195.810	186.014	1116.082
Muscle-myogen of rabbit (male)	34.45	356.861	195.654	184.915	1109.493
,, rabbit (female)	34.60	358.414	195.810	186.014	1116.082
Muscle-myosin of rabbit (male)	34.35	355.825	194.119	184.407	1106.443
,, rabbit (female)	34.45	356.861	194.654	184.916	1109.493
Muscle-myogen of ox	34.50	357.379	195.076	185.316	1111.898
,, of cow	34.65	358.932	195.945	186.143	1116.851
Muscle-myogen of codfish (male)	34.20	354.271	195.006	183.349	1100.099
,, codfish (female)	34.30	355.307	193.704	184.013	1104.078
Muscle-myosin of codfish (male)	34.05	352.717	192.208	182.591	1095.545
,, codfish (female)	34.15	353.753	193.052	183.394	1100.362
Muscle-myogen of wild duck (male)	34.10	353.235	192.504	182.873	1097.238
,, wild duck (female)	34.25	354.789	193.485	183.805	1102.829
Muscle myosin of wild duck (male)	34.00	354.789	192.485	183.805	1102.829
,, wild duck (female)	34.25	354.789	192.485	183.805	1102.829

$$\frac{C \log A - \log (A-X)}{C' \log A - \log (A-X')}$$

	$A-X$	$\log (A-X)$	$\log A = 3.1012428$ $\log A - \log (A-X')$
HCl only	131.641	2.119395	0.981847
Serum albumin of ox	169.032	2.22797	0.873273
of cow	162.175	2.209983	0.891260
Serum globulin of ox	158.459	2.199919	0.901323
of cow	153.637	2.186494	0.914749
Muscle myogen of cock	153.637	2.186494	0.914749
,, of hen	146.455	2.165705	0.923309
Muscle myosin of cock	150.639	2.177933	0.923309
,, of hen	146.455	2.165705	0.935538

Muscle myogen of rabbit (male)	133.344	2.185667	0.915575
,, of rabbit (female)	146.455	2.165705	0.935538
Muscle myosin of rabbit (male)	156.053	2.165705	0.935538
,, of rabbit (female)	153.344	2.185668	0.915575
Muscle myogen of ox	150.639	2.177933	0.923309
,, of cow	145.686	2.163418	0.937825
Muscle myogen of codfish (male)	162.438	2.210689	0.890554
,, of codfish (female)	158.459	2.199916	0.901323
Muscle myosin of codfish (male)	166.992	2.222746	0.878497
,, of codfish (female)	162.175	2.209983	0.891260
Muscle myogen of wild duck (male)	165.299	2.218267	0.882975
,, of wild duck (female)	159.708	2.203322	0.897920
Muscle myosin of wild duck (male)	169.032	2.227970	0.893273
,, of wild duck (female)	159.708	2.203322	0.897920

	HCl in free state (mg)	HCl in combined state (mg)	Ratio of male & female
Serum albumin of ox	16.219	2.016	100
,, of cow	16.553	1.683	83
Serum globulin of ox	16.740	1.495	100
,, of cow	16.989	1.246	83
Muscle myogen of cock	16.219	1.246	100
,, of hen	17.375	0.860	69
Muscle myosin of cock	17.148	1.087	100
,, of hen	17.375	0.860	79
Muscle myogen of rabbit (male)	17.004	1.231	100
,, of rabbit (female)	17.375	0.860	70
Muscle myosin of rabbit (male)	16.863	1.373	100
,, of rabbit (female)	17.004	1.231	90
Muscle Myogen of duck (male)	16.399	1.836	100
,, of duck (female)	16.676	1.559	85

Muscle myosin of duck (male)	16.219	2.016	100
„ of duck (female)	16.399	1.836	91
Muscle myogen of ox	17.148	1.087	100
„ of cow	17.417	0.818	75
Muscle myogen of codfish (male)	16.539	1.696	100
„ of codfish (female)	16.740	1.495	88
Muscle myosin of codfish (male)	16.316	1.919	100
„ of codfish (female)	16.553	1.682	88

In the case of the male serum-albumin, globulin, muscle myogen and myosin, the quantity of HCl combined is always greater than in the case of the female.

V. Total nitrogen and free amino nitrogen contents of myosin and myogen

The free amino nitrogen and total nitrogen were estimated by the same treatment as described in the first report and the following results obtained.

TABLE VI.

Protein, animals & sexes	Total nitrogen	% of free amino nitrogen	% of free amino N on bases of total nitrogen	Ratio
Myogen Boar	15.407	1.2057	7.8252	100
Sow	16.014	1.0237	6.3922	81
Male wild duck	15.270	1.1949	7.8246	100
Female wild duck	15.396	1.0342	6.7172	85
Male codfish	16.019	1.9680	12.2848	100
Female codfish	15.702	1.6546	10.5377	85
Male hatahata	14.498	1.1975	8.2598	100
Female hatahata	14.247	0.9078	6.3719	77
Myogen Boar	15.908	1.8838	11.8415	100
Sow	15.475	1.6948	10.9316	92
Male wild duck	15.742	1.3220	8.3980	100
Female wild duck	16.191	1.0444	6.4506	77
Male codfish	16.235	1.9827	12.1677	100
Female codfish	15.652	1.5217	9.722	79
Male hatahata	—	2.8643 (100)	—	—
Female hatahata	13.799	2.4337 (85)	17.6645	—

According to the table, the content of free amino nitrogen of the male myosin and myogen is always superior to that of the female. The quantity of the female is about 80–90% that of the male. This constitutes another remarkable difference between the proteins of the sexes.

VI. Separation and determination of amino acids of myosin and myogen

The authors undertook these experiments using Van-Slyke's advanced method as described in the first report and obtained the following results. The following figures are % of total nitrogen. (In the table, α and β myogen were prepared by partial precipitation with acid).

TABLE VII.

The amino acids of myosin and myogen.

Animals and sexes	Boar	Sow	Male wild duck	Female wild duck	Codfish		Hatahata	
					Male	Female	Male	Female
Myosin								
Total nitrogen	15.4079	16.0147	15.2705	15.3962	16.0198	15.7017	14.4980	14.1470
Ammonia N	0.9191	0.9030	0.7032	0.7423	0.8716	0.9375	0.8847	0.9552
Melanin N	0.2512	0.2264	0.2036	0.2451	0.2688	0.3103	0.2712	0.2714
Diamino N	4.7710	4.6804	5.1571	4.8229	5.2267	4.6538	5.2624	4.8304
Arginin N	2.5517	1.9660	2.5076	2.3320	3.0627	2.4281	2.6420	2.1710
Histidin N	0.5685	1.3203	0.9501	1.1283	0.6143	0.8536	0.4026	0.8650
Lysin N	1.4666	1.1566	1.5074	1.1853	1.3356	1.1639	1.9484	1.5415
Monoamino N	9.4666	10.1249	9.2066	9.5359	9.6527	9.8001	8.0797	8.1900
Cystin N	0.2454	0.2375	0.1920	0.1773	0.2141	0.2083	0.2694	0.2529
Myogen					(α)—Codfish—(β)			
Total N	15.9084	15.4751	15.7419	16.1908	15.8450	15.6280	16.2349	15.6524
Ammonia N	0.8455	0.8926	0.7588	0.9126	0.8775	0.8951	0.8152	0.8720
Melanin N	0.2214	0.2100	0.2108	0.2837	0.1692	0.1899	0.2407	0.2511
Diamino N	5.2477	4.6978	5.1507	5.0044	5.4315	4.6396	5.5808	4.5578
Arginin N	2.7565	1.7907	2.4239	2.1944	2.8386	2.3008	2.8889	2.2707
Histidin	0.6077	1.2455	0.5296	0.6737	0.3804	0.9920	0,4927	0.5426
Lysin N	1.6480	1.4437	1.9076	1.5550	1.5016	1.1004	1.9363	1.4922
Monoamino N	9.5938	9.6747	9.6219	9.9901	9.3668	9.9004	9.5982	9.9715
Cystin N	0.2355	0.2179	0.2896	0.2813	0.2609	0.2464	0.2629	0.2523
Myosin			(Total nitrogen %)					
Ammonia N	5.9651	6.1381	4.6050	5.1461	5.4408	5.9707	6.1022	6.7046
Melanin N	1.6303	1.4137	1.3333	1.5920	1.6779	1.9762	1.8706	1.9050

Diamino N	30.9646	29.2256	33.7717	31.3253	32.6265	29.6388	36.2974	33.9047
Arginin	16.5610	12.2792	16.4212	15.1466	19.1182	15.4639	18.2232	15.2383
Histidin N	3.6897	8.2443	6.2218	7.3284	3.8346	5.4364	2.7769	6.0715
Lysin N	9.1213	7.2221	9.8713	7.6987	9.3372	7.4126	13.4391	10.8198
Mono amino N	61.4399	63.2225	60.2901	61.9367	60.2548	62.4143	55.7298	57.4858
Cystin N	1.5927	1.4830	1.2573	1.1516	1.3365	1.3260	1.8582	1.7751
Myogen					(α)−Codfish−(β)			
Ammonia N	5.3148	5.7680	4.8203	5.6365	5.5380	5.7467	5.0213	5.5710
Melanin N	1.3917	1.3570	1.3391	1.7522	1.0678	1.2151	1.4826	1.6042
Diamino N	32.9870	32.3572	32.7197	30.9089	34.2790	29.6877	34.3753	29.1189
Arginin N	17.3273	11.5715	15.3978	13.5534	17.9148	14.7223	17.7944	14.5070
Histidin N	3.8200	8.0484	3.3643	6.0139	5.2408	6.3476	3.0348	3.4666
Lysin N	10.3593	9.3292	12.1180	9.6042	9.4768	7.0412	11.9268	9.5334
Monoamino N	60.3065	62.5179	61.1229	61.7023	59.1152	63.3504	59.1208	63.7054
Cystin N	1.4804	1.4081	1.8397	1.7374	1.6466	1.5767	1.6194	1.6119

TABLE VIII.

Ratios of nitrogen contained in different amino acids as found in female and male muscle proteins. The maximum quantity is taken as 100.

Animals and sexes	Boar	Sow	Male wild duck	Female wild duck	Codfish		Hatahata	
					Male	Female	Male	Female
Myosin								
Arginin N	100	74	100	93	100	81	100	83
Histidin N	45	100	85	100	70	100	46	100
Lysin N	100	79	100	78	100	89	100	81
Monoamino N	97	100	97	100	96	100	97	100
Myogen					(α)	Codfish	(β)	
Arginin N	100	67	100	88	100	82	100	81
Histidin N	47	100	56	100	83	100	88	100
Lysin N	100	90	100	79	100	74	100	80
Monoamino N	96	100	99	100	93	100	93	100

Thus it is seen that in male myosin and myogen there is a predominance of arginin and lysin form nitrogen, while in the female there is an excess of monoamino and histidin form nitrogen. The quantities of arginin nitrogen in the female proteins are about 85% those of the male, lysin nitrogen 82%, while the monoamino nitrogen of the male muscle proteins is about 95% of the female, and histidin nitrogen ca. 65%.

VII. The composition of acetyl proteins

For the purpose of studying the constitutional differences between the female and male serum-albumin, globulin, muscle-myogen and myosins, the authors acetylated these proteins and determined their composition.

According to Troensegaard's method, protein was acetylated as follows:—0.4 g. of proteins were kept over night with 10–15 ccm of glacial acetic acid. The solution was heated in a boiling water bath with a reflux condenser. Three ccm of acetyl chloride was added through the condenser and the bath was kept boiling. After 3 hours, 3 ccm of the reagent was added as formerly, 3 ccm more of the reagent were added twice at intervals of 3 hours and 1 hour respectively. Thus after the addition of 12 ccm of acetyl chloride, the boiling was stopped when a solution of acetyl protein was obtained. Ten ccm of acetic anhydride were added to the solution and the mixed solution was evaporated to about 10 ccm under a diminished pressure. Again, 10 ccm of acetic anhydride were added. To the mixture was added 1 g of newly fused and pulverized natrium acetate accompanied by a good shaking. The mixture was heated at 132–135°C. for 3 minutes in an oil bath. After its cooling, 10–15 ccm of dry chloroform were added and the whole was stood over night.

The chloroform solution freed from insoluble matter by filtration was then poured into dry ether. By this treatment, the acetyl protein produced was precipitated; the precipitate was washed twice and dried over sulphatic acid under diminished pressure. A very hygroscopic brown powder was prepared by successive crushings.

The nitrogen content of the compound was estimated by Kjeldahl's method and the acetyl group by Wenzel's method.

TABLE IX.

Content of nitrogen in different acetylized proteins.

Kinds of protein	Water %	N.-content (mg)	N. percentage	Ratio betw. the two sexes.
Serum globulin of ox	2.0519	3.0759	3.1402	100
〃 of cow	2.3898	2.7621	2.8298	90

Serum albumin of male horse	9.9678	3.0132	3.3404	100
〃 of female horse	9.0325	2.69928	2.9840	89
Muscle myosin of bull	3.6552	3.51534	3.6485	100
〃 of cow	3.6789	3.20147	3.2245	88
Muscle myogen of rabbit (male)	1.1825	4.14308	4.1928	100
〃 of rabblt (female)	3.0782	3.15125	3.2515	78
Muscle myosin of rabbit (male)	9.8850	4.14308	4.5626	100
〃 of rabbit (female)	10.0025	3.2625	3.6187	80
	Acetyl group (mg)	% of acetyl group	$\frac{\text{Acetyl}}{\text{N.}}$	Ratio betw. sex
Serum globulin of bull	52.9674	5.40744	17.22	100
〃 of cow	53.9795	55.3021	19.54	114
Serum albumin of male horse	52.8703	58.5026	17.51	100
〃 of female horse	53.8742	59.5636	19.96	114
Muscle myosin of bull	46.7152	48.4857	13.29	100
〃 of cow	48.9189	50.7877	15.75	119
Muscle myogen of rabbit (male)	45.8826	46.4332	11.07	100
〃 of rabbit (female)	50.6058	52.2151	16.06	145
Muscle myosin of rabbit (male)	45.8433	50.4826	11.06	100
〃 of rabbit (female)	50.1933	55.6444	15.38	139

As the nitrogen content of tho acetyl compound of male serum-globulin, albumin, muscle myogen and myosin surpasses that of the female and the acetyl group is the reverse, a very significant difference is observed in the proportions of acetyl group to nitrogen. This difference is also a characteristic of the proteins of the two sexes.

Conclusion

The most important points resulting from the foregoing experiments may be summarized as follows:—

(1) The ash and phosphorus contents of female myosin and myogen are always greater than those of male. This seems to explain the greater retention of phosphoric acid in the female body.

(2) The iso-electric point of thc female myosin and myogen is always rmoe acidic i.e. the pH value is lower than that of the male.

(3) The specific rotatory power of male myosin and myogen is always greater than that of female. This fact constitutes another of the remarkable differences between the proteins of the two sexes.

(4) In the case of male muscle myosin and myogen as well as serum albumin and globulin, the quantity of HCl combined is always greater than in the case or female.

(5) The content cf free amino nitrogen of the male myosin and myogen is always superior to that of the female. The quantity of the latter is about 80–90% of the former. This constitutes one more remarkable difference between the proteins of the sexes.

(6) Thus it is seen that in male myosin and myogen there is a predominance of arginin and lysin form nitrogen, while in the female there is an excess of monoamino and histidin form nitrogen. The quantities of arginin nitrogen in the female proteins are about 85% of those of the male, lysin nitrogen 82%, while monoamino nitrogen of the male muscle proteins is about 95% of the female, and histidin nitrogen ca. 65%.

(7) As the nitrogen content of the acetyl compound of male muscle myosin and myogen as well as serum albumin and globulin surpasses that of the female and the acetyl group is the reverse, a very significant difference is observed in the ratios of the acetyl group to nitrogen.

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