Chemical Studies on Sex Differences of Proteins in Animals and Plants (Second Report) Sex Differences of Muscle-fibre (Sarkolemm)

By

T. Tadokoro, M. Abe and **S. Watanabe**

One of the authors, T. Tadokoro and his colaborators have reported previously that the blood serum of different animals showed specificity in its absorption band in an ultraspectrum. They have also shown among plant proteins, the difference of chemical properties between the oryzenins, of common and of glutinous rice. Oryzenin is the principal protein of rice. The oryzenin of glutinous rice has a more acidic nature than that of common therefore the former showed weak combining power with HCl while the specific rotatory power and the refractive index of the glutinous is lower than that of the common. In elementary composition the content of nitrogen of the glutinous rice oryzenin is less and of sulphur is greater than that of the common. The content of arginin and lysin in the diamino acid nitrogen of the glutinous is less than that of the common, while the histidin and cystin contents are the contrary. The oryzenin of glutinous rice contained more OH-groups which would be replaced by acetyl and methyl group and also is easily decomposed by the enzymatic actions.

The same investigation of the chemical specificity of animal proteins was undertaken with serum albumine, globuline and myosin and myogen of muscles. When serum albumine and globuline were dissolved in alkali and titrated with HCl, the maximum point of turbidity and surface tension of the solution varies with the different species of animals. The specific rotatory power of myosin and myogen is highest in rabbit and next in cow and lowest in hen. Histidin contents of diamino nitrogen of myosin and myogen varies with the different species of animals. Thus the author and colaborators proved chemically the specificity of different animal and plant

[Jour. Facul. Agric., Hokkaido Imp. Univ., Sapporo, Vol. XXIII, Pt. 1, Sept., 1928]

proteins which were identified earlier by the biological method as the immunilogical reaction.

Further, in the authors' former report, concerning the sex differences of animal proteins, it was stated that the serum albumine, globuline, muscle myosin and myogen of male animals showed always a high specific rotatory power compared with that of female.

Phosphorus, free amino nitrogen and histidin contents of diamino nitrogen of these proteins of male animals are always higher than the corresponding content of female proteins. In the following investigation, the authors intended to prove the same chemical differences by sex in the muscle fibre (Sarkolemm) of additional animals.

1. Materials and their water and ash contents

In the preparation of muscle fibres, fresh meat was taken and freed from skin, tendon and fat and mashed in a mortar. The ground mass was mixed with two volumes of physiological NaCl solution, shaken for two hours and settled 24 hours in an ice chamber. The mixture was filtered with a linen cloth, the muscle juice pressed out and the process was repeated three times with NaCl solution. At last the pressed cake was treated with distilled water as mentioned above until the pressed liquid showed no chlorine reaction. The residual fibre was mounted into 70% alcohol for twenty-four hours, next into 95% and absolute alcohol then in ether and preserved in a H_2SO_4 desiccator. The water and ash contents of these samples were determined by the ordinary method.

TABLE I.

The water and ash contents of muscle fibers.

Kind of animals	Cock Hen	Bull Cow	Rabbit	Pig	Wild duck	Hata-hata	Codfish
Water %							
Male	7.369	12.032	8.712	6.368	6.124	9.145	5.171
Female	7.443	12.676	8.803	9.199	6.778	8.292	5.470
Ash %							
Male	0.712	0.704	0.627	0.772	0.639	0.715	0.369
Female	1.058	1.836	0.631	0.881	0.697	0.708	0.423
Ratio of ash of female to 100 of male	148	119	101	114	109	99 ?	114

2. Determination of pH value and acidity which is produced by muscle fibers in neutral salt solution

The frequently noticed development of acidity in neutral salt solutions in the presence of material from which no detectable quantity of acid can be extracted by pure water can be observed in the following materials. It is well known fact that one kind of acid soil (5) which contains no water soluble acids, produced an acidity in neutral salt solution as KCl etc. Mukherjee (6) stated that the same phenomenon was observed in the case of hydrated silica and Ascher and Usher (7) also observed it in the colloidal suspensoid of stearic acid in neutral salt solutions.

Many authors explained this phenomenon by assuming (1) a simple chemical interaction between the salt and a definite acid or mixture of acids, (2) a preferential absorption of a base of the salt or (3) an exchange between the kathions of the salt and hydrogen ions previously absorbed by the material. The second explanation assumed the selective absorption of hydroxyl ions from water, and can evidently be employed, alternatively with the third, to account for acidity produced by materials which are not regarded as actual or potential acids. The first, or "chemical" theory has been objected to on the ground that, if the materials dealt with contain acids, the latter must be both insoluble, since the water extracts are strong mineral acids; but no acids of this character are known. These effects may properly be regarded as arising from an ordinary chemical interaction of the ion type, qualified by the circumstance that one of the ions is non-diffusible; and their occurence is a logical consequence of the "surface dissociation" theory. This theory was developed by Pauli in connexion with colloidal suspensions, and advocated by McBain in the case of electrokinetic phenomena and it does not require the assumption of selective absorption. If the molecules in the surface of an insoluble acid in contact with water are imagined to undergo dissociation, and if the hydrogen ions so produced are osmotically active and have a range of movement determined by the electrostatic field due to the residual acid ions on the surface, such a system is separated from the external liquid by an imaginary envelope which marks the extreme range of movement of the hydrogen ions and is equivalent to a membrane permeable by any ions except the acid anions embedded in the surface of the

insoluble acid. When the external liquid is pure water, this on separation from the insoluble acid, should not have acquired hydrogen ions from the latter, since they are electrostatically tethered to its surface; but if a neutral salt is present in the water, an exchange should taken place between some of the hydrogen ions within the envelope and an electrically equivalent number of kathions in the external liquid. Thus the acidity should vary directly with the concentration of the salt within the limits denoted by the principle of "membrane equilibrium."

Our experiments with muscle fibre powder which was prepared by the above described method, were carried out in neutral KCl and Na_2SO_4 solution. The difference of acidity between the two sexes was determined. KCl and Na_2SO_4 (Merck's best) were recrystallized 2 times from pure distilled water of pH 6.9 and dried in a H_2SO_4 desiccator. These salts were dissolved in pure distilled water (pH 6.9) and 1/1, 1/2, 1/5, 1/10 and 1/20 normal solutions were prepared. A

TABLE II.

The normality of acid, produced by muscle fibre in salt solution.

Normal. of salt sol. Kind of animals	N/1	N/2	N/5	N/10	N/20	Average	Ratio
Bull (KCl)	0.0313	0.0285	—	0.0280	0.0275	0.0288	100
Cow ,,	0.0247	0.0243	—	0.0201	0.0205	0.0224	78
Cock ,,	0.0224	0.0201	—	0.0187	0.0173	0.0196	100
Hen ,,	0.0173	0.0173	—	0.0159	0.0145	0.0163	83
Hatahata							
Male ,,	0.0108	0.0108	0.0105	0.0120	0.0129	0.0114	100
Female ,,	0.0098	0.0102	—	0.0111	0.0120	0.0108	94
Codfish							
Male ,,	0.0089	0.0085	0.0087	0.0074	0.0081	0.0083	100
Female ,,	0.0076	0.0076	0.0071	0.0063	0.0068	0.0071	86
Hatahata							
Male (Na_2SO_4)	0.0127	0.0124	0.0116	0.0112	0.0132	0.0122	100
Female ,,	—	0.0114	0.0110	0.0101	0.0116	0.0110	90
Bull ,,	0.0225	0.0234	0.0240	0.0270	0.0285	0.0251	100
Cow ,,	0.0204	0.0201	0.0201	0.0225	0.0231	0.0212	84
Cock ,,	0.0180	0.0183	0.0195	0.0186	0.0180	0.0185	100
Hen ,,	0.0144	0.0150	0.0156	0.0159	0.0156	0.0153	83

definite quantity of muscle fiber powder was mixed with a definite quantity of different salt solutions, shaken and settled 24 hours. For the estimation of pH value, the mixture was treated with a potentiometer of Cambridge system using quinhydrone electrode and for acidity, the mixture was filtered with a dry filter and 10 cc of the filtrate was titrated with alkali solution using phenolphthalein as indicator.

(*a*) *Titration with NaOH solution*:—One third or half g of muscle fiber was mixed with 14–15 cc of KCl or Na_2SO_4 solution, shaken and settled 24 hours and filtered through a dry filter. Ten cc of the filtrate was titrated with 1/100 or 1/200 n-NaOH solution and the following figures were calculated as the normality of acid per one g of the muscle fiber.

The development of acidity in neutral salt solutions in the presence of muscle fibres, may be observed in the above table. It will be seen that the male muscle fibre always produces more acid solutions than female. Next, to measure the hydrogen ion concentration of the solution, the following experiment was undertaken.

(*b*) *Estimation of pH value*:—One half gram of muscle fiber powder was mixed with 10 or 15 ccm of KCl or Na_2SO_4 solution, shaken and settled 24 hrs. The hydrogen ion concentration of the mixture was estimated, using Pt-quinhydrone electrode. The following figures show milli volts of e.m.f. between the quinhydrone and the HgCl-electrodes at 15°C. In these experiments, the limit of error is ± 0.4 milli volts. The pH value was calculated by the following formula using correcting factors to temperature 1.011 m.v. at 15°C and 1.015 m.v. at 14°C. $\mathrm{pH} = \frac{0.4568\,(\text{saturate}) - \pi\,(18°\text{C})}{0.0577}$.

The pH value of the salt solution produced by the male muscle fiber is always less than that of female and the male produced greater acidity than the female. The same experiments were undertaken with the oryzenin, the main protein of rice. The oryzenin of common rice produced greater acidity than that of glutinous and showed a pH value of 6.15 while glutinous oryzenin showed a value of 6.47.

TABLE III.

The figures showed milli volts in salt solutions at 15°C.

Normality of salt sol. Kinds of animals	N/1	N/2	N/5	N/10	N/20	Sample G./ccm
Bull (KCl)	110.6	109.4	—	110.8	123.6	0.5/15
Cow ,,	101.6	100.6	—	100.4	107.6	0.5/15
Cock ,,	104.2	102.0	—	104.6	120.4	0.5/15
Hen ,,	95.4	99.2	—	100.8	107.8	0.5/15
Bull (Na_2SO_4)	119.2	125.8	127.6	133.4	141.6	0.5/15
Cow ,,	113.2	117.0	119.2	121.6	122.8	0.5/15
The figures showed pH value, calculated from the above data.						
Bull (KCl)	5.98	6.00	—	5.93	5.75	5.93
Cow ,,	6.14	6.15	—	6.16	6.03	6.12
Cock ,,	6.09	6.13	—	6.08	5.81	6.03
Hen ,,	6.25	6.18	—	6.15	6.03	6.15
Sample 0.5 g./10 ccm						
Boar ,,	6.63	6.55	6.42	—	—	6.53
Sow ,,	6.75	6.57	6.47	—	—	6.60
Hatahata						
Male ,,	7.20	7.15	7.10	7.01	6.83	7.07
Female ,,	7.22	7.19	7.17	7.07	6.86	7.10
Male wild duck ,,	6.53	6.28	6.20	6.07	6.02	6.22
Female wild duck ,,	6.65	6.42	6.40	6.45	6.23	6.43
Codfish						
Male ,,	6.38	6.37	6.36	6.35	6.37	6.37
Female ,,	6.62	6.57	6.53	6.46	6.52	6.54
0.5 g. in 10 cc						
Bull (Na_2SO_4)	5.82	5.70	5.67	5.57	5.43	5.64
Cow ,,	5.93	5.86	5.82	5.78	5.76	5.83
0.5 g. in 10 cc						
Cock ,,	5.88	5.87	5.94	5.99	5.94	5.96
Hen ,,	5.96	6.07	6.00	6.14	6.05	6.04
Boar ,,	5.89	6.00	6.02	5.99	—	5.98
Sow ,,	6.13	6.06	6.16	6.12	—	6.12
Codfish						
Male ,,	6.23	6.23	6.27	—	6.14	6.17
Female ,,	6.27	6.27	6.27	6.28	6.31	6.28
Hatahata						
Male ,,	6.45	6.48	6.42	6.45	6.51	6.46
Female ,,	6.63	6.58	6.51	6.50	6.53	6.55

3. Contents of nitrogen, sulphur and phosphorus

The contents of nitrogen, sulphur and phosphorus of the muscle fiber were estimated by the oridinary methods:— nitrogen by Kjeldahl's, sulphur by Denis-Benedict's and phosphorus by volumetric method. The following figures are percentages of dry matter.

TABLE IV.

The nitrogen, sulphur and phosphorus contents of muscle fiber.

Kind of animals	N%	Ratio	S%	Ratio	P%	Ratio
Bull	16.413	100	0.708	100	0.169	100
Cow	16.750	102	0.682	96	0.171	102
Cock	15.655	100	0.643	100	0.247	100
Hen	16.073	103	0.479	75	0.261	106
Rabbit						
Male	15.474	100	0.739	100	0.182	100
Female	16.107	104	0.728	98	0.148	81 ?
Boar	15.3771	100	0.8334	100	0.2084	100
Sow	15.4860	101	0.8066	97	0.2674	128
Wild duck						
Male	15.1122	100	0.7167	100	0.1725	100
Female	15.5552	103	0.6730	94	0.2026	117
Hatahata						
Male	16.1948	100	0.6599	100	0.6704	100
Female	16.0173	99 ?	0.6439	98	0.4120	61 ?
Codfish						
Male	14.9604	100	0.8447	100	0.0712	100
Female	15.0864	101	0.7996	95	0.1000	140

The amount of phosphorus existing in the ash of muscle fiber was observed by the authors to be about 1/5 that of sulphur. There were tendencies to find the higher sulphur content and the lower phosphorus content in the male muscle fiber as compared with the female.

The same tendency was observed in the case of oryzenin of the two broad classifications of rice. The oryzenin of glutinous rice is more acidic than that of common and shows rich phosphorus content and poor sulphur content (2).

TABLE V.

Sulphur and phosphorus content of oryzenins and ratio existing between them in various kinds of rice.

Kind of rice	Sulphur %	Phosphorus %	Ratio of S of glutinous to common	Ratio of P of glutinous to common
Akita				
common	0.892	0.166	100	88.4
glutinous	0.779	0.188	93.2	100
Etchu				
common	0.828	0.162	100	87.1
glutinous	0.777	0.186	87.4	100
Hokkaido				
common	0.745	0.153	100	87.6
glutinous	0.695	0.175	93.9	100
Hyogo				
common	0.635	0.167	100	88.3
glutinous	0.596	0.189	93.8	100
Saitama				
common	0.809	0.103	100	60.3
glutinous	0.771	0.168	95.2	100
Ibaragi				
common	0.773	0.101	100	57.6
glutinous	0.681	0.174	88.0	100
Echigo				
common	0.749	0.095	100	57.6
glutinous	0.654	0.164	87.0	100

4. Separation and determination of amino acids

Differences of the amino acids of proteins of muscle fiber between male and female were determined by Van-Slyke's advanced method which was described in our first report. The following results were obtained.

TABLE VI.

Amino acids of muscle fibers.

Animals	Nitrogen NH_3	N. of Melanin	N. of Monoamino	N. of Diamino	N. of Arginin	N. of Histidin	N. of Cystin	N. of Lysin	N. of free amino	N. of non free amino
Bull	0.9919	0.2169	8.8531	6.3512	3.2883	1.0445	0.2319	1.7865	3.1887	3.1625
Cow	1.0352	0.2300	9.2736	6.2112	3.0481	1.5416	0.1168	1.5047	2.8976	3.3138
Cock	0.9380	0.2114	8.6774	5.8583	3.1120	0.3428	0.2591	2.1144	3.2658	2.5625
Hen	0.9197	0.2102	9.1517	5.7919	2.8214	1.0571	0.1167	1.7967	2.9711	2.8208
Boar	0.9074	0.2286	8.4366	5.8045	2.9479	0.7312	0.1390	1.9864		
Sow	0.9817	0.2447	8.6042	5.6554	2.4839	1.3874	0.1322	1.6469		
Hatahata										
Male	0.8195	0.2004	9.8546	5.3203	2.7417	0.4022	0.1717	2.0047		
Female	0.9693	0.1766	9.8196	5.0518	2.4533	0.4240	0.2159	1.9586		
Male rabbit	0.9031	0.2024	9.8007	5.5069	2.9517	0.4832	0.1842	1.8878		
Female rabbit	0.9106	0.2342	10.2647	5.3405	2.5306	0.9484	0.1374	1.7241		
Male wild duck	0.9549	0.2501	9.0255	4.8817	2.5679	0.6291	0.2557	1.4290		
Female wild duck	0.9307	0.2694	9.5066	4.8485	2.4781	0.9407	0.2832	1.1465		
Male codfish	0.8818	0.2065	8.9071	4.9650	2.8387	0.6285	0.2215	1.2763		
Female codfish	0.9164	0.2364	9.1521	4.7815	2.5501	0.7815	2.2031	1.2468		
	The following figures are nitrogen percentage.									
Bull	6.0433	1.3215	53.9392	38.6959	20.0346	6.3838	1.4129	10.8846	19.4278	19.2681
Cow	6.1803	1.3731	55.3648	37.0818	18.1976	9.2086	0.6973	8.9833	17.2976	19.7839
Cock	5.9917	1.3504	55.4286	37.2294	19.8785	2.1897	1.6551	13.5061	20.8609	16.3635
Hen	5.7218	1.3077	56.9366	36.0338	17.5531	6.5767	0.7260	11.1780	18.4845	17.5493
Boar	5.9010	1.4866	54.8647	37.7477	19.1707	4.7551	0.9039	12.9179		
Sow	6.3393	1.5801	55.5612	36.5194	16.0719	8.9591	0.8537	10.6348		
Hatahata										
Male	5.0603	1.2374	60.8504	32.8519	16.9295	2.4835	1.0602	12.3787		
Female	6.0516	1.1026	61.3062	31.5396	15.3166	2.6471	1.3479	12.2280		
Male rabbit	5.5023	1.2332	59.7127	31.5519	17.9838	2.9440	1.1223	11.5018		
Female rabbit	5.4364	1.3982	61.2818	31.8836	15.1081	5.6621	0.8203	10.2931		
Male wild duck	6.3187	1.6550	59.7233	32.3030	16.9922	4.1629	1.6920	9.4559		
Female wild duck	5.9832	1.7319	61.1153	31.1696	15.9310	6.0475	1.8206	7.3705		
Male codfish	5.8942	1.3803	59.5378	33.1876	18.9748	4.2011	1.4806	8.5312		
Female codfish	6.0743	1.5670	60.6646	31.6941	16.9033	5.1802	1.3463	8.2644		

TABLE VII.

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

	Monoamino-N		Arginin-N		Lysin-N		Histidin-N	
	Female	Male	Female	Male	Female	Male	Female	Male
Bull & cow	100	97	90	100	82	100	100	69
Cock & hen	100	97	88	100	82	100	100	48
Pig	100	98	88	100	82	100	100	83
Hatahata	100	99	90	100	98	100	100	94
Rabbit	100	97	84	100	89	100	100	52
Wild Duck	100	97	93	100	87	100	100	68
Codfish	100	98	98	100	96	100	100	81

Thus it is seen that in male muscle fiber there is a predominance of arginin and lysin nitrogen, while in the female muscle fiber there is an excess of monoamino and histidin nitrogen.

The same tendency was observed in the case of oryzenin of common and glutinous rice. The oryzenin of glutinous rice is more acidic than that of common. It is rich in mono-amino and histidin nitrogen contents, and has less arginin and lysin nitrogen.

TABLE VIII.

	Monoamino-N		Arginin-N		Lysin-N		Histidin-N	
	glut.	comm.	glut.	comm.	glut.	comm.	glut.	comm.
Akita	100	94	84	100	59	100	100	58
Etchu	100	95	80	100	76	100	100	66
Hokkaido	100	98	102?	100	61	100	100	66
Hyogo	100	96	76	100	47	100	100	57
Saitama	100	97	72	100	45	100	100	57
Ibaragi	100	95	73	100	54	100	100	57
Echigo	100	96	75	100	53	100	100	57

Summary

From the results of the above experiments we can summarize the following differences in physico-chemical properties between the muscle fibers, so called "Sarkolemm" of male and female animals:—

(1) The development of acidity in neutral salt solutions was observed in the presence of muscle fibers of numerous animals. The acidity of the solutions which was produced by the muscle fibers of bull, cock, boar, male rabbit, male wild duck, male codfish and male hatahata (Arctoscopus japonicus) is always greater than that of the corresponding female muscle fibers.

(2) The existence of phosphorus in the muscle fibers of different animals was observed by the authors to be about 1/5 of the amount of sulphur. There were, however, tendencies to find the higher sulphur and the lower phosphorus content in the male muscle fibers of the bull, cock, boar, rabbit, wild duck, codfish and hatahata as compared with the corresponding female muscle fiber.

(3) Differences of the quantity of amino acids present in proteins of muscle fiber comparing male and female were determined by Van-Slyke's method. Thus it is seen that in male muscle fiber of the different animals there is a predominance of arginin and lysin nitrogen, while in the female muscle fiber there is an excess of monoamino and histidin nitrogen.

(4) The development of acidity in neutral salt solutions in the presence of male and female muscle fibers, showed a corresponding tendency in the case of the oryzenin of common and glutinous rice. The pH value of the salt solution produced by the male muscle fiber and by oryzenin of common rice is always less than produced by female muscle fibers and by the oryzenin of glutinous rice. That is to say the former two produced a greater acidity than that of the latter two.

(5) Difference of the amino acid, sulphur and phosphorus content were observed between male and female muscle fiber with the same tendency as in the oryzenin of common and glutinous rice. Thus it is seen that in male muscle fiber and oryzenin of common rice, there is a predominance of arginin and lysin while in the female muscle fiber and oryzenin of glutinous rice there is an excess of monoamino acid and histidin nitrogen.

There were tendencies to find that the higher sulphur content and

lower phosphorus content exist in the male muscle fibers and oryzenin of common rice. Then of course the contrary would hold as to sulphur and phosphorus content in the female muscle fibers and oryzenin of glutinous rice.

References

(1) Tadokoro:—Journ. Biol. Chem., Tokyo, III, 2, 1924.
(2) Tadokoro, Nakamura and Watanabe:—Journ. Coll. Agr., H.I.U. XIX, 3, 195.
(3) Tadokoro, Abe and Watanabe:—Journ. Coll. Agr., H.I.U. XIX, 2, 1927.
(4) „ :—Journ. Agr. Chem. Soc. Japan, III, 10, 37, 1927.
(5) Daikuhara: Bull. Imp. Centr. Agr. Exp. Sta. Japan, II, 1, 1914.
(6) Mukherjee:—Journ. Ind. Chem. Soc. India, II, 191, 1925.
(7) Ashar and Usher:—Journ. Chem. Soc. London, 1875-1882, 1927.