CHEMICAL STUDIES ON SEX DIFFERENCES OF PROTEINS IN ANIMALS AND PLANTS, (First Report).

Sex Differences of Muscle and Serum-proteins.

By

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Introduction.

In general, the chemical properties of animal proteins are quite different, since, originating in various animals or in the same kind of animal, the various proteins are formed in different organs and body tissues according to their physiological functions. The physiological function of any definite organ in the same family of animals is quite similar in all cases and the organ seems to form the same kind of protein. But the sexual products of male and female which are principally composed of protein are not the same. In the male the sperm is composed principally of nucleic acid and strong basic protamin and histon (Steudel[1] reported that the head of spermatozoa is composed 71.8 % of nucleic acid and 28.2 % of protamin). In the female the egg is principally composed of cytoplasm which contains vitellin and albumin. Hugounencq[2] determined the amino acid distribution in the hydrolytic products of protamin of sperm and showed that only 1/4 is monoamino acid while the principal component is diamino acid of which 3/4 is arginin. The protein of egg nucleus is "Clupeovin" which contains only 5 % diamino acid and 95 % monoamino acid. Those facts are a convincing explanation of the strong basic nature of the male proteins and the acidic nature of the female. Because the iso-electric point of vitellin and ovoalbumin is near 4.5 pH value, these have a great acidic content and are also rich in monoamino acid in their hydrolytic products. Also the iso-electric point of casein of milk is 4.7 pH and contains much monoamino acid in its hydrolytic products. Therefore there is a great difference in protein products between the two sexes. The basic and acidic nature of the proteins are shown in the following table:—

TABLE I

Amino Acids of Various Proteins.

	Vitellin		Ovoalbumin		Casein			Histon
Author	Abderhalden & Hanter[3]	Osborne[4]	Abderhalden[3]	Hausmann[5]	Abderhalden[3]	Hausmann[5]	Van Slyke[6]	Kossel[5]
Glycocoll	1.10	0.00	—		—			
Alanin	—	0.75	2.10		0.90			
Valin	2.40	1.87	—	Mono-amino-acid.	—	Mono-amino-acid.	Mono-amino-acid.	Mono-amino-acid.
Leucin	11.00	9.87	6.10		10.50			
Prolin	3.30	4.18	2.25		3.10			
Phenylalanin	2.80	2.54	4.40		3.20			
Aspartic acid	0.50	2.14	1.50	67.8	1.20	75.98	62.94	38.4
Glutaminic acid	12.22	12.95	8.00		10.70			
Tyrosin	1.60	3.37	1.10		4.30			
Histidin	—	1.90	—	Diamino acid.	—	Diamino-acid.	6.21	Diamino-acid.
Arginin	—	7.46	—		—		7.46	
Lysin	—	4.81	—	21.33	—	11.71	10.30	40.5
Ammonia	—	1.25	—	8.53	—	3.37	10.27	—

Further if we consider the chemism of sperm formation of the male we know that the process of protein production is accomplished in a very short period. For example, as reported by Paton and Dunlop,[7] the weight of sperm of salmon is increased from 14.5 g in May and June to 260 g in October or November, and also as reported by Leucart,[8] the weight of testicle of sparrow is increased about 200 times from January to April. The physiological manner of sperm formation of salmon has been described by Burian[9]: When salmon enters fresh water to increase its sperm, it takes no food but still generates a large quantity of nucleoproteid from muscle which contains little nucleoproteid. So after having finished its formation, the muscle of its back is decreased markedly. During the entire time the salmon's blood contains much albumose and pepton and also twice the usual amount of globulin. Thus the process of producing purin base from materials of less content seems to be the synthesis of imidazol rings as in the case of histidin formation in animal bodies. In the same way a new-born animal rapidly generates much nucleoproteid from egg and milk protein which contains only a small quantity of purin base. From the above facts, muscle and serum proteins which are the direct sources of sexual protein products, seem to show different physico-chemical properties com-

paring male and female. To show this the following experiments were undertaken:

(A) Sex Differences of Muscle Proteins.

The muscle formation of animals is influenced by many physiological functions which are observed to be different in male and female. The metabolism of matter in the male is higher than that in the female. Benedict[10] measured the difference as 5–6 %. Laurence and Riddle,[11] and Goettler and Baker[12] reported that the female body is always richer in body fat than the male as shown in the following table:

TABLE II

Fat Content of Domestic Fowls and Human.

	Domestic Fowl			Human	
	Cock	Hen with no egg	Hen producing egg	Man	Woman
Fat Content	15.44	17.87	27.80	141.4	226.0

Further, Sekine and Akiyama[13] determined the amino acid content, especially of diamino acids in the hydrolytic products of fish muscles and observed the following differences between male and female:

TABLE III

Diamino Acids of Fish Muscles.

	Engraulis jap.		Clupea mel.		Epinephalus ts.		Thunnus al.	
	Male	Female	Male	Female	Male	Female	Male	Female
Total-N	16.53	16.71	16.21	16.23	16.98	16.94	16.44	16.08
Cystin-N	1.26	0.99	0.61	0.27	1.12	0.85	1.80	1.74
Histidin-N	4.49	7.95	3.48	6.81	6.34	7.31	2.38	3.01
Arginin-N	7.15	5.96	7.14	6.48	6.71	6.61	7.88	6.55
Lysin-N	7.67	7.33	9.01	6.22	5.38	4.80	9.94	9.80

As to the muscle protein formation the quantity of fat present in the body has great influence upon the nature of the protein. Because amino

acids which are the components of protein are the amidation products of fatty acid, if the fat formation is great in animal bodies more acidic amino acid would be formed which would give also a more acidic nature to the muscle proteins. The authors considered that the muscle proteins of a female body should be more acidic than those of a male body.

(1) Preparation of Myosin and Myogen.

Fresh muscles, taken within 5 hours after death were ground in a mortar, mixed with the same volume of 0.6 % NaCl solution, shaken vigorously and extracted for 48 hours in an ice chamber. This mixture was filtered with a linen cloth under pressure and filtered again through a paper pulp to obtain a clear liquid. The filtrate was mixed with 3/4 volume of saturated ammonium sulphate solution and myosin was precipitated. The precipitate was poured into a bladder in water and dialysed for about 14 days in a water current and, after being freed from salt, the pure myosin was obtained by the addition of alcohol. The myosin was washed with alcohol and ether by a centrifugal machine and dried in a H_2SO_4 desiccator.

The filtrate from which myosin had been separated, was warmed to 51°C. and the precipitated residual myosin was freed; then the solution was saturated with ammonium sulphate whereupon myogen was precipitated. The myogen was treated as above and dried in a H_2SO_4 desiccator.

(2) Water, Ash and Phosphorus Content of Myosin and Myogen.

Both samples were analysed by the ordinary method and their ash and phosphorus contents estimated in percent of dry matter in the following table:

TABLE IV

The Water, Ash and Phosphorus Contents of Myosin and Myogen.

		Bull	Cow	Cock	Hen	Male Rabbit	Female Rabbit
Myosin	Water	4.6899	6.4400	9.2873	8.8294	7.3446	8.7928
	Ash	0.4208	0.4368	0.3932	0.5008	—	—
	Ratio	100	103	100	127	—	—
	Phosphorus	0.0520	0.0688	0.0575	0.0631	0.0696	0.1198
	Ratio	100	132	100	109	100	172

		Bull	Cow	Cock	Hen	Male Rabbit	Female Rabbit
Myogen	Water	8.1585	5.8091	7.4446	7.3940	8.8263	8.4384
	Ash	0.4589	0.4604	0.4657	0.5671	0.4317	0.5482
	Ratio	100	101	100	120	100	127
	Phosphorus	0.0806	0.1036	0.0881	0.1072	0.1093	0.1480
	Ratio	100	127	100	121	100	135

In the above table the ash and phosphorus contents of female myosin and myogen are always greater than those of male. This fact is one of the remarkable differences between the proteins of the two sexes.

(3) Maximum Point of the Surface Tension and of the Turbidity of the Protein Alkaline Solution in Titration with HCl Solution.

One twentieth g of each protein was dissolved in 10 cc of 1/50 n-NaOH solution. After standing 24 hours at room temperature 1 cc of the protein solution was diluted with 9 cc of redistilled water. The maximum point of the surface tension and of the turbidity was estimated by Nouy's apparatus and Duboscq's nephelometer respectively. The following numbers are cc of HCl solution necessary to bring the surface tension and turbidity of the protein solutions to their maximum.

TABLE V

cc of HCl necessary to bring the Surface Tension and Turbidity of Myosin and Myogen Solutions to their Maximum.

	Myosin				Myogen			
	Male Rabbit	Female Rabbit	Bull	Cow	Male Rabbit	Female Rabbit	Bull	Cow
1	3.60	3.20	4.10	3.90	3.40	3.15	3.95	3.80
2	3.65	3.45	—	—	3.65	3.05	—	—
Ratio	100	91	100	95	100	88	100	96

From the above results the attainment of the maximum point of the surface tension and turbidity of the male protein alkaline solution always requires a much larger quantity of acid than does that of the female.

(4) The Specific Rotatory Power of Myosin and Myogen.

The rotatory power of an organic compound is due to the presence of asymmetric carbon atoms in its molecule and it is increased by the complexity of the molecule, by saturation in molecular structure, and by the special atomic groups. Therefore it is highly pertinent in this investigation to examine the rotatory power of protein alkaline solution. One tenth g of each kind of protein was dissolved in 10 cc of 1/10 n-NaOH solution. After the indicated time, the solution was examined by means of a Haensch-Schmidt's half-shadow polariscope.

TABLE VI

Specific Rotatory Power of Myosin and Myogen Alkaline Solution of Various Animals.

		5–6 Hours			48 Hours		
		Reading	D	Ratio	Reading	D	Ratio
Myosin	Bull	-2.60	-70.79	100	—	—	—
	Cow	-2.30	-63.52	90	—	—	—
	Cock	-4.00	-69.20	100	(0·2g in 10 cc.) (24 Hours).		—
	Hen	-3.90	-67.47	97			—
	Male Rabbit	—	—	—	-2 00	-103.80	100
	Female Rabbit	—	—	—	-1.75	-90.83	87
Myogen	Bull	-3.00	-84.76	100	—	(24 Hours)	—
	Cow	-2.75	-75.67	89	—	—	—
	Cock	-2.60	-72 89	100	—	—	—
	Hen	-2.30	-64.45	88	—	—	—
	Male Rabbit	-1.80	-93.42	100	-2.20	-114.18	100
	Female Rabbit	-1.60	-83.04	89	-2.00	-103.80	91
	Male Rabbit	-1.65	-85.64	100	-2.10	-108.80	100
	Female Rabbit	-1.50	-77.85	90	-1.80	-93.42	89

In the above table, the specific rotatory power of male myosin and myogen is always greater than that of female. This fact constitutes also one remarkable difference between the proteins of the two sexes.

(5) Total Nitrogen and Free Amino Nitrogen Contents of Myosin and Myogen.

It is a widely accepted assumption that the substances giving a biuret reaction, i. e. those combining two or more amino groups of -CONH-, -CSFH-, -C(NH)NH-, or -CHNH- contain a small quantity of free amino nitrogen. At the same time, Van Slyke and Birchard estimated the quantities of free amino nitrogen of all kinds of proteins and proposed that they are proportional to lysin nitrogen of protein. As Wilson[14] has asserted that Sörensen's method[15] excelled Van Slyke's for the determination of free amino nitrogen, the authors adopted the former for the purpose.

One tenth g of protein was dissolved in 25 cc 1/5 n-NaOH solution. To 20 cc of the solution were added 10 cc of neutral formalin (50 cc of formalin of purchase was titrated with 1/5 n-NaOH solution, using 1 cc of 0.1 % phenolphthalein as indicator until the solution was coloured slightly pink). The solution was titrated with 1/20 n-HCl solution until it became colourless. A blank control experiment was carried out and the necessary corrections were made. 1 cc of 1/5 n-NaOH solution corresponds to 2.8 mg of free amino nitrogen. The total nitrogen was determined by Kjeldahl's method.

TABLE VII

Total Nitrogen and Free Amino Nitrogen.

		Total nitrogen	% of free amino nitrogen	Ratio	% of free amino nitrogen on the basis of total nitrogen	Ratio
Myosin	Bull	16.0229	2.0711	100	12.920	100
	Cow	15.8820	1.8371	88	11.567	89
	Cock	16.7980	1.8520	100	11.025	100
	Hen	16.4712	1.7199	92	10.441	94
	Male Rabbit	15.9865	2.8000	100	17.514	100
	Female Rabbit	16.2568	2.2750	81	13.994	79
6 Hours Myogen	Bull	14.8722	2.3247	100	15.631	100
	Cow	14.8690	1.9151	82	12.879	82
	Cock	15.5508	2.0420	100	13.131	100
	Hen	15.0734	1.8141	88	12.035	91
	Male Rabbit	15.4523	2.3100	100	14.949	100
	Female Rabbit	15.3653	1.9250	83	12.528	83

		Total nitrogen	% of free amino nitrogen	Ratio	% of free amino nitrogen on the basis of total nitrogen	Ratio
24 Hours	Male Rabbit	16.0548	2.7300	100	17.004	100
	Female Rabbit	16.4660	2.4500	89	14.879	87
	Male Rabbit	15.4523	3.1500	100	20.385	100
	Female Rabbit	15.3653	2.8000	89	18.222	89
	Male Rabbit	16.0318	3.6750	100	22.923	100
	Female Rabbit	15.8855	3.1500	85	19.830	86

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 latter is about 80–90 % of the former. This constitutes another remarkable difference between proteins of the sexes.

(6) Separation and Determination of Amino Acids of Myosin and Myogen.

A difference between the sexes in the amino acid of the two proteins as to both quantity and kind may be expected from the data of the above free amino nitrogen content and other analyses which show that the structures of the protein molecules differ markedly.

The authors thus undertook the following experiments according to Van Slyke's advanced method[16]. To a given quantity of sample was added 20 times its weight of 20 % HCl, the mixture was boiled and hydrolyzed for 8 hours on the sand bath under a reflux condenser. Next, almost all the HCl of the solution was driven off under a diminished pressure at below 40°C. The residue was made slightly alkaline by the addition of 10 % suspension of $Ca(OH)_2$. The ammonia nitrogen liberated was distilled into a standard H_2SO_4 under a diminished pressure at below 40°C. The remaining fluid was filtered and separated from melanin substances. The melanin nitrogen was determind by Kjeldahl's method. The filtrate was acidified with HCl and evaporated under a diminished pressure at below 40°C. To the concentrated filtrate were added 18 cc of concentrated HCl and 15 g of phosphotungstic acid and the diamino acids were precipitated. After 48 hours' undisturbed standing, the precipitate was filtered by suction and treated with a mixture of amylalcohol and ether. Using Van Slyke's micro-apparatus the free amino nitrogen was determined.

TABLE VIII

The Amino Acids of Myosin and Myogen.

	Total-N	Ammo-niaN-	Mela-nin-N	Di-amino-N	Argi-nin-N	Histi-din-N	Lysin-N	Mono-amino-N	Cystin-N
Myogen (Rabbit)									
Male	16.0318	1.5308	0.2091	5.3533	2.4495	1.4322	1.2818	8.9386	0.1898
Female	15.8855	1.4993	0.2005	5.4482	2 3014	1.8182	1.1406	8.7375	0.1880
Male	15.4523	0.6588	0.1882	5.9313	3.0493	1.5011	1.1111	8.6740	0.2698
Female	15.3653	0.6865	0.1797	6.0150	2.8121	2.0238	0.9159	8.4841	0.2632
Male	16.0540	0.9571	0.2625	5.7889	3.4735	0.8736	1.2022	9.0463	0.2396
Female	16.4660	0.9723	0.2588	8.0767	3.1109	1.7324	1.0295	9.1582	0.2039
Myosin (Rabbit)									
Male	15.9868	0.9608	0.2826	5.8277	3.3076	1.1520	1.2919	8.9157	0.1762
Female	16.2568	0.9831	0.2893	6.0255	3.1020	1.7577	0 9995	8.9589	0.1662
Myogen (Cock)	15.5167	0 7934	0.1877	5.1180	2.8149	0.5126	1.5962	9.1034	0.1945
(Hen)	15.0134	0.8292	0.1705	5.4136	2.7281	0.9882	1.5141	8.9143	0.1832
Myosin (Cock)	16.7980	0.8182	0.2178	5.8316	3.0028	0.8738	1.6772	9.9306	0.2778
(Hen)	16.4712	0.8399	0.2338	5.7153	2.8576	1.1007	1.4872	9.6822	0.2698
Myogen (Bull)	15.3880	0.8269	0.2063	4.8140	2.6648	0.3798	1.5135	9.5408	0.2559
(Cow)	15.3713	0.8361	0.2177	4.8884	2.5529	0.6351	1.4798	9.4291	0.2206
Myosin (Bull)	16.0292	0.8118	0.2237	5.0531	2.8579	0.4776	1.4831	9.9406	0.2345
(Cow)	15.8820	0.8403	0.2269	5.4620	2.6049	1.1379	1.4069	9.3528	0.2282

(*Total Nitrogen %*)

Myogen (Rabbit)									
Male	100.000	9.5485	1.3043	33.3918	15.2790	8.3350	7.9945	55.7554	1.1839
Female	100.000	9.4382	1.2622	34.2967	14.4674	11.4457	7.1801	55.0030	1.1835
Male	100.000	4.2634	1.2179	38.3846	19.7336	9.7144	7.1905	56.1340	1.7460
Female	100.000	4.4679	1.1695	39.1466	18.3016	13.1712	5.8579	55.2160	1.7130
Male	100.000	5.9615	1.6350	36.0571	21.6353	5.4414	7.4881	56.3464	1.4924
Female	100.000	5.9049	1.5717	37.1426	18.8929	10.5210	6.2523	49.5457	1.2393
Myosin (Rabbit)									
Male	100.000	6.0099	1.7678	36.4532	20.6900	7.2059	8.0810	55.7691	1.1022
Female	100.000	6.0473	1.7796	37.0645	19.0812	10.8121	6.1482	55.1086	0.8223
Myogen (Cock)	100.000	5.1132	1.2097	32.9838	18.1411	3.3035	10.2870	58.6684	1.2535
(Hen)	100.000	5.5231	1.1357	36.0585	18.1711	6.5821	10.0850	59.3756	1.2202
Myosin (Cock)	100.000	4.8696	1.2954	34.7160	17.8759	5.2018	9.9845	59.1178	1.6538
(Hen)	100.000	5.0992	1.4194	34.6987	17.3491	6.6826	9.0291	58.7826	1 6380
Myogen (Bull)	100.000	5.3737	1.3407	31.2843	17.3174	2.4682	9.8356	62.0016	1.6630
(Cow)	100.000	5.4394	1.4163	31.8021	16.6082	4.1317	9.6270	61.3422	1.4351
Myosin (Bull)	100.000	5.0645	1.3731	31.5243	17.8293	2.9796	9.2525	62.0160	1.4630
(Cow)	100.000	5.2909	1.4289	34.3911	16.4016	7.1647	8.8585	56.8901	1.4368

According to the table the content of histidin form nitrogen of the female myosin and myogen is always superior to that of the male. Both ratios are as follows:

TABLE IV

The Ratio of Histidin as Found in Table VIII.

Myogen, Rabbit	Male	100	100	100	Cock	100	Bull	100
	Female	127	135	193	Hen	198	Cow	189
Myosin, Rabbit	Male	100			Cock	100	Bull	100
	Female	150			Hen	128	Cow	240

(B) Sex Differences of Serum Proteins.

The great physiological differences of sexual products between male and female are caused by change of protein nature in blood. Smith[17] reported that in the ripening of the reproductive organs in a matured spider, the fat content of blood and liver is greater in the female than in the male. Geyer[18] stated that the female blood of *Ocneriadispar L.* contains a green colouring matter which seems to be derived from chlorophyll, while the male blood contains a yellowish xanthophyll. This difference perhaps might be caused by the difference of reducing action of the two kinds of blood. So Dewitz[19] observed that the reducing power of the female blood of insects is powerful compared with that of the male, and Manoiloff[20] and Bernatzki[21] each proposed special reactions to distinguish the difference of sex in blood. Thus the blood shows many sexual differences. Therefore the authors suppose that the serum proteins may also vary by sex. The following experiments were undertaken.

(1) Preparation of Serum-globulin and Serum-albumin and their Water, Ash and Phosphorus Contents.

A clear blood serum was diluted with twice the volume of water, after being saturated with $MgSO_4$ powder, allowed to settle over night, then filtered and washed with saturated $MgSO_4$ solution. The precipitate was dissolved in water, dialysed for 14 days, again precipitated with alcohol, washed with alcohol and ether, and dried in a H_2SO_4 desiccator. Globulin

thus prepared was analysed by the ordinary method.

Serum-albumin was precipitated from the filtrate by the addition of acetic acid. The precipitate was dissolved in water, dialysed for 14 days, again precipitated with alcohol, washed with alcohol and ether, dried over H_2SO_4. Albumin thus prepared was also analysed by the ordinary method.

Water, ash and phosphorus contents in percent of dry matter were estimated as in the following table:—

TABLE X

Water, Ash and Phosphorus Contents of Serum-globulin and Serum-albumin

		Bull	Cow	Horse	Mare	Man	Woman	Boy	Girl
Globulin	Water	9.5277	10.6007	6.3467	7.2569	7.8313	10.5469	7.4534	10.5368
	Ash	0.3624	0.4270	0.4344	0.5380	0.4238	0.4490	1.2277	1.3879
	Ratio	100	118	100	124	100	106	100	113
	Phosphorus	0.0318	0.0375	0.0527	0.0756	0.0444	0.0546	0.0763	0.0912
	Ratio	100	118	100	143	100	122	100	119
Albumin	Water	11.1888	11.4820	12.4726	10.9741	9.9213	7.5431	11.4583	11.3636
	Ash	0.5265	0.5500	0.5441	0.6252	0.6050	0.7069	0.9028	1.1607
	Ratio	100	104	100	114	100	116	100	128
	Phosphorus	0.0447	0.0689	0.0563	0.0759	0.0531	0.0704	0.1077	0.1112
	Ratio	100	154	100	135	100	132	100	103

In the above table the ash and phosphorus contents of the female serum globulin and albumin are always greater than that of the male. This is also a remarkable difference between the proteins of the two sexes now under examination.

(2) Maximum Point of the Surface Tension and of the Turbidity o the Protein Alkaline Solution in Titration with HCl Solution.

In this experiment, all the procedures are the same as in the case of myosin.

TABLE XI

cc of HCl necessary to bring the Surface Tension and the Turbidity of Protein Alkaline Solution to their Maximum.

	Serum-globulin						Serum-albumin			
	Man	Woman	Horse	Mare	Bull	Cow	Horse	Mare	Bull	Cow
1	2.35	2.05	4.75	4.50	4.45	4.15	5.10	4.75	4.70	4.45
2	2.40	2.10	4.85	4.55	4.40	4.10	5.00	4.65	—	—
3	—	—	4.95	4.35	—	—	4.95	4.60	—	—
Ratio	100	87	100	91	100	93	100	93	100	94

From the above table it will be seen that the attainment of the maximum point of the surface tension and turbidity of the male protein solution always needs a greater quantity of acid than that of the female.

(3) The Specific Rotatory Power of Serum-globulin.

In this experiment all the procedures are the same as in the case of myosin.

TABLE XII

The Specific Rotatory Power of Serum-globulin. (After five hours)

(0.2 g of the sample was dissolved in 10-15 cc of 1/10 n-NaOH solution.)

	Bull		Cow		Horse		Mare	
	Reading	α_D	Reading	α_D	Reading	α_D	Reading	α_D
	−4.15	−79.35	−3.70	−71.60	−3.05	−84.51	−2.90	−81.14
Ratio		100		90		100		95

In the above table, the specific rotatory power of the male serum globulin is always higher than that of the female and this fact constitutes also a remarkable difference between the two sexes.

(4) The Total Nitrogen and the Free Amino Nitrogen Contents of Serum-globulin and Albumin.

In these experiments all the procedure is the same as in the case of myosin.

TABLE XIII

Total Nitrogen and Free Amino Nitrogen of Serum Proteins.

		Total nitrogen	% of free amino nitrogen	Ratio	% of free amino nitrogen on the basis of total nitrogen	Ratio
Globulin	Bull	15.7974	1.3153	100	8.326	100
	Cow	15.6751	1.0841	82	6.916	83
	Horse	15.5701	1.4799	100	9.504	100
	Mare	15.3142	1.3510	91	8.821	92
	Man	—	1.5749	100	—	—
	Woman	—	1.2834	80	—	—
	Umbilicus (Boy)	—	1.4825	100	—	—
	„ (Girl)	—	1.4397	97	—	—
Albumin	Bull	15.1814	1.7025	100	11.212	100
	Cow	15.3685	1.3444	78	8.747	78
	Horse	15.3771	2.0474	100	13.314	100
	Mare	15.4396	1.6984	82	10.900	82
	Man	—	2.3624	100	—	—
	Woman	—	2.0139	85	—	—
	Umbilicus (Boy)	—	1.6652	100	—	—
	„ (Girl)	—	1.6427	98	—	—

According to the above table the free amino nitrogen content of the serum globulin and albumin of the female blood is always inferior compared to that of the male. The quantity of the former corresponds to about 70–98 % of the latter.

SUMMARY.

From the results of the above experiments we can summarize the following differences in physico-chemical properties between the myosin, myo-

gen, serum globulin and serum albumin of male and female animals:

(A) Differences by sex of myosin and myogen in muscle protein were observed in the following physico-chemical properties:

(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 attainment of the maximum point of the surface tension and turbidity of alkaline myosin or myogen solution of the male always needs a greater quantity of acid than that of the female.

(3) 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% that of the former.

(4) 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.

(5) 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 latter.

(B) Differences by sex of serum globulin and albumin were observed in the following physico-chemical properties:

(6) The ash and phosphorus contents of the female serum globulin and albumin are always greater than those of the male, the quantity of the former being greater by about 6–43% that of the latter.

(7) In order to reach the maximum point of the surface tension and turbidity, the alkaline globulin and albumin solutions of the male always need a larger quantity of acid than those of the female.

(8) The specific rotatory power of the male serum globulin is always greater than that of the female the latter corresponding to about 90–95% of that of the former.

(9) The free amino nitrogen content of the serum globulin and albumin of the female blood is always less than that of the male. The quantity of the former is about 78–98% of that of the latter.

REFERENCES.

(1) Steudel :—Zeitschr. Physiol. Chem. 472, **73**, (1911).
(2) Hugounencq :—Compt. rend. 1062, **138**, (1904).
(3) Abderhalden :—Zeitschr. physiol. Chem. 24, **46**, (1905).
(4) Osborne & Jones :—Amer. Journ. Physiol. 153, **XXIV**, (1909).
(5) Czapek :—Biochem. 28, **II**, (1920).
(6) Van Slyke :—Zeitschr. physiol. Chem. 24, **46**, (1905).
(7) Paton & Dunlop :—Report of the Royal College of Fishery Board, Edinburugh, Scotland. (1898).
(8) Leucart Hermann :—Handbuch der Physiologie 6, **II**, 78, (1881).
(9) Burian :—Chem. Spermatozoen, Wiesbaden, 818, (1906).
(10) Benedict & Emmes :—Journ. Biol. Chem. 20, (1915).
(11) Laurence & Riddle :—Amer. Journ. Physiol. 41, (1916).
(12) Goettler & Baker—Journ. Biol. Chem. 25, (1916).
(13) Sekine & Akiyama :—Journ. Imp. Fish. Inst. Japan, 31, **1**, (1926).
(14) Wilson :—Journ. Biol. Chem. 191–201, **56**, (1923).
(15) Sörensen :—Biochem. Zeitschr. 45–101, **7**, (1908).
(16) Van Slyke :—Journ. Biol. Chem. 15–85, **10**, (1911–1912).
" " 281, **22**, (1915).
(17) Smith :—Quart. Journ. Micro. Sci. 57, (1911).
(18) Geyer :—Zeitschr. Wiss. Zool. 105, (1913).
(19) Dewitz :—Zentralbl. Physiol. 145. **22**, (1908); 215, **26**, (1912).
(20) Manoiloff :—München Mediz. Wochenschr. 1784, (1924); 2186, (1924).
(21) Bernatzki :—Biol. Zentralbl. 727, **46**, (1926).

CONTENTS.