PHYSICO-CHEMICAL STUDIES ON THE SPECIFICITY OF MUSCLE AND SERUM PROTEINS OF DIFFERENT ANIMALS, (First Report).

By

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Introduction.

The term "Specificity" in biology means a direct relation of cause and effect between the substances, such as the toxin-antitoxin relation, the latter being produced only by the former, or a special disease being produced by certain organisms. Also the precipitin, anaphylaxis, agglutinins and opsonins are special physiological reactions. They were adopted for the determination of species of animals. These specificities are varied by physical changes, such as heating, cooling, coagulation of substance or by the chemical changes.[1]

The specificity of enzymatic action has been also observed by many authors in accord with the kinds of enzyme and the physico-chemical properties of substratum.[2] The different proteins showed varying physico-chemical properties according to their amphoteric natures with different combining power with acids or bases, and according to their amino acid composition, such as monoamino-, diaminoacids or polypeptide linkage. For example, nucleic acid shows a strong acidity, protamin and histon a strong basicity, and in hydrolytic products of casein and edestin a large quantity of monoamino acid appears, while histon and protamin are rich in diamino acid. In optical properties, some protein solutions show a dextro rotation while other proteins a laevo rotation. These differences of physico-chemical properties were observed in different varieties of proteins.[3]

On the contrary it has been observed that the specificity of protein showed different biological reactions in the same kind of protein. Thus all immuno-reactions in blood serum as mentioned above were reported by many authors. Uhlenhuth and Händel[4] and Trommdorff [5] stated that this difference was also observed between two near species, such as horse and mule, or sheep and goat (but it was difficult to distinguish between mouse

and rat). Those differences were observed not only in the blood serum, but also in many organs and physiological products, such as spermatozoa in different animals.

The specificity of this difference has been verified recently from the study of physico-chemical reactions by many authors. Amongst them Radmann and Hamberger[6] described the different phenomena of discharge of blood corpuscles by different species of animals. Abderhalden[7] observed a difference of sinking velocities of blood corpuscles in serum among different species of animals and stated that the sinking velocity of cow's corpuscles was the greatest and that of rabbit's situated between those of horse's and human, the latter being the smallest. Brown[8] differenciated the specificity of species by the system of crystallization of haemoglobin. Straub and Meier[9] stated that the red blood corpuscles of different species of mammalia possessed different iso-electric points respectively. The authors[10] also distinguished the absorption spectrum of blood serum between horse, dog and rabbit as follows:—

TABLE I

Wave Length of Absorption Spectra of Blood Serum.

Concentration of Serum	Original	1/2	1/4	1/8	1/16	1/32	1/64	1/128	1/256
Horse's Serum	4750	—	—	—	—	—	—	—	—
	4250	—	—	—	—	—	2990	2990	2860
	3825	—	—	—	—	—	2600	2650	2750
	3120	3070	3030	3020	2990	2995	2494	2480	2480
Dog's Serum	—	—	4080	—	—	—	—	—	—
	5800	5350	3952	—	—	—	—	—	—
	—	4905	3301	—	—	2820	—	—	—
	—	4200	3105	—	—	2625	—	—	—
	—	—	3005	3001	2990	2485	2480	2480	2480
Rabbit's Serum	—	5800	5740	—	—	—	—	—	—
	—	5100	4320	4250	4060	—	—	—	—
	—	4500	3905	3900	3950	2770	—	—	—
	—	—	3500	—	—	2770	—	—	—
	—	—	3100	3090	2850	2490	2480	2480	2480

The specificity of pure protein of plants also has been observed by many authors, such as Osborne and Wells,[11] who distinguished by biological reactions between pure globulin of hemp, flax, squash and other plant

seeds, and Zade[12] who observed the difference between the proteins of leguminous and gramineous plant seed. Further Lichtenstein[13] stated the same fact between the proteins which had been prepared from different kinds of yeast.

Though there are no investigators who discussed the specificity of pure protein from the physico-chemical standpoints, the authors are able to abstract from the previous experiments the following information related to this point. The results of Ehrström, Kossel, Kutscher and Dakin[14] on protamin and histon indicated the following differences:

TABLE II

Some Amino Acids found in Various Protamins and Histons.

Author		Protamin			Histon		
		Salmin	Clupein	Sturin	Lota-h.	Thymus-h.	Gadus-h.
Kurejeff[15]	$^{\alpha}D$	-80.8	-83.07--85.49	-60--58.8	—	—	—
Kossel	Histidi N	16.1	17.3	14.4-17.2	4.12	1.79	3.30
Ehrström & Kossel	Arginin N	66.8	68.3	57.5-63.4	23.44	25.17	26.90
	Lysin N	13.6	11.0	17.7-23.3	3.69	8.04	8.50
	Diamino N	96.5	96.6	94.5-97.1	31.25	35.00	38.70

Furthermore the results of Osborne, Abderhalden and Hopkins on albumin and globulin, and of Schultz, Abderhalden and Osborne on globulin and other proteins indicated that the difference of amino acid content of their hydrolytic products was due to the different species of plants and animals which were the origin of the proteins. A constitutional difference of protein derived from various species of plants or animals was observed from the difference in those amino acids which are considered very important in the chemistry of nutrition. The following differences in the elementary composition of casein which was prepared from human and cow's milk, especially in sulphur content, have also been observed.[16]

TABLE III

Elementary Composition of Casein of Human and Cow's Milk.

Element	Carbon	Hydrogen	Nitrogen	Sulphur	Phosphorus	Author
Casein of Cow's Milk	54.00	7.04	15.60	0.77	0.85	Lehmann
	53.07	7.13	15.65	0.76	0.80	Ellenberger
Casein of Human Milk	52.20	7.30	—	1.10	0.70	Wroblewski
	54.90	7.20	15.30	1.10	0.50	Ellenberger

Carnot[17] stated that the solubility of fibrin in acid solution is quite different in the horse, sheep and pig. Lewis[18] distinguished the specific rotatory power between pseudoglobulin, euglobulin and albumin of horse and human blood serum. The authors, as reported, have already found out the following differences between oryzenin prepared in the same manner from common and glutinous rices (the reader should refer to the Journ. Coll. Agr., Hokkaido Imp. Univ 2, XIV; 2, XVI).

(1) The abundance of asymmetric carbon, and of the COR group in the molecule and the high degree of saturation cause the higher specfic rotatory power of alkaline solution of the common rice oryzenin compared with the glutinous.

(2) Employing Cohnheim's method, the quantity of HCl combined with oryzenin of the common rice was greater than with that of the glutinous. This is due to the abundance of amino group which combines with HCl more easily in the former than in the latter.

(3) That the iso-electric point of the common rice oryzenin is near to the basic side and that of the glutinous is near to the acidic side indicates that in the former the amino group predominates over the carboxyl group, while the reverse is true in the latter.

(4) The refractive index of the common rice oryzenin is higher than that of the glutinous. This fact indicates that the former is constituted more densely than the latter.

(5) The greater quantity of nitrogen in the elementary composition of the common rice oryzenin is the reason for its basic nature. The higher ratio of carbon to oxygen of the glutinous rice oryzenin as compared with that of the common shows the predominance of acidity and of carboxyl groups in the former.

(6) The ash content of the common rice oryzenin is higher than that of the glutinous. There is a tendency to higher sulphur content and lower phosphorus content in the common rice oryzenin and vice versa in the glutinous rice oryzenin.

(7) The determination of amino acids after Van Slyke indicates that the common rice oryzenin excels in respect to content of ammonia-, arginin- and lysin-nitrogen, and the glutinous in quantities of monoamino-, histidin- and cystin-nitrogen. The quantity of free amino nitrogen in the common rice oryzenin predominates over that of the glutinous.

(8) The glutinous rice oryzenin was decomposed more rapidly than the other by pancreatin. This is because the polymerization degree of the common rice oryzenin is more complicated than the glutinous.

(9) Jodoprotein was prepared after the method of Blum and Straus and it was observed that the glutinous rice oryzenin combines with more iodine than the common. This is due to the abundance of histidin which possesses the imidazol ring.

(10) The silver salt of oryzenin was prepared. In quantity of nitrogen the common rice oryzenin predominated, while in quantity of silver the reverse held. This is due to the fact that the glutinous has more carboxyl groups than the common.

(11) An acetylation of oryzenin was carried out. The nitrogen content of the common rice acetyl oryzenin predominates over that of the glutinous, but in regard to the acetyl group the reverse holds. This seems to be on account of the abundance of the hydroxyl group which is replaced by the acetyl group in the latter.

(12) Amongst the many hundred varieties of common rice in all parts of Japan, two types are distinguished generally: those employed for the fermentation of *sake*, and those not so employed. The ash content of the *sake* rice is between 79 and 95 % of that of the latter. The specific rotatory power of the *sake* rice is lower than that of the latter, being between −64 and −72, while the latter is between −74 and −88. The iso-electric point of the *sake* rice is more acidic than that of the latter. The *sake* rice oryzenin is deficient in the quantities of ammonia, melanin and lysin nitrogen, but excels in histidin and cystin nitrogen. The content of free amino nitrogen of the *sake* rice oryzenin is inferior to that of the types not so used.

The authors intended to find out the differences o fphysico-chemical properties of myosin and myogen, the muscle proteins, and serum globulin among various kinds of animals. The following experiments were undertaken.

I. Preparation of Samples.

Fresh muscles, which must be used within five 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 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 under water and dialysed for about 14 days. After being freed from salt the 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 myosin was warmed to 51°C. and the precipitated residual myosin was freed. Then the solution was saturated with ammonium sulphate and myogen was precipitated. The myogen was treated as above and dried in a H_2SO_4 desiccator.

For the preparation of serum globulin; a clear blood serum was mixed 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 of serum globulin 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.

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

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 the turbidity were determined by Noüy's apparatus and Duboscq's nephelometer respectively. The following numbers are cc of HCl solution which were needed to bring about the maximum surface tension and turbidity of the protein solutions. In the titration, 1/200 n-HCl solution was used.

TABLE IV

Maximum Point of the Surface Tension and of the Turbidity.

(*Myosin*)

cc of HCl	3.5	3.55	3.6	3.65	3.7	3.75	3.8	3.85	3.9	3.95	4.0	4.05	4.1	4.15
Male Rabbit	—	Max.	—	—	—	—	—	—	—	—	—	—	—	—
„	—	—	Max.	—	—	—	—	—	—	—	—	—	—	—
Bull	—	—	—	—	—	—	—	—	—	—	Max.	—	—	—
„	—	—	—	—	—	—	—	—	—	—	—	Max.	—	—

(*Serum Globulin*)

cc of HCl	2.3	2.4	2.5	2.6	...	4.0	4.1	4.3	4.4	4.5	4.6	4.7	4.8	4.9	5.0	5.1
Man	—	Max.	—	—	...	—	—	—	—	—	—	—	—	—	—	—
„	—	—	Max.	—	...	—	—	—	—	—	—	—	—	—	—	—
Bull	—	—	—	—	...	—	Max.	—	—	—	—	—	—	—	—	—
„	—	—	—	—	...	—	Max.	—	—	—	—	—	—	—	—	—
Male Horse	—	—	—	—	...	—	—	—	—	—	—	—	Max.	—	—	—
„	—	—	—	—	...	—	—	—	—	—	—	—	—	Max.	—	—

From the above table, a clear difference was observed between the myosin and the serum globulin of different kinds of animals.

III. The Specific Rotatory Power of Myogen.

The rotatory power of an organic compound is caused by the presence of asymmetric carbon atoms in its molecule. It is increased by complexity of the molecule, by saturation in molecular structure, and by special atomic groups. Therefore, it is very important 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 and after a definite period, the solution was examined by a Haensch-Schmidt's half-shadow polariscope.

TABLE V

The Specific Rotatory Power of Myogen.

	Bull	Cock	Male Rabbit
Reading	-3.00	-2.60	-1.80~-1.65
Specific Rotatory Power	-84.76	-72.89	-93.42~-85.64

In the above table, the specific rotatory power of male myogen is different in the various kinds of animals.

IV. Determination of Amino Acids of Myosin and Myogen.

To a given quantity of sample was added 20 times its weight of 20 % HCl, the mixture was boiled and hydrolysed for 8 hours on a 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 determined by Kjeldahl's method. The filtrate was acidified with HCl and evaporated under a diminished pressure at below 40°C. To the concentrated solution 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 the mixture of amylalcohol and ether according to Van Slyke's modified method. The diamino acid nitrogen was determined separately. Using Van Slyke's microapparatus the free amino nitrogen was determined.

TABLE VI

Some Diamino Acids found in Myosin and Myogen of Various Male Animals.

Figures are total nitrogen %.

	Myosin			Myogen		
	Arginin-N	Histidin-N	Lysin-N	Arginin-N	Histidin-N	Lysin-N
Male Rabbit 1	—	—	—	15.2790	8.3350	7.9945
„ 2	—	—	—	19.7336	9.7144	7.1905
„ 3	—	—	—	21.6333	5.4414	7.4881
„ 4	20.6900	7.2059	8.0810	—	—	—
Cock 1	—	—	—	18.1411	3.3033	10.2870
„ 2	17.8759	5.2018	9.9845	—	—	—
Bull 1	—	—	—	17.3174	2.4882	9.8356
„ 2	17.8293	2.9796	9.2525	—	—	—

According to the table, the content of histidin, arginin and lysin of myosin and myogen is different according to the kind of animals. Especially is the content of histidin quite divergent.

RÉSUMÉ.

From the above results of the experiments, a comparison between various animals of the specificity of myosin, myogen and serum globulin may be stated in regard to the following physico-chemical properties.

(1) The difference of the maximum point of surface tension and of the trubidity of alkaline protein solution showed different iso-electric points of protein comparing bull, horse, rabbit and man.

(2) The specific rotatory power showed also a difference between myogen of bull, cock and male rabbit.

(3) The content of histidin, arginin and lysin varied between myosin and myogen of bull, male rabbit and cock.

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