ON THE NATURE AND ORIGIN OF PROTEINS*

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The purpose of this paper is to suggest an hypothesis which integrates some of the newer investigations on the chemical and physical properties of proteins, thus indicating a direction for future studies on the nature and origin of these substances. The newer developments of the theory of the structure of the protein molecule, based on the studies of Svedberg[58], Cohn[16], Astbury[1], and others, will not be discussed. It is desirable, however, to indicate some of the fallacies in the generally prevailing views on the nature and origin of proteins and to consider some of the experimental studies which are not in concordance with these ideas. Such considerations should permit the development of an hypothesis that better fits the experimental results, and should lead to further advances in the field of protein chemistry, that is, in the field of protoplasmic development and interrelationship.

The Development of the Idea of Many Discrete Proteins

If we glance through a chemical text-book[48] of the middle of the last century, we find that the studies of Mulder, Liebig, and their contemporaries had led chemists to believe that there were relatively few proteins. The reason for this belief was that proteins were characterized almost completely by their elementary chemical composition, supplemented by the observation of a few crude physical properties. The difference in the elementary composition of one protein from another is often less than is the analytical error and, therefore, these earlier investigators were left no choice but to believe that only a few classes of proteins existed in the plant and animal world.

The pioneer investigations of Ritthausen[46] on plant proteins began to change this conception. Ritthausen was able to extract from seeds of biologically related plants, numerous proteins which, although they had the same gross physical properties, could be differentiated by more careful methods and even by their elementary chemical composition. Furthermore, he was able to answer the criticisms concerning his methods of preparation by showing

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that the proteins obtained by extraction with dilute acids and alkalies were not denatured products but were similar in chemical composition and in physical properties to the proteins obtained from the same sources by extraction with neutral salts. He was able to further his contention that the proteins which he isolated from seeds were in the same chemical and physical state as those occurring naturally. In a few instances, he saw a crystalline protein deposited in the plant seed which, on extraction, gave a protein of the same crystalline structure. The significance of the investigations of Ritthausen was brought out by Osborne[42], whose carefully prepared homogeneous crystalline vegetable proteins have been the model for all subsequent investigations on the chemistry and physics of "pure" isolated proteins[12].

Let us turn back to the middle of the last century and sketch the investigations on the animal proteins. These substances are seemingly much more easily affected by dilute acids and alkalies and consequently the methods of protein preparation introduced by Ritthausen[46] were seldom successfully applied. Animal proteins could be obtained by fractional precipitation with neutral salts, and in a few cases homogeneous, reproducible, crystalline proteins were prepared, for example, the albumins from horse serum and egg-white. The separation by means of neutral salts of various proteins from animal organs was pursued principally by Denis[17], Hofmeister[26], Halliburton[22], Hammarsten[23], and Fürth[18]. Thus it was believed that there were many proteins existing in blood serum, muscle, egg-white, etc. which could be differentiated by variations in solubility in neutral salts and by coagulation temperatures, and also that all proteins in a single tissue differed from one another in chemical composition and immunological properties[64].

The isolation of so many products from plants and animals made one wonder whether so many different proteins could exist in nature. This doubt was dispelled by the presentation of the Hofmeister-Fischer peptide hypothesis (cf. Vickery and Osborne[61]) which showed that a simple calculation[31] of the number of permutations and combinations of the twenty or more amino acids which compose the usual protein would easily account for an almost infinite number of proteins in nature. A recent author[19] has said, "The fact that each species has a different set of proteins is not the surprising thing. The surprising thing is that nature is able to

control the synthesis of proteins within a single species, so that the same protein is synthesized by all members of the species."

Thus, with the refinements in analytical technic—chemical, physical, and immunological—it was shown that instead of only a few proteins distributed among the individuals of many species, these substances were sufficiently complex to account for the presence of several specific proteins in each tissue of every living organism. However, at least two chemists, Kossel[29] and Siegfried[52], attempted to show that these alleged innumerable proteins were to a certain extent interrelated. Miescher[34] and Weiss[63] had observed that in the salmon the muscle protein, which is relatively poor in arginine, is metabolized in the pre-spawning season in two ways; the larger portion of the muscle-protein amino acids is oxidized to supply energy to the starving fish, while the smaller portion, consisting of the "hexone bases," arginine, histidine, and lysine, but principally of arginine, is converted into the arginine-rich germ cells. From a long series of investigations on the amino acid composition of the fish sperm heads, Kossel[29] believed that the basic amino acids, and especially arginine, were of fundamental importance in the structure of the protein molecule.

Kossel called the breakdown of the muscle protein, which is low in basic amino acid content, to germinal protein rich in these amino acids, "biological reduction." It was likewise implied that tissue proteins were built upon a protamine nucleus by the introduction or addition of mono- and dicarboxylic amino acids to this basic nucleus. The basic nucleus hypothesis was supported by the experiments of Siegfried[52], who found that mild acid hydrolysis of such common proteins as casein and gelatin yielded products of low molecular weight (di- or tripeptides) which contained proportionally much larger amounts of the basic amino acids, especially arginine and lysine, than did the original protein[20].

These "nucleus" hypotheses were not generally accepted, however, and the usual belief, as expressed in the 1928 review of Vickery and Osborne[61], was that there are a great many unrelated chemically distinct proteins in living tissues. Many of these proteins could be obtained in a pure crystalline form from plants and animals. Thus each animal, organ, tissue, and cell was conceived to be made up of a number of unrelated distinct proteins which were capable of extraction if the appropriate solvent could be found and

whose composition would be approximately the same after isolation as it was in the living cell or tissue fluid. Naturally, the properties of the protein in the cell and out of it might be different, but this could be attributed to the influence of the other cellular constituents, non-protein as well as protein, on a complex colloidal amphoteric electrolyte.

Physicochemical Studies on Isolated Proteins

It is not necessary to review more than a few of the pertinent papers dealing with the physical and physicochemical properties of isolated proteins. Hardy[24] and Mellanby[33] studied the solubility and electrical properties of serum proteins, especially the globulins. Hardy*, in 1905, expressed his opinion against the growing idea that the various distinct proteins which had been isolated were discrete individuals preexistent in the serum. He said:

"On the whole the balance of probability is against it [that serum is a mixture of certain proteins in solution] and in favor of there being in serum some (possibly one) complex proteid which breaks down readily into fractions whose composition and properties depend upon the degree of dilution and the reagents used. . . . It [the globulin fraction] can be split by saturation with neutral salt or by acidification into fractions differing in properties according to the mode of separation."

Chick[15] also observed that some purified proteins fail to obey the phase-rule laws. These ideas were largely neglected until the extensive studies of Sørensen[56] and others† forced protein chemists to revise their views on the nature of the isolated protein molecule. Sørensen has shown that certain of the commonly isolated "homogenous" crystalline proteins, i.e., horse serum albumin, are probably composed of reversibly dissociable component or coprecipitation systems. An extension of this hypothesis would

* "Hardy early displayed an interest in the equilibrium of proteins in the blood and retained it to the end of his life. He always tended to think that in the circulating blood some unitary protein-complex exists, but he studied the recognized fractions and the globulin fraction in great detail." Obituary Notice: William Bate Hardy (1864-1933). F. G. H.: Biochem. J., 1934, *28*, 1149.

† See Haugaard and Johnson[25]; Jones and Csonka[28]; Linderstrøm-Lang[32]; Sandor and Bonnefoi[49]; Sandstedt and Blish[50]; Sinclair and Gortner, and Wiles and Gortner[58]; and Taylor[59].

explain the numerous proteins, or better termed, coprecipitation systems, of differing physical, chemical, and immunological properties, which can be isolated from cells. On the other hand, certain proteins such as insulin, prepared by different methods and from different cells, have been shown to possess identical physical and chemical properties, and are probably distinct individuals[27, 39, 40]. Furthermore, the sedimentation phenomenon of proteins at a definite pH range in Svedberg's ultracentrifuge has shown[58] that proteins can be roughly classed as having a molecular weight of some simple multiple or submultiple of 34,500. These studies indicate that there are probably some fundamental physical laws which govern the size, and consequently some of the properties, of proteins. If the particle size of serum or plasma is studied in the ultracentrifuge, the amounts of albumins and globulins (small and large particles) in a single serum can be changed by aging and even by simple dilution with Ringer's solution or distilled water[36, 37, 38]. This experiment illustrates the extreme lability of this tissue protein which is able to rearrange its component coprecipitation systems under the mildest chemical and physical treatment—a phenomenon which in the light of our ideas of the sensitivity of protoplasm is by no means unexpected.

Chemical Studies on Isolated Proteins

The extensive analyses of Fischer, Osborne, Vickery, and others for the amino acids yielded by proteins need not be mentioned except as they are necessary for the discussion. Gróh and Faltin[21] fractionally precipitated horse serum with ammonium sulfate at 30, 40, 50, 60, 70, and 80 per cent of saturation. They observed that the extinction coefficients and tryptophane content of the successive protein fractions decreased in a regular order. This was explained by them on the basis of a theory similar to that of Sørensen. They believed, also, that if serum were sufficiently diluted, the components would be completely dissociated and that they could be fractionated by neutral salts. The isolated components could then be studied physically and chemically.

Block[2, 4] observed that cattle serum could be fractionated by various neutral salts to yield albumins and globulins of differing basic amino acid composition. Furthermore, the molecular ratio

of lysine to arginine increased in the protein fractions with increasing solubility of the protein in the neutral salt solution (Table I).

TABLE I

BASIC AMINO ACIDS OF CATTLE SERUM ALBUMINS AND GLOBULINS

Protein	*Nitrogen per cent*	*Histidine per cent*	*Arginine per cent*	*Lysine per cent*	*Molecular ratio arginine:lysine*
Globulin, $MgSO_4$	14.33	1.06	5.4	4.3	10:9
Globulin, 15 per cent Na_2SO_4	16.46	1.12	5.5	6.5	10:14
Globulin, 20 per cent $(NH_4)_2SO_4$	15.73	1.16	5.3	7.9	10:18
Globulin, NaCl	15.90	1.30	6.7	12.2	10:22
Globulin, 40 per cent $(NH_4)_2SO_4$	16.53	1.80	6.2	12.1	10:23
Globulin, 30 per cent Na_2SO_4	15.66	1.28	5.2	10.9	10:25
Albumin, 60 per cent $(NH_4)_2SO_4$	15.67	2.76	6.2	13.6	10:26
Albumin, NaCl	15.36	1.23	5.2	15.6	10:36
Albumin, Na_2SO_4	15.23	1.05	6.3	22.0	10:42
Albumin, $MgSO_4$	14.71	3.22	5.0	39.6	10:92

These studies lend weight to the conception that *serum does not contain several independent proteins*; that the *fractions isolated by physicochemical methods are not preexistent in the serum but are produced by the technic employed in their preparation.* Here we have analytical evidence that the proteins which could be isolated from serum by simple treatment with neutral salts do not preexist as definite chemical entities in serum but rather that serum is composed of easily associable and dissociable components. Chemical analysis of these coprecipitation systems affords us some insight into their structure: the arginine content of all the protein fractions remained remarkably constant at about 5.7 per cent, while the lysine yielded on hydrolysis varied from 4.3 per cent in the most insoluble fraction to 39.6 per cent in the most soluble one. Thus we could conclude that the basic amino acid composition of serum albumins and globulins depends, in a measure at least, on the mode of preparation, and that the lysine-to-arginine ratio of the albumin is always higher than that of the globulin. Therefore, if two sera

from the same species were analyzed for their yield of albumin and globulin by the same procedure, we should expect, in the light of the knowledge discussed so far, that the albumin and globulin produced from both sera would have the same chemical composition. And if there were a difference in the relative proportions of the albumin to the globulin in the sera, we should believe that the serum containing the greater amount of albumin would yield the higher lysine-to-arginine ratio on hydrolysis of the total serum protein.

Although there is insufficient evidence, at present, for a final pronouncement, we believe that the phenomenon of the decomposition of a tissue protein by such mild reagents as neutral salts or simple dilution is not specific for blood plasma or serum. Yanagi[68] has shown that the soluble plasma protein of muscle can be converted or broken down, by neutral salts, into proteins or protein fractions having different physical properties which, heretofore,

TABLE II

SOME STRUCTURAL UNITS OF MAMMALIAN HEMOGLOBINS

Hemoglobin	*Horse*	*Sheep*	*Dog*
Amount of Protein Analyzed	3.37	3.31	2.26
Nitrogen (per cent)	16.70	16.83	16.40
Sulfur (per cent)	0.39	0.73	0.57
Iron (per cent)	0.33	0.32	0.33
Cystine (per cent)	0.4	0.6	1.2
Arginine (per cent)	3.2	3.5	3.3
Histidine (per cent)	7.5	7.5	7.4
Lysine (per cent)	8.1	7.7	8.1
Molecular Ratio: Iron:Sulfur:Cystine	25:50:7	25:100:14	25:75:21
Molecular Ratio: Iron:Arginine: Histidine:Lysine	1:3:8:9	1:3:8:9	1:3:8:9
Partial Formulae	$A_{18}H_{33}L_{37}Fe_4S_9C_2$*	$A_{18}H_{33}L_{36}Fe_4S_{18}C_5$*	$A_{18}H_{33}L_{37}Fe_4S_{13}C_8$*

*A means one molecule of arginine
H " " " " histidine
L " " " " lysine
Fe " " " " iron
S " " " " sulfur
C " " " " cystine

were considered as separate, unrelated substances occurring *per se* in muscle[43].

The easily crystallizable mammalian oxyhemoglobins offer a source of protein for an investigation of comparative biochemistry. It has previously been shown that although the molecular weight and iron content of certain mammalian hemoglobins are the same, other physical and chemical properties, such as crystal form, and cystine and sulfur contents, are not[62]. In the light of our investigations of the basic amino acid composition of tissue proteins (to be described below) we undertook a study of the basic amino acids of crystalline horse, sheep, and dog hemoglobins. Table II presents a summary of our data together with some information derived from a recalculation of analyses presented in the literature[6, 62]. These data demonstrate that the sulfur and cystine contents of these three proteins are quite inconstant. In fact, both the cystine and the sulfur yielded by each of these hemoglobins are simple multiples of the other two, although the molecular ratio of iron to sulfur to cystine varies in all three proteins. On the other hand, the molecular ratio of the basic amino acids, arginine, histidine, and lysine was 3:8:9 for all three hemoglobins[6].

Chemical Studies on Tissue Proteins

The investigations described above on the serum protein fractions—albumins and globulins—indicated to us that a simultaneous investigation of the albumin-globulin ratio by a standard technic and a study of the amino acid composition of the same sera should be carried out. The data summarized in Table III show that, although the albumin-globulin yield of two sera from the same animal at different times or even from normal and pathological people can vary 1000 per cent, the lysine-to-arginine ratio of the whole serum protein composite remains remarkably constant[9, 10]. In order to stress this striking constancy in the basic amino acid composition of the total serum protein, the term *orosin* (from the Greek, ὀρός, serum) has been introduced to designate the *total coagulable protein of the serum*[5]. Thus we believe that mammalian serum is not composed of albumins and globulins of varying composition which have no chemical relationship between themselves, but that mammalian orosin is composed of two or more unstable coprecipitation systems in mutual equilibrium. However much these coprecipitation systems may differ among themselves in physical and

TABLE III

CHEMICAL CONSTITUTION OF OROSINS

Source of protein	*Amount of serum protein*	*Albumin*	*Globulin*	*Ratio albumin to globulin*	*Amount of protein hydrolyzed*	*Nitrogen*	*Histidine*	*Arginine*	*Lysine*	*Molecular ratio arginine to lysine*
	%	%	%		gm.	%	%	%	%	
Human serum, normal.....	6.99	4.24	2.75	1.51:1	2.67	14.7	1.0	4.3	6.2	10:17
Human serum, normal.....	7.09	4.41	2.68	1.58:1	2.67	14.7	1.0	4.5	6.9	10:18
Human serum, nephrosis	3.35	0.44	2.91	0.51:1	1.54	14.5	0.9	4.8	6.8	10:17
Human urine, nephrosis					3.00	15.2	0.9	4.4	6.9	10:18
Dog 1, serum, normal.....	7.80	4.07	3.73	1.10:1	2.10	14.5	0.9	4.7	7.2	10:18
Dog 1, serum, protein deficient diet.....	4.27	1.61	2.66	0.61:1	1.46		0.9	4.9	6.8	10:17
Dog 2, serum, normal.....	6.88	4.29	2.59	1.65:1	2.14	14.7	0.7	4.9	7.3	10:18
Dog 2, serum, gliadin diet	4.54	1.26	3.28	0.38:1	1.68		1.3	4.5	6.4	10:17
Dog 3, serum, normal.....	6.60	3.31	3.29	1.00:1	1.61	14.6	0.8	4.9	7.0	10:17
Cow serum, normal.....	7.80				9.20	14.2	1.2	4.8	6.9	10:17
Avian serum, av. of hen, duck, turkey						15.7	2.2*	5.8	5.1	10:11

*Corrected for the average loss of histidine incident to this method of determination.

chemical characteristics, the mother substance, orosin, always has a remarkably constant basic amino acid composition[5, 9].

Our investigations have shown that the chemical composition of the albumins and globulins prepared by a variety of salts varies with the reagent employed. But sera from the same species of animal, or even from the same animal under different dietary conditions, precipitated by the same procedure, will produce albumins and globulins of different chemical composition[9, 10]. Thus we may conclude that the concentration of the orosin, as well as the changes

in the physical and chemical state of the non-protein substances in the blood, may have an effect on the amount and composition of a coprecipitation system precipitated by a neutral salt.

The data in Table III indicate that the basic amino acid composition of the mammalian orosins can be characterized by an arginine-lysine molecular ratio of 10:18. This ratio is not necessarily the same for orosins of other widely different origins. For example, we have shown[5] that the orosins prepared from bird sera (turkeys, chickens, and ducks), although constant for this order, have an arginine-lysine molecular ratio of 10:11. A recalculation of some evidence presented in the literature indicates, on the other hand, that the cystine and tyrosine yields of chicken and mammalian orosins are quite similar[45].

In contrast to the species difference in the basic amino acid yield of orosins, the keratins, whether of vertebrate or invertebrate origin, have been shown to yield a relatively constant proportion of these amino acids[3, 11] (Table IV). Furthermore, it can be seen that

TABLE IV

BASIC AMINO ACIDS AND CYSTINE YIELDED BY VARIOUS KERATINS

Source of tissue protein	*Amount hydrolyzed*	*Nitrogen*	*Cystine*	*Histidine*	*Lysine*	*Arginine*	*Molecular ratio histidine:lysine: arginine*
	gm.	per cent	per cent	per cent	per cent	per cent	
Human hair	255.4		16.5	0.50	2.5	8.0	1:5:14
Human finger nails	4.36	14.9	12.	0.49	2.4	8.4	1:3:15
Human skin (66) stratum corneum	50.0	15.1	2.3	0.59	3.09	10.09	1:6:15
Sheep wool	198.1	16.57	10.0	0.66	2.3	7.8	1:4:11
Egg shell membrane (13)		15.0	6.4	0.58	2.09	7.36	1:5:15
Cattle horn	5.0	15.1		0.64	2.8	9.4	1:5:13
Goose feathers	167.0	15.5	6.4	0.35	1.0	4.8	1:4:14
Snake epidermis	83.1	17.1	5.3	0.48	1.4	5.4	1:3:12
Silk fibroin	440.0	19.0	0.0	0.07	0.25	0.74	1:4:9
Gorgonia flabellum	41.8	15.6	5.5	0.48	2.7	6.4	1:7:13
Plexaurella dichotoma	14.3	15.6	3.2	0.43	3.0	5.4	1:7:12

during the embryological development of hair and nail from skin, the relative proportions of the basic amino acids yielded by these tissues remained constant. Thus the differences in these tissue proteins must occur in the other amino acids; for example, cystine varies from 0 to 16 per cent among the keratins. In fact, several investigators[44] have shown that the cystine obtained from finger nails of pellagrous persons was less than that yielded by hydrolysis of normal nails.

We have extended these investigations to include studies on the basic amino acid content of the protein fraction of such complicated organs as mammalian liver and even of entire plants and animals—yeasts, rats, and guinea pigs. These data are given in Tables V, VI, and VII. The rat, cat, and pig liver proteins were prepared by washing the freshly removed warm livers with ice-water. The organs were then boiled in water to coagulate the protein, ground up and extracted with hot water, alcohol, acetone, benzene, and ether. The defatted residues were then dried to constant weight. It will be seen that the arginine, histidine, and lysine contents of these mammalian livers were approximately constant (Table V).

TABLE V

THE BASIC AMINO ACIDS PRESENT IN MAMMALIAN LIVER

Source of tissue protein	*Amount hydrolyzed*	*Nitrogen*	*Histidine*	*Arginine*	*Lysine*	*Histidine:arginine:lysine*
	gm.	%	%	%	%	
Pig liver	2.00	14.0	1.5	5.2	4.5	1:6:6
Cat liver	2.00	13.9	1.5	5.0	4.5	1:6:6
Rat liver	2.00	14.1	1.8	5.4	5.6	1:5:7
Rat liver	1.40	13.1	1.4	4.4	4.7	1:6:7

The rats were grown under quite different experimental conditions. The one group was reared on a standard complete ration, the other was from the same stock but was lacking in mineral salts[54]. The animals were exsanguinated after death. It will be seen in Table VI that although the water, ash, and fat contents of these two animals were quite different, the nitrogen, cystine[65], arginine, and lysine contents of the total protein, when corrected for ash, were

constant. The guinea pig was a normal animal and was not bled after death. Although the actual percentage of arginine and lysine obtained after hydrolysis was lower than that obtained from rat tissue, the molecular ratio of arginine to lysine in these two rodents was the same (Table VI).

TABLE VI

SOME CONSTITUENTS OF WHOLE ANIMALS

	Total animal					*Composition of protein residue*				
Animal	*Wet weight*	*Dry weight*	*Ash*	*Fat*	*Protein*	*Nitrogen*	*Cystine*	*Lysine*	*Arginine*	*Molecular ratio cystine:lysine:arginine*
	gm.	%	%	%	%	%	%	%	%	
Normal rat	275.7	40.6	3.13	18.25	18.54	15.2	1.2	4.7	5.4	1:6:6
Low salt rat	136.6	31.2	2.25	7.20	20.61	15.3	1.3	4.7	5.4	1:6:6
Normal guinea pig		30.2	3.81	7.86	18.2	12.6		3.4	4.5	6:6
Whole egg (14)	39.	6.3				15.0	1.9	5.3	6.3	1:5:5

A recalculation of the data presented by Calvery and Titus[14] indicates that the molecular ratio of lysine to arginine in the white and the yolk of the hen's egg is 10:13 and 10:8 respectively. The molecular ratio of lysine:arginine in the whole egg is 1:1, or identical to the ratio obtained for whole rat or guinea pig. This is in striking contrast to the arginine:lysine ratio of 1:2 which was obtained on hydrolysis of different yeasts.

In collaboration with Dr. Charles N. Frey of the Fleischmann Laboratories, experiments on yeast were undertaken[7]. An attempt was made to change the relative proportions of arginine to lysine in a pure strain of a low protein yeast, grown under strictly controlled conditions, on three different nutritional media, the nitrogen of which was supplied as organic, inorganic, and organic and inorganic nitrogen respectively. In each case, the molecular ratio of arginine to lysine was 1:2 (Table VII). The analysis of another distillers' yeast of a high protein type grown on a natural medium also indicated the same molecular ratio for these amino acids. Thus,

TABLE VII

BASIC AMINO ACIDS OF YEAST

Type of yeast	*Arginine*	*Lysine*	*Molecular ratio arginine:lysine*
	mg.	mg.	
Low-protein organic nitrogen	115	160	1:2
Low-protein inorganic nitrogen	112	159	1:2
Low-protein organic and inorganic nitrogen	121	162	1:2
High-protein organic and inorganic nitrogen	120	158	1:2

we may conclude that, under the conditions of our experiments, the molecular ratios of the basic amino acids of yeasts cannot be changed by nutritional means.

Discussion

In attempting to summarize the evidence presented we can picture the isolation of proteins as follows:

> Protoplasm → tissue protein → isolated proteins, the composition and properties of which depend in a measure on the reagents employed in their isolation.

The decomposition of protoplasm to tissue protein can be achieved easily in some instances and only with difficulty in others. For example, if blood is allowed to clot, the serum could be called an impure tissue protein-orosin-solution. If the serum is treated with alcohol and ether, the tissue protein, orosin, can be isolated in a "pure" form. Certain epidermal tissue proteins, the keratins, on the other hand, are more difficult to separate from their protoplasmic concomitants.

Likewise, the second step in the reaction, the formation of the isolated, often crystalline, proteins from tissue protein, takes place easily in the case of the orosins; in the case of the keratins only by quite drastic treatment. The stability of the isolated proteins also shows all degrees of variability. Sørensen's experiments on a crystalline horse serum albumin indicate that it is readily decomposed by treatment with neutral salts under certain conditions. Northrop and others[27, 39, 40] have shown that other crystalline proteins, such as pepsin, are remarkably stable and probably do not

dissociate to any appreciable extent. There is also evidence that a crystalline protein having no enzymatic activity can be converted into a potent crystalline proteolytic enzyme[41].

Many investigations on isolated proteins tend to stress the differences between these substances. Our studies, especially those on the basic amino acids of mammalian hemoglobins, have shown that these substances, although they might differ considerably in sulfur and cystine content, still have a strong bond of similarity; i.e., the molecular ratio of the amino acids, arginine, histidine, and lysine is constant. The studies of Kossel[29] on the protamines and the calculations of Larmour[30], obtained from data in the literature on the arginine and lysine ratios of many proteins, also stress the importance of the basic amino acids in protein structure.

The comparative biochemistry of the tissue proteins has not been so extensively investigated, but our studies on the orosins[5, 9, 10] and keratins[3, 11] indicate that, even though the non-basic amino acids may vary widely, the relative proportions of the basic amino acids among analogous or homologous tissue proteins remain approximately fixed.

The investigations entailing entire animals are not sufficiently complete at present to warrant an opinion concerning the constancy or inconstancy of their amino acid composition. However, our results, in contrast to some very unconvincing experiments in the literature[47, 51], indicate that the total amino acid composition of any one species of plant or animal is constant, in spite of great variations in diet and physical well being.

We have presented evidence for the hypothesis that the protoplasm of a specific organism, tissue, cell mass, or certain derivatives thereof, is composed of a labile nitrogenous chemical aggregate—tissue protein—which yields arginine, histidine, and lysine in molecular ratios that are approximately fixed and characteristic for that tissue as it exists in various classes of animals. *Such labile tissue proteins are converted into the commonly isolated proteins by physicochemical treatment.* Experimental evidence makes it very improbable that these proteins or protein fractions exist independently in the living tissue, even though some of them can be obtained in a highly stabilized crystalline form.

Such an hypothesis will explain the constancy of the arginine-lysine ratios in various mammalian orosins despite the fact that they show profound differences in the yields of the individual serum

protein fractions, albumin and globulin. It also suggests a fundamental chemical similarity among homologous (sera, hemoglobins) and analogous (keratin) structures. Our investigations indicate that tissue proteins, for example, orosin and keratin, are built upon an "Anlage" of arginine, histidine, and lysine. This suggestion differs from earlier proposals, for example, those of Kossel[29] and of Siegfried[52], that postulate that the characteristic proteins which can be isolated from natural sources contain a central protamine nucleus around which additional amino acid groups (polypeptides) are joined. Our view, on the other hand, stresses the *primary importance of the basic amino acids in the genetic and embryological development of the tissue protein as it exists in protoplasm.*

Summary

The major points which are brought out in this paper are:

(1) Proteins which are isolated from plants and animals do not, in the great majority of cases, exist as such in the living organism but are *derived substances*, artificially produced from the *tissue proteins* by the reagents used in their preparation.

(2) The *tissue proteins* of plants and animals of the *same species* which have been nourished upon diets widely differing in composition yield the same proportions of amino acids—arginine, histidine, lysine and cystine.

(3) The homologous and/or analogous *tissue proteins* of different species of animals yield approximately the same proportions of the basic amino acids, arginine, histidine, and lysine even though other substances such as sulfur and cystine may vary greatly.

(4) The primary importance of arginine, histidine, and lysine in the genetic and embryological development of the tissue protein as it exists in protoplasm is pointed out.

Bibliography

1 Astbury, W. T.: Some problems in the X-ray analysis of the structure of animal hairs and other protein fibers. Tr. Faraday Soc., 1933, *29*, 193.

2 Block, R. J.: The basic amino acids of serum proteins. J. Biol. Chem., 1933, *103*, 261.

3 Block, R. J.: The basic amino acids of keratins. The basic amino acid content of human finger nails and cattle horn. J. Biol. Chem., 1934, *104*, 339.

4 Block, R. J.: The basic amino acids of serum proteins. II. The effect of heating to fifty-eight degrees. J. Biol. Chem., 1934, *104*, 343.

5 Block, R. J.: The basic amino acids of serum proteins (orosins). IV. A note on some proteins of the egg. J. Biol. Chem., 1934, *105*, 455.

6 Block, R. J.: The basic amino acids of three crystalline mammalian hemoglobins. Further evidence for a basic amino acid "anlage" of tissue proteins. J. Biol. Chem., 1934, *105*, 663.
7 Block, R. J.: Read before the meeting of the American Chem. Soc. at Cleveland, September, 1934.
8 Block, R. J.: Unpublished experiments.
9 Block, R. J., Darrow, D. C., and Cary, M. K.: The basic amino acids of serum proteins. III. A chemical relationship between serum proteins of various origins. J. Biol. Chem., 1934, *104*, 347.
10 Block, R. J., Darrow, D. C., and Cary, M. K.: Unpublished experiments.
11 Block, R. J., and Vickery, H. B.: The basic amino acids of proteins. A chemical relationship between various keratins. J. Biol. Chem., 1931, *93*, 113.
12 Bodansky, M.: Introduction to Physiological Chemistry. J. Wiley & Sons, New York, 1930.
13 Calvery, H. O.: Some chemical investigations of embryonic metabolism. J. Biol. Chem., 1932, *95*, 297.
14 Calvery, H. O., and Titus, H. W.: The composition of the proteins of eggs from hens on different diets. J. Biol. Chem., 1934, *105*, 683.
15 Chick, H.: The apparent formation of euglobulin from pseudo-globulin and a suggestion as to the relationship between these two proteins in serum. Biochem. J., 1914, *8*, 404.
16 Cohn, E. J.: Die physikalische Chemie der Eiweisskörper. Ergebn. d. Physiol., 1931, *33*, 781.
17 Denis, P. S.: Mémoires sur le sang. Paris, 1859.
18 Fürth, O.: Lehrbuch der Physiologischen und Pathologischen Chemie. Leipsig, 1925.
19 Gortner, R. A.: Outlines of Biochemistry. J. Wiley & Sons, New York, 1929.
20 Grassmann, W., and Lang, O.: Zur Kenntnis der Kyrine. Biochem. Ztschr., 1934, *269*, 211.
21 Gróh, J., and Faltin, E.: Über die Fraktionierung der Serumeiweisskörper und die Adsorptionsspektra der Fraktionen. Ztschr. f. physiol. Chem., 1931, *199*, 13.
22 Halliburton, W. D.: A Text-book of Chemical Physiology and Pathology. London and New York, 1891.
23 Hammarsten, O.: A Text-book of Physiological Chemistry. [Translated by J. A. Mandel.] J. Wiley & Sons, New York, 1908.
24 Hardy, W. B.: Colloidal Solutions. The Globulins. J. Physiol., 1905, *33*, 251.
25 Haugaard, G., and Johnson, A. H.: On the fractionation of gliadin. Compt. rend. d. trav. du lab. Carlsberg, 1930, *18*, No. 2.
26 Hofmeister, F.: Uber Bau und Gruppierung der Eiweisskörper. Ergebn. d. Physiol., 1902, *1*, 759.
27 Jensen, H., and Evans, E. A. Jr.: The chemistry of insulin. Physiol. Rev., 1934, *14*, 188.
28 Jones, D. B., and Csonka, F. A.: Precipitation of soy bean proteins at various concentrations of ammonium sulfate. J. Biol. Chem., 1932, *97*, xxix.

29 Kossel, A.: Ueber den gegenwärtigen Stand der Eiweisschemie. Ber. deut. chem. Ges., 1901, *34*, 3214.
———: Einige Bemerkungen über die Bildung der Protamine im Tierkörper. Ztschr. f. physiol. Chem., 1905, *44*, 347.
——— and Staudt, W.: Beiträge zur Kenntnis der basischen Proteine. Ztschr. f. physiol. Chem., 1927, *171*, 156.
——— and Schenck, E. G.: Untersuchungen über die basischen Eiweisstoffe, ein Beitrag zu ihrer Entwicklungsgeschichte. Ztschr. f. physiol. Chem., 1928, *173*, 278.
———: The Protamines and Histones. [Translated by W. V. Thorpe.] Monographs on Biochemistry, Longmans, Green & Co. London and New York, 1928.
30 Larmour, R. K.: Correlations between the total nitrogen of the bases and arginine nitrogen and between total nitrogen of the bases and lysine nitrogen of various proteins. Proc. Roy. Soc., Canada, 1928, *22*, 349.
31 Leathes, J. B.: Function and design. Science, 1926, *64*, 387.
32 Linderstrøm-Lang, K.: On the fractionation of casein. Compt. rend. d. trav. du lab. Carlsberg, 1929, *17*, No. 9.
33 Mellanby, J.: Globulin. J. Physiol., 1905, *33*, 338.
34 Miescher, F.: Histochemische und physiologische Arbeiten. Leipsig, 1897.
35 Mitchell, H. H., and Hamilton, T. S.: The Biochemistry of the Amino Acids. Am. Chem. Soc. Monograph Series, New York, 1929.
36 von Mutzenbecher, P.: Die analyse des Serums mit der Ultrazentrifuge. Biochem. Ztschr., 1933, *266*, 226.
37 von Mutzenbecher, P.: Die Fraktionation des Serums. Biochem. Ztschr., 1933, *266*, 250.
38 von Mutzenbecher, P.: Dissoziationen und Assoziationen der Serummoleküle. Biochem. Ztschr., 1933, *266*, 259.
39 Northrop, J. H.: Crystalline Pepsin. I. Isolation and tests of purity. II. General properties and experimental methods. IV. Hydrolysis and inactivation by acid. J. Gen. Physiol., 1930, *13*, 739-80.
40 Northrop, J. H., and Kunitz, M.: Crystalline trypsin. J. Gen. Physiol., 1932, *16*, 267-348.
41 Northrop, J. H., and Kunitz, M.: Isolation of a crystalline protein from pancreas and its conversion into a new crystalline proteolytic enzyme by trypsin. Science, 1933, *78*, 558.
42 Osborne, T. B.: The Vegetable Proteins. Monographs on Biochemistry. Longmans, Green & Co., New York, 1924.
43 Parnas, J. K.: The chemistry of muscle. Luck's Ann. Rev. Biochem. Stanford, II, 1933.
44 Payne, S. A., and Perlsweig, W. A.: Cystine content of finger nails in pellagra. J. Clin. Invest., 1933, *12*, 899.
45 Reiner, M., and Sobotka, H.: Tyrosine and cystine content of serum proteins. J. Biol. Chem., 1933, *100*, 779.
46 Ritthausen, H.: Die Eweisskörper der Getreidarten, Hülsenfrüchte und Ölsamen. Bonn, 1872.
47 Roche, A.: Recherches sur la constitution des proteines musculaires. Bull. Soc. chim. biol., 1934, *16*, 270.

48 Rogers, J. B., and Rogers, R. E.: Turner's Elements of Chemistry. 7th Ed. Philadelphia, 1846.

49 Sandor, G., and Bonnefoi, A. E.: Précipitation des albumines et des lipides du sérum sanguin par les sels neutres en fonction du pH. Bull. Soc. chim. biol., 1933, *15*, 1472.

50 Sandstedt, R. M., and Blish, M. J.: The nature and identity of wheat glutenin. Cereal Chem., 1933, *10*, 359.

51 Schenck, E. G., and Wollschitt, H.: Untersuchungen über die Abhängigkeit des Baues der Gewebeeiweissstoffe von der Stoffwechselform des Organismus. Arch. f. exper. Path. u. Pharmakol., 1933, *173*, 260.

52 Siegfried, M.: Zur Kenntnis des Glutokyrins. Ztschr. f. physiol. Chem., 1903, *43*, 44. Zur Kenntnis der Hydrolyse des Eiweisses. Ber. Verhandl. sächs, Ges. Wissensch. Math.-physische Klasse, 1903, *55*, 63.

53 Sinclair, W. B., and Gortner, R. A.: Physicochemical studies on proteins. VII. The peptization of gliadin by solutions of inorganic salts. Cereal Chem., 1933, *10*, 171. Wiles, W. H., and Gortner, R. A.: VIII. The rotatory dispersion of three gliadin preparations peptized by different solutions. Cereal Chem., 1934, *11*, 36.

54 Smith, A. H.: Privately communicated.

55 Smith, E. C.: On the coagulation of muscle plasma. Proc. Roy. Soc., London, *B*, 1934, *114*, 494.

56 Sørensen, S. P. L.: The constitution of soluble proteins. Compt. rend. d. trav. du lab. Carlsberg, 1930, *18*, No. 15.

57 Speakman, J. B., and Hirst, M. C.: The constitution of the keratin molecule. Trans. Faraday Soc., 1933, *29*, 148.

58 Svedberg, T.: Molecular weight analysis in centrifugal fields. Science, 1934, *79*, 327.

59 Taylor, A. E.: On the polymerization of globulin. J. Biol. Chem., 1906, *1*, 345.

60 Vickery, H. B.: Recent contributions to the theory of protein structure. Yale J. Biol. & Med., 1932, *4*, 595.

61 Vickery, H. B., and Osborne, T. B.: A review of the hypothesis of the structure of proteins. Physiol. Rev., 1928, *8*, 393.

62 Vickery, H. B., and White, A.: The proportions of cystine yielded by hemoglobins of the horse, dog, and sheep. Proc. Soc. Exper. Biol. & Med., 1933, *31*, 6.

63 Weiss, F.: Untersuchungen über die Bildung des Lachsprotamins. Ztschr. f. physiol. Chem., 1907, *52*, 107.

64 Wells, H. G.: The Chemical Aspects of Immunity. Am. Chem. Soc. Monograph Series, 2nd Ed., New York, 1929.

65 White, A.: Privately communicated.

66 Wilkerson, V. A.: The chemistry of human epidermis. I. Amino acid content of the stratum corneum and its comparison to other human keratins. J. Biol. Chem., 1934, *107*, 377.

67 Wu, H., and Yang, E.: A theory of denaturation. Chinese J. Physiol., 1931, *5*, 321.

68 Yanagi, K.: Ein Beitrag zum Studium der Eigenschaften des Plasmaeiweisses von Quergestreiften Muskel. J. Biochem. (Japan), 1931-32, *14*, 305.