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On the Structure of the Protein Molecule *

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In 1920 the author initiated an investigation of the proteins according to new methods, proceeding from the working hypothesis that the protein molecule essentially consists of heterocyclic ring systems which are easily split by acids, alkalies and enzymes, thereby producing the amino acids [1].

For the past 50 years the most important method of investigating the structure of the proteins has involved boiling with strong mineral acids — a method which, to say the least, seems rather one-sided in its treatment of these substances that are so important to life.

One thing that the previous theories have been unable to account for is the great similarity of elementary composition displayed by the proteins. Calculated on the basis of 10 carbon atoms, all proteins have approximately the same formula $C_{10}H_{16}N_{2.6}O_{3.2}S_{0.1-0.03}$ (12) [2], and this alone must justify an investigation on whether the protein should not have a structure that is more regular than indicated by the polypeptide theory.

The present investigation, in which is employed a reductive cleavage of the protein molecule, has shown the justification of this idea.

There have been isolated and identified four piperazines, piperidine, α-methyl pyrrolidin, and analyses have been made of a long series of high molecular weight cleavage products, obtained by stepwise degradation of widely different proteins. The results show that about 70 % of the nitrogen of the proteins is present in systems having the formulae $C_{10}H_{16}N_2O_{3.4}$ and $C_{11}H_{18}N_2O_{3.4}$ and that the former is by far the more predominant one.

These sub-units offer an explanation of why all proteins have the uniform

* With regard to preparation, fractionation, analyses, identification of isolated bases and preparations, reference is made to various papers in scientific publications and handbooks and to the author's monograph: *On the Structure of the Protein Molecule* (Einar Munksgaard, Copenhagen, and Humphrey Millford, Oxford University Press, London). In the copies of the monograph sent out after May 1947 pp. 55 and 56 have been replaced by other pages with a somewhat amended text. Reference to the monograph is by the page number in paranthesis ( ).

elementary composition, a fact that cannot be realized when the protein is boiled with concentrated hydrochloric acid.

If the proteins simply were composed of long aliphatic chains the cleavage method employed should produce large amounts of aliphatic amines, amides and amino alcohols — but of such components it has been possible to demonstrate only isoamyl-amine, and that has perhaps its origin in a reaction with the amyl-alcohol employed.

It is the author's hypothesis that the peculiar properties of the individual proteins are to some degree accountable for by the varying position of the oxygen atom in macro- and micro-sub-units.

A brief description is here given of the cleavage method and fractionation employed, followed by a report of analytical results. A more detailed description is to be found in the author's monograph (see also Troensegaard [3]).

Anhydrous solvents have been used for the proteins, especially anhydrous methanol-KOH and glacial acetic acid. Proteins like gelatin, casein and globin are transferred to dry amyl-alcohol and here subjected to a hydrogenation with metallic sodium at a temperature of 130—145° (113) [3]. Ether and water in the form of ice are added to the reaction mixture, and the ether-amyl-alcoholic phase separated from the aqueous phase. The reaction products in the two phases are fractionated according to basicity and solubility.

From the ether-amyl-alcoholic phase one obtains the fractions B_1, B_2, C_1 and C_2; from the aqueous phase D_1, D_2 and D_3.

The D_2- (the proteol) and C-fractions contain 60 % of the protein nitrogen. 12 % are found in the D_3-fraction which chiefly contains intact protein. So it must be assumed that about 70 % of the entirely hydrogenated protein are found in the D_2- and C-fractions. The remainder of the nitrogen is present in the B-fractions as well as in the form of ammonia split off in the hydrogenation (A-fraction).

The *B-fractions* consist of weakly basic substances which are not bound by primary sodium phosphate. They turn very dark upon oxidation and contain, *inter alia*, pyrrole- and indigo-like compounds. For details, see the monograph (21—22).

The C_1- and C_2-fractions are strong bases which can be extracted by shaking with primary sodium phosphate. The C_1-bases, nearly all of them oxygen-free, have their origin in the C_2-bases since they can be prepared from the latter by hydrogenation. They can be extracted from the aqueous solution with ether after saturation with K_2CO_3. The C_2-bases are obtained by extraction of the same solution by shaking with alcohol. After neutralization of the C_2-fraction with hydrochloric acid it can be fractionated into 2 groups which are soluble and insoluble in acetone.

The *D_1-fraction* is only a small fraction which is obtained by extraction with ether. It gives the pyrrole reactions, but is decomposed in the course of a day or so.

The *D_2-fraction* is obtained from the aqueous phase by salting out in alcohol with K_2CO_3. It is the largest fraction and must represent the »nucleus» of the protein. It has been given the name »proteol».

The *D_3-fraction* contains the methanol-soluble part of the aqueous phase and consists, as mentioned, presumably of protein which is but slightly affected by the hydrogenation.

The *ammonia* formed must, *e. g.*, originate from the guanidine group of the arginine and from the free amino group of the lysine.

Many proteins which are soluble in methanol-KOH cannot be transferred to amyl-alcohol before being acetylated. In the acetylation 2—5 % of the peripheral layer of the molecule is split off. The split-off, ether-soluble acetyl compounds are especially rich in nitrogen [4].

In Chapters 6 and 7 of the author's monograph it is shown that this acetylation has no influence on the nature of the hydrogenation products formed, inasmuch as there have been identified the same bases from *acetylated* as from *non-acetylated* protein. Several reviewers of the author's monograph have apparently *failed to read these chapters and therefore given a wrong picture of the investigation.*

It must be assumed that the hydrogenation involves not only *an elimination* of the oxygen but also *a rupture* of carbon chains by the heating in the strong sodium amylate. It must be assumed that the proteol and the part of the C_2-fraction which forms acetone-soluble hydrochlorides represent such rupture products. They have the same oxygen content as the original proteins and it must be supposed that they have not been hydrogenated.

After this *introduction* and *review* the investigation will now be reported in more detail.

The *proteol* (D_2) contains about 30 % of the nitrogen of the protein. The hydrochlorides of this fraction from serum globulin, casein and gliadin have very nearly the same elementary composition $C_{10}H_{16}N_2O_{3.2}(HCl)_{0.5}$ calculated on the basis of 2 nitrogen atoms (54).

It is very interesting that the acid combining capacity is 1 mole HCl per 4 nitrogen atoms. This indicates that the proteol must consist of systems having the elementary composition $C_{20}H_{32}N_4O_6$ or perhaps $C_{20}H_{32}N_4O_7$ which rather must be regarded as »macro-sub-units».

The empirical composition $C_{10}H_{16}N_{2.6}O_{3.2}S_{0.1—0.03}$ of the proteins is very close to that of the proteol (54). The nitrogen which is in excess in the protein

Table 1. The elementary composition of the D_2-fraction from the different proteins investigated.

Analysis no.	D_2-fraction	Empirical composition
1	Pt-salt of D_2-gliadin, methylated with methyl iodide	$C_{10.3}H_{16}N_2(CH_3)_{0.85}(OCH_3)_{0.65}O_{2.6}[PtCl_6]_{0.5}$
2	Pt-salt of D_2-gliadin, methylated with dimethyl sulphate	$C_{11}H_{17}N_2(CH_3)_{1.8}(OCH_3)_{0.8}O_{3.3}[PtCl_6]_{0.5}$
3	From gliadin	$C_{10}H_{16}N_2O_{3.4}$
3a	Hydrochloride from acetylated gliadin	$C_{9.6}H_{16}N_2O_{2.9}(HCl)_{0.5}$
4	From acetylated casein, methylated with methyl iodide	$C_{10.4}H_{16}N_2(CH_3)_1O_{3.8}$
5	Pt-salt from acetylated casein, methylated with methyl iodide	$C_{10}H_{18}N_2(CH_3)_{1.3}(OCH_3)_{0.4}O_2[PtCl_6]_{0.5}$
6	Hydrochloride from non-acetylated casein	$C_{9.8}H_{15}N_2O_{3.2}(HCl)_{0.52}$
7	Hydrochloride from acetylated serum globulin	$C_{10.3}H_{16}N_2O_{3.5}(HCl)_{0.5}$
8	Hydrochloride from globin	$C_{8.4}H_{14}N_2O_{2.4}(HCl)_{0.5}$
9	Pt-salt from methylated, non-acetylated gelatin	$C_{10.6}H_{20}N_2(CH_3)_{2.2}(OCH_3)_{0.6}O_{2.2}[PtCl_6]_{0.82}$

(Analyses nos. 1, 2 and 3 (gliadin): N. Troensegaard and E. Fischer, *Z. physiol. Chem.* **142** (1925) 54, 57.)

must be the nitrogen which is split off as ammonia in the hydrogenation, or which is found in the systems rich in nitrogen that are split off during the acetylation.

The proteol has, however, other chemical properties than those of the protein itself. As distinct from polypeptides it gives no biuret reaction, and it can be salted out of the aqueous solution in alcohol with K_2CO_3.

If the proteol is methylated by means of methyl iodide, both methoxyl and methylimide are formed. Thus it is possible to introduce from 30 up to 50 methoxyl groups and up to 50 methylimide groups for every 100 nitrogen

atoms. The methoxyl is easily split off by alkalies. The formation of methoxyl signifies that the more central part of the protein molecule contains very labile atom systems which do not occur in the aliphatic polypeptides. The proteins react only to a slight degree with methyl iodide. The hydrochloride of proteol is *insoluble* in acetone.

That the proteol must be the central part of the molecule is proved by the fact that this fraction from gliadin on acid hydrolysis yields 32 % proline [6]. The proteol of serum globulin gave $3^1/_4$ % amino acids which were soluble in absolute alcohol. Gliadin yields on acid hydrolysis 13 % proline, and serum globulin yields 2.5 % proline. These findings are in agreement with the assumption, based on enzymatic investigation, that the proline belongs to the central part of the protein molecule.

The proteol from the globular proteins has a more »compact» structure than the peripheral layers. It is difficult to split by renewed hydrogenation; a great deal of resinifying occurs, and very small amounts of ether-soluble bases are formed. This does not apply to the fibroid protein gelatin (66).

THE C_2-FRACTIONS

The C_2-fraction whose hydrochlorides are soluble in acetone

The hydrochloride of gliadin in this fraction has the composition $C_{11}H_{18}N_2O_{3.4}(HCl)_{0.9}$ (calculated on the basis of 2 nitrogen atoms). The fraction presumably consists of fragments of the median layer that encloses the proteol. That it is a question of fragments is evident from the fact that a methylation product gave a platinum salt having the elementary composition $C_{12}H_{22}N_2(CH_3)_3(2HCl,PtCl_4)$, but that only one-half of the nitrogen of the preparation was precipitated as platinum salt. On methylation this fraction gave no methoxyl, but the methylimide showed the presence of both secondary and tertiary nitrogen. On renewed hydrogenation it was in all essentials changed into the oxygen-poorer C_2-bases, the hydrochlorides of which are insoluble in acetone, and into the C_1-bases. Hence the fraction must be regarded as intermediary hydrogenation products.

The C_2-fraction whose hydrochlorides are insoluble in acetone (4 2)

This fraction can after methylation be divided into 4 subfractions. No methoxyl is formed. The methylation products from the different proteins can be divided into 2 fractions, one soluble and the other insoluble in acetone.

Table 2. Composition of the platinum salts of methylated C_2-bases.

From	Fraction	Analysis no.	Composition
Non acetylated gelatin	a	4	$C_{9.7}H_{17}O_{2.7}N_2(CH_3)_{2.7}[PtCl_6]_{0.87}$
	b	5	$C_{9.2}H_{19}O_{3.5}N_2(CH_3)_{2.1}[PtCl_6]_{0.72}$
	c	6	$C_8H_{17}O_{2.7}N_2(CH_3)_{2.2}[PtCl_6]_{0.76}$
	d	7	$C_9H_{18}O_{1.4}N_2(CH_3)_{1.9}[PtCl_6]_{0.81}$
Non acetylated casein	a	1	$C_{9.1}H_{16}O_2N_2(CH_3)_{2.4}[PtCl_6]_{0.63}$
	b	2	$C_{8.9}H_{17}O_{2.8}N_2(CH_3)_2[PtCl_6]_{0.59}$
	c	3	$C_{9.5}H_{18}O_{2.2}N_2(CH_3)_{2.2}[PtCl_6]_{0.67}$
	d		gave no Cu salt
Acetylated casein, not methylated	1	18	$C_9H_{16.5}O_{2.8}N_2$
	2	19	$C_{10.1}H_{19}O_{2.3}N_2$
Non-acetylated globin	a	8	$C_{8.3}H_{15}N_2(CH_3)_{2.1}[PtCl_6]_{0.85}$
	b	9	$C_{7.8}H_{12}O_{2.7}N_2(CH_3)_{1.8}[PtCl_6]_{0.45}$
	c	10	$C_{8.2}H_{13}O_{1.2}N_2(CH_3)_{0.6}[PtCl_6]_{0.52}$
	d	11	$C_{8.2}H_{12}O_{3.2}N_2(CH_3)_{1.1}[PtCl_6]_{0.24}$
Acetylated globin	a	16	$C_{11.6}H_{21}O_{2.5}N_2(CH_3)_{2.1}[PtCl_6]_{0.65}$
	c	17	$C_{10}H_{18}O_{1.8}N_2(CH_3)_{2.1}[PtCl_6]_{0.59}$
Acetylated gliadin	a		$C_{11.2}H_{22}O_{2.2}N_2(CH_3)_{2.4}[PtCl_6]_{0.8}$
	b		$C_{10}H_{18}O_{2.2}N_2(CH_3)_2[PtCl_6]_{0.8}$
	c		$C_{11.4}H_{23}O_2N_2(CH_3)_2[PtCl_6]_{0.8}$
	d		$C_{10}H_{19}O_{1.8}N_2(CH_3)_2[PtCl_6]_{0.8}$
Oxygen free bases from the hydrolysed C_2-bases of gliadin			$C_{8.6}H_{17.1}N_2(H_2PtCl_6)_{0.8}$
			$C_{10.6}H_{18.6}N_2(H_2PtCl_6)_{0.8}$
			$C_{9.2}H_{16}N_2(H_2PtCl_6)_{0.8}$
Acetylated serum globulin	a	12	$C_{10.6}H_{21}O_{2.3}N_2(CH_3)_2[PtCl_6]_{0.82}$
	b	13	$C_{9.7}H_{19}O_{1.6}N_2(CH_3)_{2.3}[PtCl_6]_{0.71}$
	c	14	$C_{9.5}H_{17}O_{1.6}N_2(CH_3)_{2.1}[PtCl_6]_{0.66}$
	d	15	$C_{10.2}H_{19}O_{2.1}N_2(CH_3)_2[PtCl_6]_{0.67}$
Non-distillable C_1-bases from gelatin (methylated)	Analyses nos. 11 and 12 in the chapter on gelatin		$C_{9.8}H_{20}O_{2.8}N_2(CH_3)_{2.1}[PtCl_6]_{0.7}$
			$C_{10.8}H_{20}O_{1.6}N_2(CH_3)_2[PtCl_6]_{0.7}$
Non-distillable C_1-bases from non-acetylated casein	Analysis in the chapter on casein		$C_{11.4}H_{16.7}O_2N_2(CH_3)_2[PtCl_6]_{0.74}$

(Analyses of gliadin: N. Troensegaard and E. Fischer, *Z. physiol. Chem.* **142** (1925) 45, 47.)

These 2 fractions can in turn be divided into 2 sub-fractions by precipitation with alcoholic copper acetate which only precipitates one-half of the preparation (43). The fractions have been investigated as platinum salts and the elementary composition of 30 such platinum salts is recorded in Table 2 (44) where the analyses have been calculated on the basis of 2 nitrogen atoms.

The analyses show that the bases from the non-acetylated proteins have a little higher nitrogen content than those from the acetylated proteins. As mentioned above, this is because the systems particularly rich in nitrogen partly have been removed by the acetylation — as especially evident from the analyses of the C_2-bases of globin.

A large part of the analyses show a platinum content of $(PtCl_6)_{0.8}$ per 2 atoms of nitrogen. In some of the fractions too little platinum chloride has been used, but since it is a question of high molecular weight substances the bases are nevertheless precipitated.

This circumstance points also to a considerable similarity of the structure of these cleavage products in the case of the different proteins, like gelatin, gliadin and serum globulin, included in the present investigation. In the case of gliadin exactly the same is found to apply to the *oxygen-free* base fractions of high molecular weight which are obtained by the acid hydrolysis. *One-fifth of the nitrogen is not basic.*

Since the methylated C_1-bases contain rather substantial amounts of tertiarily bound nitrogen and these bases have their origin in the C_2-fraction, it follows that tertiary nitrogen must be predominant in these cleavage products.

The C_2-bases from the different proteins contain from 15 to 20 % of amino nitrogen (van Slyke), and since the methylimide content of the exhaustively methylated bases is rather uniformly $(CH_3)_{1.1}$ for each nitrogen atom, this circumstance is in reasonably good agreement with the fact that the tertiary nitrogen is predominant, when taking into account that one-fifth of the nitrogen is non-basic and that the amino nitrogen binds more than one methyl group.

The molecules are larger than indicated by the formulae, the molecular weight being from 800 upward.

By *cautious acid hydrolysis* of these bases from gliadin, Wrede has isolated methyl piperazine, isopropyl piperazine, and pyrrolidyl carbinol (43, 30). These products show the ketopiperazine structure.

The analyses, which are recorded in the tabulation of the acetylated proteins gliadin, globin, serum globulin and casein, can with close approximation be interpreted as representing hydrogenated cleavage products from systems having an elementary composition $C_{10}H_{16}N_2O_{3.2}$ and $C_{11}H_{18}N_2O_{3.2}$, converting the hydrogen content according to the oxygen content of the C_2-bases whose hydrochlorides are soluble in acetone and from which these bases originate (42). *Most of the bases belong to the systems* $C_{10}H_{16}N_2O_{3.2}$. The amount of oxygen displaced in the reduction varies from 0 to 3 atoms per 2 atoms of nitrogen.

The *C_1-fraction* contains the bases which, as mentioned, are produced by complete hydrogenation of the C_2-bases. The marked uniformity of elementary composition which distinguishes the other fractions is not nearly so prominent here. The bases are soluble in ether.

Of *hydrogenation products* from this fraction there are, in the case of gliadin, gelatin and casein, isolated piperidine, methyl piperazine, trans-2,5-dimethyl piperazine, isopropyl piperazine, α-methyl pyrrolidine and isoamyl amine. These products too show the ketopiperazine structure (23, 33, 58, 74).

The most volatile bases from the 3 proteins just mentioned consist only of these compounds. If the protein molecule were composed of aliphatic peptide chains, one should here expect the occurrence of other aliphatic amines than isoamyl amine, but such are not found.

From the hydrogenation products of casein and gliadin several bases are prepared: $C_{11}H_{22}N_2$, $C_9H_{20}N_2$ and $C_{14}H_{22}N_2$. The first one is identified as isobutyl pyrrolidino piperazine, while the others have not yet been identified.

In the case of gliadin 10 different fractions have been analysed (33, 77, 82) with boiling points 100°/16 mm Hg—170°/0.1 mm Hg, but of approximately the same composition as the identified base $C_{11}H_{22}N_2$, isobutyl pyrrolidino piperazine. The bases contain secondarily and tertiarily but no primarily bound nitrogen. They have been analysed as gold and platinum salts. They all smell like piperazines. They must be assumed to have their origin in tricyclic sub-units of composition $C_{11}H_{16}N_2O_3$ or $C_{11}H_{14}N_2O_4$ with a central diketopiperazine ring (27). The piperazines demonstrated must be fragments of three-dimensional lattice structures. The three-dimensional structure is plausible because the piperazine of which the best yield is obtained is trans-2,5-dimethyl piperazine.

Of the C_1-fraction from casein 5 bases of high boiling point are investigated (35—39). Their composition shows that they can be regarded as *homologues of the base* $C_{10}H_{22}N_2$, thus $C_{10}H_{22}N_2(CH_2)_x$, where x is a small number. If the hydrogen is converted corresponding to the oxygen content of the preparation before the hydrogenation, one obtains the composition $C_{10}H_{16}N_2O_3(CH_2)_x$. It will be seen that it is the same sub-units which constantly reappear, but here they are attached to shorter carbon chains.

It is only to some degree possible to compare the C_1-bases from the serum proteins with those mentioned above — *though the proteol and C_2-fraction of serum globulin are in close agreement,* both as regards composition and chemical properties, with those of the other proteins.

Piperidine and isoamyl amine are found both in the case of serum globulin and in that of serum albumin, but piperazines cannot be demonstrated.

As in the case of the C_1-fraction from casein there are in that from serum globulin found a series of homologues of the type $C_{10}H_{16}N_2O_3(CH_2)_x$ or $C_{11}H_{18}N_2O_3(CH_2)_x$, where x is a small number. These homologues do not smell like piperazines but have a closer resemblance to the piperidines (98—101). Several of them are precipitated by $H_4Fe(Cy)_6$ which indicates that the nitrogen is tertiarily bound.

In the case of both serum proteins the C_1-fraction consists of many different bases of which some are very unstable. At the distillation of one of the fractions from serum albumin an oil of the composition $C_9H_{14}O$ was split off. It is presumably a *diterpene* (102—104).

In the case of both serum proteins the fractions with the highest boiling point (150—200° / 0.1 mm Hg) have yielded bases having the composition $C_{27}H_{45}N_3O_2$, $C_{24}H_{42}N_3O$ and $C_{27}H_{45}N_3$. Even though the distillation temperature was high, but little discoloration occurred. (Chapters 10 and 11.)

The content of carbon and hydrogen in two of these bases is in good agreement with that of the cholesterol, the third has 3 carbon atoms less. Although it is probable that the bases have their origin in systems containing more oxygen, it must be assumed that they represent a transitional stage of the synthesis of the sterols and that the completion of this synthesis involves a deamination in the liver. Similar bases could not be demonstrated in the hydrogenation products from the other proteins investigated.

So little is known regarding the function of the serum proteins in the organism — this is especially true of the serum albumin [7].

So far it has been assumed that the sterols are synthezised in the organism from nitrogen-free compounds such as acetaldehyde, methyl-glyoxal and glycerose [8]. Bloch, Borek and Rittenberg [9] have demonstrated the final synthesis of cholesterol in the liver. Liver slices from rats were suspended in Krebs' phosphate buffer, *acetic acid* labeled with C^{13} or D_2O was added, and the isotopes were demonstrated in the cholesterol isolated.

Thus the investigation has shown that the globular proteins gliadin, casein and serum globulin can be regarded as built up of a central nucleus (the proteol) of rather compact structure *surrounded* by systems of a uniform elementary composition. From these systems from gliadin and casein there are formed the C_2-bases which must have diketopiperazine structure. No piperazines are produced by the hydrogenation of the C_2-bases from the serum proteins.

Outside of these systems are layers particularly rich in nitrogen, which contain the hexone bases.

There must be some reason for the fact that the C_1-fraction from the serum proteins deviates so much from the C_1-fraction of the other proteins.

SUMMARY

This paper gives the results of an analytical investigation of the fractionation products from hydrogenated proteins and presents a new view on the structure of the protein molecule. The proteins are dissolved in anhydrous solvents and subjected to a hydrogenation with metallic sodium in dry amyl alcohol. By the various cleavages that follow and by fractionation it is shown that in this way one obtains a series of intermediary degradation products, which cannot be obtained in acid hydrolysis.

A large analytical material shows that 70 % of the nitrogen content can be referred to various systems having the elementary composition $C_{10}H_{16}N_2O_{3.2}$ and $C_{11}H_{18}N_2O_{3.2}$, chiefly the former. (The D_2- and C-fractions). This is very nearly the case of all proteins. The analyses are calculated for 2 nitrogen atoms in the molecule.

The remainder of the nitrogen is present in the peripheral systems especially rich in nitrogen, and in the B-fractions.

These degradation products explain, why all proteins, calculated on the basis of 10 carbon atoms, have an elementary composition very close to $C_{10}H_{16}N_{2.6}O_{3.2}S_{0.1-0.03}$ (12). As there is 0.6 nitrogen atom per 10 carbon atoms more in the protein molecule than in the above mentioned two systems, the other part of the protein molecule must be especially rich in nitrogen. Further it involves that the D_2- and C-fractions constitute about 85 % of the weight of the protein.

The actual building stones of the protein molecule must be systems of uniform composition, »sub-units» with the indicated formula. It has been possible (Wrede) to identify one of these sub-units in hydrogenated form; it proved to be isobutyl pyrrolidino piperazine, $C_{11}H_{22}N_2$.

It is found that the globular proteins investigated consist of a »compact» nucleus, »proteol», of uniform elementary composition and uniform acid combining capacity for the proteins investigated — $C_{10}H_{16}N_2O_{3.2}(HCl)_{0.5}$. The proteol is surrounded by systems of uniform elementary composition $C_{10}H_{16}N_2O_{3.2}$ and $C_{11}H_{18}N_2O_{3.2}$. In the case of gliadin and casein these systems contain diketopiperazine compounds. In addition there is a third peripheral system rich in nitrogen, which contains the hexone bases.

The most-hydrogenated decomposition products originate mainly from the C_2-fraction. There have been identified: piperidine, methyl piperazine, *trans*-2,5-dimethyl piperazine, isopropyl piperazine, isobutyl pyrrolidino piperazine, isoamyl amine and α-methyl pyrrolidine. Other aliphatic amines cannot be demonstrated. It is possible that the piperidine has its origin in the most peripheral layer.

From the serum proteins there are isolated three bases with the composition $C_{27}H_{45}N_3O_2$, $C_{24}H_{42}N_3O$ and $C_{27}H_{45}N_3$ as well as a »diterpene» $C_9H_{14}O$. It must be assumed that these compounds represent intermediary products in the synthesis of cholesterol and that the final synthesis takes place in the liver as shown by Bloch, Borek and Rittenberg.

APPENDIX

The author takes this occasion to mention an incorrect theory which frequently is called »Troensegaard's Pyrrole Theory». It has its origin in the author's first paper in *Z. physiol. Chem.* **112** (1920) 89 which gives a model formula composed of 3 pyrrole nuclei. This has been misinterpreted by someone presumably having no further knowledge of the author's work and its purpose. The fact is that on page 90 of the paper mentioned it is definitely stated that it is *not* the author's opinion that the proteins are composed of such systems — but this has escaped the attention of several other authors, thus for example, the co-workers of Carl L. A. Schmidt's book: *The Chemistry of the Amino Acids and Proteins* (1945).

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As it will be seen the above paper is a review of the experimental material from earlier papers by the same author, and especially from the book mentioned in the note p. 672. The reason why it has been decided nevertheless to publish it in this journal is that the material from the book may not be easily accessible to the reader. The author informs us that as long as copies of the 2nd edition of his book are still in stock, he will be glad to send on request a copy free of charge to any reader, who may be interested in the subject.

The Editors.