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The Human AB0 Blood Group Substances[1]

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Nearly half a century has passed since LANDSTEINER and his pupils announced that human bloods can be divided into four serological types and thus clarified earlier observations in which iso-agglutinins, the naturally occurring antibodies which agglutinate erythrocytes within the same animal species, were attributed to pathological changes of the blood. LANDSTEINER cross-tested the sera and erythrocytes of individuals in his laboratory and found that in some instances intense agglutination of the corpuscles resulted whereas in others no agglutination occurred. He considered this phenomenon of iso-agglutination to be caused by the reaction of the agglutinable substances, A and B, contained in the erythrocytes, with their corresponding naturally occurring specific iso-agglutinins in the serum. According as to whether the erythrocytes contain A, B, A and B, or neither factor, bloods can be classified into four groups and on the basis of these observations LANDSTEINER established the classical AB0 blood group system and formulated the following important rule:— "A person's serum cannot contain antibody for antigens present in his own erythrocytes." In one respect the human specific A and B agglutinable factors differ from all other blood group substances in being the only ones for which the corresponding iso-agglutinins are found naturally and in considerable strength in the serum. Indeed, it is to avoid during blood transfusion an agglutinable substance in the erythrocyte surface being brought accidently into contact with its specific iso-agglutinin in the circulation of a recipient that an understanding of the AB0 system is indispensable. On the basis of LANDSTEINER'S results there follows a definite relationship between the different kinds of human blood; these are set out in Table I.

Although a technique of blood transfusion has been evolved on the basis of LANDSTEINER'S serological studies and is in daily use in modern medical practice[3], it is only within the last few years that a more comprehensive understanding of the reactions involved in terms of chemistry and physics has been attained.

[1] Based on lectures given in Basle and Zürich in November 1946.

[2] From the Lister Institute of Preventive Medicine, London, S. W. 1.

[3] A. S. WIENER, "Blood Groups and Transfusion". Thomas, Illinois, U.S.A.

The isolation and characterization of the specific blood group agglutinable substances has been the subject of numerous investigations extending over many years and the results have, at one time or another, implicated proteins, lipoids, and carbohydrates as the agents responsible for the group specificity. It is now known, however, that the specific substances exist in the body in at least two forms. In the erythrocytes they are bound in some way to lipoidal or lipoprotein material and are not significantly soluble in water or saline but can be isolated by extraction of the corpuscles or stromata with dilute alcohol, whereas in the tissue fluids and secretions of the majority of persons the group substances are present in a water-soluble form[1]. Those individuals who secrete their group substances in a water-soluble form in the tissue fluids and secretions are termed "secretors" whereas those who do not secrete the active factors or do so very weakly are called "non-secretors"[2]. It has been established that the presence or absence of water soluble specific blood group substances in the secretions and tissue fluids of an individual is controlled by a single gene *S*, dominant in effect, which allows the secretion of the specific substance. There is no evidence of linkage between the character "secretor" and the A, B, or 0 groups.

Blood Group Substances from Erythrocytes

The earliest observations dealing with the isolation of the specific blood group substances from human erythrocytes were those of SCHIFF and ADELSBERGER[3] and of LANDSTEINER and VAN DER SCHEER[4] who obtained substances which showed group specific properties by extraction of the red-cells with dilute alcohol. HALLAUER[5], using a similar technique, described the isolation of specific substances from erythrocytes

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[2] F. SCHIFF and H. SASAKI, Z. Immunitäts. *77*, 129 (1932); Klin. Woch. *11*, 1426 (1932). – H. SASAKI, Z. Immunitäts. *77*, 101 (1932). – V. FRIEDENREICH and G. HARTMANN, Z. Immunitäts. *92*, 141 (1938). – G. HARTMANN, "Group Antigens in Human Organs". Copenhagen 1941.

[3] F. SCHIFF and L. ADELSBERGER, Z. Immunitäts. *40*, 335 (1924).

[4] K. LANDSTEINER and J. VAN DER SCHEER, J. exp. Med. *42*, 123 (1925).

[5] C. HALLAUER, Z. Immunitäts. *83*, 114 (1934).

of all three (A, B, and 0) groups. The composition of the specific material isolated by HALLAUER was found to be very similar for each group substance examined and fell within the range 43·1–43·3 C, 7·1–8·5 H, 6·8–7·9% N. The substances were rich in sulphur and phosphorus. More recently STEPANOV, KUSIN, MAKAJEVA, and KOSJAKOV[1] have largely confirmed these observations and have again isolated small quantities of the group substances from A and B erythrocytes by means of alcoholic extraction. The A-substance is described as containing 5·9% N and giving rise to 53% reducing substances and about 16% glucosamine after acid hydrolysis. The A-substance shows a *lævo* rotation, $[\alpha] -25^0$ to -30^0 and contains about 3% arginine. The materials were soluble in water and give negative tests for glycogen, pentose, uronic acid, fructose, phosphorus and sulphur. Although these substances give strong *in vitro* reactions with the homologous iso-agglutinin, they are recorded as being devoid of antigenic properties.

Blood Group Substances from Animal Tissue Fluids and Secretions

The occurrence of the blood-group substances in a water-soluble form in the tissue fluids and secretions of the body has been known for many years and an examination of the extensive literature on the subject shows that an insight into the chemical nature of the specific A, B, and 0 group substances has been attained almost entirely through studies on these water-soluble components. SCHIFF[2] found that commercial peptone of animal origin contains a substance which possesses human blood group A specificity and subsequently GOEBEL[3] isolated from this source a polysaccharide which shows intense A-activity. The most active material gave the following analytical figures:— C, 46·7, H, 6·5, N, 5·85, CH_3CO, 9·6% and yielded 73% of its weight reducing sugars after acid hydrolysis. The substance gave a weakly positive biuret reaction, a strong test for glucosamine and a negative reaction for uronic acid. The material was weakly *dextro* rotatory, $[\alpha] +11{\cdot}5^0$. Similar investigations on the A-substance in peptone were carried out independently by FREUDENBERG and WESTPHAL[4] who isolated a polysaccharide material almost identical in composition to that obtained by GOEBEL[5]. The specific rotation of the material was, however, slightly lævo rotatory. Glucosamine hydrochloride was isolated from the products of acid hydrolysis. In a later communication FREUDENBERG, WALSH, GRIESHABER, and SCHIFFER[1] take the view that the amino acids present in the A-specific material are impurities and that in any event most of the amino acids quantitatively determined by the ninhydrin procedure of VAN SLYKE and DILLON[2] were due to the CO_2 evolved from the glucosamine present. It is known, however, that under the conditions recommended by VAN SLYKE no CO_2 is evolved from glucosamine. MEYER, SMYTH, and PALMER[3], as a result of a study which involved the mucoids of pig gastric mucosa, isolated a neutral polysaccharide which possessed intense blood group A activity. The material contained equimolecular quantities of N-acetylglucosamine and galactose. The substance was considered to have a purity of about 75% on the basis of the glucosamine values and most preparations were reported to give a positive EHRLICH's diazo reaction, thus indicating the presence of histidine. All workers up to this time had detected amino acids in their most active specimens of A-substance but were uncertain whether to consider them as a true part of the specific complex or merely as contaminating substances and it was not until further investigations by LANDSTEINER and HARTE[4] established that the purified A-substance possessed an amino-acid containing complex equivalent to about 35% of the total N of the material that amino acids were recognized as true components, firmly attached to the main polysaccharide moiety of the A-substance. The purest specimens, prepared by heating commercial mucin with formamide at 150° C according to the technique of FULLER[5] for the isolation of bacterial polysaccharides, or by papain-HCl digestion of the mucin, gave analytical figures within the range C, 46·4–47·2, H, 6·6–6·9, N, 5·5–6·0, CH_3CO, 10·5–12·0% and are thus almost identical in composition with those obtained by earlier workers. LANDSTEINER and HARTE[4] observed that during the isolation and purification of the A-substance there was frequently a considerable fall in the biological activity as measured by the inhibition of iso-agglutination. It may be said here that there are two methods in general use for the determination of biological activity of the A-substance. The first is to measure the power of the substance to inhibit the iso-agglutination of A-erythrocytes by human or animal anti-A sera, the second is to determine the amount of A-substance which will prevent the hæmolysis of sheep erythrocytes by an anti-A rabbit immune serum. The hæmolysis test is generally believed to measure the "Forssman" or heterophile component of the *A*-agglutinogen, and is accepted as

[1] A. V. STEPANOV, A. KUSIN, Z. MAKAJEVA, and P. KOSJAKOV Biochimia *5*, 547 (1940).

[2] F. SCHIFF, Zbl. Bakt., 1. Abt. Ref. *98*, 94 (1930).

[3] W. F. GOEBEL, J. exp. Med. *68*, 221 (1938).

[4] K. FREUDENBERG and O. WESTPHAL, Sitzsber. Heidelberg. Akad. Wiss. Math. Naturwiss. Kl. 1940, 9. Abh.

[5] W. F. GOEBEL, J. exp. Med. *68*, 221 (1938).

[1] K. FREUDENBERG, H. WALSH, H. GRIESHABER, and A. SCHIFFER, Sitzsber. Heidelberg. Akad. Wiss. Math. Naturwiss. Kl. 1940, 3. Abh.

[2] D. D. VAN SLYKE and R. T. DILLON, C. r. Lab. Carlsberg *22*, 480 (1938). – D. D. VAN SLYKE, R. T. DILLON, D. A. MACFADYEN and P. HAMILTON, J. biol. Chem. *141*, 627 (1941).

[3] K. MEYER, E. SMYTH and J. PALMER, J. biol. Chem. *119*, 73 (1937).

[4] K. LANDSTEINER and R. A. HARTE, J. exp. Med. *71*, 551 (1940).

[5] A. T. FULLER, Brit. J. exp. Path. *19*, 130 (1938).

measuring a different, although closely related, serological property of the A-substance from that determined by the iso-agglutination inhibition technique.

It was at about this time that I became engaged on the isolation and characterization of the A B 0 blood group substances and in view of the known lability of cellular and tissue antigens as exemplified by certain bacterial antigens[1] it was recognized that considerable care would have to be taken if the blood group complexes were to be obtained in their "native" condition, that is, with their chemical, physical and immunological properties unchanged. Valuable experience had already been gained during a study of a closely related problem, the isolation of certain labile bacterial antigens[2] and the application of this knowledge enabled suitable methods to be employed which avoided extreme p_H values or high temperatures. The use of these restricted conditions for the manipulation of the large labile molecular species which carry the blood group specific characters, allowed certain irreversible changes to be avoided or reduced to a minimum and in consequence it was possible to obtain from pepsin, peptone and pig gastric mucin[3] of commercial origin a purified undegraded A-substance, and as we shall see later, the A, B, and 0 specific substances from human tissue fluids and secretions. The fractionation of commercial gastric mucin from aqueous solution by sodium sulphate, the extraction of the A-substance into 90% liquid phenol and its subsequent separation by means of fractional precipitation from cold organic solvents such as formamide, ethylene glycol, glacial acetic acid-ammonium acetate mixture, etc. resulted in an excellent yield of a serologically active polysaccharide-amino-acid complex being obtained. The material was found to be similar in composition to the earlier preparations of LANDSTEINER and of GOEBEL but a number of important differences in the physical and serological properties of our preparations were observed. The material isolated possessed in full the property shown by crude gastric mucin of inhibiting the iso-agglutination of A-erythrocytes by natural anti-A (α) agglutinins, retained completely the high viscosity of the original mucin, was apparently homogeneous when examined at p_H 4·0 and 8·0 in the Tiselius electrophoresis apparatus, and retained the original property of the crude mucin of forming an elastic gel on the addition of borate buffer at p_H 8·5. The addition of this reagent will reveal at once, according to the formation or not of an elastic gel, whether the material has been degraded during the course of its preparation.

The undegraded substance shows a relatively high viscosity, η, 2·8 at a concentration of 0·5% in saline. Analytical figures for different preparations of the most active and electrophoretically homogeneous material vary within the limits C, 44·8–45·8, H, 6·5–6·9, N, 5·9–6·1 (DUMAS). CH_3CO, 9·0–10·0%. Hydrolysis of good specimens of the substance with HCl gives rise to about 53% reducing sugars, 32–34% hexosamine, determined by a modification of the method of ELSON and MORGAN[1], 4·6–4·8% amino N (VAN SLYKE) and 2·4–2·6% amino acid N. It was observed, however, that besides the blood group A activity, the preparations always showed human blood group 0 character and that this activity increased in parallel with the increase in A activity during the isolation and purification of the A-substance. It was found, however, that some preparations of A-substance isolated from pseudomucinous ovarian cyst fluids (to be described later) were practically devoid of 0-specificity. Furthermore, the best specimens of human A-substance which showed no 0-character possessed two or three times the activity of the electrophoretically homogeneous pig gastric mucin "A-substance". It is to be emphasized however that, in common with most workers, we prepared our "A-substance" from commercial specimens of pig mucin, peptone or pepsin and that it was not known to what extent the properties of the A-substance in these materials were changed by the process of manufacture or depended on the source of the starting material. Our experience with the blood group substances in saliva and gastric juice, in confirmation of the earlier observations of others[2], established differences in the group specificity of the secretions in man and it seemed not improbable that similar serological differences occurred in the mucin preparations derived from individual pig's stomachs. An examination of the serological properties of the mucoids isolated from single stomachs was therefore undertaken and in the first series of 24 stomachs examined 14 were found to possess A-character and showed relatively weak or negligible 0-specificity, whereas the remaining 10 stomachs yielded a mucoid material possessing strong 0-character and showed no trace of A-activity[3]. These results established that in pig gastric mucin there can occur in place of the A-substance a mucoid material which possesses a group character similar to, or identical with, the so-called human blood group 0 factor. The 0-substance has chemical and physical properties very similar to those of A-substance and until recently no technique for the separation of these substances, the one from the other in a mixture, has been described.

[1] A. BOIVIN and L. MESROBEANU, C. r. Soc. Biol. Paris *112*, 76 (1935). – W. T. J. MORGAN, Brit. med. Bull. *2*, 281 (1944).

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[3] W. T. J. MORGAN and H. K. KING, Biochem. J. *37*, 640 (1943).

[1] L. A. ELSON and W. T. J. MORGAN, Biochem. J. *27*, 1824 (1933).

[2] F. SCHIFF and H. SASAKI. Z. Immunitäts. *77*, 129, (1932); Klin. Woch. *11*, 1946 (1932) – H. SASAKI. Z. Immunitäts. *77*, 101 (1932). – E. WITEBSKI and N. C. KLENDSHOJ, J. exp. Med. *72*, 663 (1940); *73*, 655 (1941).

[3] D. AMINOFF, W. T. J. MORGAN, and W. M. WATKINS, Nature (London) *158*, 879 (1946).

These results show conclusively that the A and 0 group character of the so-called "A-substance" isolated from commercial mucin, pepsin or peptone is due to the different (A and 0) specificities of the mucoids of individual pig's stomach linings used to make the original "pool" of material.

An entirely independent approach to the same problem, the homogeneity of the A-substance prepared from gastric mucin, has also been described by BENDICH, KABAT, and BEZER[1]. These workers concluded that the usual chemical and physical techniques for the separation of mucoids carrying the blood group specific characters were inadequate and developed an immunochemical method for estimating the absolute purity of the preparations of blood group A-substance by determining in quantitative precipitation tests the proportion of the total glucosamine in the preparation which was precipitated by excess anti-A immune-body. Values up to about 84% for the purity of a number of specimens of A-substance from individual pig stomachs were obtained. Of ten stomachs studied only seven yielded products with A-activity. All ten purified preparations, however, showed very similar properties with respect to nitrogen (5·7–6·6%), reducing sugar (55–61%), acetyl (9·1–11·3%) and relative viscosity. The mucoid we have described as possessing 0-character alone is presumably the material described as "inactive" by BENDICH, KABAT, and BEZER. The very similar chemical and physical properties and behaviour of the specific blood group substances, and of the closely related but inactive mucoids which undoubtedly occur in native tissue fluids and secretions, has up to the present time forced one to rely almost exclusively on serological techniques for their differentiation. We have found that the success or failure of special techniques elaborated to separate the mucoids in mixtures of this kind can be readily followed by determining, by means of quantitative serological inhibition tests, the ratio of the activity of the appropriate specific characters A, B, or 0. "Inactive" mucoids, that is those not possessing A, B or 0 activity, can be detected in the presence of material showing these characters by means of this type of test.

The A and 0 substances obtained from pig gastric mucin do not give a positive colour reaction[2] with Ehrlich's reagent (*p*-dimethylaminobenzaldehyde in alcoholic HCl) but give rise to a strong purple colour if they are previously heated for a short-time at 100° C with 0·05 *N* Na_2CO_3, according to the method described by MORGAN and ELSON.[2] The maximum colour develops under these conditions in about 7 minutes, according to the conditions of heating, and is equivalent to approximately 16% of N-acetylglucosamine, or about half of the total glucosamine contained in the specific substances. The course of the colour development is shown in Fig. 1 where the colour intensity, measured in a photoelectric colorimeter and expressed in terms of the equivalent amount of N-acetylglucosamine, is plotted against time of heating in 0·05 *N* Na_2CO_3 solution. The rapid destruction of the colour producing substance on heating further with alkali is clearly indicated. At the same time the specific mucoids develop 4–5% reducing sugars, expressed as glucose and determined by the copper reduction method of SOMOGYI[1], and show a rapid

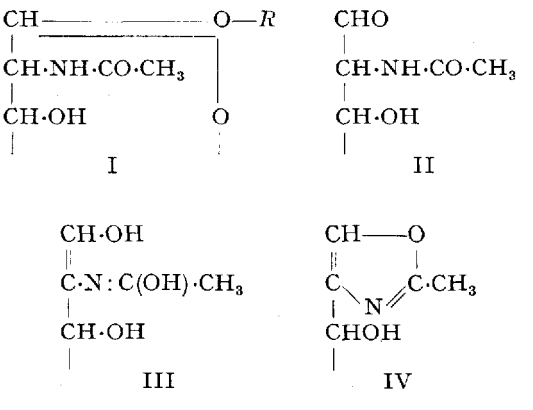
Table I

Blood group of person	Specific agglutinable substance in the erythrocytes	Specific iso-agglutinin in the serum
A	A	anti-B (β)
B	B	anti-A (α)
AB	A and B	—
0	0	anti-A (α) and anti-B (β)

Table II

Time of heating (minutes)	A-substance from pig mucin		A-substance from ovarian cyst fluid	
	Maximum dilution ($\cdot 10^3$) of A-substance giving complete inhibition	% of original activity	Maximum dilution ($\cdot 10^3$) of A-substance giving complete inhibition	% of original activity
0	1:640	100	1:1,280	100
4	1:20	3·1	1:20	1·5
7	1:5	0·7	1:5	0·3

The inhibition of iso-agglutination of human anti-A (alpha) serum by A-substance of animal and human origin after treatment with 0.05 *N* Na_2CO_3 at 100° C.



[1] E. A. KABAT, A. BENDICH, and A. E. BEZER, J. exp. Med. *83*, 477 (1946). – A. BENDICH, E. A. KABAT, and A. E. BEZER, J. exp. Med. *83*, 485 (1946).

[2] W. T. J. MORGAN and L. A. ELSON, Biochem. J. *28*, 988 (1934).

[1] M. SOMOGYI, J. biol. Chem. *117*, 771 (1937).

destruction of the serological activity as measured by the capacity of the substance to inhibit the agglutinating action of anti-A (α) on A-cells (see Table II). We believe that this mild treatment with alkali[1] causes some of the N-acetylglucosamine molecules within the specific blood group complex (I) to be changed so that a reducing aldehyde group is formed (II), which, in the presence of alkali, changes to the enolic structure (III) and eliminates water with the formation of a heterocyclic ring (IV). It is presumably a 2:4 disubstituted oxazole (or oxazoline) of this kind which con-

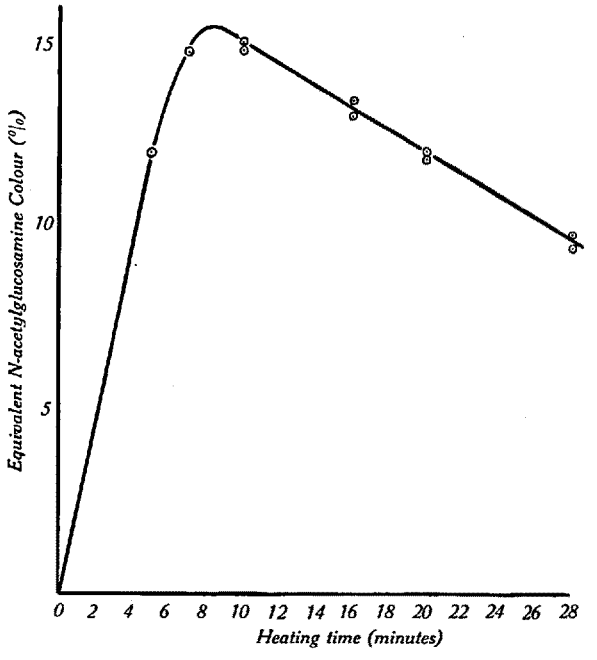


Fig. 1.

denses with the *p*-dimethylaminobenzaldehyde and gives rise to the reddish purple colouration[2]. Dialysis of the blood group A substance after it has been treated with alkali to yield the maximum colour with Ehrlich's reagent shows that about two-thirds of the total material is now able to diffuse through a cellophane membrane[3]. The material which diffuses contains almost the whole (90%) of the components that give an immediate colour with *p*-dimethylaminobenzaldehyde and which reduce SOMOGYI's copper reagent. The material retained by the cellophane membrane, on the other hand, contains the major part of the amino acid (80–90%) and the remaining part of the glucosamine (about 50%) and other carbohydrate molecules. This material is electrophoretically homogeneous at p_H 4·0 and 8·0, sediments as a single component in the ultracentrifuge (sedimentation constant, S, $1{\cdot}6 \cdot 10^{-13}$, C, 1·0% corrected to H_2O, 20° C) and possesses a probable molecular weight of about 17,000. The complex is without reducing power until after acid hydrolysis. It is not possible to estimate the total glucosamine in the diffusate since the N-acetylglucosamine which has been converted to the oxazole derivate by the action of alkali cannot be hydrolysed by acid to give again the original amount of glucosamine, for treatment of the oxazole with dilute (0·1 *N*) HCl at 18° C gives rise to considerable decomposition. The alkali labile linkages in the specific substances would appear to be glycoside linkages which are associated with C atom 1 of the acetylglucosamine units. It is to be emphasized that N-acetyl- and N-benzoylmethylglucosamides, however, are not changed under the same conditions of alkaline hydrolysis and yield neither sugars nor a colour with Ehrlich's reagent.

An examination of the A-substance after methylation and subsequent acid hydrolysis has been reported by BRAY, HENRY, and STACEY[1]. These workers recognize that under the strongly alkaline conditions employed extensive break-down of the molecular complex occurs together with considerable destruction of the specific blood group character. The methylated products dialyse readily through parchment membranes and are therefore presumably of small molecular size. Nevertheless, an essentially homogeneous, stable methylated carbohydrate residue was isolated and, after acid hydrolysis, was shown to yield 2:3:4:6 tetramethyl-*d*-mannose, 2:3:4:6 tetramethyl-*d*-galactose, 3:4:6 trimethyl-N-acetyl-α-methyl-*d*-glucosaminide and 2:3:4 trimethyl-α-methyl-*l*-fucoside. The identification of the last component indicated that *l*-fucose constitutes a terminal residue, and from the direct isolation of the trimethyl derivative of methylglucosaminide, that N-acetylglucosamine also occupies a terminal position. In view of the fact that the pepsin and gastric mucin used as a source of the A-substance in these investigations was of commercial origin, it is necessary to keep in mind that certain of the sugars identified could arise from the contaminating 0-substance[2], the monosaccharide components of which have not yet been identified.

A preliminary qualitative examination of the amino acids present in the products of acid hydrolysis of the A-substance was made by MORGAN[3] using the chromatographic method of CONSDEN, GORDON, and MARTIN[4]. The presence of 15 amino acids was in-

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[2] W. T. J. MORGAN, Biochem. J. *30*, 909 (1936); Chem. Ind. *57*, 1191 (1938). – T. WHITE, J. chem. Soc., 428 (1940). – H. WENKER, J. Am. chem. Soc. *57*, 1079 (1935). – W. H. BROMUND and R. M. HERBST, J. organ. Chem. *10*, 267 (1945).

[3] W. T. J. MORGAN, Biochem. J. *40*, XV (1946).

[1] H. G. BRAY, H. HENRY, and M. STACEY, Biochem. J *40*, 124 (1946).

[2] E. WITEBSKY and N. C. KLENDSHOJ, J. exp. Med. *72*, 663 (1940); *73*, 655 (1941). – D. AMINOFF, W. T. J. MORGAN, and W. M. WATKINS, Nature (Lond.) *158*, 879 (1946) – E. A. KABAT, A. BENDICH, and A. E. BEZER, J. exp. Med. *83*, 477 (1946).

[3] W. T. J. MORGAN, Brit. med. Bull. *2*, 165 (1944).

[4] R. CONSDEN, A. H. GORDON, and A. J. P. MARTIN, Biochem. J. (1944).

dicated. Cystine was absent. Since threonine was present in considerable concentration its amount was subsequently determined quantitatively by the method of SHINN and NICOLET[1] using the azeotropic distillation technique of MARTIN and SYNGE[2] to separate the acetaldehyde derived from threonine from a volatile aldehyde which might arise if a higher homologue of threonine such as hydroxyvaline, which would yield acetone on oxidation, is present in the specific substance. Control determinations were made on the sugar components of the A-substance so far identified i. e. *d*-galactose, *d*-mannose, N-acetylglucosamine and *l*-fucose, after they had received comparable acid treatment. The corrected figure for the threonine present in A-substance was 3·6–4·0%, an amount which indicates an abnormally high content (15–18%) of this amino acid in the amino-acid containing component. FREUDENBERG, WALSH, and MOLTER[3] claim to have isolated crystalline threonine from the acid hydrolysis products of A-substance, but no details have been published. A more recent and complete analysis of the amino acids present in pig mucin A-substance by BRAND and SAIDEL[4] gives the following values:— glycine, 1·6; valine, 0·7; isoleucine, 0·3; proline, 3·3; phenylalanine, 0·1; tryptophane, 0·2; histidine, 0·6; lysine, 1·0; aspartic acid, 0·8; glutamic acid, 1·3; serine, 1·9; and tyrosine, 0·3%. No figure for threonine is given.

Blood Group Substances from Human Sources

Most workers have encountered considerable difficulty in obtaining from human erythrocytes sufficient group specific substances for detailed immunochemical study (cf. LANDSTEINER[5], BRAY, HENRY, and STACEY[6]) and in consequence investigations have utilized more and more human tissue fluids and secretions derived from "secretors" as a source of the group substances.

As a result of the detection of the blood group factors in urine by YOSIDA[7] (1928) the isolation of the specific substances from this source has been thoroughly studied, more especially by FREUDENBERG and his co-workers. FREUDENBERG and EICHEL[8], and FREUDENBERG and MOLTER[9] from several hundred litres of the appropriate urines isolated nitrogenous polysaccharides possessing blood group specific characters. The A-substance after very thorough fractionation contained about 40% acetylglucosamine, 15% galactose, 10% acetyl, and 4·4% $C \cdot CH_3$. It was observed that A-substance which had been inactivated by gentle treatment with alkali was readily and completely reactivated by ketene. Furthermore, the hepatopancreatic juice of the snail, *Helix pomatia*, was also found to inactivate the A-substance and at the same time cause reducing substances equivalent to 25% glucose to be liberated. FREUDENBERG was unable to distinguish chemically between the specific polysaccharides obtained from the urine of persons belonging to blood groups, A, B, or 0. JORPES and NORLEN[1], and JORPES[2] reported that the action of trypsin on the blood group factor in human urine considerably decreased its anti-agglutinating activity but that the anti-lytic activity was not destroyed. These investigators believe that the anti-agglutinating and anti-lytic factors can be separated by precipitating the former factor from solution by tannin, and conclude that the anti-agglutinating factor is protein in nature whereas the anti-lytic component is probably polysaccharide. We have been unable to confirm these observations with material of high anti-lytic activity derived from pig mucin or human ovarian cyst fluid and LANDSTEINER and HARTE[3] also report that they were unable to effect a separation of the A-substance into material showing different chemical and serological properties. JORPES and THAINING[4] have again returned to the problem and have found that two fractions, which are soluble and insoluble respectively in glacial acetic and in saturated ammonium sulphate, can be obtained from their crude urine A-substance. The insoluble material neutralizes anti-A agglutinins and is destroyed by activated papain and by the action of 0·5 *N* NaOH whereas the soluble material, which neutralizes the lytic activity of an anti-A or anti-sheep cell serum, is stable under these conditions. The former material is considered to be protein in nature, the latter polysaccharide. It is now known that urine is a poor source of the specific materials and for this reason its use for the preparation of blood group substances has been largely abandoned.

Little progress can be reported on the isolation and identification of the specific A, B, and 0 substances from human gastric juice. WITEBSKY and KLENDSHOJ[5] described the isolation of 13·3 mg of material showing group B activity from 110 ml of gastric juice. The

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substance contained 1·5% N and gave rise to 75% reducing sugars. A similar substance was isolated from the gastric juice of a group 0 person[1], but owing to lack of material, detailed chemical examination was not undertaken. A recent study of the group substances from this source by BRAY, HENRY, and STACEY[2] has again emphasized the complex nature of the problem. These workers, however, described the isolation of somewhat degraded polysaccharide materials rather than the "native" specific substances. The pooled products obtained from individuals belonging to groups A, B, and 0 proved to be similar in appearance and general properties, although wide variations in analytical figures within each group are recorded. The inadequate nature of the serological data concerning the donors and the final products renders the results of limited value.

Although much immunological work has been carried out on the specific group substances in human saliva only one adequate attempt to isolate them has been recorded. LANDSTEINER and HARTE[3] recovered about 15 mg of active substance from 500 ml portions of the secretion. On a dry-weight basis the substances recovered were at least 50 times more active than the original dry saliva. The purified material obtained from "secretors" belonging to groups A, B, and 0 failed to show significantly different analytical figures. The nitrogenous polysaccharides obtained contained about 5·5% N, and gave 2·5% amino-acid N, 21–23% hexosamine, and 45–48% reducing sugars after acid hydrolysis. The materials gave negative colour reactions for tyrosine and tryptophane, but positive reactions for histidine and arginine.

PUTKONEN[4] demonstrated that the concentration of the specific substances in the saliva and gastric juice of "secretors" is higher than in other tissue fluids and secretions, but it has been shown that even in saliva and gastric juice the active substance represents only a small part of the total solid matter. Furthermore, these secretions are difficult to obtain from individual donors in useful quantities. In certain instances, however, mucilaginous fluids accumulate in man as a result of pathological changes or of tissue overgrowth, and the knowledge that pseudomucinous ovarian cysts are of frequent occurrence prompted MORGAN and VAN HEYNINGEN[5] (1944) to examine the fluid contents of these adenomata for blood group substances. An examination of nearly 100 pseudomucinous cyst fluids has revealed that, when they are obtained from women who possess the power to secrete their specific blood group substances in a water-soluble form, the fluids are a convenient and potent source of the group specific substances A, B, and 0. The volume of individual cyst fluids varies from a few hundred ml to many litres, and individual cysts from secretors belonging to group A have been found to contain several grammes of the A-substance which can be purified by methods similar to those elaborated for the isolation of the A-substance from gastric mucin of the pig or commercial peptone or pepsin. The activity of the native cyst fluid has been compared[1] with that of known specimens of salivas on a dry-weight basis and it has been shown that cyst fluids belonging to group A frequently contain more than one hundred times as much specific substance per ml as is contained in the same quantity of a highly active specimen of A-saliva.

The A-substance present in the pseudomucinous ovarian cyst fluids of secretors belonging to group A was first isolated by KING and MORGAN[2]. More recent work has yielded an electrophoretically homogeneous substance specimens of which yield analytical figures within the range 45·5–46·5% C, 6·7–6·9% H, 5·9 to 6·1% N, 8·5–9·5% CH_3CO. Acid hydrolysis gives rise to 53% reducing sugars, 33–34% hexosamine, 4·6 to 5·0 of α-amino N (VAN SLYKE), and 2·5–2·7% amino-acid N. The A-substance behaves with dilute alkali exactly as has been described for the A-substance derived from pig gastric mucin.

The active cyst fluids from secretors belonging to groups B and 0 also offer a convenient source of these factors but in our experience they contain much less of the appropriate specific substance than does a good specimen of A-cyst fluid.

The isolation and characterization of an 0-substance has been recorded by MORGAN and WADDELL[3], and it is of interest to note that the 0-specific material contains about the same amount of nitrogen, hexosamine and amino acids as the A-substance and yields after acid hydrolysis similar quantities of reducing sugars, α-amino groups (VAN SLYKE) and amino acids. Specimens of A and 0 substances, hydrolysed for 32 hrs. with 5 *N* HCl at 100° were examined at the same time and under identical conditions by the partition chromatographic method of CONSDEN, GORDON, and MARTIN[4] using phenol and collidine. The chromatograms revealed no differences with respect to number, position and intensity of the amino acid "spots". The relative intensity of the "spots" in decreasing order was as follows;— (1) threonine, (2) leucine, iso-leucine, (3) serine, valine, alanine, (4) glycine, glutamic and aspartic acids, (5) lysine, arginine, phenylalanine, (6) hydroxyproline, (7) histidine and methionine. No

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cystine was present. The specific rotation of our most active preparation of 0-substance is lævo rotatory, $[\alpha]_{54-61}$ 23± 3°, but this value cannot be accepted as final until further evidence as to the homogeneity of the preparation is forthcoming. The 0-substance on treatment with dilute alkali behaves similarly to the A-substance of animal and human origin and preparations of B-substance likewise react with Ehrlich's reagent after heating with dilute alkali for a few minutes. The extreme lability of the three specific blood group substances in the presence of alkali is a characteristic property, for it has not been encountered during the examination of several other complex polysaccharides which contain hexosamine molecules. Similar conclusions[1] have been reached by HOLTZMAN, BENNETT, BROWN, and NIEMANN as a result of their studies on the correlation between the A-specific activity and the colour produced by the action of Ehrlich's reagent on alkali treated A-substances of different origin.

The amino-acid components in the group substances may be joined by glycoside linkage to C atom 1 of some of the N-acetyl hexosamine molecules, if so it is most probably this linkage which is so extremely susceptible to the action of alkali. On the other hand the peptide linkage could occur through the free OH groups in the glucosamine component. The A-substance contains significantly more acetyl groups than can be accounted for on the basis of its content of N-acetyl-glucosamine and although 0-acetyl groups are known to be extremely labile in the presence of alkali their presence could not explain the observed development of a positive reaction with Ehrlich's reagent after treatment of the group substance with alkali, unless they were attached to C atom 1 in the hexosamine molecules.

The Specific Immunological Properties of the Group Substances

The presence of an amino-acid containing component as part of the specific blood group complexes leads one to expect that these substances would possess pronounced antigenic properties *per se*, but it has been found that the carefully purified specific materials are not strongly antigenic when tested in selected rabbits and seldom give rise to sera which show anti-A or anti-B agglutinating titres higher than five or ten times the titre of the animal's natural agglutinins before immunization.

The species of animal chosen for antigenic tests of this kind, however, is known to influence quantitatively the extent of the immune response, and as a result of experiments carried with Dr. LOUTIT during 1944 on 40 volunteers it was found that in man the injection of A-substance of both animal and human origin leads to a more regular production of anti-A agglutinin than had been observed in the rabbit. In certain instances extremely high agglutinin titres (1:50,000) were obtained in group 0 individuals whereas other group 0 persons receiving the same amount of the same blood group preparation showed no significant increase above their original natural anti-A (α) agglutinin levels. The use of partially purified A and B substances of animal origin to induce the formation of anti-A and anti-B agglutinins in man has been described by WITEBSKY, KLENDSHOJ, and MCNEIL[1] and by the injection of small doses of human saliva into individuals of the appropriate blood groups, WIENER, SOBLE, and POLIVKA[2] obtained agglutinating sera which were suitable for the typing of unknown bloods. Similar results have been obtained by CHRISTIAENS, SEVIN, and CORNILLOT[3]. Rabbits immunized with a purified group 0 substance frequently give rise to quite useful anti-0 agglutinating sera which after absorption with A_1B cells react at a dilution of 1:1000 with 0 cells but give no significant agglutination at any dilution with some A_1B cells[4].

The predominantly polysaccharide nature of the specific group substances suggested that they could be converted into full antigens by means of a simple method which had been used originally to convert certain non-antigenic specific bacterial polysaccharides[5] and plant polysaccharides, such as kanten, gum arabic and cherry gum[6] into full antigens, and indeed artificial complexes were made from A-substance of animal[7] and human origin[8] by means of this method and were found to be potent antigens. A partially purified human B-substance likewise gave rise to an antigenic complex[9]. Anti-A and anti-B sera produced in rabbits by means of these artificial antigens were exceedingly potent and very specific[9, 10], and a useful *alpha* (α) reagent which agglutinates A_1 and A_1B cells and not A_2 or A_2B cells can be readily prepared from the anti-A serum. The use of these high titred anti-A immune sera for the detection of weakly reacting erythrocytes such as those of A_2B and A_3 and their use

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as reagents in certain clinical studies involving the technique of differential agglutination is indicated[1].

Enzymic Decomposition of the Group Substances

SCHIFF and WEILER[2] observed that the normal fæces of persons of all groups and of certain animals contain an enzyme which readily inactivates the A and B blood group substances. The enzyme is also present in saliva[2] and is found in the saliva of secretors and non-secretors alike[3]. The enzyme does not appear to be derived from the bacteria found in the mouth and it has been suggested by SCHIFF and BURÓN[4] that the enzyme is secreted by the glandular cells. LANDSTEINER and CHASE[5] reported that A-substance was decomposed by an organism, *Pullulomyxa Botrytis*[6], which was isolated by MORGAN and THAYSEN[7], and shown to be active in decomposing a number of specific polysaccharides of bacterial origin.

SCHIFF[8] observed that cultures and culture filtrates of some strains of *Cl. welchii* possess an enzyme which inactivates the blood group A substance contained in peptone and human saliva. The decomposition was considered specific for A-substance. We have also examined culture filtrates from *Cl. welchii* Types A and B and have found them to contain, after suitable purification and concentration, enzyme systems which rapidly destroy the specific serological characters of the purified A, B, and 0 substances. Concentrated culture filtrates obtained from Type-A strains contain these enzymes together with collagenase (K toxin[9]), hyaluronidase and α- and θ-toxins[10]. The most potent enzyme preparations have been obtained from *Cl. welchii*, Type-B filtrates.

It was observed[11] that heating the culture filtrate for 1 hour at 56° C inactivated the enzyme which destroyed the A and B specificity of human and animal group substances whereas the enzyme responsible for the destruction of the corresponding 0-character remained unimpaired. The results of some preliminary experiments show that the destruction of the serological activity of the A and B substances by the enzyme preparations is prevented by an antiserum produced against a crude *Cl. welchii*, Type-A filtrate and which was known to contain α-antitoxin, θ-antitoxin, anti-collagenase and anti-hyaluronidase and certainly possessed antibodies against other unidentified antigenic components present in the original culture filtrate. This multivalent antiserum, however, failed to inhibit the action of the thermostable enzyme responsible for the destruction of the 0-substance.

A few observations have already been made on the chemical changes brought about by the action of a mixed enzyme preparation obtained from *Cl. welchii*, Type A, on the "A-substance" of pig mucin, and of the heat stable 0-active enzyme on purified human 0-substance. In the former instance after the group substance had been rendered serologically inactive by the appropriate enzyme, reducing sugars were present and primary amino groups, as measured by VAN SLYKE's method, formed, and in each instance a small part of the specific substance was found to diffuse through a cellophane membrane. So far we have not succeeded in destroying the group specificity of the intact erythrocyte by means of these enzyme preparations but the corresponding serologically active stromata, especially those belonging to group 0 can be readily inactivated. The experiments are being extended in an attempt to relate the different chemical changes observed to the action of single enzymes, and the specific serological characters with known chemical constitution.

Genetical Considerations

Geneticists have established that the four classical blood group characters A, B, AB, and 0 are inherited in man according to Mendelian laws, and BERNSTEIN's theory of inheritance postulates that the blood group of the individual depends on the presence of two of three allelomorphic genes any of which are capable of occupying the same two loci on the chromosome pair which carries them. Group 0 is due to the operation of a pair of these genes, *00*, recessive in effect whereas the groups A and B are each produced by the substitution of gene *A* or *B* (using the letters defining the blood group character to designate the gene), at the locus of *0*. The dominance of gene *A* or *B* over *0* is almost complete and the result of the presence of a single gene *A* or *B* is nearly or quite indistinguishable from that induced by the presence of a double gene dose, *AA* or *BB*.

With this knowledge of the blood group relationships it is possible to consider the significance of the presence or absence of the so-called 0-substance in the secretions and tissue fluids of persons of different genotypes, more expecially of those individuals belonging to the genotype A_1B. For example it is known that A_1B persons who secrete the A and B factors in a water-soluble form, also secrete 0-substance, whereas according to BERNSTEIN's theory, persons of genotype

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[10] C. L. OAKLEY, Bull. Hygiene *18*, 781 (1943).
[11] W. T. J. MORGAN, Nature *158*, 759 (1946).

A_1B do not contain the *0*-gene and are therefore unable to form 0-substance. What then is the explanation for the presence of 0 factor in the secretions? It has been suggested that the so-called 0-substance is a basic material which suitably modified by the A or B genes developes the additional character A or B respectively. It is therefore of interest to consider the secretion of the 0-substance in the light of HIRSZFELD's[1] recent explanation for the reactivity of erythrocytes with anti-A, anti-B, and anti-0 sera. HIRSZFELD subdivides group A bloods into "pleiades" A_j, A_r, A_m, A_2, A_3, A_4, and A_5 arbitrarily chosen and arranged in increasing order of reactivity with anti-0 serum. A similar relationship is said to hold for B-cells. It is claimed that the genotypes A_jA_j, A_jA_r, A_jA_m, A_jA_2, and $A_j\,0$ constitute a single phenotype which reacts strongly with anti-A and weakly with anti-0 reagents. Individual genes correspond to each stage of mutation from 0 to A and the gene A_j confers less 0-character on the erythrocytes than gene A_r which in turn confers less 0-reactivity than genes A_m, A_2, A_3, etc. The individual genes which have reached a similar degree of mutation and which are therefore close together in the series of transitional forms, for example, A_j and A_r, show the phenomenon of partial dominance, whereas those further apart, such as A_j and *0*, show absolute dominance. Thus the erythrocytes of individuals of the homozygous genotype A_jA_j and of the heterozygous genotype A_j0 react equally well with an anti-0 serum because the gene A_j which confers a weak but definite 0-character to the cells, suppresses more or less completely the effect of any gene tending to confer stronger-0 character, such as A_r, A_m, A_2 or the *0*-gene itself. The completion of mutation from 0 to A or B is recognized in the rare gene forms A_c and B_c which are completely lacking in 0-character and individuals containing A_c or B_c only in the genotype will fail to develop 0-substance and will in consequence possess erythrocytes which are unable to react with anti-0 agglutinin. The tissue fluids and secretions will, for the same reason, be free from 0-substance. In the blood serum of such rare individuals the appearance of natural anti-0 agglutinins can be expected and indeed in man a naturally occurring anti-0 agglutinin is very occasionally observed. It follows, therefore, that persons possessing all other forms of the A or B gene, such as A_j or B_j, A_m or B_m, etc. will possess a genetic constitution which will induce the formation of some 0-character and this will occur irrespective of the heterozygous or homozygous nature of the genotype. It will be at once apparent that this concept of gene structure will explain satisfactorily the presence of more or less 0-substance in the erythrocytes and in the secretions and tissue fluids of individuals of the genotypes A_2A_2, A_1A_2, A_2B and A_1B where the "pleiade" of the person is not A_c or B_c. For some time past we have been examining on a quantitative basis the relationship of the amount of the group substances (A, B, and 0) in a given secretion to the genotype of the individual and as a result of the investigations we are now able to classify secretors belonging to group A as A_1 or A_2 on the basis of the amount of A and 0 substance secreted in the tissue fluids or secretions. Similarly, it is possible to differentiate individuals belonging to the genotypes A_1B and A_2B by the same method. It is evident that the results of a biochemical approach along these lines will express in chemical terms one aspect of the specific functions of a gene, will reveal certain characteristic chemical differences in individuals and thus contribute to the understanding of the genetics of human blood groups.

Considerations of this kind open up a new field of investigation for the immunochemist. The isolation of the blood group substances as homogeneous molecular species from individuals of known genotype is their immediate problem and is one that is proving exceedingly difficult. In view of the importance of determining the precise differences in these closely related gene products for our understanding of the mechanism of gene action, however, it is a problem worthy of very serious study. Until this is accomplished statements as to the detailed composition and structure of these important biological materials must be accepted with reserve. The progress towards an understanding of the specificity of the ABO system of human blood groups in terms of chemistry and physics reported in these lectures although strictly limited, is not inconsiderable. Perhaps some of the points discussed will suggest directions for future work. It is evident that there must be a greater appreciation by the chemist of the finer biological differences that exist in the blood group substances of individuals of different genotypes if homogeneous molecular species characteristic of the blood group are to have their chemical constitution related to the specific immunological properties of the substances. Furthermore, if such material, derived not from "group A blood", "group B saliva", "a group A tumor", etc. but from the blood, saliva and tumors of individuals of accurately known genotype, is finally obtained, it will be suitable for examination in the light of genetical data and a valuable contribution to the chemical aspects of human genetics will have been made.

Résumé

Cet article rend compte des essais d'isolation des substances spécifiques des groupes sanguins de l'homme. Elles sont déterminées comme étant des complexes mucoïdes ou polysaccharides-aminoacides et l'on traite de quelques-unes de leurs propriétés physiques, chimiques et immunochimiques. En isolant ces substances qui forment des

[1] L. HIRSZFELD and R. AMZEL, Ann. Inst. Pasteur *65*, 251, 386 (1940).

complexes moléculaires homogènes, on estime pouvoir obtenir, si l'on opère sur le sang d'individus de génotype connu, un matériel permettant de déterminer l'influence des gènes *A*, *B* et *0* sur les caractères physiques des produits de gènes très voisins. Les résultats des recherches entreprises dans ces directions constitueront une précieuse contribution à nos connaissances sur la chimie des substances génétiques humaines.

Über die optimale Zusammensetzung der Nahrung

Von E. ALBERT ZELLER,[1] Basel

Während der Kriegszeit lag dem Ernährungsphysiologen die Pflicht ob, diejenigen Unterlagen zu beschaffen, die es den zuständigen Behörden ermöglichten, die Einfuhr, Produktion und Verteilung der Nahrungsmittel so zu gestalten, daß die zugeteilten Rationen alle lebensnotwendigen Nährstoffe in ausreichenden Mengen enthielten[2]. Diese Arbeit wurde durch den Mangel von allgemein anerkannten und physiologisch begründeten Maßstäben sehr erschwert. Es soll hier unter Zuhilfenahme einiger neuerer Beispiele das Problem der bestmöglichen Ernährung und dessen noch nicht durchschaubare Vielschichtigkeit darzustellen versucht werden.

Vorerst seien drei Termini technici eingeführt. Als *Minimum* kann diejenige Menge eines Nährstoffes definiert werden, die den Ausbruch einer ausgeprägten Mangelkrankheit verhindert. So sollen beispielsweise 7 mg Nikotinsäure-amid gerade genügen, um das Entstehen der Pellagra zu verhüten[3]. Doch ist offensichtlich die mit der Nahrung zugeführte Menge dieses Vitamins nicht allein für das Zustandekommen der Mangelkrankheit verantwortlich, da die Milch trotz ihres sehr geringen Niacingehalts[4] pellagraverhindernd wirkt. Es scheint das Minimum somit keine konstante Größe darzustellen.

Vor fünfundzwanzig Jahren entwickelte E. V. MCCOLLUM[5] den Begriff des *Optimums*, der durch einige Versuche von H. C. SHERMAN dem Verständnis nahegebracht werden kann: Weiße Ratten wurden ausschließlich mit Weizen und Trockenmilch gefüttert. Während 63 Generationen gediehen die Tiere ausgezeichnet[6], so daß die Kost als adäquat und weit über dem Minimum stehend bezeichnet werden muß. Und doch ist diese Nahrung, wenn das erreichbare Alter als Kriterium gewählt wird, nicht optimal. Wurde ihr nämlich etwas Butter beigefügt, oder einfach das Verhältnis zwischen Weizen und Trockenmilch geändert, so stieg die durchschnittliche Lebensdauer deutlich an. Damit ist gerade auch die *adäquate* Zufuhr festgelegt worden, die in gewissen Fällen mit den *restricted intake requirements*[1] übereinstimmen mag, und die den weiten Bereich zwischen Minimum und Optimum unterteilt.

Synthese und Resorption von B-Vitaminen im Dickdarm

Einst hoffte man, ein einfaches Verfahren für die Bestimmung des Vitamin-C-Optimums zu besitzen. Die Methode bestand darin, vor und nach Belastung des Organismus mit Ascorbinsäure die Ausscheidung derselben durch die Niere zu verfolgen. Die folgenden Ausführungen sollen aber am Beispiel der B-Vitamine zeigen, daß die alleinige Kenntnis von der Exkretion eines Vitamins oder andern essentiellen Nährstoffes nur einen beschränkten Einblick in den Stoffwechsel und Bedarf solcher Stoffe erlaubt.

Nicht immer ist die in der Nahrung vorhandene Menge an B-Vitaminen in erster Linie für die Ausscheidung verantwortlich zu machen, da beispielsweise bei Wiederkäuern das Mehrfache der im Futter zugeführten B-Vitamine in der Milch gefunden wird. Diese Erscheinung ist nach den eingehenden Untersuchungen amerikanischer Autoren[2] auf die ausgezeichnete Vitaminbildung durch die Mikroorganismen des Pansens zurückzuführen. In den auf den Magen folgenden Dünndarmabschnitten werden die Vitamine resorbiert und auf diese Weise vom Wirt ausgenützt.

Auch die Flora des menschlichen Dickdarms ist zur Synthese von B-Vitaminen fähig, wie die großen im Kot ausgeschiedenen Mengen beweisen. Im Gegensatz zum Wiederkäuer findet die Bildung aber im Dickdarm statt, also *hinter* den gut resorbierenden Darmabschnitten. Es muß daher die Frage behandelt werden, ob die im Dickdarm aufgebauten B-Faktoren in den Körper des Wirts übertreten[2]. Das reichliche Vorkommen dieser Stoffe im Kot zeigt ja gerade, daß jedenfalls ein großer Teil der Resorption entgeht.

[1] Vortrag, gehalten am 23. Oktober 1946 in der Naturforschenden Gesellschaft in Basel.

[2] Sir JOHN ORR und D. LUBBOCK, Feeding the people in war-time. London 1940. – A. FLEISCH, Ernährungsprobleme in Mangelzeiten. Basel 1947. – E. A. ZELLER, Die menschliche und tierische Ernährung in Mangelzeiten. Schweiz. landwirtschaftl. Mh. *23*, 109 (1945). – H. KAPP, «Ärztliche Erfahrungen mit der Kriegsernährung in der Schweiz», Exper. *3*, 11 (1947).

[3] L. JUSTIN-BESANÇON und A. LWOFF, Vitamine antipellagreuse et avitaminoses nicotiniques. Paris 1942. Seiten 102–108.

[4] Niacin = Nikotinsäure = Antipellagravitamin = PP-factor.

[5] E. V. MCCOLLUM, The newer knowledge of nutrition, 2nd ed. New York 1926. Seite 46.

[6] H. C. SHERMAN, Chemistry of food and nutrition, 6th ed. New York 1941. Seite 517, und persönliche Mitteilung.

[1] Food consumption levels. London 1944. Seite 31.

[2] Zusammenfassung: V. A. NAJJAR und R. BARRETT, "The synthesis of B Vitamins by intestinal bacteria", Vitamins and Hormones *3*, 23 (1945).