LXVII. CHOLEIC ACIDS.

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I. THE BIOCHEMICAL SIGNIFICANCE OF THE CHOLEIC ACID PRINCIPLE.

PHYSICAL properties in homologous series are functions of molecular size. When constants like melting-point, solubility, viscosity, surface tension effects, acid dissociation constant and others are plotted against molecular weight or against the number of carbon atoms, the resulting diagrams indicate regular gradual changes of these physical properties, *e.g.* the solubilities of the ethyl esters in the acetic acid series were found to follow an inverse geometrical progression with the length of the carbon chain [Sobotka and Kahn, 1931]. Some properties follow an oscillatory course between those homologues with an even number of carbon atoms and those with an odd one. Their physical and chemical properties will be reflected by their biochemical behaviour. Diffusion through membranes, toxicity towards cells, specific pharmacological effects, and even the probability of their formation and occurrence depend on the combined effects of simpler physical constants.

Nevertheless, not all biochemical properties of homologous substances can be explained on the basis of these additive factors. The synthesis of fatty acids from carbohydrates may be associated with preferential formation of fatty acids whose number of carbon atoms is a multiple of six. Another factor which might induce periodical, instead of gradual changes, in the biochemical properties of homologous fatty acids and their derivatives is their "co-ordinative valency," as revealed by the study of the choleic acids.

The central position of one atom or ion surrounded by a number of molecules has been recognised as the general architectural principle of inorganic molecular compounds. This number must be such as to allow for a symmetrical arrangement around the pivotal atom and the co-ordination numbers most commonly encountered are 4, 6, 8, occasionally 2 and 3, while the numbers 5 and 7 would not permit symmetrical constellation. Potential higher co-ordination numbers are 12 and 20 [Hüttig, 1920] but no evidence has been adduced for the existence of such huge symmetrical molecular aggregates.

Organic molecular compounds are of a much simpler structure, the prevailing molecular ratio being 1 : 1 in almost every instance. The choleic acids form the most remarkable exception. Wieland and Sorge [1916] recognised that natural "choleic acid" was a molecular compound consisting of 8 molecules of

desoxycholic acid and 1 molecule of stearic or palmitic acid. While all bile acids are known to influence the physical condition of lipoids in the dissolved state, desoxycholic acid seems to have a special chemical affinity for fatty acids and other substances, giving rise to the formation of crystalline molecular compounds. This principle, designated the "Choleic Acid Principle," shed new light on the significance of the bile in metabolism. Wieland and Sorge recognised that molecular compounds of desoxycholic acid of this type may be formed not only with ethyl alcohol, ether, acetone, acetic and higher fatty acids, but also with substances like benzene, phenol, naphthalene, camphor and others, rendering them water-soluble in the form of alkali choleinates, a fact of no mean physiological and pharmacological importance.

Choleic acids in homologous series. Rheinboldt and his co-workers [1926, 1929] studied the choleic acids consisting of aliphatic acids, and their esters, with desoxycholic acid or Boedecker's apocholic acid. They reached the following conclusions. (1) Choleic acids are built on the co-ordination principle and show the same molecular ratios as inorganic co-ordination compounds and as postulated by geometric considerations. They are "co-ordination compounds of higher order." (2) One molecule of acetic acid combines with a single molecule of desoxycholic acid, 1 molecule of propionic acid with 3 molecules of the bile acid, butyric to caprylic acids with 4. For pelargonic to myristic acids the co-ordination number is 6, for pentadecylic acid upwards 8. The same numbers obtain for analogous acids with double bonds. (3) An alcohol with n carbon atoms has the same co-ordination number as the acid with $(n + 1)$ carbon atoms. (4) In alkyl esters of aliphatic acids the co-ordination number peculiar to the longer of the two chains prevails.

A graphic representation of the data mentioned under (2) yields a terrace-shaped diagram (Fig. 8). In the group of choleic acids between butyric and caprylic acids one may expect a steady increase of "co-ordinative unsaturation" while a new cycle will be resumed with pelargonic acid-choleic acid. Thus a formal resemblance to the periodic system of elements is suggested.

Since the bearing of co-ordinative valency upon biochemical problems is being studied in this laboratory, it seemed desirable to secure data on the co-ordination numbers in other homologous series of biochemical significance. In Part II the choleic acids derived from saturated aliphatic dicarboxylic acids are described.

Choleic acids of isomerides. Other aspects of the Choleic Acid Principle are offered by the study of choleic acids derived from isomeric substances. The choleic acids of the isomeric valeric acids, to be dealt with below (Part III), exemplify the influence of structural isomerism. Similar considerations actuated experiments to decide whether or not the bile acids permit one to differentiate between optical antipodes. Successful experiments in this direction will be reported in a subsequent paper [Sobotka, 1931; Sobotka and Goldberg, 1932]. We are also studying the relation of *cis-trans* isomerism on choleic acid formation.

An investigation of ethyl acetoacetate-choleic acid [Sobotka and Kahn, 1932] and the choleic acids of related tautomeric substances yielded results of twofold interest. We could demonstrate that ethyl acetoacetate in its molecular compound with three molecules of desoxycholic acid is completely enolised. This finding suggests a participation of the bile acids in the oxidation of β -keto-acids in the liver. On the other hand, the rate of the molecular dissociation of choleic acids into their molecular constituents subsequent to solution may be estimated in these choleic acids by titration of the liberated enol form of the acholic constituent¹. These measurements illustrate the stability of choleic acids from a different angle.

Stability of choleic acids. Several features in the behaviour of the bile acids towards lipoids and other water-insoluble substances are shared by the saponin group. But while many of these polycyclic substances enter into molecular compounds, for instance digitonin with cholesterol (1 : 1), the formation of molecular compounds of higher order seems to be limited to the desoxycholic and apocholic acids found in or derived from animal bile². Whether this peculiarity signifies a specially high degree of affinity present in smaller measure in other bile acids, or whether it is an individual distinctive feature of bile acids with two hydroxyl groups, is not known; but it offers an approach to quantitative investigations on the influence of bile acids on lipid metabolism. One must bear in mind however that these substances differ not only in their co-ordination numbers, but in their stabilities. Choleic acids of equal co-ordination number will vary through the volatility of the acholic constituent, through their solubility in various solvents, the rate of their molecular dissociation (see above), and through their stability in the presence of substances competing for co-ordinative linkage with the different bile acids.

Multiple proportions in co-ordination compounds. It is imaginable that two choleic acids differing in their co-ordination numbers are formed from one acholic constituent. This possibility has been exemplified by Rheinboldt, König and Flume [1929] in the case of camphor. We are able to add a few instances where choleic acids were formed in two proportions (Part III). The conditions for the formation of these compounds, their stabilities and their place in lipid metabolism remain to be investigated.

II. CO-ORDINATION COMPOUNDS OF POLY-METHYLENE DICARBOXYLIC ACIDS WITH DESOXYCHOLIC ACID.

The general method of synthesis of molecular and dicarboxylic acid compounds with desoxycholic acid is as follows. Very pure desoxycholic acid and the acholic component are dissolved in a small amount of hot absolute ethyl alcohol. The crystals obtained upon cooling are separated and dried under

¹ The term *acholic* constituent is suggested for any chemical substance combining with a bile acid to form a choleic acid.

² Cholic acid crystallises with 1 molecule of alcohol.

reduced pressure in an Abderhalden pistol. The substance is then recrystallised from ethyl alcohol until it shows a constant composition. This method may be applied in all cases where the solubility of the desired choleic acid in alcohol is less than that of alcohol-choleic acid. It failed in the present series with glutaric and pimelic acids since the first crop of crystals consisted of a mixture of alcohol-choleic acid and the dicarboxylic acid-choleic acid. On subsequent recrystallisation pure alcohol-choleic acid was obtained. In all other instances however a synthetic product of constant composition was obtained.

Analysis of choleic acids. Choleic acids, synthesised from two known components, may be isolated free from solvent by properly instituted drying procedures. Nevertheless, elementary analysis does not yield conclusive evidence as to the proportional shares of the two components, owing to the small differences in elementary composition.

The xylene method of Wieland and Sorge [1916], extensively used by Rheinboldt [1926], is based on the dissociation of the aliphatic-choleic acid in xylene yielding the insoluble xylene di-choleic¹ acid *plus* the free aliphatic acid. The latter is subsequently isolated from its xylene solution and titrated. While Rheinboldt applied this procedure with advantage to the analysis of monocarboxylic acid-choleic acids, the insolubility of dicarboxylic acids in xylene creates a new difficulty and one must resort to a more involved method including the isolation of the calcium salt [Rheinboldt, König and Otten, 1929, p. 262].

Whenever the acholic constituent has either a low acid equivalent or no acid character at all, the acid equivalents computed for choleic acids with various co-ordinative ratios are sufficiently far from each other to allow a definite decision regarding this ratio. Since the critical acid equivalents in the most unfavourable case of the present series (octodecamethylenedicarboxylic acid-choleic acid) were more than 2 % removed from each other, the determination of acid equivalents was the method of choice.

Another method of ascertaining the molecular ratio in a co-ordinative compound is based on the melting-point diagram [Rheinboldt, 1925; Rheinboldt and Kircheisen, 1926; Pfeiffer, 1927]. Various mixtures of the two components are fused together and after complete homogeneity is attained, the melting-point is determined. In most instances, a maximum can be observed in the melting-point diagram, when a compound exists, but never when no compound is formed. The composition of the compound is given by the abscissa corresponding to this maximum. It is practical to plot as abscissae molecular percentages instead of percentages by weight.

This method offers independent corroboration of the analytical results. But the interpretation of the melting-point curve will fail in cases where it has no sharp maximum or none at all. It is necessary, then, to resort to the thawing-point curve [Auftau-Diagramm, Rheinboldt, 1925]. This curve is

¹ We propose the terms *di-choleic acid*, *tri-choleic acid*, etc., to designate molecular compounds containing two, three, etc. molecules of desoxycholic acid.

constructed from the temperatures at which the mixture will start to liquefy, while the melting-point curve is defined by complete disappearance of the solid phase.

The substances A and B (Fig. 1) form a compound of the molecular ratio 1 : 2. There are five points where the melting-point curve and the thawing-point curve coincide, i.e. where a sharp melting-point is observed, the two pure components, their molecular compound, and the two eutectic mixtures ($A + AB_2$) and ($AB_2 + B$). With any other mixture, there is a greater or smaller range of temperature between thawing-point and melting-point, where a solid and a liquid phase coexist. Everything is liquid in the field above the melting-point curve and everything is solid below the thawing-point curve. The solid substance A coexists with the liquid mixture of variable composition in the wing-shaped field enclosed by the two curves leading from the melting-point of pure A to that of the eutectic ($A + AB_2$). In the similarly shaped field between ($AB_2 + B$) and pure B , solid B forms the solid phase of the biphasic mixture. The two fields above the central parts of the thawing-point curve and below the centre span of the melting-point curve symbolise the existence of solid AB_2 in a melt, containing an excess of A or B , for the temperature ranges given.

Fig. 1.

Fig. 1. Melting- and thawing-point diagram of a system $A + B$ with a homogeneously melting compound AB_2 . (According to Rheinboldt.)

Fig. 2.

Fig. 2. System $A + B$ forming a compound AB_2 which does not melt homogeneously. (According to Rheinboldt.)

Diagrams of the general type represented by Fig. 1 are obtained in the case of many choleic acids, e.g. in Fig. 3 exemplifying the system sebacic acid + desoxycholic acid. A maximal melting point is found at 181° for a mixture of 20 mol. % sebacic acid + 80 mol. % desoxycholic acid. The compound AB_4 has a very wide range of existence and the melting-point curve reaches a flat but definite maximum. The eutectic mixtures approach the pure compounds so closely in composition that the melting-point curve cannot be verified experimentally for the branches A to ($A + AB_4$) and ($AB_4 + B$) to B . But the melting-points of the eutectic mixtures are revealed by the ordinates of the two horizontal branches of the thawing-point curve. These two horizontals are designated $m-n$ and $o-p$ in Figs. 1 and 2 and will be referred to as eutectic levels.

In some instances, curves of another type are obtained (Fig. 2). The maximum of the melting-point curve is hidden and the compound does not melt sharply and homogeneously, but the component with the lower melting-point melts at a faster rate, while the coexisting solid phase is enriched in substance B . Such a curve was observed with pimelic acid, one of the lower odd-numbered acids with which the preparation of pure choleic acids by recrystallisation failed. The co-ordinative affinity of these acids is so weak that the acholic constituent leaves the crystal structure and dissolves or melts without simultaneous liquefaction of the other partner, namely without complete disruption of the desoxycholic acid pattern. This interpretation is in effect the same as

that given above, when the failure to prepare certain choleic acids was explained by their greater solubility as compared to alcohol-choleic acid. An analysis of the thawing-point curve may be helpful for the determination of the co-ordination number in these instances.

The following variations and peculiarities were noted in the choleic acids studied.

(1) The melting-point of the molecular compound is higher than that of either constituent. This condition was encountered with sebacic acid (Fig. 3) and all higher acids studied in the series.

Fig. 3.

Fig. 3. System sebacic acid + desoxycholic acid.

Fig. 4.

Fig. 4. System succinic acid + desoxycholic acid.

(2) Both components melt at a higher temperature than their compound in the instance of succinic acid *plus* desoxycholic acid (Fig. 4). However, the compound has a definite maximum melting-point and the thawing-point of 162° with the same percentage composition is 8° above the higher one of the two eutectic levels (154° and 150°). A peculiar irregularity in the succinic acid-desoxycholic acid thawing-point curve remains to be explained. There is an indication of a third eutectic level between 15 and 7 % succinic acid at 163°, implying the existence of a second less stable molecular compound of the possible composition 1 : 6.

Fig. 5.

Fig. 5. System adipic acid + desoxycholic acid.

Fig. 6.

Fig. 6. System azelaic acid + desoxycholic acid.

(3) The melting-point of the compound is between the melting-points of the constituents as with adipic and azelaic acids (Figs. 5 and 6). This is also assumed in the example given in Fig. 1. While a definite turning point appears in the melting-point curve of azelaic acid at the composition 1 : 4, the adipic acid curve is almost horizontal between 33 and 15 % adipic acid. It is for the thawing-points only that a decision can be reached confirming the molecular ratio 1 : 3. The conditions in adipic acid-choleic acid resemble those illustrated in Fig. 2 and in pimelic acid-choleic acid.

In the case of pimelic acid (Fig. 7) the melting-point of the compound is situated between those of its components, but there is no initial decrease in the melting-point on the branch from the melting-point of the compound toward the higher-melting component. In any mixture containing an excess of the higher-melting component this component will be less soluble in the melt than the molecular compound. Solid *B* (desoxycholeic acid) will coexist with the liquid phase throughout the triangular field enclosed by the eutectic horizontal *o-p* and the two curves leading upwards to the melting-point of *B*. The melting curve of pimelic acid-choleic acid conforms closely with the schematic case of Fig. 2. The thawing curve at 20 and 17.5 % pimelic acid (molecular ratio 1 : 4 and 1 : 4.7) shows an elevation over the lower thawing-point 131° at 14.7 % pimelic acid which represents the eutectic level *o-p*. It is doubtful whether deviations from the theory as in this instance, and in the case of succinic acid discussed above, are due to experimental errors, impurities and the like. We are inclined to believe that these exceptions are caused by the interference of molecular compounds of different composition and lower stability. These compounds are completely dissociated at melting-temperature but they may play a rôle at lower temperatures thus causing the complications mentioned. Such interferences are more apt to be displayed by molecular compounds of higher order like choleic acids than by the molecular compounds with the ratio

Fig. 7. System pimelic acid + desoxycholeic acid.

1 : 1 or 1 : 2 which formed the basis for the development of the melting-point and thawing-point theories. The application of Weissenberger's studies [1924, 1925] on depression of vapour tension in molecular compounds to choleic acids with volatile acholic components might aid in the elucidation of this point.

As mentioned previously, the range of existence of the choleic compounds commonly approaches so closely the 0 and 100 % ordinates that the minimum melting-points of the two eutectic mixtures ($A + AB_n$) and ($AB_n + B$) cannot be verified. A perusal of Rheinboldt's experiments [1926, 1928, 1929] shows that this holds for all choleic acids investigated excepting the compounds of camphor with desoxycholeic and apocholic acids with the molecular ratio 1 : 1. This is in contrast with the numerous systems like picric acid + naphthalene where all features demanded by the theory may be verified [Rheinboldt, 1925]. But even when the melting-point curve shows the theoretical drop, with increasing percentages of bile acid being added to the pure fatty acid, as in the cases of succinic and adipic acids, the low inflection points of the melting-point curves at 168° with 50 % succinic acid and at 141° with 85 and 81 % adipic acid are so far from the thawing-point curves as to exclude the interpolation of sharply-melting eutectic mixtures. Here too the deviation from the theoretical course seems to be more deeply rooted than in experimental fallacies. It may be noted that no eutectic level but a straight diagonal runs from 110° to 140° in the thawing diagram of adipic acid. A slight slant of the *m-n* branch obtains also with pimelic acid, while all the other systems studied lack this irregularity.

Table I is a synopsis of these results and the findings by the acid equivalent titrations. The melting-point of the acholic component is given for comparison

with the melting-point of the choleic acids. Except in the case of pimelic acid, all co-ordination numbers were confirmed independently by the estimation of the acid equivalent. We confined ourselves to the latter method in the case of

Table I. *Melting-points, acid equivalents and co-ordination numbers of dicarboxylic acid-choleic acids.*

Acid	Formula	Melting-point ° C.		Acid equivalent				Co-ordination number
		Free acid	Choleic acid	Found	Calculated			
					(N - 1)	N	(N + 1)	
Succinic	CO ₂ H(CH ₂) ₂ CO ₂ H	186	171	228	170.1	225.7	259.0	2
Adipic	CO ₂ H(CH ₂) ₄ CO ₂ H	154	165.5	271.5	232.7	264.6	285.2	3
Pimelic	CO ₂ H(CH ₂) ₅ CO ₂ H	105	—	—	—	—	—	4
Suberic	CO ₂ H(CH ₂) ₆ CO ₂ H	142	172.5	285.5	270.2	289.9	304.5	4
Azelaic	CO ₂ H(CH ₂) ₇ CO ₂ H	109	172	296.5	273.0	292.2	306.5	4
Sebacic	CO ₂ H(CH ₂) ₈ CO ₂ H	133	181	294	275.8	295.2	308.5	4*
	CO ₂ H(CH ₂) ₉ CO ₂ H	111	172	299	278.6	297.6	311.1	4
	CO ₂ H(CH ₂) ₁₀ CO ₂ H	129	183.5	322	312.5	323.0	330.7	6
Brassylic	CO ₂ H(CH ₂) ₁₁ CO ₂ H	116	181	323.5	314.5	324.8	332.2	6
	CO ₂ H(CH ₂) ₁₂ CO ₂ H	126	186.5	326	316.5	326.5	333.8	6
Thapsic	CO ₂ H(CH ₂) ₁₄ CO ₂ H	125	188	332	320.5	330.0	336.9	6
	CO ₂ H(CH ₂) ₁₆ CO ₂ H	125.5	191.5	333.5	324.6	333.5	340.1	6
	CO ₂ H(CH ₂) ₁₈ CO ₂ H	126.5	192	335	327.1	335.3	341.6	6

* Cf. Wieland [1928].

Fig. 8. Relation between length of aliphatic chain and co-ordination number.

suberic acid, enneamethylenedicarboxylic acid and the four acids with high molecular weight. While the melting-point diagram allowed no decision for pimelic- and adipic-choleic acids, the ratio 1 : 3 could be ascertained for the latter both by the thawing-point diagram and the acid equivalent. Preference

was given to the ratio 1 : 4 over 1 : 5 for pimelic acid-choleic acid for the theoretical reason that 5 never occurs as co-ordination number and that the homologues following pimelic acid combine definitely in the ratio 1 : 4 with bile acid.

Fig. 8 gives a graphic representation of the correlation between number of methylene groups and co-ordination number in the present series. The co-ordination number for glutaric acid is inserted arbitrarily with little hope that a glutaric acid-choleic acid may be synthesised, as preliminary experiments showed that such a compound must be extremely labile. The analogous terrace-shaped diagram given by Rheinboldt [1926, 1929] and based on his own and some of Wieland's data is added beneath to facilitate comparison.

Possible relations between these periodic changes in co-ordinative valency and biochemical behaviour were discussed in Part I.

EXPERIMENTAL.

Source of material. The following acids of the polymethylenedicarboxylic series were synthesised. Enneamethylenedicarboxylic acid, decamethylenedicarboxylic acid and hexadecamethylenedicarboxylic acid were prepared according to Chuit [1926] by reduction of diethyl azelate, diethyl sebacate and diethyl thapsate with sodium in alcoholic solution following Bouveault's procedure. The resulting diols were converted into 1 : 9-dibromononane, 1 : 10-dibromodecane and 1 : 16-dibromohexadecane respectively and subsequently into the corresponding dinitriles which were saponified by alcoholic alkali to yield the dicarboxylic acids. Dodecamethylenedicarboxylic acid and octadecamethylenedicarboxylic acid were prepared from 1 : 10-dibromodecane and 1 : 16-dibromohexadecane respectively with ethyl sodiomalonate and saponification followed by partial decarboxylation of the resulting dodecane (or octadecane)-tetracarboxylic acid. Pimelic acid was prepared by reduction of salicylic acid with sodium in amyl alcohol [Einhorn and Willstätter, 1894; Einhorn and Lumsden, 1894]. We are indebted to the firm M. Naef et Cie., Geneva, for a specimen of brassylic acid. Some of the commercial acids were purified by vacuum distillation of the diethyl ester or of the free acid itself.

Pure desoxycholic acid was prepared from acetic acid-choleic acid or from commercial desoxycholic acid in the following manner. 100 g. of the starting material which should be strictly free from cholic acid are dissolved in 500 cc. hot ethyl alcohol. The filtered solution is concentrated to less than 200 cc. on an electric hot-plate and the syrupy residue poured with constant stirring into 1500 cc. of cold ether. A fine sandy powder settles within a few minutes and is filtered off with suction. If the amount of alcohol poured into the ether has been kept low, only a few grams of bile acid will be left in the ethereal filtrate at this stage. The ether retains all lipin impurities. The crystalline ether-choleic acid is dissolved in one or two parts of absolute alcohol and recrystallised. At this stage, alcohol-choleic acid should be obtained in long transparent prisms. They are carefully separated from the mother-liquor and may be recrystallised once more to show a melting-point of about 118°–120°. These procedures should not involve a loss of more than 40 g.

This alcohol-choleic acid is sufficiently pure for syntheses in alcoholic solution. For the determination of thawing-points and melting-points it is

essential to provide the purest desoxycholic acid. This is obtained from the pure alcohol-choleic acid of which 60 g. are taken up in 100 cc. of alcohol and poured into a wide beaker containing 1 litre of boiling water. Boiling is maintained for several hours, water being added to replace losses by evaporation [*cf.* White, 1929, p. 1171]. The pasty white mass changes into heavy hard lumps as alcohol is given off. These lumps are separated, roughly dried, ground and finally dried to constant weight. The melting-point of this product was 176° (corrected, see below), its thawing-point was 171–172°. Its purity was further checked by its optical activity.

It is worth noting that various lots of alcohol-choleic acid differ in the tenacity with which the alcohol is retained under reduced pressure and high temperature. On the other hand, it was observed that desoxycholic acid, free from solvents, would increase in weight slightly when kept for a few months in a desiccator over phosphorus pentoxide, simultaneously its thawing-point would drop below 170°. Therefore, free desoxycholic acid used in this work was always freshly prepared from a stock of alcohol-choleic acid.

Syntheses of choleic acids. As a rule, 2 g. of the dicarboxylic acid were added to a warm solution of 8 g. desoxycholic acid in 20–30 cc. of alcohol. The choleic acid separated on cooling at varying speed as a crystalline powder. This was separated and dried in an Abderhalden drying apparatus at 80° in a high vacuum, until a weighed specimen had reached constant weight. Its melting-point and its acid equivalent were then determined. A portion of the specimen was recrystallised from ethyl alcohol and these procedures were repeated two or three times when necessary. In several instances the first or second fraction showed a higher melting-point than the subsequent ones. This holds particularly for the lower members of the series. As discussed in the theoretical part, these choleic acids are less stable and will crystallise in presence of an excess of the acholic component only. When recrystallisation is attempted from alcohol or other solvents, the solvent-choleic acid gradually replaces the desired choleic acid, thus leading to products of lower melting-point and higher acid equivalent approaching, with alcohol as solvent, 438 (ethyl alcohol-choleic acid). Even less labile choleic acids cannot be recrystallised except within a certain limit of excess of either constituent. The crystal fraction with the highest melting-point was used for the determination of the acid equivalent.

Determination of acid equivalents. The acid equivalents were determined by dissolving 50–100 mg. of substance in 4–5 cc. of methyl alcohol and titrating with 0.05 *N* aqueous NaOH with bromothymol blue as indicator. In some instances an excess of alkali was used and titrated with 0.05 *N* HCl. Both methods yield identical values. For the calculation of the acid equivalents, the mol. wt. of desoxycholic acid was assumed to be 392.32, *e.g.* the acid equivalent for sebacic acid-tetracholeic acid is obtained from

$$\frac{(392.32 \times 4) + (202.14 \times 1)}{4 + 2} = 295.2.$$

The results of these analyses are summarised in the theoretical part.

Melting- and thawing-points. The mixtures of varying molecular composition are prepared as follows. 5 mg. or more of the acholic component are weighed with an accuracy of 0.01 mg., if possible on a micro-balance, into a glass thimble made from a light test-tube and measuring about 12 mm. in diameter and 20 mm. in height. An amount of desoxycholic acid is added approximating as closely as possible to the amount computed for the intended molecular ratio. The thimble is placed on a hot-plate in a 30 cc. beaker which serves as an air-bath; 2-3 drops of methyl alcohol are added. This solvent was chosen because it is less firmly held *in vacuo* than ethyl alcohol or acetone. The mixture dissolves in the methyl alcohol and while homogeneity is attained part of the solvent evaporates. Great care has to be exercised to avoid spattering even for a few moments after removal from the hot-plate. As many as four thimbles may be carefully introduced at the same time into a double-jacketed Abderhalden pistol and dried for 1 hour at 80° in the case of dicarboxylic acid-choleic acids. After cooling, the scaly mixture is broken up and triturated with a heavy rounded rod serving as a pestle.

For the observation of the thawing- and melting-points, we use a Thiele-Anthes melting-point flask [Rheinboldt, 1925]. The sulphuric acid is agitated by a current of dried air. The thermometer is calibrated in the melting-point apparatus used against a set of Anschütz thermometers which are immersed above the meniscus. In this manner corrected melting-points are obtainable. As the corrections in the essential range of temperature reach 3-4° a uniform correction is very desirable.

The substance is tightly packed in a column 6-8 mm. high by means of a fine glass rod in the melting-point tubes, which should not be too narrow. The melting-point tube is placed so that its tip is situated in front of and in contact with the bulb of the thermometer. The substance is observed through a magnifying glass. The melting-point tube may be rotated for close scrutiny at the upper end protruding through a side-tube of the Thiele apparatus. The temperature is raised at a rate not exceeding 5° per minute but at the critical temperatures a considerably lower rate will be found advantageous. While one observer watches the substance, a second observer follows the rise of the mercury on the thermometer scale which is turned at a right angle from the first observer. The second observer records the observations made by the first one.

The thawing-point of a mixture is defined by its incipient liquefaction. If thorough homogeneity of the sample is attained, the beginning of liquefaction is manifested by the simultaneous appearance of droplets at many points on the walls of the melting-point tube. These droplets coalesce, and usually within a few degrees rise the crystalline powder will lose its lustre. This transition is described by Rheinboldt [1925] as a change from a silky to a velvet-like appearance and we found that this simile is very apt to describe the phenomenon in many cases. At this point, the substance will shrink, giving rise to horizontal fissures. After this, the mass changes into a few drops separated by air bubbles. A few degrees hence will suffice for the substance to reach its melting-point.

The opaque fluid clears, starting at the periphery and from the bottom, but it should not be pronounced melted until the last traces of a crystal skeleton have disappeared in the centre or towards the interfaces of the air bubbles. Care should be taken not to confuse these last crystals with foam occluded in the viscous fluid.

SUMMARY.

The synthesis and analysis of choleic acids is discussed and exemplified by the molecular compounds of desoxycholic acid with aliphatic dicarboxylic acids. The co-ordinative valences of these acids were ascertained by the determination of the acid-equivalents of the choleic acids and by a study of their melting- and thawing-point diagrams. The theory of these diagrams is discussed, deviations are enumerated, and their explanation is attempted.

The co-ordination number of succinic acid is 2, of adipic acid 3, of pimelic, suberic, azelaic, sebacic and enneamethylenedicarboxylic acids 4, and that of decamethylenedicarboxylic, brassylic, dodecamethylenedicarboxylic, thapsic, hexadecamethylene- and octadecamethylene-dicarboxylic acids 6.

III. STRUCTURAL ISOMERISM AND CO-ORDINATIVE VALENCY. MULTIPLE CO-ORDINATION NUMBERS.

This part deals with the relationship between chain-isomerism and co-ordination number in the desoxycholic acid compounds of the valeric and the butyric acids.

The choleic acids of these acids were prepared by direct synthesis, *e.g.* 5 g. of pure desoxycholic acid were dissolved in 20 cc. of the aliphatic acid and kept at room temperature or in the ice-chest. A crop of crystals separated and was dried *in vacuo* over KOH until constant weight and constant acid equivalent was reached. The other method of synthesis, namely in alcoholic solution (see Part II, p. 564), led to the formation of choleic acids in the case of *n*-butyric and *n*-valeric acids only. The branched isomerides, and in addition propionic acid, fail to form choleic acids in alcoholic solution, apparently because of the less stability and greater solubility of their molecular compounds, as was previously discussed in the case of glutaric acid. In every one of these instances alcohol-choleic acid was obtained as the main product.

The co-ordination number of all these choleic acids was found to be 2 except of *n*-butyric and *n*-valeric acids which combined with 4 desoxycholic acid molecules in alcoholic solution in agreement with Wieland and Sorge's results. However, a *n*-butyric-dicholeic acid was obtained by direct synthesis. Thus, two choleic acids differing from each other in their co-ordination numbers 2 and 4, are formed from butyric acid, as in the case of camphor (1 and 2).

Tiglic, sorbic and oleic acids display the same co-ordinative valency as the saturated acids of the same number of carbon atoms. Crotonic acid, however, combined with desoxycholic acid in the molecular ratio 3 : 1 while the maximal

co-ordination number of butyric acid is 4. The influence of the double bond on co-ordinative valency is greater in a small molecule and it seems to alter the character of configuration. Crystallographic measurements and especially crystal analysis by X-rays [Herzog, Kratky and Kuriyama, 1931] will increase our understanding of these conditions.

A third method for the synthesis of choleic acids consists of the addition of a water-insoluble acholic constituent to an aqueous solution of sodium desoxycholate. In the case of ethyl butyrate, the thin layer of ester placed on top of the bile salt solution solidifies within a short time. The needle-shaped crystals isolated from this layer consist of ethyl butyrate-choleic acid. It is noteworthy that the sodium ion is left behind in the aqueous phase. This is an instance of hydrolytic adsorption where the contact with a non-electrolyte phase alters the concentration of the electrolytes of the aqueous phase and eventually influences the p_H . Such processes have a bearing on the p_H -adjustment of bile which is being studied in this laboratory [Ottenberg and Kahn, 1932].

Ethyl butyrate-choleic acid prepared in this manner shows a molecular ratio 4 : 1 in agreement with Rheinboldt's rules for the co-ordination number of esters. A second ethyl butyrate-choleic acid may be obtained from ethyl butyrate *plus* desoxycholic acid in alcoholic solution. This substance contains 2 molecules bile acid for each molecule of ester.

Table II. *Acid equivalents and co-ordination numbers of choleic acids.*

Acid	Found	Acid equivalent			Co-ordination number	Method of synthesis
		Calculated				
		(N - 1)	N	(N + 1)		
Propionic	288	233.2	286.2	312.8	2	Direct
<i>n</i> -Butyric	{ 335	316.3	331.5	341.5	4	Alcohol
	292	240.2	290.9	316.3	2	Direct
<i>iso</i> Butyric	296	240.2	290.9	316.3	2	Direct
<i>n</i> -Valeric	329	319.8	334.3	343.9	4	Alcohol or direct
<i>iso</i> Valeric	294	247.2	295.6	319.8	2	Direct
Methylethylacetic	296	247.2	295.6	319.8	2	Direct
Trimethylacetic	297	247.2	295.6	319.8	2	Direct
Crotonic	313	290.2	315.8	331.1	3	Alcohol
Ethyl butyrate	{ 452	508.4	450.4	431.0	2	Na-desoxych.
	420	431.0	421.3	415.5	4	Alcohol

None of the choleic acids mentioned above shows a definite melting-point, as they all gradually lose their acholic constituent between 140° and 150°. None the less, they are definite chemical entities, as they are stable and of constant acid equivalent at normal temperature and pressure and even during prolonged storage in a desiccator over alkali hydroxide. They are odourless despite the great volatility and strong odour of their acholic constituents.

The following experiment illustrates the influence of temperature on the co-ordination number. 1149 mg. ethyl alcohol-choleic acid (molecular ratio 1 : 1) contains 120.5 mg. alcohol. When heated in an Abderhalden drying apparatus at 80° *in vacuo*, it lost 64 mg. (56 % of the alcohol) within 15 minutes.

The other half of the alcohol was tenaciously retained and the additional loss after 4 hours under the same conditions was 7.5 mg. only. When heated at 95° for 15 and 30 minutes and at 110° for 1 hour the losses were 55.5 % and 62 %. The product obtained by heating alcohol-choleic acid *in vacuo* to 80–100° has therefore the composition of an alcohol-dicholeic acid. Mörner [1924] investigated the stability of acetic acid-choleic acid at normal temperature and pressure over H₂SO₄ and CaO. The volatilisation of the acholic component in this choleic acid proceeds gradually without perceptible sudden retardation after the loss of one-half or any other fraction of its percentage of acetic acid.

SUMMARY.

The co-ordination number of isomeric butyric and valeric acids is 2 with the exception of *n*-butyric and *n*-valeric acids which display a maximal co-ordinative valency of 4.

Butyric acid and ethyl butyrate form two choleic acids each, depending on the method of synthesis with molecular ratios 4 : 1 and 2 : 1. A second ethyl alcohol-choleic acid of molecular ratio 2 : 1 is formed at elevated temperatures.

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