ON VARIATIONS OBSERVED IN THE COMPOSITION OF
SODIUM GLYCOCHOLATE PREPARED BY DIFFERENT
METHODS

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Having had occasion to determine the molecular weight of sodium glycocholate in connection with other work, the results obtained were of so unexpected a character that it was thought worth while to investigate the matter more closely.

The material employed (and referred to above) was Merck's 'sodium glycocholate.' The method of preparation as carried out by the makers is as follows :—

Ox bile is treated with a solution of lead acetate. The precipitated lead salts are decomposed by sodium carbonate and the residue extracted with alcohol. The alcohol is evaporated off and 'sodium glycocholate' results.

It is evident that 'sodium glycocholate' is at least a mixture of sodium glycocholate and sodium taurocholate. The makers themselves state that 'the substance is used for medicinal purposes only and is no chemically pure body.' From the generally accepted view, however, of the relative proportions of the constituents of ox bile, one would expect this preparation to be at least 90 per cent. pure sodium glycocholate. An examination of the substance, however, showed this to be by no means the case.

The empiric formulae and percentage composition of sodium glycocholate and sodium taurocholate usually stated are due to Strecker,¹ and are as follows :—

	Formula	Molecular weight	% N	% Na
Sodium glycocholate	$C_{26}H_{42}NO_6Na$	487	2.87	4.72
Sodium taurocholate	$C_{26}H_{44}NSO_7Na$	537	2.60	4.25

In a preparation of the mixed salts, therefore, one would expect values for the percentage composition to lie between those given in the table, but approximating more closely to those for sodium glycocholate since this is present in much greater proportion than sodium taurocholate.

The 'sodium glycocholate' having been first tested for absorbed moisture (found 2.6 per cent.) and for the presence of inorganic impurities (*e.g.* sodium chloride and sodium carbonate—which were found to be absent), the following systematic examination was carried out :—

I. Determination of the molecular weight in aqueous solution—

(a) By the lowering of the freezing point.

(b) By the rise of boiling point.

II. Determination of the molecular weight in alcoholic solution by the rise of boiling point.

III. Determination of the percentage of sodium.

IV. Determination of the percentage of nitrogen.

I.—DETERMINATION OF THE MOLECULAR WEIGHT IN AQUEOUS SOLUTION

(a) *By the lowering of freezing point of water :—*

Mass of the solvent = 22.5 grams			
Mass of 'sodium glycocholate' in grams	Freezing point (Beckmann therm.)	Δ	Molecular weight
0.0	2.78	—	—
0.1125	2.71	0.07	134
0.5332	2.46	0.32	139
0.8664	2.26	0.52	139

The mean molecular weight in water is therefore 137.

1. Strecker, *Liebig Ann. d. Chem.*

(b) Molecular weight by the elevation of boiling point :—

Mass of solvent = 26.30 grams

Mass of 'sodium glycocholate' in grams	Boiling point (Beckmann therm.)	Rise of boiling point	Molecular weight
0.0	1.918	—	—
0.1765	1.940	0.022	158
0.5265	2.009	0.091	114 ?
0.9205	2.049	0.131	140

First and last determination, mean, 149

The values obtained for a molecular weight by the elevation of boiling point when *water* is the solvent can scarcely be taken as conclusive in themselves, owing to the comparative difficulty of carrying out a determination with this solvent. The above results must, therefore, be regarded as surprisingly good corroboration of the more trustworthy determinations obtained by the freezing point method. Since we are dealing with an alkali salt in aqueous solution it is evident that dissociation has taken place, and the above solutions are sufficiently dilute to cause approximately complete dissociation. Assuming the substance to be a mono-sodio derivative we obtain the result that the *undissociated* molecular weight is 278. To check this, a determination was carried out in alcohol.¹ This requires great care in order to get rid of traces of moisture both in the alcohol and the bile salt—for this latter is exceedingly hygroscopic.

II.—MOLECULAR WEIGHT OF THE 'SODIUM GLYCOCHOLEATE' BY THE ELEVATION OF THE BOILING POINT OF ALCOHOL

'Sodium glycocholate' in grams	Rise of boiling point	Molecular weight
0.2208	0.037	282
0.2820	0.047	283

Mean, 283

1. Solutions of soaps in alcohol give molecular weights corresponding to non-dissociation. It was therefore thought (and the results justify the assumption) that no dissociation of the bile salt would take place in this solvent.

The good agreement between the results obtained for the substance in the undissociated state in alcohol and that calculated from the determination in aqueous solution, points apparently to the possibility of error in the usually accepted empirical formula and molecular weight.

III.—PERCENTAGE OF SODIUM IN 'SODIUM GLYCOCHOLEATE'

The sodium was estimated in the ordinary manner by first incinerating the substance and finally estimating as sulphate.

The following results were obtained :—

'Sodium glycocholate' in grams	Sodium sulphate in grams	Percentage of sodium
[0.7585	0.1894	8.09]
1.0491	0.2813	8.6
1.0125	0.2702	8.6

Mean, 8.6

Assuming the substance to be a *mono*-sodium derivative—

A molecular weight of 283 requires 8.12 % sodium

"	"	487	"	4.72	"
"	"	537	"	4.28	"

The above results seem to afford fairly conclusive evidence in favour of the smaller value for the molecular weight. An alternative hypothesis, however, might be possible. The formulae of the bile acids may be mainly according to those of Strecker, but the acids may be dibasic and hence their salts may contain two sodium atoms, viz., $C_{26}H_{41}NO_6Na_2$ and $C_{26}H_{49}NSO_7Na_2$. This would almost double the sodium percentage, which would be in agreement with that found. Also a disodium salt in dilute aqueous solution would give rise to three ions approximately; thus yielding a molecular weight— $3 \times 140 = 420$. This, although a much higher value, differs considerably from the accepted value. The fact that this is at complete variance with the actual value observed in alcohol renders the hypothesis practically untenable.

To further test the values obtained for the molecular weight the percentage of nitrogen was determined by Kjeldahl's method.

IV.—NITROGEN PERCENTAGE

'Sodium glycocholate' in grams	Percentage nitrogen
0.832	1.03
1.087	0.90
Mean, 0.965	

Assuming one nitrogen atom in the molecule of salt—

The nitrogen percentage required for molecular weight 283 is 4.94

"	"	"	"	487 " 2.87
"	"	"	"	537 " 2.60

The experimental values for the nitrogen are therefore at utter variance with all the molecular weights, the discrepancy being most marked in the case of the molecular weight actually determined.

All this conflicting evidence can be explained only by assuming that either Strecker's formula is quite wrong, or that there are other substances present whose existence has been overlooked.¹

In fact if we assume that over 50 per cent. of the 'sodium glycocholate' consists of sodium salts of nitrogen-free acids of molecular weight much smaller than sodium glycocholate, the anomalies observed would be accounted for.

To form an approximate idea of the proportion of true sodium glycocholate in 'sodium glycocholate' a quantity of the latter was hydrolysed with alkali, and the cholic acid produced was estimated by Lassar-Cohn's² quantitative method. The yield was 3 grammes of cholic acid from 8.5 grammes of 'sodium glycocholate,' the theoretical yield being 6.4 grammes of cholic acid. This points to the percentage of true sodium glycocholate being about 50 per cent. of the material.

To settle the question, a quantity of ox bile was obtained and from it (1) the bile salts were prepared by Plattner's method, and (2) 'sodium glycocholate' prepared according to Merck's method. The results of the analyses of the substances prepared by these two methods are given below.

1. NOTE: It may be mentioned that the presence of sodium oleate was tested for by formation of the lead salt and extraction with ether—negative result.

2. Lassar-Cohn, *Ber. d. deut. chem. Gesell.*, 26, Part I, p. 146 (1893).

(I) THE BILE SALT PREPARED ACCORDING TO PLATTNER'S METHOD

This method, as is well known, simply consists in evaporation of the bile on the water bath and extraction of the residue with alcohol, thereby removing the sodium glycocholate and sodium taurocholate.

(a) *Determination of the molecular weight of the bile salt thus obtained.*

Mass of solvent (water) in grams	Mass of solute in grams	Δ	Molecular Weight weight
27.70	0.5890	0.15°	262
31.55	0.5830	0.13°	263
			Mean, 262.5

Assuming that the substance is about 80 per cent. dissociated at this dilution in water, we find for the undissociated molecular weight the value 472. This is somewhat low but is in fair agreement with the value 487 required for the sodium glycocholate according to Strecker.

(b) *Determination of Nitrogen (Kjeldahl).*

Grams. of substance	N in grams	Nitrogen percentage
1.123	0.02856	2.55

The value required for Strecker's sodium glycocholate is 2.8 per cent.

(c) *Determination of the sodium content.*

Substance in grams	Weight of sodium sulphate	Sodium percentage
1.1908	0.1931	5.2

The value required for sodium glycocholate is 4.7 per cent.

This examination of the product obtained by Plattner's method confirms the usually accepted formulae and constitution of sodium glycocholate *when this body is obtained pure*. The small discrepancies noted above can again be explained by assuming the presence in small quantities of that substance (or substances) which appear in large quantities in Merck's preparation.

(2) ANALYSIS OF PRODUCT OBTAINED BY MERCK'S METHOD OF PREPARATION

A quantity of fresh ox bile was treated according to Merck's method, the product yielding the following results on analysis :—

Percentage of Sodium :

Substance in grams	Sodium sulphate in grams	% Na
1.1412	0.2152	6.11
1.0097	0.1902	6.10
		Mean, 6.1

Percentage of Nitrogen :

Substance in grams		Percentage nitrogen
0.4426	...	2.66
0.8979	...	2.40
		Mean, 2.53

We have here again in the case of the sodium content the discrepancies already noted. The values for nitrogen, however, as will be seen, lie not far from those for pure sodium glycocholate. There can be little doubt but that we are in all cases dealing with the same contaminating substance, though the amount of this substance appearing in the final product depends on the conditions of manipulation.

The most unexpected thing about this substance is the exceedingly large amount of it evidently present in ox bile ; especially when one remembers that the exhaustive work of Lassar-Cohn¹ and others point to the fact that in ox bile the sodium glycocholate and sodium taurocholate are present in much greater proportion than all the other salts together. For example, after hydrolysis of the glycocoll and taurine derivatives, Lassar-Cohn¹ found that ' 100 litres of ox bile yield 4.790 grammes of cholic acid and 405 grammes of other acids.'

1. Lassar-Cohn, *Ber. d. deut. chem. Gesell.*, 26, Part I, p. 146, (1893).

The important question is, of course—what is this hitherto undetected substance or substances? In this connection it may be mentioned that an attempt was made to ascertain whether there was any formation of double salt between the pure sodium glycocholate and some of the precipitants or products of precipitation. Thus, a quantity of the material obtained by Plattner's method was dissolved in water and treated with excess sodium acetate. Lead acetate was then added, and the product decomposed with sodium carbonate and evaporated to dryness. In the presence of sodium acetate it was thought that possibly a double salt of the form $(C_{26}H_{42}NO_6Na + C_2H_3O_2Na)$ might have been produced and removed by the final extraction with alcohol. The substance obtained, however, proved to be simply sodium glycocholate, an analysis of the material yielding 5.0 per cent of sodium.

Referring to Merck's preparation of 'sodium glycocholate,' the analyses already given allow one to calculate the mean molecular weight of the substance (or substances) whose presence has been suspected.

Thus assuming that the unknown body is nitrogen free, the determination of nitrogen recorded leads at once to the conclusion that Merck's preparation consists of 35.7 per cent. sodium glycocholate and 64.3 per cent. unknown substance (or substances).

Now if

M = the undissociated molecular weight of pure sodium glycocholate = 487

M' = the mean undissociated molecular weight of the mixture = 282

m = the mean undissociated molecular weight of the unknown substance (or substances)

it follows that :

$$\frac{100}{M'} = \frac{35.7}{M} + \frac{64.3}{m}$$

whence $m = 227$, and as this is a sodium salt or salts (presumably a mono sodium derivative) the mean molecular weight of the acid (or acids) is 205.

In the saturated fatty series of acids the following members occur—

Lauric acid	$C_{12}H_{24}O_2$	molecular weight	200
Tridecylic „	$C_{13}H_{26}O_2$	„ „	214
Myristic „	$C_{14}H_{28}O_2$	„ „	228

If we are dealing with a single substance in the case of the unknown salt the value obtained for the molecular weight points strongly to the presence of sodium laurate. It is much more probable, however, that we are really dealing with more than one body. In this connection one may mention the acids whose salts are present in ox bile according to Lassar-Cohn,¹ viz., glycocholic, taurocholic, stearic, palmitic, oleic, myristic and some ‘amorphous acids.’ According to Lassar-Cohn, as already stated, the first two acids are present in amount about 10 times that of the remaining acids added together. It is very suggestive, however, that myristic acid should have been found as a constituent of ox bile, although Lassar-Cohn’s analyses point to its being present in extremely small quantity.

Myristic acid, as already stated, has a molecular weight of 228, which is not so very far removed from the mean value 205 of the unknown constituent. And indeed this mean value would be readily realised by myristic acid containing a small proportion of some acid of much lower molecular weight, as for example valeric acid (molecular weight 102), or caproic acid (molecular weight 116), both of which are known to be produced in animal metabolism.

SUMMARY

The result of the foregoing investigation has been to confirm Strecker’s formula for sodium glycocholate when this is obtained in the pure state. At the same time, evidence is adduced to show that in the ordinary methods of extraction of mixed bile salts (*i.e.* sodium glycocholate + sodium taurocholate) one does *not* always obtain a mixture consisting entirely (or almost entirely) of these two salts.

1. Lassar-Cohn, *loc. cit.*

The resulting product appears to contain as well, varying amounts of the sodium salts of nitrogen-free fatty acids of very much smaller molecular weight which may, in certain cases, be present to the extent of over 50 per cent. of the 'mixed bile salt' preparation.

It is suggested that sodium myristate is the chief representative of these lower fatty salts, this body being probably present in ox-bile to a very much greater extent than the analyses of Lassar-Cohn seem to show.