3. The method is readily applicable to the micro scale (1.0–10 μ g. of nitrogen), when no special apparatus is required.

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Preparation of Highly Potent Intrinsic Factor Mucoprotein

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Knowledge of the chemical identity of intrinsic factor (Castle, 1929) remained incomplete for a relatively long time. It was realized to be a protein because it was thermolabile, precipitated by ammonium sulphate and retained in an ultrafilter. Only recently, however, has it been shown to be a mucoprotein. Evidence for this has emanated from several laboratories. Glass, Boyd, Rubinstein, Vigals & Chevalley (1951) noted that the glandular mucoprotein fraction from gastric juice had a number of properties similar to intrinsic factor, which they suggested might belong to the same chemical class. Preparations of glandular mucoprotein from human gastric juice produced clinical improvement when administered orally with cyanocobalamin to patients with pernicious anaemia (Glass, 1953). This activity was so low when compared with other clinically potent preparations that it could only be inferred that glandular mucoprotein and intrinsic factor were not the same (Welch & Heinle, 1951; Castle, 1953; Schilling, 1954; Williams, Ellenbogen & Esposito, 1954). The latter, therefore, was either an impurity in the glandular preparations or part of a complex and not necessarily a mucoprotein. Also preparations of glandular mucoprotein made at different times varied in chemical composition (Glass, 1955).

Independently, in our own laboratory, evidence was produced which pointed to the probable

mucoprotein nature of intrinsic factor (Latner, Ungley, Cox, McEvoy-Bowe & Raine, 1953). By means of a preparative paper-strip electrophoresis technique, it was possible to fractionate human gastric juice and to demonstrate that the segment of paper containing the intrinsic factor activity stained with periodic acid-Schiff's reagent, a histological stain used to demonstrate mucopolysaccharides and mucoproteins. Later the electrophoresis technique was used to fractionate a crude concentrate of pig intrinsic factor (Latner & McEvoy-Bowe, 1953; Latner, 1955) and to obtain active solid material. The potency tested by the faecal-excretion test with radioactive cyanocobalamin (Heinle, Welch, Scharf, Meacham & Prusoff, 1952; Callender, Turnbull & Wakisaka, 1954) indicated (Latner, Merrills & Raine, 1954a) that the material was more active than other preparations then described. Chemical analysis demonstrated that the fractions obtained in this way were largely composed of mucoprotein.

We have also briefly described another technique of preparation of potent mucoprotein material from dried pig-stomach powder (Latner, Merrills & Raine, 1954b). This proved to be of the same order of activity as our best material prepared by electrophoresis. Moreover, it was homogeneous after paper-strip electrophoresis at different pH levels, as well as in the ultracentrifuge (Latner, 1955). On

the basis of these findings we concluded that the material was pure; we now know this to be incorrect.

More recently, Williams *et al.* (1954) prepared a mucopolypeptide in a state of high purity which had well-marked intrinsic factor activity when given to patients with pernicious anaemia in relapse.

Recently, Andresen (1954) reported the preparation of a complex of a mucoprotein with cyanocobalamin which was clinically active in patients with pernicious anaemia. The complex was at first thought to be pure because of its behaviour in the ultracentrifuge. Dr Andresen very kindly supplied us with some of his material, and we found it did not behave as a pure substance during passage through an ion-exchange column or on paper electrophoresis. Observations with the classical electrophoresis technique also gave evidence of heterogeneity (Andresen & Skouby, 1955), and it was claimed that further purification yielded the pure complex. Diffusion data seemed to support this claim (Holdsworth & Otteson, 1955). Unfortunately, the pure substance had not been tested for intrinsic factor activity.

Further investigation of our own material (Latner *et al.* 1954*b*) by ion-exchange column techniques also gave evidence that it was not a single substance. This has led to the development of a preparative column technique which has resulted in an even more active preparation (Latner, Merrills & Raine, 1955). These new findings have somewhat delayed detailed publication of our methods of preparation, but we now feel we have carried the fractionation sufficiently far to merit such a report.

METHODS

Biological activity. The potency of the material was assayed by the faecal-excretion technique (Heinle *et al.* 1952; Callender *et al.* 1954). Radioactive cyanocobalamin (0.5 μ g.) was given to a patient with pernicious anaemia in remission, and the amount of radioactivity present in the faeces estimated over the next week or so, until a specimen was obtained which contained only 1% of the original dose. In this way it was possible to determine the amount of vitamin absorbed. The test was then repeated with the same amount of radioactive vitamin and a known amount of the intrinsic factor preparation. The increase in intestinal absorption over that with the vitamin alone was a measure of the intrinsic factor activity.

Blood-group substance activity. The agglutination-inhibition tests (Morgan & King, 1943) were carried out on A₁ erythrocytes and anti-A serum with blood-group substance A as standard.

Column chromatography. Amberlite IRC50 analytical-grade ion-exchange resin was dried overnight in a hot-air oven at 45°, to render the particles brittle, and ground in a ball mill until it would pass through a 100-mesh sieve. The sieved powder was stirred with water at room temperature and allowed to settle for 15 min., and the supernatant cloudy fluid decanted off. By repeating this procedure

twice more it was possible to remove most of the material which had been ground too finely. The resin was then purified according to the general directions given by Hirs, Moore & Stein (1953).

The resin was now equilibrated with a solution containing 0.2 g. mol. of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and 3 ml. of *N*-NaOH/l., giving a pH of 4.9 (buffer A). A short column of resin, 3 cm. $\times$ 3 cm., supported on a no. 4 porosity sintered-glass disk, was used for the preparative work. The column was formed by pouring a slurry of resin with the buffer in four portions, air pressure of 10 lb./sq.in. being applied at the top of the column after each addition in order to pack the resin. Material for chromatography was dissolved in the buffer A for application to the column and initial elution was also carried out with buffer A. Later fractions were eluted with a solution at pH 5.2 containing 1.0 g. mol. of $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ and 70 ml. of *N*-NaOH per litre (buffer B). For both eluent buffers, the A.R. phosphate used was recrystallized twice from water, in order to bring the ultraviolet absorption to a level low enough to allow estimation of the protein content of the fractions by absorption at 275 μ .

Electrophoresis. The paper-electrophoresis technique has already been described (Latner, 1952). The staining technique, however, has been elaborated to demonstrate proteins and mucoproteins on the same strip. The strips previously stained with Naphthalene Black 12B 200 (I.C.I. Ltd.) were scanned (Latner, Molyneux & Rose, 1954) and photographed. Each stained strip was then immersed in 0.5% periodic acid for 5 min. with occasional shaking and rapidly washed in 70% (v/v) ethanol. The strip was now covered with a reducing solution of 5% $\text{Na}_2\text{S}_2\text{O}_3$ and 5% KI in 0.01 *N*-HCl, left for 8 min. and again rapidly washed in 70% (v/v) ethanol. It was now immersed in Schiff's reagent (Lillie, 1948) for 20 min. and then placed for 2 min. in 0.1 *N*-HCl containing 0.01% of $\text{Na}_2\text{S}_2\text{O}_4$; after two rapid washings with acetic acid it was dried in air. The mucoproteins appeared as blue-purple bands. During the procedure the original Naphthalene Black staining was dissolved out completely.

Paper chromatography. Ascending two-dimensional paper chromatography was carried out by the technique of Datta, Dent & Harris (1950). For the amino acids, the solvent systems phenol/water/ NH_3 and collidine-lutidine/water/diethylamine were used. For the sugars, the ammonia and diethylamine were omitted. The temperature was controlled at 22°. For amino acids, chromatography was carried out on 0.25 mg. of material after hydrolysis in sealed tubes with 6 *N*-HCl for 10 hr. at 100°. The amino acids were detected by dipping the papers in an acetone solution of ninhydrin (Toennies & Kolb, 1951). For sugars, chromatography was carried out on 1 mg. of material after hydrolysis in sealed tubes with 0.5 *N*-HCl for 12 hr. at 100°. The sugars were detected by dipping the papers into a solution of aniline hydrogen phthalate in acetone.

Total nitrogen. Determined by the Kjeldahl method with the micro-diffusion apparatus of Conway (1939).

Hexosamine. After hydrolysis in sealed tubes with 3 *N*-HCl at 100° for 15 hr., the Belcher, Nutten & Sambrook (1954) modification of the method of Elson & Morgan (1933) was used.

Tyrosine and tryptophan. Estimated from the ultraviolet absorption in 0.1 *N*-NaOH, by means of the equations of Goodwin & Morton (1946).

Fucose. Determined by the Cy R 10 reaction of Dische & Shettles (1948).

Preparations

All aqueous solutions were maintained at 5°, either in a cold room or in a refrigerated centrifuge.

Preparation of crude concentrate. This was prepared from dried stomach powder (Armour) by a technique similar to one already described (Williams *et al.* 1954). The extract of the dried powder in 2% (w/v) NaCl was, however, immediately raised to 55% saturation with $(\text{NH}_4)_2\text{SO}_4$. The resultant precipitate was centrifuged off and dialysed in running water until free from ammonium salts. The dialysed solution was freeze-dried and gave a powder which was a mixture of the materials obtained separately by Williams *et al.* (1954) at 33 and 55% saturation.

Preparation of intrinsic factor mucoprotein. A smooth suspension of the crude concentrate was obtained by adding 200 ml. of a 2% NaCl solution in small successive portions to 10 g. of the powder whilst stirring. The suspension was allowed to stand for 30 min., and then centrifuged in an angle centrifuge (M.S.E.) at 13000 rev./min. (20000 *g*) for 1 hr. The resultant clear supernatant liquid (174 ml.) was decanted off and fractionated by a procedure almost identical with that of Prusoff, Welch, Heinle & Meacham (1953). A.R. $(\text{NH}_4)_2\text{SO}_4$ (45.5 g.) was added in small portions at a time, with constant stirring to produce a saturation of approximately 35%. After standing for 30 min. the mixture was again centrifuged at 20000 *g* for 30 min. The supernatant liquid (184 ml.) was decanted off and a further 23 g. of $(\text{NH}_4)_2\text{SO}_4$ added to it in small portions with stirring, thus bringing the percentage saturation to approximately 55%. The precipitate which formed was separated by high-speed centrifuging for 30 min., dissolved in 10 ml. of distilled water, and the solution ultrafiltered through a sac made from Visking membrane until its volume was reduced to approximately one-fifth. Distilled water (8 ml.) was now added with mixing and ultrafiltration continued until the liquid in the sac was again small in volume. It was demonstrated that during the last stage of ultrafiltration very little ammonia was present in the ultrafiltrate. The residue in the ultrafilter was again diluted to 10 ml. with distilled water and the heavy precipitate which had formed during ultrafiltration removed by high-speed centrifuging. It was known from a parallel experiment that the supernatant contained material which showed only a single boundary in the ultracentrifuge (Fig. 1) after removal of very small amounts of precipitate at pH 2.0 and 5.5. Since there was a possibility that the lower pH might cause some chemical change, and since the precipitates obtained in the parallel experiment were almost negligible in quantity, it was decided to omit these precipitations. $\text{NaH}_2\text{PO}_4 \cdot 2\text{H}_2\text{O}$ (0.31 g.) was dissolved in the supernatant liquid and the pH adjusted to 4.9 by the addition of a few drops of *N*-NaOH. This solution was applied to the column of IRC50, already equilibrated with buffer solution A. The rate of flow was adjusted so that the material took approximately 45 min. to soak into the column. Buffer solution A was then applied as eluent at a flow rate of 10 ml./hr., and the effluent fractions were collected in volumes of 10 ml. by means of an automatic fraction collector. After 26 fractions had been collected, the eluent solution was changed to buffer solution B at pH 5.2. The effluent was collected for a further 24 fractions, each 10 ml. in volume. The absorption at 275 μm . was determined for each fraction in a spectrophotometer (Unicam SP. 500).

In this way, the results shown in Fig. 2 were obtained. It will be seen that part of the material applied to the column appeared in the effluent soon after the hold-up volume, and that a second peak appeared during elution with buffer solution B. This peak covered fractions 29–36, with a little trailing. Fractions 2, 3 and 4 were pooled, saturated with $(\text{NH}_4)_2\text{SO}_4$, allowed to stand for 30 min. in the cold room and the resultant precipitate was centrifuged off. This was dissolved in 10 ml. of distilled water, and concentrated to a small volume by ultrafiltration through Visking membrane. A further 10 ml. of distilled water was added and ultrafiltration continued. This process of ultrafiltration and

Fig. 1. Sedimentation diagrams of material similar to that applied to column. From above downwards, 20, 40 and 60 min. after full speed, 54000 rev./min. (1% solution in phosphate buffer, pH 8.0; $I = 0.2 + 0.15\text{M-NaCl}$).

Fig. 2. Extinctions at 275 μm . of fractions (10 ml.) from column of IRC 50 (3 cm. $\times$ 3 cm.; hold-up volume = 9.0 ml.).

dilution was repeated until the ultrafiltrate no longer contained ammonia demonstrable by Nessler's reagent. The small residue in the ultrafilter was made up to 10 ml. with distilled water, centrifuged until completely clear, and freeze-dried. This yielded 60 mg. of a white powder, which we shall refer to as 'peak one material'.

Fractions 30-34 inclusive were similarly combined, precipitated with $(\text{NH}_4)_2\text{SO}_4$, dissolved in distilled water, and ultrafiltered. After being clarified by centrifuging and freeze-drying, the ultrafilter residue yielded 38 mg. of an off-white powder. Preliminary observations (Latner, 1955) had indicated that this would have intrinsic factor activity, and it was decided to attempt to fractionate it further by precipitation with cold ethanol. Throughout this fractionation the temperature was maintained as near to -10° as possible. 25 mg. of the material was dissolved in 2.5 ml. of distilled water, cooled in ice and rapidly poured with stirring into an equal volume of cold 40% (v/v) ethanol. The resultant mixture was allowed to stand for 1 hr., during which time a precipitate appeared almost immediately and gradually became more dense. The precipitate was centrifuged off in the refrigerated-angle centrifuge for 30 min. at 10 000 rev./min., and the supernatant rapidly decanted into another tube. The precipitate was washed with 1 ml. of cold 20% (v/v) ethanol, centrifuged off, and freeze-dried, yielding 14 mg. of a faintly buff-coloured powder. This material will be subsequently referred to as 'intrinsic factor mucoprotein'.

To the supernatant solution from the original ethanol precipitation, a further 3.3 ml. of cold ethanol (final concentration 50% v/v) was gradually added with stirring. The solution was allowed to stand for 30 min., during which time a precipitate gradually formed, which was centrifuged off at low temperature, washed with 1 ml. of cold 50% ethanol, again centrifuged, and freeze-dried, yielding 3 mg. of a faintly buff-coloured powder. The supernatant from the 50% ethanol precipitation was also freeze-dried, yielding 1 mg. of white powder.

RESULTS

Biological activity

The results obtained in the faecal-excretion tests are shown in Table 1. The 'peak one material', not shown in Table 1, proved completely inactive. A dose of 0.5 mg. of the intrinsic factor mucoprotein produced a mean increase of absorption of $0.15 \mu\text{g.}$ of cyanocobalamin. For comparison, results obtained in the same patient with material which had

previously been tested clinically (Thompson & Latner, 1955) are also shown. At a dosage of 2 mg. this gave a mean increase in absorption of $0.25 \mu\text{g.}$ Provided that the result obtained is below the maximum of which the patient is capable, the increase in absorption is proportional to the dose of intrinsic factor material administered (Baker & Mollin, 1955; Callender & Evans, 1955). Our patient had a maximum absorption of $0.4 \mu\text{g.}$ of cyanocobalamin under the conditions of the test. Therefore 0.5 mg. of the clinically tested material would have brought about a mean increased absorption of $0.06 \mu\text{g.}$ This is approximately two-fifths of the figure obtained with 0.5 mg. of the intrinsic factor mucoprotein. The latter was, therefore, approximately two and a half times as active as our earlier clinically tested material.

Blood-group substance activity

The blood-group substance activity of the intrinsic factor mucoprotein proved to be extremely low. It was only 3% or less of the activity of blood-group substance A.

Paper electrophoresis

The results of paper electrophoresis of the intrinsic factor mucoprotein (0.3 mg.) in 0.05M sodium phosphate buffer at pH 8.0 and 6.4 are reproduced in Fig. 3. For comparison the results obtained with equal amounts of the Andresen (1954) protein-cyanocobalamin complex and pure γ -chymotrypsin are also shown. The latter are included to show that the spread of the intrinsic factor mucoprotein band at each pH level was not more than would be expected from a protein of similar composition (cyanocobalamin complex) or of low molecular weight (γ -chymotrypsin). The paper strips relating to intrinsic factor materials were stained for mucoprotein, but the resultant picture differed only in colour from that obtained with Naphthalene Black staining alone. The chymotrypsin was stained with Naphthalene Black only. It will be seen that at both pH values the intrinsic factor mucoprotein gave a major single band appearing on the cathode side of the line of application. There was also

Table 1. *Results obtained with intrinsic factor mucoprotein (IFM) and clinically tested material (CTM) in faecal-excretion tests with radioactive cyanocobalamin (B_{12})*

Material administered	Absorption of B_{12} ($\mu\text{g.}$)	Increase due to intrinsic factor ($\mu\text{g.}$)
0.5 $\mu\text{g.}$ of B_{12}	0.03 0.07 0.05	—
0.5 $\mu\text{g.}$ of B_{12} + 0.5 mg. of IFM	0.21 0.19	0.15
0.5 $\mu\text{g.}$ of B_{12} + 2.0 mg. of CTM	0.32 0.27	0.25

a scarcely detectable narrow band just on the anode side. Judging from the intensity of staining, this represented at the most some 5–10% of the original material. It is interesting to note that the intrinsic factor–cyanocobalamin complex contained a relatively large amount of mucoprotein impurity.

Although the 50% alcohol precipitate seemed nearly as biologically active as intrinsic factor mucoprotein, its behaviour on electrophoresis indicated an appreciably greater proportion of impurity.

Fig. 3. Results of paper-strip electrophoresis in 0.05M sodium phosphate buffer (Whatman no. 100; 16 hr.; 120v). (a) Intrinsic factor mucoprotein at pH 6.4; (b) intrinsic factor mucoprotein at pH 8.0; (c) cyanocobalamin–protein complex at pH 8.0; (d) chymotrypsin at pH 8.0.

Fig. 4. Two-dimensional chromatogram obtained with carbohydrate constituents of intrinsic factor mucoprotein.

Chemical analysis

The chromatogram obtained from the carbohydrate constituents of intrinsic factor mucoprotein is illustrated in Fig. 4. Fucose, galactose and mannose were detected as well as a fourth spot which was either glucosamine, or chondrosamine, or both. The identification of these spots has been confirmed by adding known sugar solutions separately to samples of hydrolysate. The hexosamine spot gave the Elson & Morgan reaction.

The amino acids detected were: alanine, serine, glycine, threonine, valine, methionine, leucine/isoleucine, cysteine, tyrosine, phenylalanine, proline, arginine, lysine, aspartic and glutamic acids. There was possibly a spot in the position which would have been occupied by hydroxyproline. This was so faint, however, that we were not really sure of its presence. In any case we feel that if present in such small amount it may have arisen from a small quantity of a collagen derivative present as a contaminant.

Quantitative analysis of the intrinsic factor mucoprotein gave the results shown in Table 2. The figures have been corrected to dry weight. The values for fucose, tryptophan and tyrosine are consistent with a calculated minimum molecular weight of between 12000 and 16000. In the ultracentrifuge, material similar to that fractionated on the column showed only a single component (Fig. 1). Even after a prolonged period of sedimentation, 1.5 hr. at approximately 250 000 g, the boundary had not separated completely from the meniscus and consequently no attempt was made to calculate a sedimentation coefficient. The behaviour is consistent with a molecular weight of the order of 15000 or rather less.

DISCUSSION

The intrinsic factor mucoprotein we have described appears essentially homogeneous when tested by paper-strip electrophoresis at different pH levels, as

Table 2. Analytical data of intrinsic factor mucoprotein

Moisture content was determined by heating at 105° for 18 hr.; other analyses are corrected to dry weight.

Component	Content (%)	Calculated minimum mol.wt.
Moisture	3.5	—
Sodium (flame photometer)	<1.0	—
Potassium (flame photometer)	Not detected	—
Nitrogen	11.2	—
Hexosamine	5.8	—
Tyrosine	2.4	7 500
Tryptophan	1.3	16 000
Fucose	1.4	12 000

well as when examined by column chromatography. Since it was prepared from a fraction not obviously heterogeneous in the ultracentrifuge, the isolated mucoprotein is probably of similar homogeneity; an assumption supported by the fact that there has been no breakdown on the column of IRC 50, since the molecular weight has not been changed by this procedure. Unfortunately, the small amount of material still in our possession precludes observation of its sedimentation behaviour.

Although it would appear that the mucoprotein is now highly purified, we hesitate to claim the exact degree of purity, since we were previously (Latner *et al.* 1954*a*) misled by the physical behaviour of similar material. It is interesting to note that in contrast to the earlier (Latner *et al.* 1954*b*) material the blood-group substance activity of the present intrinsic factor mucoprotein is slight. It would appear that the activity demonstrated in the earlier preparation was mainly, if not entirely, due to contamination. Other workers have demonstrated that the blood-group substance activity of potent concentrates does not run parallel to intrinsic factor activity (Baal, Wijmenga & Hart, 1955).

Our earlier fraction gave minimal clinical responses after ten daily doses of 1 mg., given together with 5 µg. of cyanocobalamin, and maximal responses when the daily dose of intrinsic factor material was increased to 4 mg. (Thompson & Latner, 1955). We have evidence that maximum response was also obtained when the daily dosage of the concentrate was reduced to 3 mg. The faecal-excretion tests with radioactive cyanocobalamin indicated that this clinically tested material had two-fifths of the activity of intrinsic factor mucoprotein. In so far as it is possible to calculate, one could anticipate therefore that intrinsic factor mucoprotein would produce maximal clinical response, under our conditions of testing, at a daily dose of the order of 1.0 mg. This is of the same order of activity as that found for the complex prepared by Andresen (1954).

That the mucoprotein is now several times more active than our previous best material does not necessarily mean that the latter has been concentrated approximately threefold. At various stages of purification, fractions have been found to be more biologically active than their yield would lead us to expect. It seems highly probable that one or more of the impurities in intrinsic factor concentrates inhibit cyanocobalamin absorption in the intestine. It is known that the vitamin combined with certain proteins may be rendered unavailable to an animal species from which the proteins were not derived (Coates *et al.* 1955). It is interesting to note that the 'peak one material' that we obtained by column chromatography had no intrinsic factor

activity but inhibited absorption of cyanocobalamin in the rat (Latner & Merrills, unpublished observations). This type of inhibition rather than a species specificity of intrinsic factor might explain the depression of intestinal absorption of the vitamin in this animal produced by human gastric juice and a pig intrinsic factor concentrate (Clayton, Latner & Schofield, 1955).

Three substances with intrinsic factor activity have now been prepared from pig stomach in a state approaching purity. The molecular weight of the mucopolypeptide (Williams *et al.* 1954) is of the order of 5000, that of the cyanocobalamin-protein complex 79 000 (Holdsworth & Otteson, 1955) and that of our own mucoprotein is approximately 15 000. It is possible that intrinsic factor activity is possessed by a number of different substances in the pig-stomach mucosa, but it is by no means unlikely that these substances may be derived from each other. The mucopolypeptide may well be produced from the mucoprotein by the splitting off of the protein moiety. This would be in accord with the fact that the hexosamine content of each of the two substances bears an inverse relationship to the molecular weight. The intrinsic factor mucoprotein has almost the same hexosamine content as the complex and very similar biological activity. This suggests that the mucoprotein might be the structural unit of the protein moiety of the complex.

SUMMARY

1. A mucoprotein has been obtained from dried pig-stomach powder by a technique involving ammonium sulphate precipitation, chromatography on a column of IRC50 and fractionation with cold ethanol.
2. It was homogeneous after paper-strip electrophoresis at pH 6.4 and 8.0, and the fraction from which it was prepared was almost homogeneous in the ultracentrifuge.
3. The mucoprotein would appear to have a molecular weight of approximately 15 000.
4. A dose of 0.5 mg. proved effective in the faecal-excretion test with radioactive cyanocobalamin.

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Species Differences in the Conjugation of Free Bile Acids with Taurine and Glycine

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Several investigations indicate that no free bile acids are normally found in the bile, but only acids conjugated with taurine and glycine (Haslewood & Sjövall, 1954; Norman, 1954). It is also known that the bile acids are reabsorbed mainly in the conjugated form from the intestine (Norman, 1955; Portman & Mann, 1955).

In different species the bile acids are found conjugated with taurine and glycine in various proportions (Haslewood & Sjövall, 1954; Haslewood, 1955). Thus rabbit and pig bile contain almost exclusively glycine-conjugated acids, whereas rat

and bird bile contain only taurine-conjugated acids. In man and primates both conjugates are found in variable proportions.

Bergström & Gloor (1954) found that mainly taurine-conjugated acids were formed in human-liver homogenates when both taurine and glycine were available for conjugation with free bile acids. The same preference for taurine has been demonstrated in rat-liver homogenates (Bremer, 1955a). The enzyme system of rat liver performing the conjugation of free bile acids with taurine and glycine is confined to the microsomal particles of the liver cell (Bremer & Gloor, 1955; Bremer, 1956). The isolated microsomes perform this conjugation

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