SECRETION OF ORGANIC ANIONS IN THE FORMATION OF URINE AND BILE

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It is obvious to anyone even superficially acquainted with the fields of urine and bile formation that not only has our knowledge in these two fields progressed at very different rates, but also that the attention these fields receive from research workers is very unequal. The reasons are at least partly obvious. Urine is one of the biological fluids most easily obtainable. Probably more important, however, is the circumstance that it is formed by an organ the anatomy of which tempts the observer to divide the process of urine formation into discrete operations. No similar incentive to thought and formulation of hypotheses exists with regard to bile formation; and it is probably chiefly for this reason that knowledge and methods have progressed more rapidly in the field of renal physiology and have only subsequently been applied to the study of bile formation. Usually, however, these fields are treated as separate, even when their similarity would invite a joint treatment. An attempt to review a limited part of the process of urine formation together with its counterpart in bile formation necessarily leads to considerable repetition. As this circumstance points to the close relationship of the essential problems in the two cases it is hoped that these deficiencies will be overlooked. The treatment chosen has made it natural to put emphasis on certain aspects of the secretion of organic acids in the kidney, because in the reviewer's opinion they may shed some light on bile formation, whereas other aspects, in themselves perhaps more important, have been neglected.

There is, however, an abundance of competent reviews on renal physiology, and any one interested can easily find a guide to any special topic. Smith's monograph (131) surveys the whole field in such a way as to provide a suitable starting point. References to papers previous to 1950 are therefore cited here only in special cases, a procedure further justified by the fact that the more limited topic of renal tubular transport was reviewed at about the same time by Taggart (140) and Beyer (13) and that the latter's review in this journal contains a very considerable list of references. Almost yearly reviews in the Annual Review of Physiology have usually given a very satisfactory picture of

the progress in renal physiology and the most recent by Lotspeich (88) gives an unusually thorough and illuminating discussion of the mechanisms of tubular excretion. Reviews limited to special topics of tubular transport will be mentioned in their context.

The problems connected with bile formation have not been reviewed so frequently. In addition to reviews of bile composition (134), bile acids (66) and enterohepatic circulation of bile acids (80), the very full review of choleresis and choleretics by Bizard and Vanlerenberghe in 1956 (19), and the discussion of bile formation by Brauer (28) in the same year seem to be especially useful.

The criteria for active transport in the kidney and liver are usually not very strict. No account is normally taken of the possible influence of electric forces on ion equilibria. In many cases, however, the concentration ratios obtainable are such as to make any considerable influence by this factor unlikely. In isolated renal tubules (57) and in the bile capillaries (73) the concentration in the lumen of the tubules may be more than a thousand times that outside. The tacit (and perhaps sometimes erroneous) assumption is made that other substances, which do not show such high concentration ratios, are also actively transferred. As the majority of the substances treated in this review appear to be handled by the same transport mechanism, the phenomena discussed may with some confidence be assumed to pertain to active transport.

Methodology

a. Renal tubular transport. The methods used in renal physiology are reviewed in Smith's monograph (131), with due emphasis on the clearance method. This method is by far the most generally used when trying to elucidate the handling of any substance by the kidney. As the urine is formed through the action of two fundamentally different processes, glomerular filtration and tubular transfer (excretion and reabsorption), the influence of the glomeruli is assessed by the simultaneous study of the excretion of a substance which is considered uninfluenced by tubular events. Glomerular filtration rate is generally equated with the clearance of inulin, *i.e.*, the virtual volume of plasma completely cleared of inulin by the kidney in one minute. Any substance having a clearance (corrected for plasma binding) higher than the simultaneous inulin clearance is considered to be excreted by the tubules, and substances having a lower clearance than inulin are classified as reabsorbed by the tubules.

To obtain information (if possible) about any quantitative restriction of secretory transfer (maximal tubular rate of transfer, T_m), the amount secreted is measured at different plasma concentrations. In order to obtain reliable results it is in practice usually necessary to keep the plasma concentration constant by continuous infusion during a not too short period of time. The plasma concentration is thus changed stepwise to give the necessary range of concentrations.

Kidneys completely lacking glomeruli, such as are found in some marine fishes, are uniquely suited for the study of tubular excretion (51, 58, 90, 92, 126). Another means of avoiding the complicating factor of glomerular filtra-

tion is to take advantage of the renal portal circulation found in all vertebrates except mammals. As early as 1878, Nussbaum (100) tried to make use of the renal portal circulation of the frog kidney to study tubular excretion. His method, which involves the tying of the renal arteries, has been criticized (probably erroneously) on the ground that reestablishment of glomerular circulation is possible. In any case the dexterity required probably makes the common use of this method impossible.

Later Cullis (46) and Bainbridge *et al.* (4) used an artificial perfusion of the frog kidney, trying to establish a technique which permitted essentially separate perfusion of glomeruli and tubules. Many later authors, amongst whom Höber and his associates (71, 72, 121) should be mentioned, have used this method. A critical review of the method is given by Shannon (127).

The advantage of supplying a compound only to the tubules may be retained without having recourse to artificial perfusion, by supplying the substance primarily to only one of the kidneys, using the other kidney to estimate the influence of recirculation. Such a method has been developed (135, 136, 138), using unanesthetized chickens.

The urine is collected separately from the two kidneys. The compound to be examined is injected into one leg. The blood from the leg flows at least partly through the intertubular capillaries of the ipsilateral kidney, where in the case of tubular excretion part of the injected amount is transferred to the urine. The remainder goes to the heart, and is then eventually excreted equally by the two kidneys. The surplus excreted in the urine on the injected side compared to the uninjected side is the amount extracted during one passage through the kidney. When divided by the amount injected this surplus gives the apparent tubular extraction fraction. Correction for blood by-passing the kidney may be made, and true extraction fractions may be calculated. The method is thus a quantitative method. (Incidentally, it should be emphasized that the avian kidney is a metanephros, since the misapprehension is sometimes encountered that it is a mesonephros.)

Tubular secretion may in some instances be studied more advantageously under circumstances where only small parts of the kidney are used. Chambers and his coworkers (*e.g.*, 37, 38) used *in vitro* cultures of proximal tubules of the mesonephros of the chick embryo to study the accumulation of dyes, especially phenol red. The influence of metabolic inhibitors was also studied.

Studies of the accumulation of dyes in the lumen of the proximal tubules of excised frog kidneys (72, 114) or thin slices of frog kidneys (57) kept in oxygenated saline have also contributed to the knowledge of tubular secretion. Still better visibility is afforded by teased preparations of flounder tubules, where the accumulation of phenol red or chlorphenol red is striking (57).

The accumulation of *p*-aminohippuric acid in slices of the renal cortex of various mammals has also been studied in a considerable number of investigations, especially in connection with the study of cellular metabolites and metabolic inhibitors (45).

b. Biliary excretion. The clearance procedure has been used in several cases in bile studies. It would seem that the fact that there is no glomerular filtration and the use of a reference substance is thus obviated, would make clearance studies on the liver easier than on the kidney, and indeed, the liver should be as useful in such studies as the aglomerular kidney. There are, however, several reasons which rather tend to make the opposite true. The collection of bile is by no means as simple as the collection of urine. This circumstance is responsible for the fact that whereas most investigations are concerned with the urinary *output*, the *uptake* of dyes by the liver has been far more often investigated than the biliary output. Though this is no drawback when the goal is to estimate the blood flow through the liver (24, 36, 77, 86, 98, 108, 118, 119, 123, 149), the results are of doubtful value when used to describe the secretory transfer.

As discussed below, the substances of most interest in bile studies are accumulated to a considerable extent in the cells, which tends to make the secretory response to a change in plasma concentration sluggish. This fact would make the use of fairly long periods of constant plasma concentration desirable, an aim achieved with relative ease at low concentrations, when the maximal rate of uptake by the liver is higher than the infusion rate. In the case of the kidney, usually no difficulties are encountered at levels higher than those necessary to saturate a tubular transport mechanism, since when the infusion rate is increased, the nonspecific glomerular excretory route will establish a new equilibrium at a higher plasma concentration. The urinary excretion of most substances which have been studied by workers in the bile field is slight, and therefore, when the rate of infusion is increased beyond the capacity of the secretory mechanism of the liver, the plasma concentration may increase continuously and fairly rapidly. Though methods have been devised to calculate the maximal rate of uptake from this rate of increase of the plasma concentration (87, 94), the procedure is by no means simple and accurate.

In connection with studies on the perfused frog kidney, Höber and his coworkers developed similar methods for the study of bile formation (69, 71, 73). Fish livers have also been used (68). In these cases oxygenated saline seems to be reasonably satisfactory as a perfusate, but in the artificial perfusion of mammalian livers more complicated methods are necessary. An apparently efficient method of perfusing rat livers has been developed by Brauer *et al.* (32), and the preparation has been used in a large number of investigations concerning bile formation and the uptake of bromsulphthalein. These problems have also been studied in the perfused dog liver (2). Rat liver slices in oxygenated saline have also been used to study the uptake of bromsulphthalein (29).

The uptake and biliary excretion has been fairly extensively studied by means of fluorescence microscopy since the pioneer work of Ellinger and Hirt (55). The high concentrations of fluorescein obtained make the liver very suitable for this type of work, and both rats (65) and frogs (62) have been used in recent studies.

Compounds secreted into bile and urine

As will be further discussed below, many of the substances which are actively transferred by the liver cells or the renal tubular cells compete for transport, depressing each other's secretion when they are offered to the cells in large amounts. This is the main argument for the assumption that certain substances share a secretory mechanism. The force of this argument may be very differently assessed, but generally its validity is admitted. As far as the kidney is concerned, most authors consider that most organic acids secreted by the tubules share one mechanism, and it may be tentatively assumed that the same is the case in the liver.

It is then very natural to ask the question: which are the substances normally secreted by these mechanisms? With regard to the mammalian kidney the question remained unanswered for a considerable time. Phenol red, the secretion of which was first unequivocally demonstrated (93, 124), did not possess any normally occurring analogue, and thus as late as 1939 Shannon in his important review (127) suggested that the mechanism resulting in the secretion of phenol red might "be considered to be results of incidents in the cells' genetic history" and lack a normal function. In 1945 Smith *et al.* (132) showed that a number of compounds related to hippuric acid were secreted by the human kidney. The inference was strong that hippuric acid would be similarly handled, and in 1946 the tubular excretion of hippuric acid in the chicken was reported (135). Later the secretion of several glucuronides and sulphuric esters of phenols was reported (136, 137; *cf.* also 33, 75). Both hippuric acid and glucuronides and sulphuric esters are normal though relatively minor components of mammalian urine.

With regard to the bile, the bile acids are of course the most characteristic, and usually (apart from water) quantitatively the most important constituents of the bile.

In mammals, birds, and reptiles glycocholic and taurocholic acids are the compounds normally occurring in bile (for review *cf.* 66). Investigations using clearance methods are lacking, owing to difficulties connected with their determination in plasma but their high concentration in bile can hardly be explained except by their active transfer from the cells to the bile.

The glycocholic acids like the hippuric acids are glycine conjugates. The taurocholic acids have no counterpart in normal urine, but they are sulphonic acids, and thus related to many of the dyes, such as phenol red, which are known to be secreted by the renal tubules.

In "lower" vertebrates, in addition to taurocholic acids the bile contains sulphuric esters of "bile alcohols" such as scymnol in the dogfish, ranol in the frog and pentahydroxybufostane in the toad (66).

Next to the bile acids, bilirubin is the most characteristic component of bile. Through recent investigations it has been shown that the naturally occurring compounds are a monoglucuronide and especially in the bile a diglucuronide of bilirubin (17, 122).

It may seem unwarranted thus to collect facts from widely different vertebrate groups, but there is abundant evidence that the secretory mechanism for aromatic acids in the kidney is in most respects very similar in all vertebrate groups (*cf.* 127), and the same seems to be true with regard to the secretion of the bile components.

The naturally occurring compounds which are secreted into the urine and into the bile clearly belong to groups of compounds which are, broadly speaking, the same in both cases.

This similarity between the two transport mechanisms is emphasized when other compounds known to be actively transferred by the two organs are taken into account. The majority of these compounds are in both cases dyes, mostly sulphonic acids, *e.g.*, phenol red and its derivatives, which are efficiently secreted in both cases (1, 73, 93, 117, 139), and fluorescein, which is abundantly secreted into the bile (65) but has also been reported to be secreted into the mesonephric tubules of the chicken embryo (37), to take only two of the best investigated cases. Bromsulphthalein, the secretion of which into the bile has been the object of so many investigations (*e.g.*, 117, 118), has also been reported to be transferred by the renal tubules in man (21). Penicillin is very efficiently secreted by the kidney (15, 111) and has also been stated to be secreted into dog bile (42). The same applies to *p*-aminohippuric acid (42, 132). In both these cases the biliary excretion is slight and has been doubted (28), but considerable biliary excretion of *p*-aminohippuric acid is reported in the goosefish (58).

In the kidney, phlorizin and (at least under certain circumstances) phlorizinglucuronide are secreted by the tubules (33). In phlorizinized dogs considerable amounts of phlorizin (or phlorizinglucuronide) are excreted into the bile (78, 79). In this connection the biliary excretion of the glucoside esculin (62) may be mentioned. There appear to be no investigations showing whether this represents unchanged esculin or not.

Cinchophen is excreted into the bile to a considerable extent (3, 9, 11, 25, 26, 76). Its mode of renal excretion has not been investigated, but the fact that it depresses competitively the secretion of phenol red (48, 155) suggests an affinity for the tubular excretory mechanism.

Competition for secretion has been shown between most of the groups of compounds which are excreted by the tubules in the kidney (*cf.* 14, 131, 138, 140). The evidence for competition in the biliary excretion of these compounds is reviewed later. In anticipation of this discussion, it may be mentioned that there is reasonably satisfactory evidence that several of the organic acids which are secreted into the bile compete for transfer, when they are supplied in sufficient amounts.

In summary it may be said that the most reasonable interpretation is that the organic acids excreted by the tubules in the kidney are handled by one mechanism. This mechanism will hereafter be referred to as the hippurate transport mechanism. Similarly, it seems fairly well established that the aromatic acids secreted into the bile are also handled by one mechanism, which will be called the bile acid transport mechanism. The similarities shown be-

tween these two mechanisms with regard to the groups of compounds and, in some cases, even the compounds transferred, are such as to indicate strongly that the two mechanisms are similar in their essential construction. However, many single compounds known to be efficiently secreted by one mechanism are transferred only inefficiently or not at all by the other mechanism, which shows that the mechanisms are not completely identical.

Uptake and intracellular storage of secreted compounds

The transfer of a substance through a secreting cell may logically be divided into three phases: uptake (passage from the plasma, or rather, extracellular fluid into the cell); passage through the cell (with or without accumulation); and passage from the cell to the tubular lumen. Early workers on the kidneys, from Heidenhain (70) and at least into the 1920's, tended to emphasize the accumulation in cells as a sign of secretory transfer through these cells (for a review *cf.* 127). However, in the minds of most later workers in this field the phenomena of accumulation and secretion became dissociated, and it was concluded by Shannon (127) that "if cellular storage takes place preliminary to, or in the process of its tubular transfer, the localized solute must be considered to be in dynamic equilibrium with the concurrent plasma concentration."

This view was to a considerable extent based on the observation that both in the frog renal tubule (72, 114) and in the cultured mesonephric tubules of chicken embryos (37, 38), phenol red is concentrated to a very high degree in the tubular lumen, without any noticeable concentration in the cells. This observation has been amply confirmed and extended by observations *in vitro* on frog kidney slices and teased flounder tubules (57, 60). These observations clearly show that the important and constantly observable concentration difference occurs at the luminal border of the tubule cells, where, accordingly, the most important part of the mechanism of transfer seems to be located. This fact has led to a tendency to disregard to some extent both the mechanism of entrance of transferred substances and the possibility of storage in the tubule cells. The occurrence of intracellular accumulation of transferred substances has, however, been reported by several authors (*e.g.*, 44, 59, 82, 109, 138). Of considerable interest in this connection is the fact that this intracellular accumulation exhibits competitive phenomena, as was suggested by Josephson and Kallas (82) and shown by the studies of Forster and Copenhaver (59). The latter authors, working with rabbit renal cortex slices and the method of Cross and Taggart (45), were able to show that the intracellular accumulation of phenol red and chlorphenol red which may result in a concentration ratio cell/substrate of more than 100 is competitively inhibited by *p*-aminohippuric acid and probenecid (Benemid), and is prevented by dinitrophenol or cold (44). The authors concluded that the movement of phenol red into the cells of the renal proximal tubules was an active process, subject to competitive inhibition, and that this was the first step in the over-all transfer of this and other secreted substances.

In the liver the secretion into the bile is to a considerable and pronounced

extent dissociated from the uptake (*e.g.*, 30, 31, 34, 42, 65, 150). Whereas the uptake of bromsulphthalein and fluorescein occurs as soon as the dye is added to the plasma, the excretion is delayed, and is at first considerably slower than the rate of uptake. This leads to a considerable accumulation of dye, which was originally ascribed tentatively to Kupffer's cells (34), but has since been shown to be mainly due to the hepatic parenchymal cells (65, 97). The storage results in a considerably higher concentration in the cells than in plasma, and the intracellular concentration may reach very high levels (29, 31, 65). Brauer and Pessotti (29), from their studies on the uptake of bromsulphthalein by the perfused rat liver and by rat liver slices, concluded that the uptake of dye *in vitro* is independent of metabolic processes (as it is uninfluenced by fluoride, cyanide, and mercuric ions) and is an essentially physico-chemical process. They suggest that the uptake is attributable to binding of the dye by intracellular proteins. This interpretation, attractive by reason of its simplicity, does not apply generally to *in vivo* conditions, since Hanzon (65) found that the uptake of fluorescein is considerably slowed down by lowering of the temperature, and at temperatures in the neighbourhood of 20°C, no apparent dye uptake (or biliary elimination) could be observed.

In vivo observations on dogs by Brauer *et al.* (30, 31) and by Cook *et al.* (42) similarly show a considerable hepatic storage of bromsulphthalein. When infusion of the dye is terminated, this store is gradually depleted by biliary secretion. Similar phenomena have been observed with bromcresol green in the chicken (139).

The picture of dye uptake and secretion may tentatively be described as follows, taking the processes in the reverse order.

a. The transfer from the cell to the lumen is probably the result of an active process, dependent on the metabolic activity of the cell. The evidence reviewed above shows that a very considerable concentration difference may arise across the cellular surface bounding the tubular lumen, or the bile capillary. The observations pertaining to the kidney were made *in vitro*, but the high concentration in the urine and the small amount of phenol red possibly stored in the kidney during phenol red secretion (133), shows that this is true *in vivo* too, at least with respect to some compounds. It does not seem very probable that the membrane limiting the tubular lumen has a high degree of passive permeability to substances which are efficiently transferred, but actual observations are scarce. In the toadfish, Shannon (126) reports that the maximal rate of tubular transfer is uninfluenced by plasma concentrations of phenol red higher than the simultaneous urine concentration. The possibility of resorption, active or passive, of some secreted compounds is discussed later.

b. The ions of a substance which is transferred by the cells may, during its existence in the cell, be either free or bound. Accumulation in the cell may result either from an active uptake exceeding the rate of active output or by binding within the cell. Considerable passive accumulation in rat liver slices (29) tends to show that storage due to binding plays a rôle in the liver. By analogy with the binding of ions to plasma proteins (84), the greater the quan-

tity of dye already bound, the slower the rate of uptake by the cellular components is likely to be, until complete saturation occurs. Storage and excretion should then compete for the free dye entering the cell (and different substances may be expected to compete for storage). Equality between uptake and output will not be reached until the storage capacity is saturated. When the supply of dye diminishes the store will slowly be depleted, making possible for some time an output exceeding the uptake. The stored amount and the output would be expected to fall off in a more or less parallel manner, both decreasing gradually. If, on the other hand, the store consisted of free dye one would expect a relatively undiminished excretion (close to the maximal transfer rate) for some time, and thereafter a relatively abrupt fall. This is not the case (30, 31, 139) and consequently the major part of the amount stored in the hepatic cells is more probably bound to cellular components, in agreement with the view put forward by Brauer and Pessotti (29). With regard to the renal proximal tubule the evidence is more difficult to evaluate. In slices of renal cortex Forster and Copenhaver (59) found considerable intracellular accumulation with a series of phenol red derivatives. The accumulation was more pronounced with compounds which had been shown to have a slow rate of tubular transfer (138). These slowly transferred compounds were "trapped" in the cells which were cooled or treated with dinitrophenol, whereas rapidly transferred compounds left the cells rapidly under these circumstances (44). There is fairly close correlation between plasma protein binding and the slow rate of transfer of these phenol red derivatives (Sperber, unpublished observations). This may be an indication that binding to cellular components is important in these latter experiments, and that the mechanism of cellular accumulation is perhaps not very different in kidney and liver.

c. An unlimited permeability of any considerable part of the cell surface would of course be fatal to the biochemical integrity of the cells of the liver or kidney. Some sort of transfer mechanism would seem to be necessary for the entrance of the relatively large ions which are discussed here. This does not mean that this transfer mechanism must be able to effect an active transfer. A facilitated diffusion mechanism (146, 147) would be sufficient, and such a mechanism would account for competitive phenomena. The observations previously mentioned suggest, however, that an active mechanism is at work. Whether this also means that the movement of these substances through the basal surface is unidirectional to the same extent as through the luminal surface seems uncertain, and it appears likely that "stored" ions may leave the cell through the basal surface as well as through the luminal surface.

Competition due to quantitative limitations with regard to the transfer processes and storage "space" appears possible in all the three steps described.

If it is assumed that the processes of uptake and output are both active and rate-limited and that passive movements are quantitatively unimportant, it may be of some interest to note that, provided the maximal rate of uptake is higher than the maximal rate of output, the output will be rate-limiting for the over-all process, and accumulation of free dye may occur. If on the other

hand the rate of output has a higher limit, the uptake will be limiting for the over-all process. In this latter case accumulation of free ions will not occur in the cell, but any accumulation should be due to some mechanism making the ions unavailable to the output mechanism.

The three steps separately discussed above may be integrated into one mechanism by assuming that the compound to be transferred is coupled to a cellular component at the outer surface, and that the complex formed is dissociated at the luminal surface, as assumed by Shannon (127), Beyer (13) and Chinard (41). This means that the amount of the compound which is stored in the cell is equal to the amount bound to the cellular substance necessary for the transport. Chinard (41) assumes that the molecular weight of the cellular component may be calculated from the time needed for the resulting complex to diffuse across the tubule cell, which time is equated with the delay apparent when *p*-aminohippuric acid is compared with a compound excreted exclusively by the glomeruli. This explanation would require that all substances transferred by the same mechanism should show about the same delay (the cellular component is calculated to be much heavier than the transferred substance). From studies on the tubular excretion of phenol red and phenol red derivatives in the chicken (138) it seems quite clear that this is not the case, bromphenol blue, for instance, having a considerably longer delay than phenol red. It appears likely that transient unspecific binding to cellular components may give the observed effect.

Competition and maximal rate of transfer

With regard to the kidney it has been shown for several of the substances which are secreted that there is an upper limit to the amount that can be secreted per time unit (124, 127, 130, 131, 133); *i.e.*, they show a maximal tubular rate of transfer (T_m). This circumstance is usually considered to be causally connected with the phenomenon of competition for secretion.

The conception of a T_m has sometimes been criticized on the ground that the tubular transfer rate is not independent of the available excess of the substance which is transported (6, 52).

In some cases a reduction of tubular secretion has been brought about by supplying the tubules with a very heavy load of *p*-aminohippurate (6, 52) or diodrast (6, 104). This has been interpreted either as the result of interference with the transport mechanism, or as an indication that back diffusion of the secreted compound occurs. Both interpretations may be connected with a possible effect of high concentrations of the secreted compounds on the secreting cells. Such an effect would not seem surprising in view of the high concentrations involved. The effects of high *p*-aminohippurate loads may be reversed, at least in the dog, by supplying acetate (120).

In any case it does not appear that the usefulness of the concept of a T_m is seriously impaired by the above-mentioned circumstances.

The early investigations revealed only moderate differences with regard to the maximal transfer rates of different compounds, expressed in moles (39,

131). However, different substances may show exceedingly different maximal tubular transfer rates (138).

Taking this into account, the simplest possible picture of the mechanism would be that each molecular species transported by one mechanism is characterized by the maximal number of molecules which can be transported in the time unit. When two molecular species are transported simultaneously each molecule will still occupy its appropriate share of the mechanism, and when the supply of molecules to the mechanism exceeds the maximal transfer rate the molecules supplied will compete with each other.

This model predicts that when the arterial plasma concentration of a substance rises, the tubular excretion of the substance is described by two straight lines, one passing through the origin, and the other parallel to the abscissa. This mode of treatment has been used by Smith (130); on the other hand, the formula of Shannon (127) gives these two straight lines as the limiting case, but otherwise gives a curvilinear relation between plasma concentration and secretory transfer. For qualitative purposes, at least, the former treatment seems sufficient, and its simplicity is a considerable merit.

Using this model it is easy qualitatively to describe what would happen if at the start of the infusion of a compound another compound were already being supplied to the transport mechanism at a constant rate, and a short discussion of this case will be appended here mainly to provide a background for a later discussion of the effect of some choleretics.

In Fig. 1 (top) is depicted the simplest case, when the transport mechanism is used by one endogenous substance and another substance (called the exogenous substance) is supplied in addition. The two substances involved have the same affinity for the mechanism, and have the same maximal transfer rate, N molecules (or ions) per time unit. When the supply of the exogenous substance to the tubules is increased, the number of molecules of this substance transferred increases linearly, until the sum of the transferred molecules of both substances is equal to N. Further increase in supply results in a continuous decrease of the number of transferred molecules of the endogenous substance, and a gradual approach of the number of transferred molecules of the exogenous substance towards its transfer maximum (= N). The total number of molecules transferred at first rises and then remains constant at the transfer maximum. When the maximal transfer rates of the two substances are different the results will be qualitatively similar, with a gradual approach towards the maximal transfer rate which would exist from the exogenous substance alone. The total number of molecules transferred will, however, show somewhat more complicated phenomena. Two simple cases may be discussed briefly. In the one case (Fig. 1, middle) the endogenous substance is supplied to the tubules at a rate equal to one fourth of its maximal transfer rate N. With no supply of exogenous substance all of it will be transferred. The exogenous substance is supposed to have a maximal transfer rate N/4. All molecules of the exogenous substance will be transferred until ¾ of its transfer maximum is occupied by its molecules. At higher supply rates

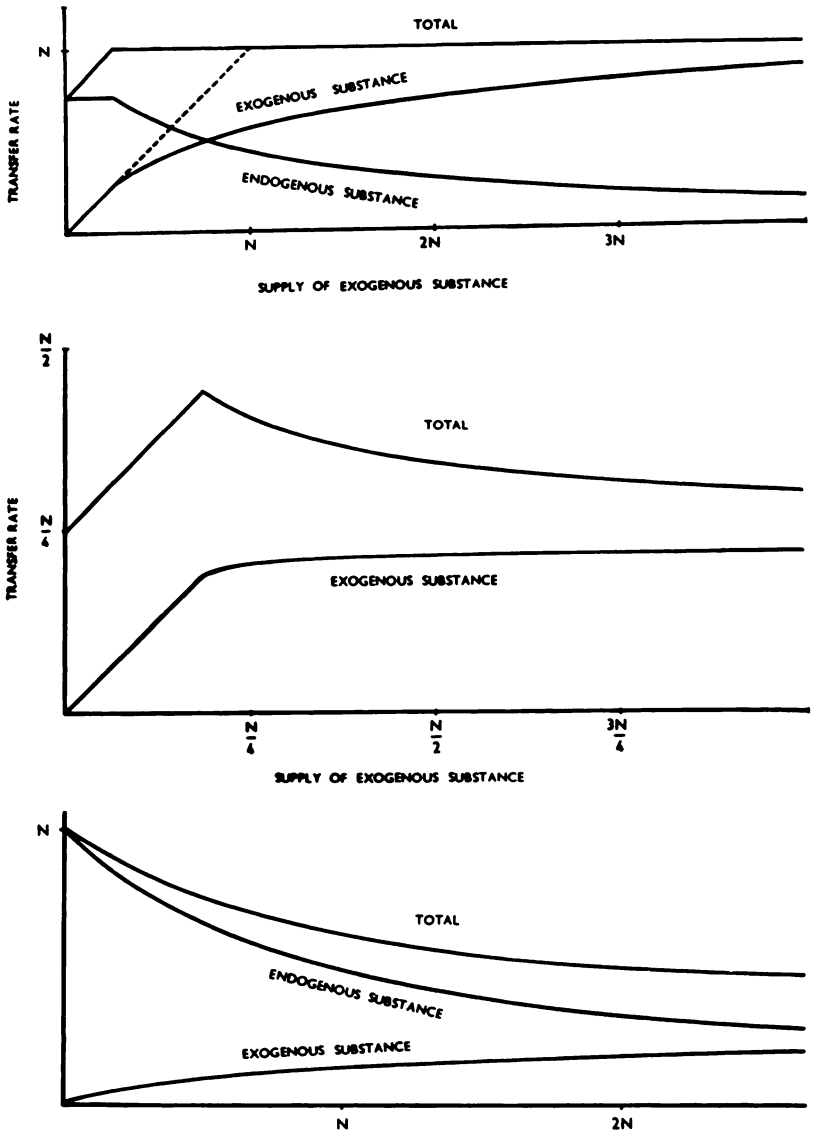

FIG. 1. Transfer of two compounds by one transfer mechanism. In the three cases schematically depicted one of the substances ("endogenous") is supplied to the mechanism at a constant rate; the supply of the other ("exogenous") is varied. The maximal rate of transfer of the endogenous substance is N molecules/time unit. *Top:* Rate of supply of endogenous substance ¾ N. Maximal transfer rate of exogenous substance N. *Middle:* Rate of supply of endogenous substance N/4. Maximal rate of transfer of exogenous substance N/4. *Bottom:* Rate of supply of endogenous substance N. Maximal rate of transfer of exogenous substance N/4.

competition will occur. At this point the total number of molecules transferred will be 7/16 of N. Since, during competition, each molecule of the exogenous substance will replace four of the endogenous, the total number of molecules transferred will decrease towards N/4.

In Fig. 1 (bottom) the supply of the endogenous substance is equal to its maximal transfer rate, but otherwise the case is similar to that in Fig. 1 (middle). It is quite clear that the total number of molecules transported will fall progressively from N towards N/4, as the supply of the exogenous substance is increased.

The facts and concepts pertaining to quantitative limitations of secretory transfer into the bile are far less easy to survey.

At low rates of infusion of a compound which is excreted exclusively, or almost exclusively, by the liver (usually bromsulphthalein has been used), a stable plasma concentration is eventually obtained. When this has been achieved, the rate of uptake of bromsulphthalein is also stable. If on the other hand the rate of infusion is increased beyond a certain limit, the plasma concentration rises successively, indicating that the liver is unable to keep pace with the increased supply of the dye.

This circumstance has been used to estimate the maximal rate of uptake by the liver in the dog by Mason *et al.* (94) and in the rabbit by Lewis (87) and Taleisnik (145). Similar phenomena occur when single injections are used instead of continuous infusion (*e.g.*, 77, 98), though the interpretation of the results of such studies is much more difficult.

The most natural explanation of a maximal rate of uptake is that it is causally related to a maximal rate of biliary excretion. However, the connection between the uptake and the excretion of bromsulphthalein has been somewhat obscure until recently. The biliary excretion shows a considerable delay compared with the uptake, as first emphasized by Cantarow and Wirts (34). Brauer and Pessotti (30) further showed that the amount excreted in the bile is less than that taken up by the liver. Later investigations by Brauer *et al.* (31) solved this puzzle by showing that part of the bromsulphthalein is converted in the liver into colourless compounds which are also excreted into the bile.

Only in a few cases has the rate of biliary excretion been studied over a wide range of plasma concentrations. Haywood *et al.* (68) investigated the biliary excretion of eriocyanin during artificial perfusion of the trout liver. They found a maximal rate of excretion at a dye concentration of 0.0005%. Lower, but also higher concentrations gave a lower dye output.

Cook *et al.* (42) studied the biliary concentration and output of various substances in the anesthetized dog during widely different plasma concentrations, obtained by continuous infusion. For bromsulphthalein these investigators found a maximal rate of biliary excretion at a plasma concentration of about 0.2 mM/l, with lower transfer rates at higher plasma concentrations. For other substances investigated no maximal rate of transfer was observed. In this case, as in the previous, the fall-off in excretion coincided with a fall in bile flow.

Hanzon (65) reports a maximal rate of biliary excretion for bilirubin in the

anesthetized rat. The value of this observation is to some extent diminished by the fact that it may reflect a maximal rate of biochemical transformation of the injected compound, since this is not identical with the compound normally excreted in the bile (17, 122).

Sperber (139), working with unanesthetized chickens, reports that phenol red and bromcresol green show maximal rates of biliary excretion. No fall in transfer rate has been observed at plasma concentrations higher than those necessary to achieve saturation of the biliary excretion mechanism. However, the maximal plasma concentrations used are only about five times higher than the saturation limit.

Though the evidence available is not conclusive, it does indicate that a limited transfer rate is a characteristic of the bile acid transport mechanism. The maximal rate of transfer is either practically independent of plasma concentration above the saturation limit (as in the kidney), or the rate of transfer is depressed by excessive plasma concentrations of the transported compound. This latter case is also sometimes met with in the kidney.

Brauer and Pessotti (30) deny the existence of a strict limitation of the rate of uptake, and they were unable to find any certain indication of a maximal excretion rate in the unanesthetized dog. On the other hand they emphasize that the *concentration* of bromsulphthalein in bile tends to approach a limit. These authors (28, 30) and also Hanzon (65) are inclined to consider this biliary concentration limit as the important quantitative limitation of the transfer system.

Such an upper concentration limit is also evident from the data for bromsulphthalein given by Cook *et al.* (42), and there is a similar tendency in the experiments which are the basis of Sperber's short communication (139).

Brauer and Pessotti (30) and Hanzon (65) consider that this biliary concentration limit explains competitive phenomena insofar as substances excreted into the bile depress one another's concentrations. This is not evident to the reviewer. There is no apparent reason why the concentrations reached by two substances should influence each other, if the rate of transfer of each is unlimited except by the concentration in the bile. On the other hand, if the rate of transfer has an upper limit, and the two substances are handled by the same mechanism, they will compete if the supply of the substances exceeds the maximal transfer rate. Thus when the amount transferred is decreased for each substance, the concentrations will also decrease, provided the bile flow is not decreased in proportion. Further, if the flow of bile is more or less proportional to the number of molecules or ions transferred (*cf.* p. 128), the biliary concentration of a substance may be lowered by the simultaneous excretion of another substance (resulting in an increased bile flow), even if this substance is not handled by the same mechanism, or when the amounts transferred are below the maximal transport capacity.

It is obvious that competition, evident by diminished rate of excretion as the result of simultaneous transfer of other compounds, may logically be connected

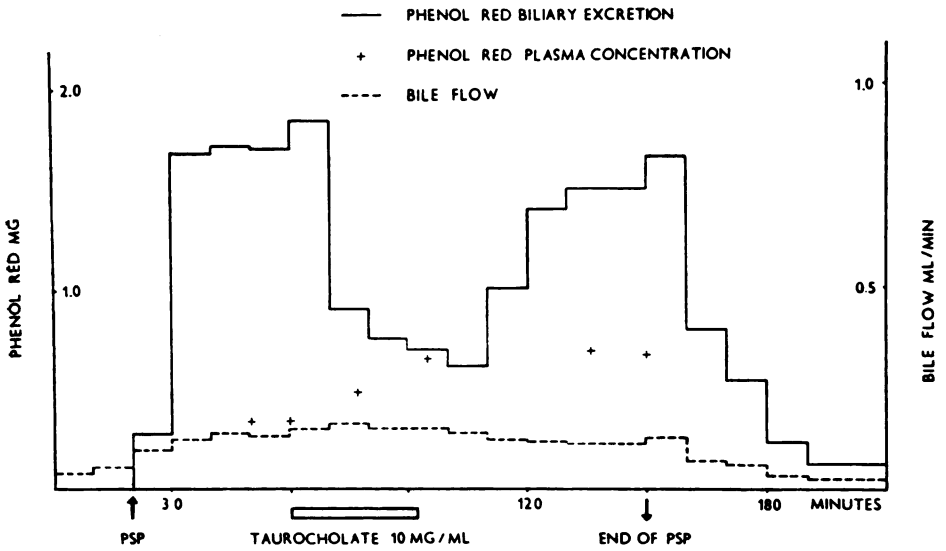

FIG. 2. Influence of taurocholate on the biliary excretion of phenol red in a chicken. 1 ml/min of a phenol red (PSP) solution was infused from 20 min to 150 min. From 60 min to 92 min the infusion contained in addition 10 mg/ml sodium taurocholate.

with the concept of a maximal transfer rate, and be regarded as supplementary evidence for the existence of a quantitative limitation of this type.

Reinhold and Wilson (113) and Bradley and Ivy (26) found that the endogenous excretion of cholate was reduced when large amounts of dehydrocholic acid were administered. Similarly cinchophen depresses the cholate excretion despite increased bile flow (25, 26). The excretion of bromsulphthalein was depressed by dehydrocholic acid (35, 151) and cholate (151). The mechanism of the cholate effect is perhaps somewhat obscure, since at least part of the cholate is conjugated before its excretion in bile (81). This also applies to the depressive effect on fluorescein excretion by bilirubin and cholate in the rat (65). Mendeloff *et al.* (98) report that the hepatic uptake of Rose Bengal is reduced by bromsulphthalein, but the excretion into the bile has not been examined.

Taurocholic acid and glycocholic acid depress the secretion of phenol red in the chicken (Fig. 2), and bromcresol green depresses the biliary excretion of taurocholate (139).

There are numerous reports that the administration of choleretic substances (bile acids, dehydrocholic acid) increases the excretion of other substances into the bile. This is not necessarily contradictory to the views expressed here, since doses of such a compound which are too small to effect saturation of the secretory mechanism will not be expected to have a depressing effect, and the output of diverse substances may even increase as a result of the flushing out of the

bile ducts by increased bile flow (or possibly as a result of diminished back diffusion).

Compounds which are both secreted and reabsorbed in the kidney

The view that a substance may be both secreted and reabsorbed by the renal tubules was stated by Barclay, Cooke and Kenney in 1947 (5). The clearance method is not well suited to the investigation of this possibility, and the cases enumerated by them did not appear very convincing to most investigators at that time. Later it was clearly shown that potassium is both reabsorbed and secreted. A number of substances handled by the hippurate mechanism may also belong in this category, and some examples are discussed below.

Carinamide (4′ carboxyphenylmethanesulfonanilide), introduced by Beyer (12) as an agent to depress the tubular secretion of penicillin, was originally thought to be unaffected by tubular secretion and reabsorption. It was then natural to believe that its depressing effect on penicillin secretion was of another type than that ascribed to *p*-aminohippuric acid (15), where competition for transport was clearly involved. It was, however, later shown that carinamide may be both secreted to some extent by the tubules (50, 138) and reabsorbed by them (102). It would then seem probable that the inhibitory effect on penicillin secretion is due to competition for secretion. Later probenecid (Benemid) was developed for the same purpose (*cf.* 13, 16), and it was assumed that this compound, too, was representative of substances which are not secreted but nevertheless, through affinity with a component of the transfer mechanism, are able competitively to inhibit tubular secretion. Benemid has a very low clearance, indicating almost complete reabsorption. This circumstance of course makes it impossible to decide if there is any tubular secretion of the compound. In this case the interpretation given by Beyer is possible, but it seems equally possible that the inhibitory effect on tubular secretion is an indication that Benemid is secreted, though the rapid reabsorption makes this circumstance impossible to detect. The same applies to 3-hydroxy-2-phenyl-cinchoninic acid, which depresses phenol red and penicillin secretion (48, 155) but has a very low clearance (155).

Pantothenic acid has been reported to be reabsorbed at low plasma levels in man (116) and the dog (153), but at high plasma concentrations it is secreted by the tubules in man (22, 116), and its secretion is depressed by carinamide (116) and Benemid (22).

Uric acid is efficiently excreted by the tubules in birds (96, 125) and reptiles (91), and tubular secretion has been reported in the perfused frog kidney (89). (For reviews of the literature on gout *cf.* 18 and 144.) In man, however, the clearance of uric acid is normally considerably lower than the inulin clearance (*cf.* 131), and the clearance rises with increasing plasma concentration, indicating an active reabsorption with a maximal rate of reabsorption (8). This would seem to put the handling of uric acid in mammals and nonmammalian vertebrates into completely different categories.

The reabsorption of urate in man may be depressed by a variety of agents,

among which may be mentioned carinamide (144), diodrast (23, 144), Benemid (129), phenol red and cinchophen (144) and salicylate (129, 144). As far as Benemid and carinamide are concerned, the simplest explanation would seem to be, that these two substances, which are reabsorbed by the tubules, are handled by the same reabsorptive mechanism as uric acid, and that their effect on uric acid represents competition for a common transfer mechanism. With regard to the other compounds mentioned, it is striking that so many compounds apparently secreted by the hippurate mechanism should influence the reabsorption of uric acid. So is the fact that Benemid and carinamide, which are reabsorbed by the tubules and pronouncedly influence uric acid reabsorption, are potent inhibitors of the hippurate mechanism. These circumstances would seem to suggest that the hippurate secretion mechanism and the mechanism for reabsorption of uric acid are closely related to each other.

It has also been shown that low dosages of Benemid and acetyl-salicylic acid decrease the excretion of uric acid, whereas high dosages increase the uric acid clearance (154). One of the explanations put forward to explain these findings is that uric acid is both secreted and reabsorbed by the tubules in man (154; *cf.* 105), and that both the tubular mechanisms involved are sensitive to Benemid and acetyl-salicylic acid.

The possibility that uric acid may be secreted by the tubules in man is strengthened by the report of a single case of hypouricemia in man, presumably due to tubular excretion of uric acid (107; *cf.*, however, 129), and by reports of tubular secretion of uric acid in the Dalmatian coach hound (16, 152), apparently depressed by Benemid (16). Rabbits excrete endogenous uric acid at a rate below or comparable to glomerular filtration rate, but uric acid clearance rises to about twice the glomerular filtration rate when the plasma concentration of uric acid is elevated (106). Further, Poulsen has shown that salicylic acid and Benemid depress uric acid secretion (105) in the rabbit. He concludes that uric acid is probably both reabsorbed and secreted by the tubules in the rabbit and in man.

In the chicken *p*-aminohippurate, in amounts sufficient to saturate the transfer mechanism, depresses the secretion of uric acid (Sperber, unpublished experiments).

The secretion of uric acid by the renal tubules seems to be well established, and may be common to most vertebrate groups. It seems natural to assume it is handled by the hippurate mechanism.

Another substance which may, according to the circumstances, show net reabsorption or net secretion, is salicylate (47, 64, 74). In this case an additional complication is introduced by the fact that the urinary pH is of decisive importance. When the urinary pH (in man) is below 6.5 (64), most of the salicylate filtered is reabsorbed. When the urine pH rises after administration of bicarbonate the excretion of salicylate increases, and the clearance becomes higher than the glomerular filtration rate (20, 47, 64). Dalgaard-Mikkelsen (47) does not accept the interpretation that this is due mainly to diffusion of unionized salicylic acid through the wall of the renal tubules, and the statement that the

tubular excretion of salicylate is depressed by Benemid (64) tends to show that this explanation is insufficient.

Phlorizinglucuronide, according to Braun *et al.* (33), is normally reabsorbed, but shows tubular secretion under the influence of Benemid. This is, in some respects, the opposite situation to that of uric acid, and the interpretation is difficult.

This also applies to the excretion of diodrast and *p*-aminohippurate in the newt, in which Kinter (83) reports both reabsorption and secretion of these substances.

Another type of investigation may be exemplified by the study of Huang and Knoefel (75) on the excretion of glucuronides and glycine conjugates of different aminobenzoic acids. Some of these conjugates are excreted by the tubules, others are reabsorbed. Such observations could be explained by assuming simultaneous secretion and resorption, quantitatively different for different compounds.

In summary, evidence seems to be accumulating that a considerable number of substances more or less clearly shown to be secreted by the hippurate mechanism are also reabsorbed in the tubules. That the evidence is difficult to interpret, and may, at least in some cases, turn out not to support the above interpretation, is by no means surprising, since the methods available are not well suited for the demonstration of simultaneous secretion and reabsorption.

Choleretic influence of secreted compounds

When administered in considerable quantities *p*-aminohippurate may have a diuretic effect, explainable as an osmotic diuresis; that is, the occurrence of unreabsorbable ions in the tubular urine prevents reabsorption of water, which is considered to be more or less passive in the proximal tubule (*cf.* 131). As the transport capacity of the tubules is relatively slight, the tubular part of this effect is not considerable, but the major effect is due to the filtration of considerable quantities of the compound.

Whereas the *diuretic* effect of compounds copiously secreted by the hippurate mechanism is unimportant, many of the substances handled by the bile acid mechanism are known as *choleretics*.

The recent thorough review on choleretics by Bizard and Vanlerenberghe (19; *cf.* 63) summarizes the available evidence, and no attempt will be made here to give a complete review. The following points may, however, be emphasized.

Taurocholic and glycocholic acids are generally active as choleretics, though different compounds are not equally active (*e.g.*, 10, 49, 113). The unconjugated cholic acids are also active (7, 10, 35, 99, 113), but usually no figures are given showing the extent to which they are conjugated before excretion (see, however, 80). Different compounds are unequally active. There can be little doubt that these compounds, or products formed from them by the liver, are excreted in the bile. In most other cases little is known about the biliary excretion of choleretic substances. Cinchophen has a pronounced choleretic action (25) and is excreted in the bile to a considerable extent (25, 26). Fluorescein is a powerful cho-

leretic in the rat, and is abundantly secreted into the bile (65). Phenol red and several of its derivatives have a choleretic effect and are efficiently secreted (139). Derivatives of phlorizin, at least partly the glucuronide, are abundantly secreted into the bile (in dogs) and provoke a considerable increase in bile flow (79).

It is clear that an important group of choleretics consists of substances which are excreted into the bile. The most natural explanation is that they are choleretic because they appear in the bile, and that their choleretic effect is due to their osmotic effect. This explanation has been indicated for dehydrocholic acid by Reinhold and Wilson (113), for cinchophen by Berman *et al.* (3, 9, 11), for phlorizinglucuronide by Jenner and Smyth (79), and for phenol red by Sperber (139).

That the presence in the bile is necessary is also evident from the diagrams of some experiments on rats by Hanzon (65), which show that when the excretion of fluorescein is depressed by simultaneous administration of bilirubin the choleretic effect of fluorescein is abolished. The choleretic effect should then, at least as a first approximation, be proportional to the increase in the number of osmotically effective particles in the bile.

As the majority of these substances are probably secreted by a common mechanism, with a limited transport capacity, the general discussion presented earlier may provide at least a qualitative indication of the phenomena to expect. One important circumstance in this connection is that there is almost always a basal biliary excretion of tauro- and glycocholic acids. The cases which may be expected, then, are those shown in Fig. 1. According to Fig. 1 (top) the approach to the transfer maximum for a substance may be very gradual, and this fact may partially explain why it has been relatively difficult to obtain satisfactory experimental evidence for a maximal transport capacity in the liver. A low level of bile acid secretion would seem to be advantageous, when attempting such experiments.

Fig. 1 (middle) and 1 (bottom) indicate that the total number of ions transferred into the bile, and presumably the choleretic effect of an exogenous compound, may be influenced both by the basal level of bile acid secretion and by the dosage employed, in a relatively complicated manner. Indeed the same substance administered in large amounts *may* have a weaker choleretic effect than a smaller dosage, and the same dose may at one time effect a choleretic response and at another an anticholeretic.

This point has been experimentally studied (139). In the chicken bromcresol green normally has a considerable choleretic effect, but when the bile flow is originally high, whether this is due to high endogenous bile acid secretion or to infusion of taurocholate, bromcresol green may have a pronounced anticholeretic action. In the anesthetized rat phenol red (which has a high transport maximum) is an efficient choleretic. Bromcresol green, which has a considerably lower transport maximum, reduces the initial high rate of bile flow.

There are, however, several circumstances which at first sight would tend to show that this simple explanation is incorrect.

It is well known that the different bile acids are not active choleretics in simple proportion to their excretion in bile. Taurocholic acid and glycocholic acid, for

example, are found in the bile in a much higher molecular concentration than is dehydrocholic acid (or the product which may be excreted into the bile after administration of this compound). However, most of the bile acids are associated to a considerable degree in solution, and solutions of their salts show an appreciably smaller freezing-point depression than would be expected from the concentration (54, 110, 115). They are then less osmotically active than dehydrocholic acid, which shows a normal osmotic behaviour (53). This point was emphasized by Reinhold and Wilson (112, 113) as early as 1934, and the inverse relationship between choleretic activity and surface activity had already been pointed out in 1924 by Neubauer (99).

Bilirubin has usually been reported to be inactive as a choleretic, despite its occurrence in the bile. This might be explicable as resulting from depression of bile acid excretion by the bilirubin, but at least a subsidiary circumstance is the fact that bilirubin either forms larger complexes in the bile, or is combined with other bile components to larger aggregates (103), which would of course make its osmotic effect slight or negligible.

The bile acid transport mechanism and the formation of bile

In the case of urine formation, diuresis is usually ascribed to a diminution of reabsorption, since in glomerular kidneys the glomerular filtrate is normally formed at a considerably higher rate than the urine flow. Thus the study of diuresis has little direct bearing on the mechanism of urine formation.

Despite this it does not seem improbable that the study of choleresis could contribute materially to the knowledge of bile formation. The simplest (and in the reviewer's opinion most natural) view is that factors which augment bile flow are the same as those initiating bile formation.

It has been maintained by many authors that the substances occurring in bile may be divided into two categories, those which are secreted, and those which enter the bile by filtration or diffusion (*e.g.*, 42, 43, 69).

The idea of filtration has been refuted by Brauer (28), who emphasized that the division into these two groups is arbitrary, and that no mechanism comparable to glomerular filtration exists in the liver. If by filtration is implied a mechanism closely similar to the glomerular function in the kidney, he is undoubtedly right. However, recently it has been emphasized (101) that flow of water and dissolved substances may occur as a result of osmotic forces, and that this flow may appropriately be equated with filtration. This view has been opposed (40), but it seems clear that the concept is well founded (61, 95, 148). It appears quite possible to assume osmotic filtration as a factor in bile formation. The primary event of bile formation would be the active transfer (from the cells or through the cells) of bile acids (and possibly other, though quantitatively less important compounds) into the bile capillaries. The osmotic effect of these would result in a flow of water and dissolved molecules and ions into the bile capillaries. This flow could simply be supposed to occur through pores in the walls of the biliary capillaries. These pores would be too small to allow the escape of the bile salt ions, but large enough to allow the entrance of water molecules and, for example, sodium, potas-

sium, chloride and bicarbonate ions. The pores may be assumed to exist either in the membrane separating the liver cells from the biliary capillary, or between the cells. The close similarity between the sodium and potassium concentration of bile and plasma (85, 112), and the rapid equilibration of radioactive sodium and potassium between these fluids (27, 85), would seem to favour the latter route (other more complicated explanations of these facts are of course possible).

This very simple view is, however, not quite acceptable with regard to the fact that inulin may be found in bile in concentrations not very much inferior to the simultaneous plasma concentration (42, 67, 69). Unless this is due to some form of cellular transport (and there are no indications of this) it would seem to imply the existence of fairly large pores, which permit the passage of molecules with a molecular weight of about 5000, and which would then of course allow the passage of phenol red and bile acids with a much smaller molecular weight. If unrestricted passage of bile acid ions out through these pores were possible, the driving force of the supposed mechanism would vanish. This difficulty may be overcome by assuming that the passage of anions through the pores is restricted, a not unlikely possibility. However, the work of Pappenheimer and his coworkers (101) tends to show that some permeability to inulin may be possible, even with pores which do noticeably restrict the permeability to much smaller molecules and ions. Quite a considerable diffusion of bile acids from the bile capillaries would not seriously impair the suggested mechanism, as long as the diffusion is sufficiently restricted to give the necessary osmotic effect.

The high inulin bile/plasma ratios reported in the perfused frog liver (69), the anesthetized dog (42) and the toadfish (67) have all been found during very low bile flows when diffusion would be most important. The observations on the toadfish show a considerable decrease in bile inulin concentration under the influence of choleretics. This is tentatively ascribed to an increased resistance to the passage of inulin into the bile (67). However, the total amount of inulin excreted in the bile seems to be increased during choleresis, and thus the conditions found seem to be more easily explicable as the result of restricted diffusion according to the lines developed by Pappenheimer (101). Unpublished experiments on chickens by the author also show that the inulin bile/plasma ratio falls with increasing bile flow. Sucrose has a higher bile/plasma ratio than inulin in comparable circumstances, and this ratio is also reduced when the bile flow increases.

It is not suggested that this hypothetical mechanism is the only mechanism operative in the formation of the bile. It would seem that the bile duct system (including its finest branches) may be expected to have some modifying influence, especially with regard to biliary bicarbonate and chloride concentration, which are known to vary considerably both within and between different vertebrate groups.

Type of mechanism assumed for transfer of hippurate

The above discussion has almost exclusively concerned itself with formal and descriptive aspects. Problems such as what substances share a transfer mechanism

have a bearing on what type of mechanism is possible, or likely, but the indications are only indirect.

Another line of investigation extensively used is the study of the influence of metabolically active substances, inhibitors or metabolic intermediates. The investigators in this field have recently reviewed their findings (13, 14, 56, 128, 141, 142), and the review by Lotspeich (88) also discusses this field thoroughly. However valuable these investigations may be in the long run, their value as a guide for the formulation of a concrete hypothesis concerning the mechanism of transfer is not yet obvious.

More indisputable evidence (though of a negative character) as to what types of mechanism are possible has been obtained in two investigations by Taggart (141, 143). He used isotopically labelled *p*-aminohippuric acid to study what transformations, if any, this substance undergoes during active transfer. In one investigation (141) *p*-aminohippuric acid, with C^{14} in the carboxyl group, was infused into a dog. No change of activity in the carboxyl group occurred. Thus any mechanism including hydrolysis and resynthesis of this substance is excluded. This is of considerable interest with regard to the suggestion (28) that such processes might be involved in the biliary secretion of similar compounds.

In the other experiment Taggart labelled the carboxyl group of *p*-aminohippuric acid with O^{18}. After tubular transfer there was no change in O^{18} content. This shows that neither an amide nor a thiol ester linkage can have been formed during transfer. Neither is the formation of an ester of the type —CO—O—CH_2R considered likely. The possibility of the formation of a carboxylic-phosphoric anhydride is not excluded, and an ion exchange mechanism would also fit the observations (143).

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