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THE EXCRETION OF SALICYLATE

BY

C. R. MACPHERSON, M. D. MILNE, AND BARBARA M. EVANS

From the Department of Medicine, Postgraduate Medical School of London

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Almost all administered salicylate is removed from the body by excretion in the urine, only very small amounts being eliminated in the faeces and sweat. The benzene ring of salicyl compounds is resistant to metabolic breakdown. Many drugs which are excreted in the urine are more toxic in renal failure, and smaller doses have to be prescribed—as, for example, streptomycin, hexamethonium, salts of potassium and magnesium, and ammonium chloride. This has not been described for salicylates, and it therefore seemed of value to investigate salicylate excretion both in normal subjects and in uraemic patients.

Sodium bicarbonate reduces the toxic effects of salicylate, and this was found by Smull, Wegria, and Leland (1944) to be associated with reduction of serum salicylate concentration. Smith, Gleason, Stoll, and Ogorzalek (1946) showed that this was due to increased rate of excretion of the drug in alkaline urine. A considerable but variable proportion of urinary salicylate is conjugated with glycine as salicyluric acid or with glycuronic acid. The raised excretion in alkaline urine is due to increased elimination of free salicylate (Parker, 1948). Further details of the fractionation of urinary salicyl compounds have been investigated by Alpen, Mandel, Rodwell, and Smith (1951) using salicylic acid labelled with ^{14}C at the carboxyl carbon atom. In man, 10–85% is excreted as free salicylate, 0–50% as salicyluric acid, and 15–40% as glycuronic acid conjugates. Gutman, Yü, and Sirota (1955), comparing the clearance of free salicylate with that of inulin, found the ratio C_{sal}/C_{in} to be considerably below unity in acid urines, but to be greater than one at urinary *p*H above 7.5. In this paper we have amplified previous work by studying salicylate excretion when the urine is made alkaline by hyperventilation and by ingestion of the carbonic anhydrase inhibitor, acetazoleamide. The effects of change in urinary volume produced both by water and by osmotic diuresis have also been studied. The theoretical and practical implications of the results obtained are discussed in detail.

METHODS

Observations were made on two healthy adult males (C. R. M. and M. D. M.), an adult female patient with normal renal function who was being treated with salicylates, and two adult males with chronic glomerulonephritis and uraemia. Precautions were taken to avoid any toxic reaction in the patients, but the normal subjects, who had more prolonged salicylate intake, suffered from tinnitus, high-tone deafness, and anorexia. Sodium salicylate was taken by mouth in a dose of 0.5 g. hourly, and clearance observations were made at stable salicylate levels of 15 to 18 mg./100 ml. In the normal subjects repeated observations were made during periods of acute change in urinary *p*H produced by taking sodium bicarbonate 10 g., acetazoleamide 250 mg., ammonium chloride 4 g., or by hyperventilation at 2.5 times the basal rate for 2 hr. In the patients a single period involving ingestion of acetazoleamide only was studied. The clearances in the uraemic patients were combined with determination of inulin clearance used as a routine renal function test. The effects of acute alterations in urinary volume were studied in the two normal subjects only. Water diuresis was obtained by rapid ingestion of 1 l. tap-water, and osmotic diuresis by rapid intravenous infusion of 300 ml. 25% mannitol.

The following analytical methods were used : serum salicylate, method of Smith *et al.* (1946), determination of the non-protein bound fraction of serum salicylate being made after ultra-filtration through cellophane ; fractionation and estimation of urinary salicyl compounds, method of Smith *et al.* (1946) ; chromatography of salicylate and salicyluric acid, method of Dalgleish (1955) ; serum and urinary inulin, method of Roe, Epstein, and Goldstein (1949) ; urinary *p*H, glass electrode ; urinary osmolarity, depression of freezing point using a Beckmann thermometer.

Statistical methods used in the analysis of the results were as described by Snedecor (1946).

RESULTS

Salicylate clearances were measured at reasonably stable serum levels of 15 to 18 mg./100 ml. The ultra-filterable fraction used for calculation of the free salicylate clearance varied from 22–44% of the total, with a mean value of 32%. At these

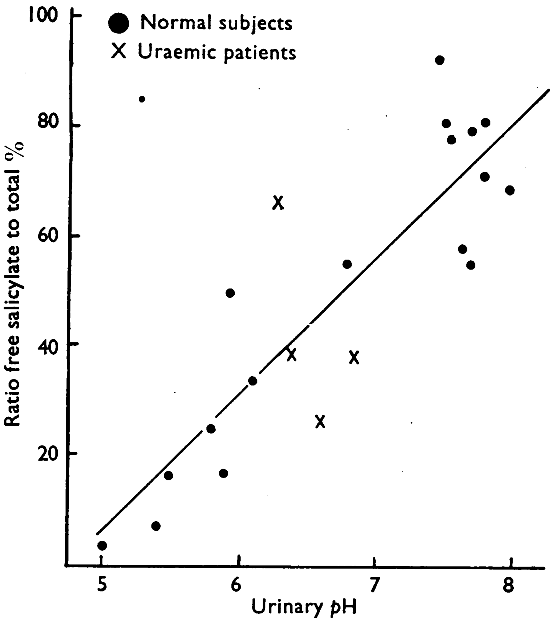

FIG. 1.—Relation between the percentage of urinary salicylate excreted as free salicylate and the urinary *p*H. The proportion of free salicylate in uraemia is within normal limits.

serum levels the excretion of combined salicylate ranged from 1.0–3.3 mg./min., with a mean value of 1.7 mg./min. There was no significant change in the excretion of combined salicylate with variation in urinary *p*H. In contrast, the excretion of free salicylate increased rapidly as the urine became more alkaline. Consequently, the free salicylate fraction was low when the urine was acid, and high when alkaline (Fig. 1). Both free and combined salicylate were excreted by the uraemic patients at low normal values, and in proportion similar to those in healthy subjects (Fig. 1). In particular, formation of salicyluric acid was not significantly reduced in either patient. This was confirmed both by chemical estimation and by chromatographic separation.

In Fig. 2, clearance values of free salicylate (C_{sal}) are plotted against urinary *p*H. The relation is obviously not linear ; but Table I shows that the logarithmic regression is linear, so that log (C_{sal}) is directly proportional to urinary *p*H (correlation coefficient + 0.92). Three different agents—sodium bicarbonate, acetazoleamide, and hyperventilation—were used to make the urine alkaline, but the effect on the free salicylate clearance was identical with each stimulus. The results obtained with acetazoleamide, which produces a systemic acidosis and an alkaline urine, are of special importance, since they

TABLE I

ANALYSIS OF VARIANCE OF RELATION OF LOG (C_{sal}) AND URINARY *p*H

*p*H values were grouped at intervals of 0·2 of a *p*H unit.

Source of Variation	Degrees of Freedom	Sum of Squares	Mean Square	F Value	P
Within *p*H groups . .	14	15·61	1·11		
Logarithmic regression . .	1	14·91	14·91	372·8	
Deviation from regression	13	0·70	0·054		
Error	49	1·95	0·040	1·35	>0·20

prove that urinary *p*H rather than systemic acid-base balance determines the free salicylate clearance. Ammonium chloride, producing a strongly acid urine, reduced the clearance almost to zero.

Clearances in the uraemic patients were in the low normal range, and on two occasions exceeded the simultaneous inulin clearance which averaged 20 ml./min. in these patients. This may occur in normal people at urinary *p*H above 7.5 (Gutman *et al.*, 1955), but in the patients was seen at *p*H 7.3 and 7.35.

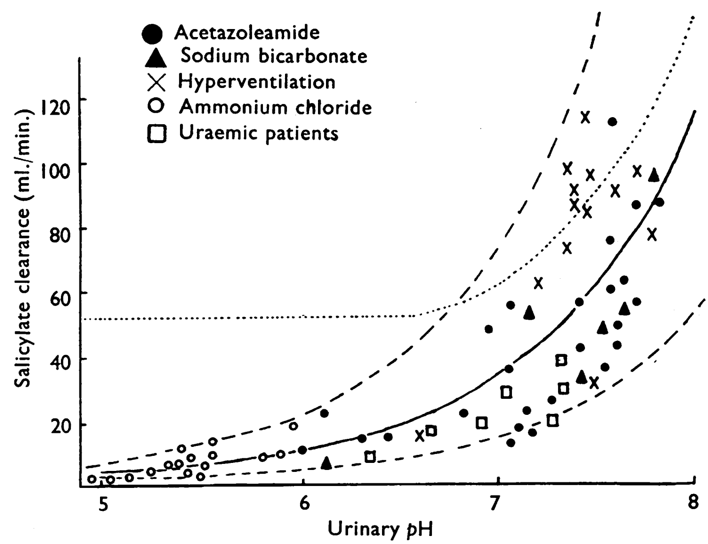

FIG. 2.—Relation between the free salicylate clearance and the urinary *p*H. The calculated regression line and the limits at twice the standard deviation are shown. The upper dotted line represents the average clearance calculated from total urinary salicylate. The log (C_{sal})/urinary *p*H relation remains unaffected whatever method is used to alter urinary *p*H.

The regression equation relating log (C_{sal}) and urinary pH was calculated to be:

$$\log (C_{sal}) = (0.52 \times pH) - 2.10$$

A similar calculation from the data of Gutman *et al.* (1955), in which sodium bicarbonate alone was used to alter urinary pH, gave the equation:

$$\log (C_{sal}) = (0.52 \times pH) - 1.92$$

The difference between the two results is not statistically significant. The regression coefficient of 0.52, which defines the rate of change of clearance with pH, will be shown later to be of great theoretical importance.

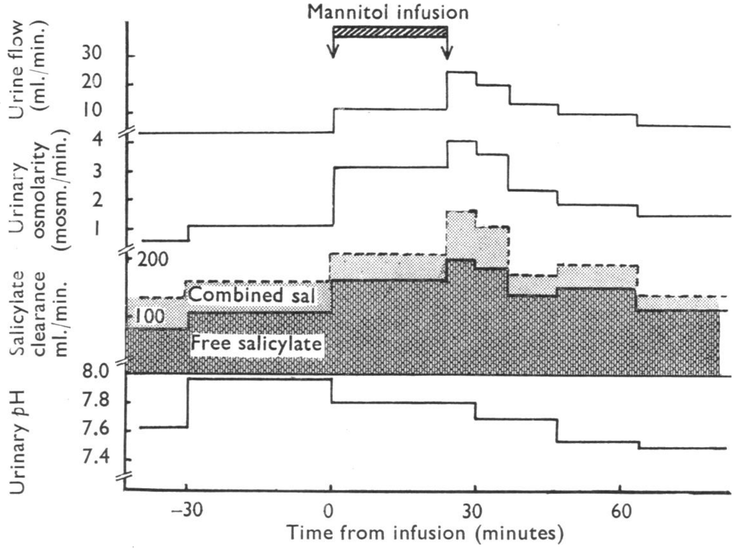

FIG. 4.—Effect of osmotic diuresis on the free salicylate clearance. The urine flow and the osmolar excretion increase proportionately more than the salicylate clearance.

Fig. 2 gives the average values of the clearance calculated from total, as well as free, salicylate. This is of less theoretical but more practical importance, since it determines the actual rate of salicylate elimination from the body. There is no significant change in excretion of pH values below 7.0, but in alkaline urine the clearance rapidly increases with rising pH.

There is considerable scatter, largely due to variation in urine flow, in the (C_{sal})/urinary pH relation. Fig. 2 gives the limits at twice the standard deviation. Clearances obtained at low rates of urine flow were lower than the values calculated from the regression equation. Conversely, during water diuresis, higher values were found. Fig. 3 shows the relation between the deviation of the logarithms of observed and calculated clearance values and the urinary minute volume. As the urinary volume rises from 0.5 to 6.0 ml./min., there is a significant increase in clearance ($P<0.01$), but at still higher rates of flow no further rise occurs. This may explain the observation of Parker (1948) that dehydration tends to raise serum salicylate levels with consequent increased toxicity. If the urine is alkaline, however, variation due to flow is small compared with that due to pH change.

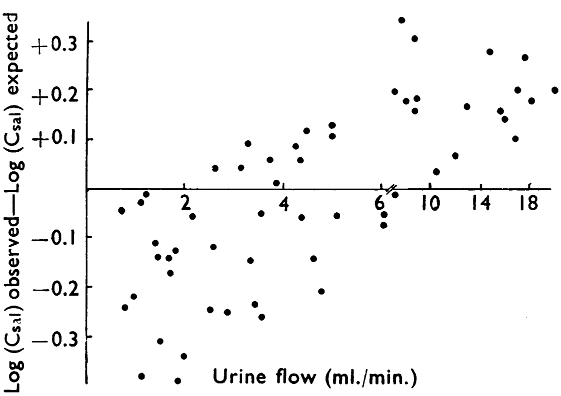

FIG. 3.—Relation between the deviation of the logarithms of the observed and calculated free salicylate clearance and the urinary minute volume. At low rates of urine flow values are less than the expected level and the reverse occurs during water diuresis. Note change of scale of abscissa at 6 ml./min.

Fig. 4 gives the values of clearances obtained during osmotic diuresis after rapid mannitol infusion. The urine was kept alkaline throughout by the ingestion of 15 g. sodium bicarbonate three hours previously. The urine volume rapidly increased from 3 to 24 ml./min., and the osmolarity from 1 to 4 mosm./min. Simultaneously, the free salicylate clearance rose sharply to twice the initial level. Osmotic diuresis therefore has a much greater effect on salicylate clearance than water diuresis. The theoretical implications of this will be discussed later.

DISCUSSION

The results show that the clearance of free salicylate is increased by alkalinization of the urine, whether this is produced by sodium bicarbonate, acetazoleamide, or hyperventilation. Thus, the main determinant of salicylate excretion is proved to be the reaction of the urine and not systemic acid-base balance. Smith (1949) suggested that the explanation lay in selective tubular reabsorption of un-ionized salicylate molecules. This theory has been shown to be quantitatively inadmissible by Dalgaard-Mikkelsen (1951). The pK_a of salicylate, which is the pH at which there are equal numbers of ionized and un-ionized molecules, is as low as 3.0. In urine of pH 5.0 about 99% of salicylate is ionized, and the proportion increases with rising pH. The concentration of the un-ionized fraction is therefore invariably low, and selective tubular reabsorption would be quite inadequate to account for the large changes in salicylate excretion.

An alternative explanation put forward by Berliner (1954) is much more satisfactory, and, in a slightly modified form, is strongly supported by our data. Berliner states, "If relatively complete impermeability to the ionized form exists and if equilibrium between tubular lumen and peri-tubular fluid occurs, the concentration of un-ionized salicylate must approach equality within and without the tubule. Under these circumstances the observed changes in excretion (including excretion, in alkaline urine, of amounts in excess of those filtered) would be predicted and no active transport of salicylate *per se* would be required." We consider that this statement should be modified in that this equilibrium is in fact far from complete, and the important influence of variation in urinary flow must be considered.

The details of this theory are more easily appreciated by reference to the diagram (Fig. 5). Only the distal tubule need be considered, since this purely physico-chemical explanation excludes participation of the glomerulus and proximal

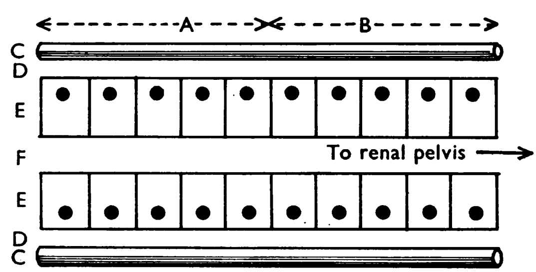

FIG. 5.—Diagrammatic representation of a length of distal tubule. In the more proximal portion, A, where urinary pH is determined, the tubule is permeable to un-ionized salicylate molecules but impermeable to the ionized fraction. In the more distal portion, B, which is concerned with determination of rate of urine flow, the tubule is almost completely impermeable both to ionized and to un-ionized fractions. Peri-tubular arterioles and capillaries are represented by C, peri-tubular fluid by D, tubule cells by E, and tubular lumen by F. Diffusion of un-ionized salicylate molecules from the peri-tubular fluid, D, to the tubular lumen, F, accounts for the marked influence of urinary pH on salicylate excretion.

tubule. A single distal tubule is represented by the tubular cells E surrounding the tubular lumen F. The tubule is bathed in peri-tubular fluid D, which approaches chemical equilibrium with an ultra-filtrate of the blood contained in the small arterioles and capillaries C. It must be assumed that the tubule is divided into two portions with widely different properties and functions, a more proximal portion A and a distal portion B. The former is concerned with adjustment of urinary pH by appropriate exchanges of hydrogen and ammonium ions for sodium in the tubular fluid according to the systemic acid-base balance. The cell membrane has selective permeability to salicylate molecules in that it is freely permeable to the un-ionized fraction, but does not allow passage of ionized salicylate. In contrast, part B of the tubule is concerned with selective reabsorption of water and is the final determinant of the rate of urinary flow. The cell membrane here is almost completely impermeable to salicylate molecules, whether ionized or un-ionized. The possibility that there is some overlap of function, or that there is a gradual and progressive change of property, is not precluded. There is histopathological evidence that in fact this latter portion of the nephron may lie in the collecting tubule rather than in the true distal tubule (Darmady, 1954).

Molecules of un-ionized salicylate diffuse freely from the peri-tubular fluid D into the distal tubular cells E, and finally into the fluid in the tubular lumen F. In any solution containing salicylate, the ratio of ionized and un-ionized salicylate molecules is determined by the Henderson-Hasselbalch equation:

$$pH - pK_a = \log \frac{[\text{ionized molecules}]}{[\text{un-ionized molecules}]}.$$

If the salicylate molecules diffuse into a highly alkaline urine a greater proportion become ionized, and therefore more salicylate must pass from the peri-tubular fluid to tubular lumen before equilibrium of the un-ionized fraction is reached. This would naturally result in a raised salicylate excretion in alkaline urine. The fact that urinary pH determines the rate of salicylate excretion rather than the final urinary salicylate concentration proves that the distal portion B of the nephron is almost completely impermeable to salicylate molecules. The slight influence of urine flow during water diuresis shows, however, that there is minimal diffusion even in this segment of the nephron.

The fraction $\frac{[\text{ionized molecules}]}{[\text{un-ionized molecules}]}$ can be shown to be directly proportional to the free salicylate clearance. The concentration of the un-ionized fraction in serum and peri-tubular fluid must vary with the non-protein bound serum salicylate, since blood *p*H is almost constant. Similarly, the concentration of ionized molecules within part A of the tubule must be proportional to free salicylate excretion in unit time, since over 99% of this is ionized. Theoretically, then, the following relation should hold:

log (salicylate clearance) = urinary *p*H minus a constant.

Experimentally it was found that:

log (salicylate clearance) = (0.52 × urinary *p*H) minus a constant.

This highly significant discrepancy can be explained by the assumption that equilibrium of un-ionized salicylate between peri-tubular fluid and tubular lumen is not complete. In view of the limited time available for diffusion complete equilibrium is in fact most unlikely to occur. By Fick's law of diffusion, the rate of flow of a diffusing substance is proportional to its concentration gradient. This means that, at increased rates of salicylate excretion in alkaline urines, there is a higher concentration gradient of un-ionized salicylate between peri-tubular fluid and the tubular lumen, and consequently equilibrium is less complete. If full equilibration occurred, C_{sal} would be expected to increase tenfold for every unit rise of urinary *p*H, whereas in fact a tenfold increase occurs with every *two* units rise of *p*H.

Further confirmation of the diffusion theory is given by the results obtained during osmotic diuresis. Current views of renal physiology suggest that the volume of isosmotic fluid in the segment of the distal tubule A is proportional to the excreted osmolar load. Infusion of mannitol (Fig. 4) temporarily increased the urinary osmolar excretion fourfold. This would increase the volume of tubular fluid available for diffusion of salicylate by a similar amount. If equilibration were complete the salicylate clearance would be expected to rise correspondingly, but the observed increase was in fact half this—that is, twofold. Again, this can be explained by the assumption of a greater concentration gradient with increased rate of salicylate excretion.

The results in the uraemic patients show that excretion of salicylate may be almost normal despite gross reduction of the glomerular filtration rate. This is again to be expected on the diffusion theory, since glomerular function is not involved. Salicylate excretion in extreme renal failure may obviously be limited by gross reduction of the renal blood flow. The kidney cannot excrete a substance at a rate greater than that at which it is delivered by the blood. This aspect was not investigated, since accurate determination of renal blood flow in uraemia necessitates renal vein catheterization (Cargill, 1949).

Similar principles have been shown to govern the urinary excretion of ammonia (Clarke, Evans, MacIntyre, and Milne, 1955). Since ammonia is a basic substance increased excretion occurs in acid urines. The direction of the concentration gradient with ammonia is modified by its intracellular synthesis. The highest concentration is within the cell, and diffusion occurs both into the tubular lumen and outwards to the peri-tubular fluid and renal capillary blood. It is probable that a diffusion mechanism also determines the excretion of gentisic acid (Batterman and Sommer, 1953), and of the bases quinine (Haag, Larson, and Schwartz, 1943), nicotine (Haag and Larson, 1942), mepacrine, chloroquine, sontoquine (Jailer, Rosenfeld, and Shannon, 1947), and procaine (Terp, 1951). The observed data regarding the excretion of these substances are, however, not nearly so complete as those relating to ammonia and salicylate.

In summary, the diffusion theory explains almost all the observed facts of salicylate and ammonia excretion. One possible theoretical objection is that a purely physico-chemical mechanism is considered to perform osmotic work by concentration of either salicylate or ammonia. In fact, however, work is performed in the maintenance of a concentration gradient of hydrogen ion between blood and urine. In addition, it is probable that intracellular energy is necessary to maintain the peculiar selective permeability of the cell membrane to the un-ionized molecule.

The results are of practical pharmacological importance in the following respects:

(*a*) Therapeutic doses of salicylate can be prescribed in cases of uraemia of moderate severity without being excessively toxic. In severe renal failure, however, excretion may be impaired by gross reduction of the renal blood flow.

(*b*) In the treatment of salicylate poisoning the aim should be to produce and maintain a urine of maximal *p*H. It is not sufficient to make the urine alkaline to litmus. The reaction should be kept about *p*H 8.0. Acetazoleamide may prove to be useful, as it causes very rapid alkalinization of the urine, and produces a systemic acidosis which may counteract the respiratory alkalosis of salicylate intoxication. This drug, however, produces only temporary alkalinization of the urine, and sodium bicarbonate is necessary to maintain a high urinary pH. The production of an osmotic diuresis by mannitol may also be useful in increasing salicylate excretion. Sufficient fluid should be given either orally or intravenously to prevent dehydration and maintain an adequate urine flow.

(c) Sodium bicarbonate should not be prescribed if salicylates are being given therapeutically. It is often considered that the prescription of a mixture of sodium bicarbonate and salicylate is exactly equivalent to the administration of a smaller dose of sodium salicylate alone. This is not correct. The aim in the therapeutic use of salicylates is to maintain a steady, non-toxic, but adequate blood level. This can be achieved only if the excretion of the drug remains constant. From the data in Fig. 2 it can be shown that log $(C_{sal}) = (0.52 \times \text{urinary } p\text{H}) - \text{a constant}$. Differentiating with respect to pH:

$$\log_e 10 \times \frac{1}{C_{sal}} \times \frac{d\,(C_{sal})}{d\,(p\text{H})} = 0.52$$

$$\frac{d\,(C_{sal})}{d\,(p\text{H})} = 0.23 \times (C_{sal})$$

Thus, the rate of change of salicylate excretion with respect to variation in urinary pH is proportional to the free salicylate clearance. Rapid variation of salicylate excretion therefore occurs when the urine is alkaline, with corresponding difficulty in the stabilization of serum levels.

Summary

1. The free salicylate clearance (C_{sal}) is related to urinary pH, log (C_{sal}) varying directly with pH.

2. The relation log (C_{sal})/urinary pH remains identical when urinary pH is altered by sodium bicarbonate, acetazoleamide, ammonium chloride, and hyperventilation. Thus, alteration in urinary pH rather than the systemic acid-base balance determines the rate of salicylate excretion.

3. Salicylate excretion in two patients with moderate uraemia was in the low normal range.

4. Water diuresis slightly increases the salicylate clearance, but osmotic diuresis has a much greater effect.

5. The observed facts are shown to be compatible with a theory that free salicylate is excreted by a process of tubular diffusion of un-ionized salicylate molecules rather than by the more familiar method of glomerular filtration and tubular reabsorption. Since the ratio of ionized to un-ionized salicylate increases with rising pH, this explains the more rapid excretion in alkaline urine.

6. The practical applications of the results in salicylate therapy and in the treatment of salicylate poisoning are discussed.

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