Effect of Complex Formation on Drug Absorption I

Complexes of Salicylic Acid with Absorbable and Nonabsorbable Compounds

By GERHARD LEVY and RICHARD H. REUNING*

The effect of complex formation on drug absorption (gastric absorption by rats) has been studied by means of two model systems: (*a*) a rapidly absorbed drug (salicylic acid) complexed with a hydrophilic, surface-active, nonabsorbed, macromolecular substance (polysorbate 60), and (*b*) a rapidly absorbed drug (salicylic acid) complexed with a much more slowly absorbed drug (caffeine). In both studies, ethanol was used as a noncomplexed marker to afford recognition of possible changes in permeability of the gastric mucosa. Results of these studies were correlated with kinetic analyses based on the stability or binding constants of the respective complexes, the difference in diffusivity between free drug and drug complex, and the absorption rate of the free drug. It is shown that the absorption rate of drugs can be modified by formation of complexes which differ from the free drug mainly in size or in lipoid-water partition coefficient.

COMPLEX FORMATION of drugs has been the subject of extensive physicochemical studies in recent years. These studies have demonstrated the potential utility of complexation as a means of increasing the solubility and stability of drugs. It is also appreciated now that unintended complex formation may occur between drugs that are administered simultaneously either in a single or in separate dosage forms. These considerations warrant an investigation of the effect of complex formation on drug absorption. Moreover, it is desirable to explore the potentialities of intentional complex formation as an approach to the modification of absorption characteristics of chemotherapeutic agents.

The physicochemical properties of drug complexes can differ in several respects from those of the respective free drugs; such differences can involve electrical charge, diffusivity, size, and lipoid-water partition coefficient, among others. These properties can have an appreciable effect on the rate of transfer of substances across biologic membranes. Studies in this laboratory, to be described in this and in subsequent reports, have been concerned with the use of model compounds and complexes to investigate various aspects of complex formation and its effect on drug absorption, as well as with the exploration of a number of potential applications of drug complexation in chemotherapy. This initial report deals with a study of the effect of complexation with an absorbable and nonabsorbable compound, respectively, on the absorption of salicylic acid from the stomach of the rat.

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* Fellow of the American Foundation for Pharmaceutical Education.

EXPERIMENTAL

Experimental Procedure in Rats.—Female Wistar rats weighing 100–150 Gm. were used. The rats were starved for 15–20 hours prior to the experiment, and water was removed from the animals 0.5 hour prior to the operation. The animals were anesthetized with ether and were maintained under anesthesia for the entire course of the experiment. A midline incision was made in the abdominal region, and the stomach was exposed. The esophagus and the small intestine were then tightly ligated immediately adjacent to the cardiac sphincter and about 1 cm. from the pyloric sphincter, respectively. Care was taken not to occlude major blood vessels. Five milliliters of drug solution, previously warmed to 37°, was injected with a syringe and hypodermic needle by inserting the needle into the intestine between the pyloric valve and the ligature on the intestine and subsequently working the needle through the pyloric valve into the lumen of the stomach. A third ligature was tied around the intestine and needle to prevent leakage on injection. As the needle was withdrawn, this third ligature was tightened further to prevent any leakage of the drug solution. After completion of the procedure, the incision was closed with wound clips. At the end of 1 hour, the entire stomach was excised and homogenized. The homogenate was then assayed for unabsorbed salicylic acid and ethanol.

Determination of Temperature of Drug Solution within the Rat Stomach.—The surgical procedure was the same as described in the above paragraph, except that along with the hypodermic needle a thermistor probe was inserted through the pyloric valve into the stomach. The thermistor probe was left in the stomach for 1 hour after the drug solution, which had been preheated to 37°, was injected. The thermistor probe was part of a thermistor type temperature recorder[1] which provided a continuous record of the drug solution temperature over the 1-hour absorption period.

[1] Tri-R Tempcord-R, Tri-R Instruments, Jamaica, N. Y.

TABLE I.—EFFECT OF COMPLEX FORMATION ON SALICYLIC ACID ABSORPTION FROM RAT STOMACH

Compn. of Solution,[a] %	Animals, No.	Salicylic Acid Absorbed,[b] %	Half-Absorption Time,[c] Hr.	Initial Complexation of Salicylic Acid,[d] %
Salicylic acid, 0.1	10	50 (6)[e]	1.0	0
Salicylic acid, 0.1 Polysorbate 60, 2	11	33 (5)	1.7	62
Salicylic acid, 0.1 Caffeine, 2.75	10	37 (4)	1.5	84

[a] 0.1 *N* HCl served as the solvent in all solutions. [b] In 1 hour. [c] Assuming absorption to be a first-order process. [d] Based on *in vitro* determinations. [e] Standard deviation in parentheses.

Determination of Fluid Volume in the Rat Stomach.—Phenol red was used as a marker to determine the extent of volume change of the drug solution in the rat stomach due to water absorption or fluid secretion. The procedure was the same as that used in the absorption experiments, except for the following modifications. The stomach was first washed with four separate portions of a phenol red solution (0.02% in 0.1 *N* hydrochloric acid) before injecting the solution for the 1-hour absorption period. Instead of the entire stomach being homogenized, the solution was withdrawn from the stomach, and the concentration of phenol red was determined. A control experiment using the same procedure on excised stomachs was also performed in order to determine the extent of binding of phenol red to gastric contents and to the mucosa during the 1-hour absorption period. Determinations were made both with a 0.02% phenol red solution and a solution containing 0.02% phenol red and 0.1% salicylic acid.

Phenol red was assayed by diluting the withdrawn solution, adding sufficient sodium hydroxide to yield a 0.01 *N* concentration of this base, filtering through a filter (Millipore), and determining the absorbance at 560 mμ with a Bausch & Lomb spectronic 20 colorimeter. Any change in the volume of the drug solution in the stomach during the hour would be reflected by a corresponding change in phenol red concentration.

Assay for Salicylic Acid.—A modification of the method of Brodie *et al.* (1) for determination of salicylic acid in plasma was used. The rat stomach and its contents were homogenized with 20 ml. water in a Waring Blendor micro-jar. The homogenate was transferred to a separator, acidified with 5 ml. of 6 *N* hydrochloric acid, and extracted with one 30-ml. and three 20-ml. portions of ethylene dichloride. Each ethylene dichloride layer was separated by centrifuging. Enough ethylene dichloride was added to the combined extracts to obtain a total of 100 ml. A 20-ml. aliquot of the ethylene dichloride extract was shaken for 5 minutes with 10 ml. of an aqueous solution of ferric nitrate (1%) in 0.07 *N* nitric acid. After centrifuging, the aqueous phase was aspirated and assayed colorimetrically for salicylic acid at 530 mμ with a Bausch & Lomb spectronic 20 colorimeter.

Using the tissue extraction method described above, tissue blanks were analyzed and were found to yield essentially negligible salicylate blank values whether or not polysorbate 60 or caffeine was present. Extraction efficiency was determined by adding known amounts of salicylic acid solution to rat stomachs, homogenizing, and then extracting and assaying as described above. Recovery was found to be essentially constant over the concentration ranges encountered, regardless of the presence or absence of polysorbate 60 or caffeine. The average recovery was 91%. In the absorption experiments, the amount of salicylic acid remaining unabsorbed was calculated on the basis of the concentration of the assayed solution, its dilution, and the correction factor for recovery.

Assay for Ethanol.—Ethanol concentration was determined by the enzymatic method of Bücher and Redetzki (2), using a small aliquot of diluted rat stomach homogenate. In this method, ethanol is reacted with alcohol dehydrogenase (yeast) and diphosphopyridine nucleotide (DPN) in a buffered medium. The enzyme catalyzes the oxidation of ethanol by DPN. Ethanol concentration is reflected by the concentration of reduced DPN which was determined spectrophotometrically at 360 mμ with a Beckman model DU spectrophotometer. Tissue blanks were determined and, although significant, were quite constant. Recoveries were found to be essentially constant over the concentration range used, the average recovery being 87%. In the absorption experiments, the amount of ethanol remaining unabsorbed was calculated from the spectrophotometric results appropriately corrected for blank value, dilution factor, and recovery.

Determination of the Extent of Binding of Salicylic Acid by Polysorbate 60.—This determination was carried out by an equilibrium dialysis method. Ten milliliters of a 2% polysorbate 60 solution in 0.1 *N* hydrochloric acid was placed in a dialysis bag.[2] The bag was then placed into a 125-ml. conical flask which contained 110 ml. of a salicylic acid solution in the same solvent. The solutions were allowed to equilibrate at 33° for 7 days in a constant temperature water bath shaker. During this time the solutions were constantly agitated. At the end of 7 days, the solutions inside and outside the dialysis bag were assayed for salicylic acid concentration using the colorimetric procedure of Trinder (3). The increase in concentration of the inside solution over that of the outside solution is a direct indication of the amount of salicylic acid bound to polysorbate 60 contained in the bag. Similar experiments were carried out using distilled water and 0.005 *N* HCl (rather than 0.1 *N* HCl) as the solvent.

Determination of the Extent of Binding of Ethanol by Polysorbate 60.—This determination was carried out by an equilibrium dialysis method similar to the one described in the preceding paragraph. Ten milliliters of a 2% ethanol solution in distilled water was placed in a dialysis bag which was suspended in 110 ml. of 2% ethanol and 2% polysorbate 60 in distilled water. The solutions were equilibrated

[2] Tomac Nylon Bags, American Hospital Supply Corp., Evanston, Ill.

for up to 15 days and assayed by the method of Pawan and Hoult (4). Control experiments were carried out to verify the permeability of the dialysis membrane to ethanol, the impermeability of the membrane to polysorbate 60, and the absence of interference of polysorbate 60 with the ethanol assay.

Determination of the Stability Constant of the Caffeine–Salicylic Acid Complex.—The solubility method described by Higuchi and Zuck (15) was used to determine the stability constant of the caffeine–salicylic acid complex at 33°.

Determination of Partition Coefficients.—Partition coefficients were determined using 0.1 *N* hydrochloric acid and isoamyl acetate as the aqueous and organic phase, respectively. Equilibration was carried out at room temperature for at least 12 hours using an automatic shaking apparatus. Salicylic acid concentration was determined by the method of Trinder (3) and caffeine concentration by the method of Axelrod and Reichenthal (5).

RESULTS AND DISCUSSION

The model drug used in these studies, salicylic acid, is absorbed from the stomach and intestinal tract mainly by passive diffusion of nonionized molecules (6, 7). It was chosen because it is rapidly absorbed, and it forms complexes with many drugs and other compounds of pharmaceutical interest. It was decided to study gastric rather than intestinal absorption for the following reasons: the pH dependence of salicylic acid absorption and the feasibility of maintaining a low and constant pH in the stomach, the more rapid absorption of one of the complexing agents (caffeine) from the intestine which resulted in lethalities during preliminary intestinal perfusion experiments, the relative constancy of gastric volume under the experimental conditions, the possibility of using relatively large volumes of drug solution, and the ease of isolation and manipulation of the stomach.

The experimental procedures differed somewhat from the methodology used by many other investigators. In order to prevent damage to the delicate gastric mucosa, the rat stomach was not washed prior to the absorption experiment; the entire stomach and its contents (rather than gastric contents alone) were assayed at the end of the absorption period, so that only drug which had entered the circulation was considered as having been absorbed.

The two complexing systems used in this investigation represent different characteristics. The salicylic acid–polysorbate 60 system consists of a well-absorbed drug and a macromolecular substance which is not absorbed as such. It may be assumed, therefore, that the micellar complex of salicylic acid and polysorbate 60 will not pass across biologic membranes. On the other hand, both components of the salicylic acid–caffeine system are absorbed, but the latter at a much slower rate than the former (6). It may be expected that the complex itself would be absorbable but at a rate somewhat different from that of either of the component drugs.

Since the *in vivo* effects of complex formation were to be correlated with *in vitro* binding or stability data, the *in vivo* temperature of the drug solutions was determined, and *in vitro* measurements were carried out at the same temperature. Continuous temperature recording in the stomach of rats under conditions similar to those of actual absorption experiments indicated an average intragastric temperature of 34° initially, which decreased to 32° toward the end of 1 hour. Consequently, all physicochemical measurements were made at 33°.

The Salicylic Acid–Polysorbate 60 Complex.—The absorption of salicylic acid was decreased from 50% in 1 hour to 33% in 1 hour in the presence of 2% polysorbate 60 (Table I). This difference was found to be statistically significant ($p < 0.01$) by the Student *t* test. Absorption of salicylic acid from the stomach can be depicted satisfactorily as an exponential process (6, 8, 9), provided that adequate corrections are made for gastric emptying or that gastric volume can be maintained essentially constant. In general agreement with the observations of Schanker *et al.* (6), we found (by using phenol red as a marker) that gastric volume in the ligated rat stomach had changed on the average by less than 7% at the end of 1 hour, under the experimental conditions. This permitted calculation of first-order rate constants and half-absorption times, although the values must be looked upon as approximations only.

Levy (10) has discussed various mechanisms by which surface-active agents may modify drug absorption. He cautioned that it is necessary to consider possible effects on the absorbing membranes, interaction of the surface-active agent with the drug, and modification of dosage form properties. Some surface-active agents can cause damage to the gastrointestinal epithelium (11), and it is therefore possible that the over-all effect of polysorbate 60 on the absorption of salicylic acid, as observed in the present study, may represent the sum of two effects: modification of membrane permeability characteristics and micellar complexation of the drug. To investigate this possibility, the absorption of ethanol without as well as in the presence of polysorbate 60 was determined. Ethanol is not bound to polysorbate 60 (as evidenced by the results of equilibrium dialysis), and modification in membrane permeability characteristics should consequently be reflected by changes in the absorption rate of ethanol. Two per cent polysorbate 60 had no measurable effect on ethanol absorption (Table II), and it may be concluded therefore that changes in salicylic acid absorption in the presence of polysorbate 60 are due solely (or at least predominantly) to complex formation.

TABLE II.—EFFECT OF SALICYLIC ACID, CAFFEINE, AND POLYSORBATE 60 ON ETHANOL ABSORPTION FROM RAT STOMACH

Compn. of Solutions,[a] %	Animals, No.	Ethanol Absorbed,[b] %	S.-D.
Ethanol, 2	5	48	8
Salicylic acid, 0.1 Ethanol, 2	4	46	6
Salicylic acid, 0.1 Polysorbate 60, 2 Ethanol, 2	5	42	7
Salicylic acid, 0.1 Caffeine, 2.75 Ethanol, 2	4	51	4

[a] 0.1 *N* HCl served as the solvent in all solutions. [b] In 1 hour.

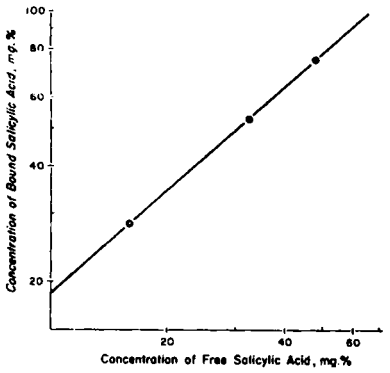

Fig. 1.—Relation between concentration of bound and free salicylic acid in solution of 2% polysorbate 60 in 0.1 *N* hydrochloric acid at 33°. Each point represents the average of four determinations.

The magnitude of interaction of salicylic acid with polysorbate 60 was determined by equilibrium dialysis and was found to be describable by the equation

$$C_b = KC_f^a \qquad \text{(Eq. 1)}$$

or

$$\log C_b = \log K + a \log C_f \qquad \text{(Eq. 2)}$$

where C_b is the concentration of bound drug, C_f is the concentration of free drug, and K and a are constants. This relationship, which is analogous to the Freundlich adsorption isotherm, applies also to the interaction of many drugs with plasma proteins (12). Results of the binding study (Fig. 1) showed that in the salicylic acid concentration range which existed in the absorption experiment (0.1% initially, decreasing to 0.067% at the end of 1 hour), the binding of drug with polysorbate 60 varied from 61.5 to 63.0%. Thus, the extent of drug complexation was essentially constant.

The gastric absorption of salicylic acid in the absence of complexing agents can be described by the exponential expression

$$\log A = \log A_0 - \frac{K}{2.3} t \qquad \text{(Eq. 3)}$$

where A_0 is the amount of drug introduced into the stomach, A is the amount remaining in the stomach at time t, and K is the first-order absorption rate constant.

When a constant fraction of drug is complexed and when it is assumed that only free drug is absorbed, absorption rate may be described by

$$\frac{dA}{dt} = -KfA \qquad \text{(Eq. 4)}$$

where dA/dt is the rate of absorption and f is the fraction of free drug. Upon integration, evaluation of constant of integration and change to common logarithms,

$$\log A = \log A_0 - \frac{K}{2.3} ft \qquad \text{(Eq. 5)}$$

The amount of salicylic acid remaining unabsorbed at 1 hour, upon administration of 5 ml. of a solution of 0.1% salicylic acid and 2% polysorbate 60, was calculated by Eq. 5. Using values of K = 0.69 hour^{-1} and f = 0.38, the theoretical amount A remaining unabsorbed is 3.8 mg. The actual average amount unabsorbed was 3.4 mg. The small difference between the theoretical and experimental value may be due to normal experimental variation, or it may be caused by competitive binding of polysorbate 60 by mucosal proteins or gastric contents. Lack of stability of polysorbate 60 in acidic gastric fluids was not a factor, since equilibrium dialysis of salicylic acid against polysorbate 60 in either 0.1 *N* HCl or distilled water yielded essentially equal results. It is suggested, therefore, that the effect of polysorbate 60 on the absorption of salicylic acid, under the experimental conditions, is due to a decrease in activity of the drug as a result of micellar complexation. It appears that the magnitude of the *in vivo* effects may be estimated satisfactorily on the basis of kinetic considerations based on *in vitro* binding constants and the absorption rate constant for the free drug.

The Salicylic Acid–Caffeine Complex.—The gastric absorption of salicylic acid is decreased from 50% per hour to 37% per hour in the presence of caffeine (Table I). The difference is statistically significant ($p < 0.01$). Since caffeine is ulcerogenic in rats [daily oral administration of caffeine for 5 days has caused superficial erosions of the glandular mucosa (13)], and since such breakdown of the mucosa:plasma barrier can result in more rapid drug absorption (14), ethanol was used as a noncomplexed marker to determine possible modification in membrane permeability characteristics. There was no evidence of increased permeability (Table II), and it may be concluded that acute administration of caffeine did not damage the gastric mucosa significantly during the 1-hour absorption period. The decreased absorption of salicylic acid cannot be ascribed to an increased pH of the solution containing caffeine, since the pH remained the same as that of the solution which did not contain caffeine.

The stability constant of the salicylic acid–caffeine complex in 0.1 *N* hydrochloric acid at 33°, determined by the solubility method (15), was found to be 40 (Fig. 2). This value compares favorably with that reported by Higuchi and Zuck (15) for the same complex at 30°—namely 44. A decrease in the stability constant at higher temperatures is to

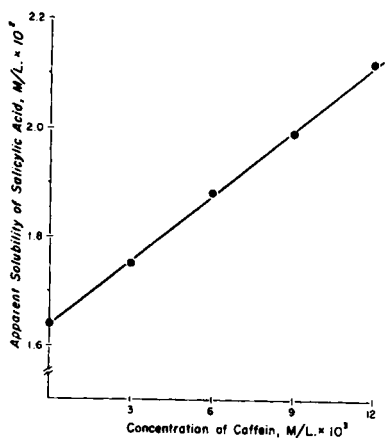

Fig. 2.—Apparent solubility of salicylic acid in 0.1 *N* hydrochloric acid at 33° as a function of caffeine concentration. Each point represents the average of at least four determinations.

TABLE III.—PARTITION COEFFICIENTS (ISOAMYL ACETATE, 0.1 *N* HCl) OF SALICYLIC ACID AND CAFFEINE ALONE AND PARTLY COMPLEXED

Initial Compn. of Aqueous Phase, %	Ratio, Organic: Aqueous Phase	Partition Coefficient
Salicylic Acid		
Salicylic acid, 0.1	5:100	163
Salicylic acid, 0.1 Caffeine, 2.75	5:100	28
Caffeine		
Caffeine, 0.2	10:1	0.27
Caffeine, 0.2 Salicylic acid, 0.275	10:1	0.37
Caffeine, 0.2 Salicylic acid,[a] 3.025	1:1	0.46
Caffeine, 0.2 Salicylic acid,[a] 8.525	1:1	0.84

[a] In these instances, most of the salicylic acid was dissolved in the organic phase prior to equilibration.

be expected. The partition coefficient (isoamyl acetate:0.1 *N* HCl) of salicylic acid is considerably greater than that of caffeine. Complexation of salicylic acid with caffeine decreases the apparent partition coefficient of the former and increases the apparent partition coefficient of the latter (Table III). This suggests one possible mechanism for the decreased absorption of salicylic acid in the presence of caffeine, as found in the present study: the salicylic acid–caffeine complex may be absorbed more slowly than salicylic acid itself and, since salicylic acid was 84% complexed in the administered solution, the over-all absorption retarding effect may be appreciable.

The theoretical rate of absorption of the salicylic acid–caffeine complex may be calculated by use of an equation developed by Higuchi and Lachman for an analogous *in vitro* system (16):

$$\frac{1}{t_{0.5}}(K_sC + 1) = \frac{1}{t_{0.5f}} + \frac{1}{t_{0.5c}}K_sC \quad \text{(Eq. 5)}$$

where K_s is the stability constant of the complex, C is the molar concentration of free caffeine, $t_{0.5}$ is the half-absorption time of salicylic acid in the presence of caffeine, $t_{0.5f}$ is the half-absorption time of free salicylic acid, and $t_{0.5c}$ is the half-absorption time of the salicylic acid–caffeine complex. Equation 5 applies in the strict sense only to systems where the complexing agent (caffeine in this case) is not absorbed, but the equation can be used satisfactorily in the present case since caffeine is relatively slowly absorbed and is used in high concentration.

The following information is used to solve Eq. 5: $t_{0.5} = 1.5$ hours and $t_{0.5f} = 1.0$ hours (both from data in Table I), $K_s = 40$ (from data depicted in Fig. 2), and $C = 0.135$ *M* or 0.101 *M* calculated from K_s using

$$(C) = \frac{(S - C)}{(S)K_s} \quad \text{(Eq. 6)}$$

where (C) is the concentration of free caffeine, (S) is the concentration of free salicylic acid, and $(S - C)$ is the concentration of the salicylic acid-caffeine complex. According to the data of Schanker *et al.* (6), caffeine is 24% absorbed in 1 hour. Our own data [based on 90 minute absorption experiments (17)] indicate an absorption rate of 27% per hour. If caffeine were not absorbed at all (which is the condition actually required for use of Eq. 5), $t_{0.5}$ is 6.3 hours. Going to the other extreme by assuming caffeine concentration to be 27% lower from the very beginning, $t_{0.5}$ is 5.0 hours. It is evident that (*a*) the equation can be used satisfactorily for estimating purposes (particularly in view of the limitations imposed by experimental variability of the *in vivo* system) and (*b*) it must be assumed that the complex itself is absorbed since the theoretical $t_{0.5}$ calculated above is much greater than the experimentally obtained value. Accepting this assumption and solving for $t_{0.5c}$ (the half-absorption time of the complex), yields a value of 1.7 hours (this value is the same if based on no absorption of free caffeine or on immediate 27% absorption). Interestingly, this value is intermediate between that of free salicylic acid (1.0 hour) and free caffeine (2.2–2.5 hours). The relative absorption rates are also consistent with the respective partition coefficients.

Despite the attractiveness of the hypothesis presented above, it is appropriate to consider an alternative mechanism to explain the experimental data. There is some indication that the gastric absorption of salicylic acid by the rat is, under the experimental conditions, rate limited by the rate of movement of drug molecules from the center of the gastric lumen to the periphery (6, 18). Assuming this to be mainly a diffusion process (rather than mixing and flow of gastric contents) and using Thovert's equation (19) to estimate the effect of molecular weight on diffusivity, it can be shown that the diffusivity of salicylic acid is 55% greater than that of the salicylic acid–caffeine complex:

$$\frac{\text{diffusivity of salicylic acid}}{\text{diffusivity of salicylic acid–caffeine complex}} = \frac{\sqrt{\text{mol. wt. of complex}}}{\sqrt{\text{mol. wt. of free acid}}} = 1.55$$

Correcting for the extent of salicylic acid complexation (84%) in the salicylic acid–caffeine system, the absorption rate constant of free drug should be about 42% greater than that in the presence of caffeine. This was indeed the case. Thus, the experimental data are equally compatible with an assumed decreased membrane permeation rate of the complex (compared with free salicylic acid) and a decreased rate of diffusion of the complex to the absorbing membranes. Further studies of this problem are in progress.

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