A HORMONE MECHANISM FOR GALL-BLADDER CONTRACTION AND EVACUATION

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Received for publication June 20, 1928

The numerous reports in the literature showing the marked efficacy of fats, egg-yolk and meat protein in emptying the gall bladder convinced us that the effect of "secretin" on the evacuation and motor activity of the gall bladder should be studied, since it is well known that these substances stimulate the pancreas, and that "secretin" (very impure solutions) promotes the formation of bile. With this objective in mind, we have performed several series of experiments, the results of which prove, we believe, that a hormone mechanism is concerned in gall-bladder contraction and evacuation.

For some time Ivy, Kloster and Lueth (1927) have been working on the purification of "secretin" for the purpose of perfecting a pancreatic function test for man. In our work we have used the preparations prepared for us by them. The solutions furnished us were "highly purified," were vaso-dilatin-free, and have had no objective deleterious action when injected intravenously in succession in anesthetized or unanesthetized animals.

In a search of the literature it has been found that several investigators have attempted to determine whether or not a humoral mechanism is concerned in gall bladder evacuation. Boyden (1926a) found that the transfusion of blood of fed or starved cats caused a partial evacuation of the gall bladder. He does not state that he tested the compatibility of the blood of the cats used. He interpreted the results of his experiments on this phase of the gall bladder question as meaning that the gall bladder is sensitive to bodily conditions which effect changes in the circulating blood. Whitaker (1926) injected doses of "secretin" prepared by the Bayliss-Starling method with negative results. Copher and Illingworth (1928) injected "secretin" with negative results; but they do not state the method by which the "secretin" used by them was prepared. Brugsch and Hors ters (1926) placed a condom balloon in the gall bladder of dogs and found that 10 cc. of a preparation of Dale and Laidlaw (1912-13) "secretin" caused a contraction and rhythmic changes of the gall bladder, and "hypo-
physin” relaxed it. They observed that histamine (10 mgm.) caused the gall bladder to contract. They concluded that histamine and “secretin” act alike, the latter being the physiological agent. They did not make a simultaneous blood pressure tracing to rule out vaso-dilatation as being a factor, which we know is a factor.

Experiments. On cats without cystic duct clamped. The cat was barbitalized, the common bile duct was cannulated, and a cannula was placed into the gall bladder through a small opening in the fundus. The cannula in the gall bladder was connected to a glass tube, which served as a manometer. The glass tube was connected to a recording tambour. A simultaneous carotid blood-pressure tracing was made. Four such experiments were performed. Within one minute after the intravenous injection of the “purified secretin” (2 mgm. of solid material), the intra-gall-bladder pressure was increased, and steadily increased until it amounted to from 2 to 3.5 cm. of bile pressure. About four minutes after the injection the bile flow was augmented. The pressure started to decline in from 1 to 1 hour in these experiments. The blood pressure, respiration and heart rate were unaffected by the injection. Normal saline controls proved negative.

On cats with cystic duct clamped. To avoid entirely the possibility that the increase in gall bladder pressure might be due to an inflow of bile, which was not likely, we clamped the cystic duct. Three experiments of this kind were performed. In all experiments the gall-bladder pressure was raised on the injection of the “secretin,” but not to the extent that occurred in the preceding experiments. This difference, we believe, was due to some interference with the blood supply of the gall bladder, which is very difficult to avoid in the cat on ligating or clamping the cystic duct.

Atropine and histamine in cats. It was found that the “secretin” preparation caused as much contraction after the injection of 1 mgm. of atropine as it did before the injection of atropine. Two such experiments were performed.

The injection of 0.5 mgm. of histamine intravenously in the cat caused a decided fall in blood pressure without changing the intra-gall-bladder pressure. This effect is different from that which occurred in the dog, which will be shown later in this paper.

On dogs with the cystic duct clamped. Dogs were anesthetized with barbital (0.200 gram per kilo body weight) or with “light” ether. The pancreatic duct was cannulated, the cystic duct was clamped above the opening of the right hepatic duct, and the fundus of the gall bladder was cannulated. The cannula was connected to a glass tube which served as a manometer, which in turn was connected to a recording tambour. The carotid was connected to a recording blood pressure manometer. In many experiments the cystic artery was included in the clamp, it being impossible to prevent this without anatomical dissection, which we wished to avoid.
When the "highly purified secretin" preparation was injected intravenously (3 mgm. of solid material) the intra-gall-bladder pressure began to rise in from one to two minutes, even before the pancreas began to secrete. The rise of pressure from a single injection has varied in over eighty experiments from 1.0 cm. of bile pressure to 11.5 cm. of bile pressure. We have found only one dog out of more than eighty which has failed to respond. Why, we do not know. The injection, as a rule, causes no change in blood pressure, heart rate or respiration. Occasionally a 5 or 10 mm. rise followed by a fall of 5 or 10 mm. in blood pressure occurs, which we believe is due to the handling of the femoral vein into which all of our injections have been made. Sometimes a 5 or 10 mm. fall will occur which may persist from one to ten minutes (figs. 1, 2 and 3).

We believe that one cause of the variation in the amount of increase in intra-gall-bladder pressure which occurs is due to the inclusion of the cystic artery in the clamp. The greatest rises have usually occurred in those animals in which it was possible to clamp the duct without at the same time clamping the cystic artery.

In some animals in which the tonic contraction amounts to only a 3 cm. rise in pressure, rhythmic contractions amounting to as much as 2.5 cm. of bile pressure occur superimposed on the tonic contraction. As a rule, when the tonic contraction is quite marked the rhythmic contractions disappear at the height of the tonic contraction.

Our experiments show that there is an optimal initial pressure necessary for an optimal response to the injection. This optimal pressure is approximately 6 cm. of bile pressure. In this connection we have found that if, due to the spilling of some bile during the insertion of the cannula into the fundus of the gall bladder, the bile does not rise in the cannula several centimeters above the gall bladder, a response to the injection may not occur; but if some normal saline (37°C.) is placed in the cannula so that it rises in the tube for 5 or 7 cm., then the injection results in a decided contraction.

The period of increased gall-bladder pressure due to one injection lasts from 10 to 60 minutes, the average being about 15 minutes. If a tonus rhythm is not being manifested by the gall bladder prior to the first injection, it usually appears during the period of the relaxation of the tonic contraction and frequently persists for the remainder of the experiment, which in the process of assaying various preparations for further purification may last as long as six to eight hours.

If the animal has been fed prior to barbitalization, or if the gall bladder has been "energized" by a meal, the injection of the "secretin" preparation causes only a slight response. This is analogous to our experience that the gall bladder frequently fails to respond after it has been caused to contract some ten or twelve times within three or four hours. The maximum rise
due to a single injection was 11.5 cm. of bile, and the maximum rise due to a series of injections was 22.5 cm. of bile, six injections being made at five or ten minute intervals. We have observed no difference in the gall-bladder response of male and female dogs.

Bile and bile salts. The intravenous injection of dilute bile and one per cent solutions of sodium glycocholate and taurocholate failed to cause gall bladder contraction.

Atropine and histamine in dogs. Even 10 mgm. of atropine sulphate will not prevent the rise in gall-bladder pressure when the "secretin" preparation is injected (fig. 5).

Histamine causes in the dog a contraction of the gall bladder which is a mirror image of the fall in blood pressure. Every agent that we have injected which causes a fall in blood pressure has caused a contraction of the gall bladder (fig. 3).

Deep anesthesia. Moderate and deep ether anesthesia as a rule prevents a contraction from occurring. Deep barbital anesthesia, we believe, decreases the response, especially if the blood pressure is low.

On unanesthetized dogs with hepatic ducts tied and common duct cannulated. Under morphine-ether anesthesia, with aseptic technique, the hepatic ducts of two dogs were tied, and a cannula was inserted into the common bile duct before it entered the duodenal wall. The cannula was connected to the exterior with a rubber tube, which was clamped to hold the bile in the gall bladder. The dogs were trained to lie quietly. From twelve to twenty-four hours later the rubber tube was connected to a glass tube, serving as a manometer, which in turn was connected to a recording tambour. A single injection of the "secretin" preparation caused the pressure to rise from 20 to 30 mm. of bile, where it was maintained for from 30 to 60 minutes, after which it gradually returned to normal. In one of these dogs we injected one dose every four minutes for five injections. A step-like rise in pressure resulted. The original pressure prior to the first injection was 18.0 cm. of bile, and after the last injection it gradually rose to 24.0 cm. of bile pressure. After about one-half to one hour it slowly returned to normal (fig. 6).

On unanesthetized dogs with the cystic duct cannulated. Under morphine-ether anesthesia, with aseptic technique the cystic duct was cannulated above the opening of the right hepatic duct in such a manner as not to spill any of the bladder bile. The cannula was connected with the outside by a rubber tube, which was clamped to prevent the loss of bile. The following day, the clamp was removed and the rubber tube was connected with a manometer and a recording tambour. Two animals were used. In one of the animals, the usual contraction of the gall bladder on the injection of "secretin" was observed, as described in the preceding paragraph. In the other animal, it was found that fluid would pass into the gall bladder,
but the cannula was so occluded that changes in intra-gall-bladder pressure could not be recorded. We decided to inject the "secretin" preparation. Following the first injection nothing occurred; after the second injection the animal salivated (denoting nausea); after the third injection, the animal vomited and could not be kept quiet. Since this was the only time that we had elicited vomiting by injecting our material, and since the animal reacted as animals frequently do when a balloon is placed in the gall bladder and distended, we concluded that the contraction of the gall bladder with the cystic duct occluded caused the distress.

Evacuation of lipiodol (iodized oil) from the gall bladder in the unanesthetized dog by successive injections. Under morphine-ether anesthesia and with aseptic technique, the gall bladder (ten dogs) was filled with lipiodol (iodized oil) after the removal of an aliquot portion of bile (from 15 to 20 cc. usually). The dog was trained to lie quietly. From eighteen to twenty-four hours later, the animal not being fed, serial roentgenograms (from 2 to 10 minutes apart) were made, one dog's dose of the "secretin" preparation (a dose known to cause gall-bladder contraction) being injected intravenously every ten minutes for one hour. A study of the roentgenograms and autopsy showed that in three of the ten dogs, all but about 3 cc. of the lipiodol had been evacuated; in three, about two-thirds of the lipiodol had been evacuated; and in three, about one-half had been evacuated. In one, only a small quantity was evacuated. This animal was given, three hours later, a meal of cream (200 cc.), egg-yolks (6) and some milk. It was found that this meal caused no evacuation. The next morning a radiological examination and a picture showed a dense shadow, the lipiodol, and a line about 2 cm. away from the lipiodol shadow, which we interpreted as the gall bladder wall. We believed that fresh bile had entered the gall bladder during the night. Three injections of the "secretin" preparation were then given with the result that the outer line was caused to move in and make contact with the lipiodol shadow. We believe that this denoted that the fresh bile had been evacuated. Autopsy revealed that the lipiodol mass was almost gelatinous in consistency, and we believe so viscous that it could not be evacuated by the gall bladder. Similar gall bladder evacuation can be accomplished in dogs under light barbital anesthesia (figs. 7, 8 and 9).

It should be pointed out that lipiodol is more viscous than normal gall-bladder bile, and hence is more difficult to evacuate from the gall bladder.

An occasional roentgenogram from some of the dogs shows that the hepatic ducts are injected with lipiodol for from 0.5 to 3 cm. This occurred more frequently in the case of the right hepatic duct than in the others. We believe that this injection of the hepatic ducts is most probably due to increased pressure on the intramural portion of the common bile duct exerted by the duodenum, preventing the outflow of the lipiodol into the duodenum.
The injections of the "secretin" preparation had no objective nor detectable effects on the animal.

When we carefully observed the gall bladder under the fluoroscope, we could see it change its contour. We only observed definite changes in the half adjacent to the neck of the gall bladder. If the dome of the gall bladder contracted, we did not see it.

On contraction of the gall bladder caused by hydrochloric acid in the duodenum. Ten experiments were performed in which the cystic duct was clamped and the gall bladder cannulated under barbital anesthesia. We introduced by means of a needle and syringe, from 10 to 40 cc. of N/10 HCl into the duodenum and observed contraction of the gall bladder in every instance. The minimal quantity with which we were able to elicit a response was 15 cc., but we believe that under more normal experimental conditions less acid would be necessary (figs. 9 and 10).

The latent period of contraction was in most instances about two minutes; in a few instances about five minutes; whereas, the latent period for the response of the pancreas varied in these experiments from 5 to 10 minutes.

On contraction of the gall bladder caused by other agents introduced into the duodenum. In no experiment were we able to cause a marked and sustained change in the gall bladder by injecting olive oil into the duodenum. The same was true of cream and egg-yolk. The injection of butter was attended with more success. But if the cream, olive oil and egg-yolk were digested with "pancreatin" previous to injection, contraction occurred.

Fig. 1. Dog under barbital anesthesia with the cystic duct clamped, fundus of the gall bladder cannulated. The upper record is a tambour tracing of the intra-gall-bladder pressure. The record just below it is the blood pressure. The lower line shows the base line of the blood pressure, the point and time of injection and drops of pancreatic juice. At 9.10 cc. of preparation S III were injected intravenously. At 10 3 cc. of preparation 80-t-p-i were injected. The latter preparation contained 1 mgm. of solid material per cubic centimeter, and comparably more secretin and less cholecystokinin than preparation S III.

Fig. 2. Dog under barbital anesthesia with cystic duct clamped and the fundus of the gall bladder cannulated. Nine cubic centimeters of preparation 1802 were injected intravenously. This amounted to four threshold doses. It contained no vaso-dilatin. The period shown on the tracing was approximately twenty minutes. The bile rose in the manometer 11.5 cm. This tracing was the result of the demonstration before the North Chicago Branch of the Chicago Medical Society.

Fig. 3. Dog under barbital anesthesia with cystic duct clamped, the fundus of the gall bladder cannulated, and the pancreatic duct cannulated. This tracing contrasts the effect of histamine and a cholecystokinin preparation.

Fig. 4. Dog under barbital anesthesia with the cystic duct clamped, the fundus of the gall bladder cannulated and the pancreatic duct cannulated. The tracing shows contraction of the gall bladder with rhythmic contractions (approximately 3 per minute) caused by the injection into the duodenum of 50 cc. of "pancreatin" digested egg yolks and cream.
One-half per cent butyric acid and five per cent soap solution when injected into the duodenum caused a contraction.

**Proof of a Hormone.** On the results of cross-circulation experiments. Four cross-circulation experiments (carotid-to-carotid) have been performed, three yielding positive results and one negative.

Fig. 5. Dog under barbital anesthesia, cystic duct clamped, fundus of gall bladder cannulated, and the pancreatic duct cannulated. The tracing shows that atropine does not prevent the action of cholecystokinin. Atropine may possibly slightly decrease its action, but we have not performed a sufficient number of experiments to definitely settle this question.

Fig. 6. Twenty-four hours previously the hepatic ducts were tied and the common bile duct was cannulated. The cannula was connected to a manometer and a recording tambour. Injections were made at the times indicated. Note the steplike rise in pressure from an original pressure of 180 mm. of bile to a pressure of 240 mm. of bile. The pressure began to decrease about 5:30.

Dogs of a like size were chosen whose bloods were shown to be compatible by agglutination tests. They were anesthetized with barbital and placed on the table side by side. The abdomen of each dog was opened, the
cystic duct clamped, the gall bladder and the pancreatic duct cannulated. The gall-bladder cannula was connected to a recording tambour. The lateral carotids were connected for a blood pressure tracing, and the two medial carotids were connected by cannulae for a cross-circulation. (See article by Ivy, Lim and McCarthy, 1925.) Simultaneous records of the blood pressure, intra-gall-bladder pressure, and pancreatic flow of both dogs were made.

In three out of four experiments the introduction of 40 cc. of N/10 HCl into the duodenum of the “first” dog caused the gall bladder of the “second” dog to contract. The gall bladder of the “second” dog, or the one that did not receive acid in the duodenum, contracted after a period of eight minutes in one animal, ten minutes in a second animal, and twelve minutes in the third animal (figs. 9 and 10).

Fig. 7. Roentgenogram 1 was made before the injection of cholecystokinin; 2 was made one-half hour later after four injections at 10-minute intervals; 3 was made one hour after 1 after seven injections. Note the injection of the hepatic duct of the right lower lobe in 3. Gall bladder filled with iodized oil 18 hours previous to the experiment.

These experiments demonstrate that the introduction of the acid into the duodenum of the “first” animal caused something to enter the blood, which passed via the cross-circulation cannula to the “second” animal, causing the gall bladder of the “second” animal to contract.

The pancreas of the “second” dog was only stimulated in one of the four experiments.

Discussion. Our results show that by the use of appropriate methods, a “purified” extract of the intestinal mucosa can be made containing a substance which on intravenous administration causes the gall bladder to contract and evacuate. That the substance is not histamine is shown by the fact that its injection causes no fall of the blood pressure. That the substance is not choline is shown by the fact that its injection is not antagonized by atropine in addition to the fact that blood pressure does not fall.
Fig. 8. 1, control at 9:20; 2, 10 minutes after first injection. Note iodized oil in duodenum; 2a, 9:33, 2 minutes after 2nd injection; 3, 9:40, 2 minutes after 3rd injection (note slight injection of right hepatic duct); 4, 9:50, 2 minutes after 4th injection (can follow duct to duodenum); 5, 10:00, 2 minutes after 5th injection; 6, 10:10, 2 minutes after 6th injection; 7, 10:20, 2 minutes after 7th injection. Injections were then stopped. One-half hour later 8 was taken. Note the change in contour denoting that the gall bladder has probably relaxed. The dog was immediately etherized. The gall bladder contained about 2 or 3 cc. of iodized oil in a viscid mass, and 4 or 5 cc. of apparently "fresh" bile.
The chemical properties of this substance, as far as they are known, will be given in a later paper.

After some consideration, we have decided to name the substance in intestinal extracts which causes the gall bladder to contract, "cholecystokinin" (that which excites or moves the gall bladder.) Some evidence will
be presented in a later paper which leads us to believe that the gall bladder contracting principle is different from "secretin".

The maximum power for the gall bladder to contract caused by injection of "cholecystokinin" under our experimental methods is 24.0 cm. of bile pressure. This figure agrees closely with the figures reported by Higgins and Mann (1926). Three hours after a meal, they observed a maximum pressure of 26.0 cm. of bile; the average pressure maintained for 30 or 40 minutes was 21.0 cm. Our average figure is approximately 10.0 cm. of bile on the anesthetized dogs, and in the three unanesthetized dogs, approximately 20.0 cm. (Mann, 1924).

We have observed rhythmic contractions of the gall bladder to occur spontaneously. They almost always occur after an injection of "cholecystokinin," if they have not been present before, and if present before are, as a rule, increased by the injection. Frequently, a small dose of "cholecystokinin" may not cause a tonic contraction, but will initiate or increase the amplitude of the rhythmic contractions. They occur at a rate of from 2 to 4 per minute (Mann, 1924). We have observed rhythmic contractions which have amounted to as much as 3.0 cm. of bile pressure.

We have interpreted the injection of, or the backing up of the lipiodol into the hepatic ducts, as being due to the pressure of the duodenum on the intramural portion of the common bile duct, or to a hypertonic sphincter of Oddi. The literature (Mann, 1924) shows that a minimum pressure of 5.0 cm. and a maximum of 67.5 cm. of water is necessary to overcome the resistance of the sphincter of Oddi, or the intramural portion of the common bile duct. It is obvious, then, whether or not the gall bladder contraction leads to evacuation of the gall bladder depends on the amount of resistance of the sphincter or intramural portion of the common duct. If the duodenum were spastic, it is quite likely that the gall bladder would not evacuate even though caused to contract by "cholecystokinin" (see Boyd, 1928). This, we believe, is the most probable explanation of the negative results obtained by Whitaker (1927) on the introduction of acid into the duodenum of man.

We have never observed the gall bladder of the dog to be completely empty, and we have not been able to empty it completely by injections of "cholecystokinin." Because of the anatomical attachments and relations of the gall bladder it is difficult to conceive of complete emptying except by some mechanism causing very gradual suction.

The results of cross-circulation certainly demonstrate a hormone action. We know of no other way of accounting for our results other than that the acid in the duodenum caused something to enter the blood. We used relatively large quantities of acid in order to get a maximum formation of the hormone. A maximum formation of the hormone is necessary for
such an experiment because of the dilution in the "recipient's" blood of only the hormone that flows through one carotid of the "donor." Under ideal conditions, which we believe we had in our experiments, there would be a minimum dilution of at least one to twenty. Even then, the hormone flows through an additional capillary bed (the head). We believe the positive results observed are unequivocal.

The observed fact that there was an apparent relation between the tension of the gall bladder, or the pressure exerted on its wall, and the amount of contraction elicited is not contrary to the physiological properties of smooth muscle. The best explanation that we can offer for the observation that the gall bladder of the "fed" dog did not respond as well as that of the "starved" dog (24 hours), is that the gall-bladder musculature had been either almost maximally excited or fatigued by the hormone produced by the meal. The amount of bile present in the gall bladder of these "fed" dogs was less than that we observed to be in most of the "starved" dogs. The "pressure head" against which the gall bladder contracted, from 5 to 6 cm. of bile, was the same in both "starved" and "fed" dogs. We have not performed experiments on the effect of varying the pressure in the gall bladder of the "starved" dogs, or with the attempt to analyse this phenomenon.

The fact that we observed no contraction of the gall bladder on the introduction of olive oil, cream and egg-yolks into the duodenum, and that a slight contraction occurred on the introduction of butter, 0.5 per cent butyric acid, 5 per cent soap solution, and that a marked contraction occurred on the introduction of digested (pancreatin) egg-yolk and cream, argues that before fats, egg-yolk, etc., stimulate the formation of a hormone or cause gall bladder contraction they must be digested. This is not necessarily true, however, because our dogs were anesthetized and in such a condition only strong or maximal stimuli may cause action. According to Boyden (1926b, 1928) the gall bladder begins to empty within fifteen minutes after ingestion of a meal. Elman and McMaster (1926) and Boyden (1928) report that the smelling or sight of fried bacon causes a change in the gall bladder and some ejection of bile, which Whitaker (1927) was unable to confirm. These observations are mentioned to call attention to the very likely possibility that other mechanisms than a hormone mechanism are concerned in causing gall-bladder evacuation.

**SUMMARY AND CONCLUSIONS**

1. An extract of the upper intestinal mucosa has been shown on intravenous injection to cause contraction and evacuation of the gall bladder. The extract (prepared by Ivy, Kloster and Lueth) is free of vaso-dilatin and has no objective toxic effect on unanesthetized dogs.
2. Cross-circulation experiments show that when acid is injected into the duodenum, something goes into the blood which causes the gall bladder to contract. We believe this observation proves a hormone mechanism for gall bladder contraction and evacuation. It is pointed out that other mechanisms may be concerned in gall bladder evacuation.

3. We propose the term "cholecystokinin" as a name for the hormone and the active substance in intestinal extracts, which causes the gall bladder to contract and evacuate.

4. The amount of pressure that results from the contraction of the gall bladder under the influence of "cholecystokinin" is for one injection a maximum of 11.5 cm. of bile pressure and a minimum of 1.0 cm., and for a series of injections an average of 10.0 cm. and a maximum of 22.5 cm. in anesthetized dogs; and in unanesthetized dogs for a series of injections it is an average of 20.0 cm. of bile and a maximum of 24.0 cm. of bile. The average duration of the contraction for a single dose is 15 minutes. Following a series of doses the gall bladder relaxes in from 30 to 60 minutes.

5. The injection of the following substances into the duodenum of the dog caused the gall bladder to contract with the animal under light barbital-ether anesthesia: 15 to 40 cc. of N/10 HCl, 30 cc. of butter, digested egg-yolk, cream and olive oil, 0.5 per cent butyric acid, and 5 per cent soap solution. Undigested olive oil, egg-yolk and cream were ineffectual.

6. Spontaneous rhythmic contractions of the gall bladder were observed to occur from 2 to 4 times a minute. A small dose of "cholecystokinin" would usually increase their amplitude to as much as 3 cm. of bile pressure; a large dose would usually cause them to disappear at the height of the contraction, but they would reappear some time during the period of relaxation. If they were not present prior to the injection, they would frequently appear during the latter part of the period of relaxation.

7. We have observed the hepatic ducts to be injected with lipiodol during the contraction of the gall bladder, the injection of the ducts being due, we believe, to increased (abnormal?) tone of the duodenum or sphincter of Oddi.

We desire to acknowledge the assistance of Dr. Grant Kloster and Mr. H. C. Lueth in performing a number of our experiments.


Demonstrations: Physiology Seminar, University of Chicago, November 23, 1928; North Shore Branch of Chicago Medical Society, April 5, 1928.
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