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Movement of free fatty acids into and out of the blood stream in normal rabbits and in rabbits injected subcutaneously with the pituitary adipokinetic substances, Fraction H and adrenocorticotropin*

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SUMMARY

Uptake and release of plasma free fatty acids by the tissues of the rabbit were investigated by measuring the arteriovenous difference in plasma FFA concentration across the perirenal fat depot, intestines and mesentery, leg, kidney, and liver. In animals given subcutaneous injection of glucose, the arteriovenous difference was negative (net release) across the intestines and mesentery, positive across the perirenal fat depot and kidney, and positive or negative across the leg and liver. After subcutaneous injection of a pituitary adipokinetic substance, either Fraction H or adrenocorticotropin (ACTH), the arteriovenous difference was negative across the perirenal fat depot, the intestines and mesentery, and the leg, while large positive differences (net uptake) were found across the kidney and liver. Stainable lipid was present in the hepatic cord cells and the cells of the renal convoluted tubules 1 hr after injection. The exchange of glucose between tissues and blood stream was not altered during adipokinesis.

It is concluded that in glucose-treated rabbits, large fat depots take up FFA from the blood stream; in contrast, following the subcutaneous injection of Fraction H and ACTH, FFA are mobilized from adipose tissue into the blood stream. During the mobilization of FFA, the kidney and liver take up FFA, which probably are synthesized into triglycerides in the hepatic cord cells and cells of the renal convoluted tubules.

In mammals, triglyceride fatty acids in adipose tissues are readily mobilized into the blood stream in the form of free fatty acids (FFA) (1, 2). The mammalian pituitary gland contains at least four substances, each of which accelerates the mobilization of FFA in certain species of animals; viz, adrenocorticotropin (ACTH), thyroid-stimulating hormone (TSH), melanocyte-stimulating hormone (MSH), and the lipid-mobilizing principle recently identified in saline extracts of the gland by Rudman and co-workers and designated Fraction H (3–6). These adipokinetic substances differ one from the other in their species specificity, but they all promote the release of FFA in vitro from the adipose tissue of susceptible species (7). Fraction H, MSH, and ACTH each have been reported to produce a substantial increase in plasma FFA concentration in the intact rabbit (5, 8). Mobi-

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lization of FFA into the blood stream of the rabbit by the subcutaneous injection of a single dose of Fraction H is followed by an increase in the triglyceride content of the liver and kidneys (9). These studies suggest that, in rabbits, parenteral administration of adipokinetic substances derived from the pituitary gland stimulates the mobilization of FFA from adipose tissue into the blood stream, and that subsequently the FFA leave the blood stream within the liver and kidney, where they are reincorporated into triglycerides.

The following experiments were done to test this hypothesis. The arteriovenous (AV) difference in plasma FFA concentration was measured across the perirenal fat depot, the intestines and mesentery, the leg, the kidney, and the liver, in untreated rabbits, and then in rabbits injected subcutaneously with pituitary Fraction H or ACTH.

MATERIALS

Animals. Male and female rabbits of mixed breeds, weighing 3,500–4,800 g, were used. The animals had free access to Purina Rabbit Chow Checkers and water, until they were anesthetized for the surgical procedures.

Adipokinetic Materials. Fraction H was prepared from pituitary glands of hogs (10). The potency of this material was such that 0.125 mg injected subcutaneously was the minimal dose that produced an 8-fold increase in the rabbit's plasma FFA concentration. Fraction H contains less than 0.8% of any of the nine recognized mammalian pituitary hormones (6). The following batches of oxycellulose-purified ACTH were utilized:

SOURCE	LOT NO.	POTENCY
Wilson[1]	105385	123 units/mg
Wilson	116283	95 units/mg
Armour[2]	216-176-1	25 units/mg

Determinations. Free fatty acids were measured by the method of Dole[3] (2); glucose by the method of Folin and Wu (12).

EXPERIMENTAL METHODS

Studies were performed on three series of animals: (1) rabbits that received no adipokinetic substances, (2) rabbits that each received a subcutaneous injection of 1.0 mg of Fraction H 1 or 2 hr before the operative procedure, and (3) rabbits that each received a subcutaneous injection of 20–50 units of ACTH 1 or 2 hr before the procedure.

Nembutal was administered intravenously until the corneal reflex just disappeared. All arterial blood samples were obtained from the abdominal aorta. Blood from the venous side of the perirenal fat depot was removed from the adrenolumbar vein at a point in its course that excluded any blood coming from the adrenal gland (13). Samples were obtained for the leg from the femoral vein, for the intestines and mesentery

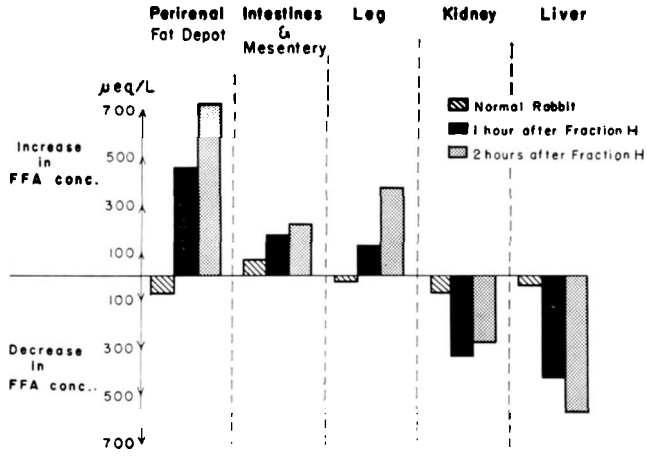

FIG. 1. Average change in plasma FFA concentration from artery to vein across five body areas in normal rabbits and rabbits after the subcutaneous injection of Fraction H. This figure summarizes the data of Tables 1 and 2.

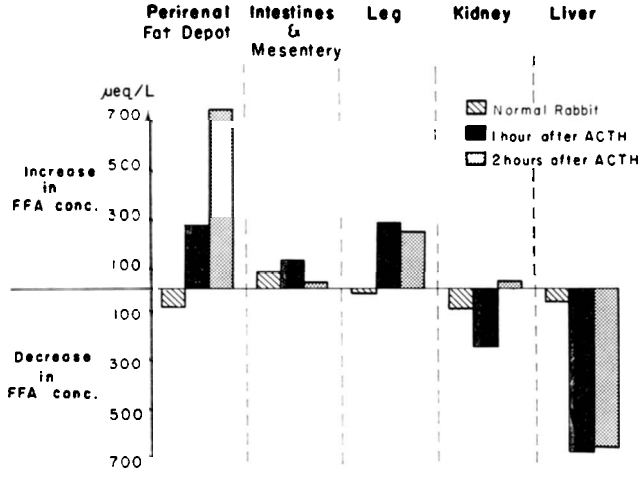

FIG. 2. Average change in plasma FFA concentration from artery to vein across five body areas in the normal rabbits and rabbits after the subcutaneous injection of ACTH. This figure summarizes the data of Table 3.

[1] Wilson Laboratories, Chicago, Illinois.
[2] Armour Pharmaceutical Co., Kankakee, Illinois.
[3] Several runs of FFA determinations were done using the method of Trout, Estes, and Friedberg (11) in parallel with the method of Dole (2). No appreciable difference was noted between the results. Thus, correction for the plasma lactic acid concentration did not appear to be necessary and the simpler method of Dolwe as employed.

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TABLE 1. ARTERIOVENOUS DIFFERENCE IN PLASMA FFA CONCENTRATION ACROSS FIVE BODY AREAS IN RABBITS INJECTED WITH GLUCOSE

Rabbit No.	Arteriovenous Difference				Plasma Concentration		
	Fat Pad	Intestines and Mesentery	Leg	Kidney	Abdominal Aorta	Portal Vein	Hepatic Vein
	μEq/liter				*μEq/liter*		
1		−66	+42		147	213	248
2		−42	0		185	227	213
3	+94			−24	188		
4		−78	+75		216	294	205
5		−120	−148		223	343	164
6				+91	237		
		−100	+119		292	392	410
7	+50			+73	244		
8		−8	−40		299	307	311
9	+89			+66	350		
10		−149	−75		361	510	216
11	+121			+156	391		
Mean	+89	−80	−4	+73	261	327	252

collectively from the portal vein, and for the kidney from the renal vein. Venous blood from the liver was aspirated from a branch of the hepatic vein located underneath or along the side of the gall bladder. Arterial and venous blood samples were not obtained simultaneously. Instead, samples were obtained in groups, each group consisting of an arterial sample and one to three venous samples, withdrawn so that no more than 2 min passed between the collection of venous and the arterial sample. Arteriovenous differences were calculated by subtracting the concentration of the substance in each of the venous samples from the concentration in the arterial sample collected within the same time period. Calculations of AV differences across the liver were not made because the relative amounts of blood supplied to this organ by the portal vein and hepatic artery in the rabbit are not known. Studies in dogs show a wide variation in this ratio from animal to animal and from time to time in the same animal (14, 15). Because of this uncertainty, only the plasma concentrations are listed in the tables. However, average AV differences across the liver are represented in Fig. 1 and 2 to demonstrate the uptake and release of FFA relative to the blood stream for all five areas studied. The data for the liver are derived by subtracting the concentration of FFA found in the hepatic vein from an average of the concentration found in the aorta and the portal vein. These figures probably represent the minimum amount of FFA being exchanged. More complex calculations, assuming a greater proportion of flow through the portal vein, do not seem justified since in most cases the values are not altered by more than 10%.

RESULTS

The mean plasma FFA concentration in blood obtained from the ear vein of 24 nonfasted rabbits was 239 μEq/liter, with a range of 91–563 μEq/liter. In a preliminary experiment in which ten of these animals were anesthetized and subjected to a laporotomy, the plasma FFA concentration in blood samples obtained from the aorta varied from 218 to 849 μEq/liter (mean 478 μEq/liter). A second series of 11 rabbits was given a subcutaneous injection of 40 ml of 5% glucose in saline 1 hr before the operation; in this group of animals, the arterial plasma FFA concentration was in a range between 147 and 391 μEq/liter (mean 260 μEq/liter). Accordingly, the animals in all subsequent experiments were given a subcutaneous injection of 5% glucose in saline 1 hr before operation in order to offset any tendency of the anesthesia and operation to cause an elevation in plasma FFA concentration.

Arteriovenous Differences in Plasma FFA Concentration Across Five Body Areas in Rabbits Injected with Glucose. The AV differences in plasma FFA concentration were calculated for the 11 control animals that received a subcutaneous injection of glucose but were not given any adipokinetic substance (Table 1). The plasma FFA concentration in these animals increased consistently only across the intestines and mesentery; the average increase was 34%. An average decrease in concentration of 32% occurred across the perirenal fat depot and across the kidney. Arteriovenous differences in FFA concentration across the leg and the liver were almost equally divided between positive and negative so that the mean percentage changes were

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TABLE 2. ARTERIOVENOUS DIFFERENCES IN PLASMA FFA CONCENTRATION IN RABBITS GIVEN FRACTION H

Rabbit No.	Arteriovenous Difference				Plasma Concentration		
	Fat Pad	Intestines and Mesentery	Leg	Kidney	Abdominal Aorta	Portal Vein	Hepatic Vein
	μEq/liter				*μEq/liter*		
			1 hr After Injection				
12	−162			+122	604		
13		+52	−40		648	596	352
14	−185			+152	732		
		−253			544	797	415
15	−605			+262	887		
16		−199	−223		947	1146	780
	−661				708		
17	−623		−184		1579		
		−233			1039	1271	461
18		−271	−30		1617	1888	1117
19	−871			+328	2156		
20	−141			+757	2833		
Mean	−464	−181	−119	+324	1191	1140	625
			2 hr After Injection				
21	−1456			−7	807		
22		−212			1396	1608	1060
			−524		804		
23		−72	−350	+365	1455	1527	1154
24		−203			1500	1703	1215
	−270		−394		1099		
25	−825			+486	1708		
		−320			1407	1727	740
26	−233			+145	1713		
27		−283	−271		1906	2189	1170
28	−779			+478	2128		
		−143			1353	1496	798
Mean	−713	−206	−385	+293	1440	1708	1024

small. There was no apparent relationship between the plasma FFA concentration and the direction or magnitude of the exchange of FFA between the blood and any of the tissues studied, but the number of observations in each group is too small to be conclusive on this point.

Arteriovenous Differences in Plasma FFA Concentration Across Five Body Areas in Rabbits Given Fraction H. Administration of 1.0 mg of Fraction H per rabbit to a second series of animals resulted in a fourfold increase in average arterial plasma FFA concentration in 1 hr, and a fivefold increase 2 hr after the injection. The principle change in the movement of FFA in these animals occurred across the perirenal fat pad (Table 2). The plasma FFA concentration increased an average of 45% across this tissue at both 1 and 2 hr after the injection, compared to the average decrease of 32% found in the controls. The percentage increases in FFA concentration across the leg and the intestines and mesentery are small compared to those found across the perirenal fat depot; however, the relative amounts of FFA contributed by each of these areas cannot be assessed because the rate of perfusion of blood through these tissues was not measured.

In contrast, the marked decreases in FFA concentration found across the kidney and liver appear to indicate that large amounts of FFA leave the blood stream to enter these organs at both 1 and 2 hr after injection. These data are summarized in Fig. 1.

Arteriovenous Differences in Plasma FFA Concentration Across Five Body Areas in Rabbits that Received ACTH. The data in Table 3 indicate that the response to ACTH is similar to that found after administration of Fraction H. Substantial increases in plasma FFA concentration occurred across the perirenal fat pad, while substantial decreases took place across the liver. The fall in concentration of FFA across the kidney was less consistent in direction and smaller in magnitude

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TABLE 3. ARTERIOVENOUS DIFFERENCE IN FFA CONCENTRATION IN RABBITS GIVEN ACTH

Rabbit No.	Arteriovenous Difference				Plasma Concentration		
	Fat Pad	Intestines and Mesentery	Leg	Kidney	Abdominal Aorta	Portal Vein	Hepatic Vein
		μEq/liter				*μEq/liter*	
			1 hr After Injection				
29		−159	+92		1359	1515	1109
	−385				721		
30		−165		+194	1414	1579	817
31	−864		−663	−42	1551		
32		−32		+51	1611	1643	1111
33	−892	+115		+395	1726		
			−204		1614		
34					1842	1715	1240
		−155			1560		
				−73	1222		
35	−307		−316	+65	2306		
36		+200			2475	2275	2066
37		−55			2496	2552	1725
38	−935		−29	−224	2591		
39	−614		−292	−185	2688		
40	−1097		−251	−140	2716		
		+37			2409	2372	1251
41	−544		−69	−513	2990		
Mean	−706	−27	−217	−47	1960	1950	1331
			2 hr After Injection				
42					957	1177	565
		−140			1037		
				+70	817		
43				+262	1826		
		+257			1723		
44		+64			1809	1744	1450
	−216			+110	1478		
45		−183	−494		1811	1994	1201
	−9			+307	1683		
46		−453	−237		2278	2730	1916
	−44				2278		
				+715	2442		
47		−118	−427		2426	2544	1857
	−812			−302	1867		
48		−178	−186		2513	2691	1624
	−554			+238	2161		
49	+186		+117	+467	2668		
Mean	−242	−107	−245	+233	1869	2147	1436

after ACTH compared with Fraction H.

Arteriovenous Differences in Blood Glucose Concentration During the Mobilization of FFA Induced by Fraction H and ACTH. The well-known interrelationship between blood glucose and plasma FFA levels (1, 2) suggested the possibility that the mobilization of FFA into the blood stream produced by Fraction H or ACTH might be an indirect effect of an alteration in glucose metabolism within one or more tissues of the body. Blood glucose AV differences were measured in rabbits during mobilization of FFA by Fraction H and ACTH, in order to learn whether or not the exchange of glucose between blood and tissues was altered by either of these agents.

Each rabbit in this experiment, as in the previous experiments, received a subcutaneous clysis of 40 ml of 5% glucose in saline 1 hr before the operation. Thus the blood glucose concentration in the aorta was elevated to a range from 130 to 360 mg %. It can be seen from the data in Table 4 that the pattern of AV differences in blood glucose concentration in the animals treated with Fraction H or ACTH was unchanged

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TABLE 4. ARTERIOVENOUS DIFFERENCES IN BLOOD GLUCOSE IN RABBITS

Rabbit No.	Arteriovenous Difference				Plasma Concentration		
	Fat Pad	Intestines and Mesentery	Leg	Kidney	Abdominal Aorta	Portal Vein	Hepatic Vein
	mg %				*mg %*		
			Control Rabbits				
50	+14			+14	130		
51	+29			+7	148		
52			+40		180		
	+33			+29	155		
53	+35			+21	194		
4		−6	+36		196	203	260
54	+16			−12	202		
55				+4	208		
5		+41			209	167	188
			+64		244		
8		+14	+59		223	209	262
Mean	+25	+16	+50	+11	190	193	237
			Rabbits Given Fraction H				
17	+4				119		
		+114			260		
21	+30			+11	133		
18		+4	+4		137	133	185
	+63			+47	138		
13		+15	+25		141	126	163
22		+20			143	123	169
23		+38	+28		156	118	190
	+52			+16	193		
14	+9			+9	163		
		+31			236	205	256
27		+24	+16		167	143	199
				+43	235		
24		+6			172	165	221
	+26		+53		199		
16		+26	+43		175	150	181
	−7				137		
28	+9			−8	211		
		+25			267		
25		+84			238	154	341
Mean	+22	+35	+28	+18	180	146	213
			Rabbits Given ACTH				
34					130	120	173
		+23			144		
				+15	170		
35	+10			+1	154		
40	+28		+17	0	167		
39	−1		−17	−1	181		
31	+4		+20	+1	200		
47		+20	+11		210	190	256
	+34			+20	267		
43					223	237	311
		+13			249		
				−6	254		
42					257	268	355
		+5			272		
				−7	298		
38	+56		+42	+48	270		
41	+33		+52	+2	294		
48		+45	+29		361	316	356
	+62			+75	403		
Mean	+28	+21	+22	+13	237	226	290

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from the pattern observed in control rabbits. The blood glucose level rose in passage through the liver, while it fell or remained substantially unchanged in transit through the other four areas studied. About half of the blood samples utilized for determination of glucose were aliquots of blood withdrawn for FFA determination. There was no apparent relationship between plasma FFA concentration or the FFA AV difference, on the one hand, and the blood glucose concentration or the glucose AV difference on the other.

Histological Studies of the Liver and Kidney Following Administration of Fraction H and ACTH. Prior reports of chemical analyses of liver and kidney tissues of rabbits 4–8 hr after the administration of Fraction H described a three- to eightfold increase in the triglyceride content of these organs (9). The livers and kidneys of animals that had received Fraction H or ACTH were examined microscopically in order to determine the precise anatomical location of the triglycerides within these organs.

Livers prepared by frozen section and stained with Oil Red O showed a diffuse, punctate sudanophilia in the hepatic cord cells of all animals as early as 1 hr after treatment. Lipid was not evident in the portal areas or in the reticuloendothelial cells. Liver tissues examined at 2 hr after the injection of ACTH or Fraction H showed the same distribution of lipid; the intensity of the stain suggested a higher concentration of lipid than at 1 hr, with some of it in the form of small- to medium-sized droplets. However, fat did not appear as a single large vacuole filling the cell, such as is seen in fatty metamorphosis.

In the kidney, a few of the convoluted tubules showed a punctate sudanophilia 1 hr after the injection of ACTH or Fraction H. The lipid-containing tubules were seen in cross section around the groups of glomeruli closest to the capsule of the kidney. At the second hour, more of the convoluted tubules appeared red; often a red-stained tubule, cut in longitudinal section, extended well down towards the medulla. The fat appeared to be confined to the convoluted tubules; it was not found in the glomerulus, in other portions of the nephron, or in the interstitial spaces.

DISCUSSION

In the group of rabbits that did not receive an adipokinetic agent, there was a net movement of FFA into the blood from the intestines and mesentery and a net movement of FFA out of the blood into the perirenal adipose tissue. These rabbits were not fasted prior to the experiment, and each was given a subcutaneous injection of glucose before blood samples were obtained. It is possible that the increase in FFA concentration across the intestines and mesentery was due to FFA entering the portal circulation directly by absorption from the food within the lumen of the intestines. Some FFA, particularly the short-chain acids, are absorbed in this manner (16), but short-chain acids do not partition into the Dole heptane extract (2). Secondly, the augmented concentration could be derived from FFA being synthesized from carbohydrates in the wall of the gut. Lastly, a structural and/or functional difference between the mesenteric and perirenal fat depots could explain these findings.

Following the administration of Fraction H or ACTH, the net movement of FFA between the blood and the perirenal adipose tissue underwent a reversal in direction. In vitro studies have demonstrated that Fraction H and ACTH stimulate hydrolysis of triglycerides in adipose tissue (7, 17). The large increases in plasma FFA concentration, found across perirenal adipose tissue in the present experiments, imply that the FFA products of this enhanced lipolysis readily pour out of the adipose tissue into the blood stream in the intact animal. Furthermore, the adipose tissue becomes visibly hyperemic in most animals 1–2 hr after the administration of Fraction H, suggesting an increased tissue blood flow (4). The combination of an increase in plasma FFA concentration from artery to vein plus an increased rate of blood flow indicates that the perirenal adipose tissue is an important source of the FFA mobilized into the blood stream by Fraction H and ACTH. Adipose tissue elsewhere in the body probably responds in a similar manner; there is sufficient adipose tissue in the leg and the mesentery to be the source of the increases in plasma FFA concentration found across these areas after the administration of Fraction H or ACTH. In fact, it is likely that the adipose tissue throughout the body is the target organ for the adipokinetic effects of Fraction H and ACTH, and that these substances act directly within all fat depots to stimulate lipolysis and the subsequent release of FFA into the blood stream.

There are at least two alternative hypotheses, each of which could account for the observed increase in plasma FFA following the administration of pituitary adipokinetic substances. Kellner, Hirsch, and Freeman have suggested that these agents might act by preventing FFA from leaving the blood stream (18). This action, alone or in conjunction with the stimulation of lipolysis, could account for the observed accumulation of FFA in the plasma. The present data indicate that there was no interference with the exodus of FFA from the blood stream. In fact, large amounts of FFA did leave the plasma and enter the liver and kidney.

Another hypothesis to explain the mechanism of FFA mobilization by adipokinetic agents is the possibility that these agents affect glucose metabolism primarily.

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Any direct interference with the availability of glucose to the animal could conceivably result in a secondary mobilization of FFA, such as is seen in starvation (1, 2). In the present experiments, it was found that the AV differences in blood glucose do not change after administration of Fraction H or ACTH, and Rudman and co-workers have shown previously that there is no change in the glucose tolerance curves of animals treated with Fraction H (19). These findings strongly suggest that Fraction H and ACTH do not mobilize FFA secondarily to a primary change in glucose metabolism.

The striking decreases in plasma FFA concentration that take place across the kidney and liver in rabbits injected with Fraction H or ACTH indicate that these two organs are the principal sites where the mobilized FFA leave the blood stream. Free fatty acids appear to be converted rapidly into triglycerides, since the triglyceride content of these organs increases five-to eightfold (9), and fat is demonstrable in the hepatic cord cells and cells of the renal convoluted tubules. It is well known that the liver can metabolize FFA in a number of ways, including incorporation into triglycerides and subsequently into lipoproteins, but little is known regarding the ability of the kidney to metabolize fatty acids. Taken together, the uptake of large amounts of FFA by the kidney, and the subsequent accumulation of triglycerides there, strongly suggest that the kidney may play an active role in the transport and metabolism of lipids.

The eventual fate of the triglycerides that accumulate in the liver and kidney is not known. Eighteen to twenty-four hours after the subcutaneous administration of 3–5 mg of Fraction H, a visible hyperlipemia develops; a similar hyperlipemia can be produced by a continuous infusion of ACTH (20). Studies are under way to learn whether or not the triglycerides in the liver, the kidney, or both are the source of the hyperlipemia observed after administration of Fraction H.

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