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187. Akira Minato, Hisashi Fukuzawa, Seiyu Hirose, and Yasuko Matsunaga*2 : Radioisotopic Studies on Percutaneous Absorption. I. Absorption of Water-soluble Substances from Hydrophilic and Absorption Ointments through Mouse Skin.*3

(*Faculty of Pharmaceutical Sciences, University of Chiba*[*1])

The percutaneous absorbability of water-soluble substances from emulsion-type ointments was demonstrated by measuring the absorption of radioactivity after application of hydrophilic and absorption ointments containing $Na^{131}I$, ^{59}Fe-EDTA, ^{59}Fe-tiron or $^{22}NaCl$ to hair-clipped mouse skin. The absorption of lipid-soluble ^{59}Fe-cupferron and ^{59}Fe-carbamate from these vehicles was quite small. The large promoting effect of polyethylene oleyl ether on the percutaneous absorption of ^{59}Fe-EDTA from polyethylene glycol 1500 through mouse skin suggested some important contribution of surface-active agents to the percutaneous absorption of water-soluble substances from hydrophilic and absorption ointments.

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Since the appearance of Overton's "lipid theory"[1] which postulated the dependence of the penetrating ability of a substance through the skin upon the degree of its solubility in lipid, numerous papers have been published reporting the percutaneous absorption of various lipid-soluble substances, *e.g.*, vitamin A,[2] vitamin E,[3] ^{14}C-hydrocortisone,[4,5] ^{14}C-cortisone,[4,6] ^{14}C-progesterone,[7] and ^{3}H-estradiol.[7] Concerning the "lipid theory," Bliss[8] has indicated that the properties and powers of the drug itself rather than the ointment vehicle are the major determining factors in absorption from the skin. Rothman[9] has stated in his text book that the lipid-soluble substances are fairly rapidly and completely absorbed through the skin, although the absorption appears to be faster if the substance is to some degree soluble also in water; and that the kind of vehicle in which it is incorporated and the solubility of the substance in the vehicle are of the secondary importance.

There are many reports, however, which demonstrated the percutaneous absorption of water-soluble and lipid-insoluble substances. Szczesniak, *et al.*[10] stated the penetration of heavy water through the skin by measuring the deuterium oxide content of the blood of rats that had been immersed in heavy water. Percutaneous absorption of water was also described by DeLong[11] who exposed a shaved area of the abdominal

*1 Yayoi-cho, Chiba (湊 顕, 福沢 寿, 広瀬聖雄, 松永泰子).
*2 Present address : National Institute of Health, Higashimurayama, Tokyo.
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skin of rats to an atmosphere of tritium-labeled water-vapor and found significant amount of radioactivity in the blood of the animals. Cyr, *et al.*[12] demonstrated the accumulation of radioactivity in the thyroid after application of white petrolatum containing $Na^{131}I$ to the hair-clipped skin of rats. Percutaneous absorption of $Na^{131}I$ was also reported by several other investigators including Miller and Selle[13] who measured the distribution of radioactivity to the thyroid and other organs after application of $Na^{131}I$ incorporated in a water-miscible ointment to the skin of guinea pigs and rabbits. Johnston and Lee[14] described the percutaneous absorption of $^{24}NaCl$ by measuring the radioactivity of the left hand and urine with Geiger counter following the inunction of hydrous lanolin containing $^{24}NaCl$ to the inner surface of upper right arm of normal human subjects. Witten, *et al.*[15] demonstrated the absorption of ^{32}P by autoradiographic analysis of the human skin to which radioactive phosphate had been applied as aqueous solution using a blotting paper disc. Percutaneous absorption of methionine was recognized by Edwards[16] who applied ^{35}S-methionine solution with gentle rubbing to the shaved area of back skin of guinea pigs and observed the appearance of radioactivity in the newly grown hair of the area where no ointment had been applied. It was reported by Schaefer, *et al.*[17] that the marked increase in urinary excretion of thiamine and riboflavin was observed following the application of these water-soluble vitamins incorporated in an oleaginous type ointment base to the shaved skin of rats.

The structure of animal skin is different from that of human skin. For example, the skin of rabbits, rats and mice, which have been used frequently in the works on percutaneous absorption, lacks sweat gland and abounds in hair and hair-follicles, in contrast to that of mankind. In addition, it is virtually impossible to apply small amount of aqueous solution uniformly to hair-clipped animal skin. Moreover, when animals are used, some additional technical cares must be taken to avoid unexpected disturbance of that part of skin where a sample has been topically applied. In spite of all these limitations, however, animals have been used in many studies on the percutaneous absorption of various substances, because of the following main reasons; the first, biologically dangerous substances including radioactive compounds are under many restrictions as to the application to human subjects; the second, the excision of normal human skin for the evaluation of any substance absorbed through the skin is hard to accomplish; the third, the absorption of medicaments by way of sweat duct of human skin has been reported to be negligible as compared with that by pilosebacious and transepidermal routes.[18~20]

Although a number of papers are found which made use of radioisotopes in the studies on percutaneous absorption, few of them are entirely satisfactory from the point of quantitative radioisotopic measurement of the amount of a substance absorbed through the skin into the body.

This paper deals with the absorption of several radioactive water-soluble substances from hydrophilic and absorption ointments through hair-clipped mouse skin.

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Materials and Methods

Ointment Bases——Ointment bases used in this study were hydrophilic ointment (O/W type) and absorption ointment (W/O type), whose compositions are shown in Table I.

Radioisotopes——$Na^{131}I$ (carrier free) and $^{22}NaCl$ (specific activity : 51 mc./mmole) were purchased from the Radiochemical Center, Amersham, and $^{59}FeCl_3$ (specific activity : 1250 mc./mmole) was obtained from the Oak Ridge National Laboratory, Oak Ridge.

Chelating Agents——Disodium ethylenediaminetetraäcetate (EDTA), ammonium nitrosophenylhydroxylamine (cupferron), disodium pyrocatechol-3,5-disulfonate (tiron) and sodium diethyldithiocarbamate (carbamate) were obtained from Wako Pure Chemical Industries, LTD., Tokyo.

Preparation of Radioactive Ointment——Radioactive ointment was prepared by mixing an ointment base with each radioactive substance on a watch-glass as designated in the legend of each table. In the case of ^{59}Fe-chelate compound, radioactive ferric chloride was reacted with slightly excess amount of each chelating agent at neutral pH and added to ointment base directly (water-soluble chelate) or after being extracted with chloroform (lipid-soluble chelate). It was known by radiometric assay that ^{59}Fe-cupferron and ^{59}Fe-carbamate were extracted almost quantitatively with chloroform or benzene from aqueous mixture at neutral pH, while $K^{131}I$, $^{22}NaCl$, ^{59}Fe-EDTA and ^{59}Fe-tiron were not extracted significantly with these organic solvents. In order to attain the possible reproducibility in water content of radioactive ointments, each ointment was kneaded on a watch-glass with a micro-spatula for exactly ten minutes, and applied on mouse skin after short-time storage in a well-closed glass container. Radioisotopic homogeneity of each ointment was confirmed by measuring the radioactivity of several portions of the ointment.

Table I. Composition of Ointment Bases

Hydrophilic ointment (g.)		Absorption ointment (g.)	
White petrolatum	25.0	White petrolatum	40.0
Stearyl alcohol	25.0	Cetanol	20.0
Propylene glycol	12.0	Polyoxyethylene oleyl ether	5.0
Sodium laurylsulfate	1.0	Water	*q. s.*
Ethyl *p*-hydroxybenzoate	0.025		
Propyl *p*-hydroxybenzoate	0.015		
Water	*q. s.*		
	100.0		100.0

Application of Radioactive Ointment——Male mature mice (dd strain) weighing 20 to 25 g. were fixed on their backs by the limbs with threads on boards, and abdorminal hair was clipped with scissors and an electric shaver, taking the best care to keep the skin undamaged. About 50 mg. of each radioactive ointment were weighed exactly and applied to 5 cm^2 area of the hair-clipped skin in an animal room (23°, 60～70 per cent humidity). Mice were kept for 20 hours under fixation on their backs in the same room and sacrificed by ether.

Determination of Per Cent Absorption of Radioisotope——The area of the skin, where radioactive ointment had been applied, was taken out with scissors, wrapped in a small sheet of paraffin-paper, and put into the bottom of a test tube. The radioactivity of each sample was then assayed with a well-type scintillation counter on account of its gamma-ray. Per cent absorption of radioisotope through the skin into the body was calculated by comparing the radioactivity of the excised skin with that of the known weight of radioactive ointment.

Results and Discussions

Absorption of ^{131}I-iodide

Although it has been generally accepted that electrolytes are impermeable through the skin, the percutaneous absorption of iodide ion has been repeatedly reported.[12,13] In the present investigation, the absorption of iodide ion through hair-clipped mouse skin was assumed on the basis of the following experimental results.

As shown in Table II, only about 31 and 68 per cent of the applied radioactivity were found with the excised skin where $K^{131}I$-containing hydrophilic or absorption

ointment had been applied 20 hours before the sacrification of the animals. In order to determine whether this disappearence of ^{131}I from the skin was due to the percutaneous absorption of ^{131}I into the body, the carcass and excreta (urine and feces excreted during 20 hours) were mixed with 10 per cent NaOH, homogenized in a Waring Blendor, and heated in a boiling water, after which its radioactivity was measured with a well-type scintillation counter. By adding this value to that of the excised skin, total recovery of ^{131}I was estimated. As seen in Table II, 96.8 and 96.2 per cent of the applied ^{131}I were recovered. Therefore, it was known that about 69.0 and 31.8 per cent of the applied ^{131}I were absorbed into the body after the application of $K^{131}I$-containing hydrophilic and absorption ointments to the hair-clipped skin of mice.

The next step was to clarify whether ^{131}I was absorbed in the water-soluble form or after the oxidation to lipid-soluble iodine as suggested by Rothman[9] according to the "lipid theory." For this porpose, a larger quantity of carrier KI or $Na_2S_2O_3$ was added to the $K^{131}I$-ointments to prevent the oxidation of ^{131}I, and the percutaneous absorption of radioactivity was measured by the same method. As shown in Table III, the addition of the KI or $Na_2S_2O_3$ resulted in no decrease in the amount of percutaneous absorption of ^{131}I. On the basis of these data, it seemed most reasonable to assume that ^{131}I was absorbed from these ointments through mouse skin in the form of water-soluble iodide ion. The larger absorption of ^{131}I in the case of Table III than that of Table II might be the reflection of the differences in the viscosity of the ointments.

Table II. Percutaneous Absorption of ^{131}I after Application of $K^{131}I$-ointments to Mouse Skin (1)

Ointment base	Radioactivity of ointment (c.p.m./50 mg.)	Number of animals	Amount of ointment applied (mg.)	Amount of ^{131}I remained in excised skin (%)	Total recovery of ^{131}I [b] (%)	Amount of ^{131}I absorbed through skin (%)
Hyd. oint.[a]	34200	3	50.5±1.1[c]	31.0±3.7	96.8±2.4	69.0±3.7
Abs. oint.[a]	36100	3	48.4±3.0	68.2±2.8	96.2±2.8	31.8±2.8

a) Each ointment base (1.0 g.) was mixed with 50 μl. of 0.001*M* $K^{131}I$.
b) Total recovery of ^{131}I was measured by the method described in the text.
c) Mean ± standard error.

Table III. Percutaneous Absorption of ^{131}I after Application of $K^{131}I$-ointments to Mouse Skin (2)

Experimental number	Ointment base	Radioactivity of ointment (c.p.m./50 mg.)	Number of animals	Amount of ointment applied (mg.)	Per cent absorption of ^{131}I
1[a]	Hyd. oint.	3050	3	48.6±1.9[c]	87.2±1.4
	Abs. oint.	3140	3	49.4±0.8	47.4±3.6
2[b]	Hyd. oint.	3080	3	50.3±2.2	80.0±1.6
	Abs. oint.	3270	2	50.1±0.5	34.2±3.6

a) A solution of 50 mμmoles of $K^{131}I$ and 50 μmoles of KI in 100 μl. of H_2O was mixed with 1.0 g. of each ointment base.
b) A solution of 50 mμmoles of $K^{131}I$ and 50 μmoles of $Na_2S_2O_3$ in 100 μl. of H_2O was mixed with 1.0 g. of each ointment base.
c) Mean ± standard error.

Absorption of ^{59}Fe-chelate Compounds

In order to confirm the absorption of water-soluble substances from hydrophilic and absorption ointments through mouse skin, use was made of the ^{59}Fe-chelate compounds, the radioactivity of which could be as easily and accurately assayed with

a well-type scintillation counter as that of ^{131}I. ^{59}Fe-EDTA was used as a stable water-soluble chelate compound and, for comparison, ^{59}Fe-cupferron, as a lipid-soluble one. These chelate compounds were mixed with hydrophilic and absorption ointments by the method described in the legend of Table IV, and thus prepared radioactive ointments were applied to the hair-clipped mouse skin to measure the amount of radioactivity absorbed in 20 hours. As shown in Table IV, as much as 66.4 and 80.2 per cent of the applied radioactivity was found to be absorbed following the application of hydrophilic and absorption ointments containing ^{59}Fe-EDTA. On the other hand, only 13.0 and 14.6 per cent absorption of radioactivity was observed in the case of ^{59}Fe-cupferron-ointments.

Table IV. Percutaneous Absorption of ^{59}Fe after Application of ^{59}Fe-EDTA- and ^{59}Fe-cupferron-ointments to Mouse Skin

Exper. number	Ointment base	Form of ^{59}Fe	Radioactivity of ointment (c.p.m./50 mg.)	Number of animals	Amount of ointment applied (mg.)	Per cent absorption of ^{59}Fe
1	Hyd. oint.	^{59}Fe-EDTA[a]	5588	4	52.9±0.8[e]	66.4±5.0[c]
		^{59}Fe-cupferron[b]	6373	4	53.4±1.0	13.0±2.0[c]
2	Abs. oint.	^{59}Fe-EDTA[a]	7072	4	51.6±1.0	80.2±4.1[d]
		^{59}Fe-cupferron[b]	6178	4	52.4±0.9	14.6±0.3[d]

a) A solution of 50 mμmoles of $^{59}FeCl_3$ and 100 mμmoles of EDTA in 100 μl. of H_2O (neutral pH) was mixed with 1.0 g. of each ointment base and 100 μl. of $CHCl_3$.
b) A solution of 50 mμmoles of ^{59}Fe-cupferron in 100 μl. of $CHCl_3$ was mixed with 1.0 g. of each ointment base and 100 μl. of H_2O.
c) Significant difference between the two mean values ($P<0.01$).
d) Significant difference between the two mean values ($P<0.01$).
e) Mean ± standard error.

Table V. Percutaneous Absorption of ^{59}Fe after Application of ^{59}Fe-EDTA- and ^{59}Fe-tiron-ointments to Mouse Skin

Exper. number	Ointment base	Form of ^{59}Fe	Radioactivity of ointment (c.p.m./50 mg.)	Number of animals	Amount of ointment applied (mg.)	Per cent absorption of ^{59}Fe
1	Hyd. oint.	^{59}Fe-EDTA[a]	6871	4	49.1±0.8[e]	54.6±2.2[c]
		^{59}Fe-tiron[b]	6118	4	52.3±1.3	35.2±2.5[c]
2	Abs. oint.	^{59}Fe-EDTA[a]	5752	4	50.5±1.0	58.4±3.5[d]
		^{59}Fe-tiron[b]	5404	4	52.4±0.8	15.8±2.3[d]

a) A solution of 50 mμmoles of $^{59}FeCl_3$ and 100 mμmoles of EDTA in 100 μl. of H_2O (neutral pH) was mixed with 1.0 g. of each ointment base.
b) A solution of 50 mμmoles of $^{59}FeCl_3$ and 200 mμmoles of tiron in 100 μl. of H_2O (neutral pH) was mixed with 1.0 g. of each ointment base.
c) Significant difference between the two mean values ($P<0.01$).
d) Significant difference between the two mean values ($P<0.01$).
e) Mean ± standard error.

Since there was some room for the possibility that water-soluble ^{59}Fe-EDTA might be absorbed from the above ointments as a results of disturbance of the skin barrier by chloroform contained in the radioactive ointments,[9,21] the absorption of ^{59}Fe-EDTA and ^{59}Fe-tiron, another stable water-soluble chelate compound, from chloroform-free hydrophilic and absorption ointments was evaluated as described in Table V. It was observed that about 54.6 and 35.2 per cent of the applied radioactivity was absorbed from hydrophilic ointment, and 58.4 and 15.8 per cent from absorption

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ointment. Therefore, it was concluded that at least some kind of water-soluble substances could be absorbed from emulsion-type ointments, such as hydrophilic and absorption ointment, through mouse skin, in agreement with the assumption that ^{131}I-iodide was absorbed from these ointment bases without prior oxidation to lipid-soluble iodine.

Stolar, *et al.*[22] observed a measurable degree of absorption of sodium salicylate from hydrophilic ointment through hair-clipped rabbit skin, although a greater degree of absorption was found when sodium salicylate was replaced by salicylic acid. In view of the pH of the skin and vehicle, they assumed that sodium salicylate might be absorbed percutaneously without prior conversion to salicylic acid. As for the human skin, however, Nogami, *et al.*[23] reported that sodium salicylate was only slightly absorbed from various ointment bases including hydrophilic and absorption ointments, whereas salicylic acid was absorbed very well from the vehicles. The cause of the apparent difference between human skin and that of mouse or rabbit concerning the absorbability of water-soluble substances from emulsion-type ointment remains to be solved.

It was also recognized that the amounts of ^{59}Fe-tiron absorbed from hydrophilic and absorption ointments were smaller than those of ^{59}Fe-EDTA from the same vehicles, although they are both stable water-soluble chelate compounds. However, the effect of difference in the viscosity of these radioactive ointments must be taken into consideration.

Table Ⅵ. Percutaneous Absorption of ^{59}Fe after Application of ^{59}Fe-cupferron- and ^{59}Fe-carbamate-ointments to Mouse Skin

Ointment base	Form of ^{59}Fe	Radioactivity of ointment (c.p.m./50 mg.)	Number of animals	Amount of ointment applied (mg.)	Per cent absorption of ^{59}Fe
Abs. oint.	^{59}Fe-cupferron[a]	3843	4	50.8±1.2[d]	10.4±2.2[c]
	^{59}Fe-carbamate[b]	3318	4	45.5±3.4	6.1±0.5[c]

a) A solution of 50 mμmoles of ^{59}Fe-cupferron in 100 μl. of $CHCl_3$ was mixed with 1.0 g. of the ointment base.
b) A solution of 50 mμmoles of ^{59}Fe-carbamate in 100 μl. of $CHCl_3$ was mixed with 1.0 g. of the ointment base.
c) No significant difference between the two mean values ($P>0.10$).
d) Mean ± standard error.

Table Ⅶ. Percutaneous Absorption of ^{59}Fe after Application of ^{59}Fe-cupferron-white-petrolatum-ointments to Mouse Skin

Ointment base	Form of ^{59}Fe	Radioactivity of ointment (c.p.m./50 mg.)	Number of animals	Amount of ointment applied (mg.)	Per cent absorption of ^{59}Fe
White petrolatum	^{59}Fe-cupferron[a]	10045	3	57.9±0.6[b]	2.7±1.0

a) A solution of 50 mμmoles of ^{59}Fe-cupferron in 50 μl. of $CHCl_3$ was mixed with 1.0 g. of white petrolatum.
b) Mean ± standard error.

Table Ⅵ shows the results of comparison of two lipid-soluble chelate compounds, ^{59}Fe-cupferron and ^{59}Fe-carbamate, in the percutaneous absorbability from absorption ointment. The date were quite small in both groups and no signifficant difference was observed. The measurement of percutaneous absorption of ^{59}Fe-EDTA from aqueous solution was impossible because of the difficulty in uniform application of the aqueous

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23) H. Nogami, J. Hasegawa, M. Hanano : This Bulletin, **4**, 347 (1956).

solution to the hair-clipped mouse skin. On the other hand, white petrolatum containing ^{59}Fe-cupferron was easily applied to the mouse skin and percutaneous absorption of radioactivity was measured. However, virtually no absorption of ^{59}Fe-cupferron from this oleaginous type ointment was recognized (Table Ⅶ).

Assuming the contribution of surface-active agents to the absorption of water-soluble substances from hydrophilic and absorption ointments through mouse skin, the effect of polyoxyethylene oleyl ether on the percutaneous absorption of ^{59}Fe-EDTA from polyethylene glycol 1500 was examined. This ointment base is not only hydrophilic but easily applicable to hair-clipped mouse skin. As shown in Table Ⅷ, the absorption of ^{59}Fe-EDTA was significantly increased by the addition of the surfactant, without which no or a trace amount of radioactivity was found to be absorbed. The highest absorption was observed in case of the third ointment base composed of polyethylene glycol 1500, white petrolatum and polyoxyethylene oleyl ether. These results suggest that surface-active agents may play some important role in the percutaneous absorption of water-soluble substances from emulsion-type ointments. Promoting effect of surfactants

Table Ⅷ. Effect of Polyoxyethylene Oleyl Ether on Percutaneous Absorption of ^{59}Fe after Application of ^{59}Fe-EDTA-polyethylene-glycol-1500-ointment to Mouse Skin

Exper. number[a)]	Ointment base	Form of ^{59}Fe	Radioactivity of ointment (c.p.m./50 mg.)	Number of animals	Amount of ointment applied (mg.)	Per cent absorption of ^{59}Fe
1	PEG 1500[d)]	^{59}Fe-EDTA	3886	4	52.6±1.2[f)]	5.4±1.7[b)]
2	PEG 1500 POE[e)]	^{59}Fe-EDTA	3873	4	50.8±0.9	21.3±1.7[b,c)]
3	PEG 1500 POE White petrol.	^{59}Fe-EDTA	3418	4	52.3±0.6	25.7±1.1[c)]

a) A solution of 50 mμmoles of $^{59}FeCl_3$ and 100 mμmoles of EDTA in 100 μl. of H_2O was mixed with 1.0 g. of PEG 1500 (Exp. 1), 1.0 g. of PEG 1500 plus 100 mg. of POE (Exp. 2), or 300 mg. of PEG 1500 plus 600 mg. of white petrolatum and 700 mg. of POE (Exp. 3).
b) Significant difference between the two mean values ($P<0.01$).
c) Significant difference between the two mean values ($P<0.10$).
d) Polyethylene glycol 1500. *e*) Polyoxyethylene oleyl ether. *f*) Mean ± standard error.

Table Ⅸ. Percutaneous Absorption of ^{22}Na after Application of ^{22}NaCl-ointments to Mouse Skin

Exper. number	Ointment base	Concent. of carrier NaCl	Radioactivity of ointment (c.p.m./50 mg.)	Number of animals	Amount of ointment applied (mg.)	Per cent absorption of ^{22}Na
1[a)]	Hyd. oint.	L[c)]	9863	4	51.8±0.8[e)]	50.8±4.1[f)]
		H[d)]	9202	4	52.0±0.6	48.4±3.5[f)]
2[b)]	Abs. oint.	L[c)]	10129	3	51.8±0.6	68.4±4.4[g)]
		H[d)]	9199	4	52.8±0.4	73.5±2.1[g)]

a) The time of duration of application was 10 hr.
b) The time of duration of application was 5 hr.
c) Each ointment base (1.0 g.) was mixed with 50 μl. of 0.001*M* ^{22}NaCl.
d) Each ointment base (1.0 g.) was mixed with 50 μl. of 0.1*M* ^{22}NaCl.
e) Mean ± standard error.
f) No significant difference between the two mean values ($P>0.10$).
g) No significant difference between the two mean values ($P>0.10$).

on percutaneous absorption of various substances have been reported by several worker.[24~27]

Absorption of $^{22}NaCl$

The percutaneous absorbability of water-soluble and lipid-insoluble substance from the emulsion-type ointments through mouse skin was demonstrated furthermore using $^{22}NaCl$ as a tracer as shown in Table K. It was also known from the results of this experiment that the 100-fold increase in the concentration of carrier NaCl produced no significant change in the rate of absorption of ^{22}Na within the experimental conditions. However, the minute difference in the rates of absorption can not be detected by this type of experiment, because the slight difference in the viscosity of radioactive ointments is inevitable.

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