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ENZYME LEVELS OF GLUCURONIC ACID METABOLISM IN THE LIVER, KIDNEY AND INTESTINE OF NORMAL AND FASTED RATS

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Abstract—Uridine diphosphate glucose dehydrogenase activity was normally highest in the liver, followed by the intestine and kidneys. The activity was significantly lower in the liver of fasted than of fed animals. β-Glucuronidase activity was higher in the liver and intestine of fasted than of fed rats. Glucuronidation was low in the kidney and intestine compared with the liver. Fasting resulted in a slight decrease in this process in the intestine only. Release of phosphate from uridine diphosphate glucuronic acid during incubation was normally highest in the intestine, fasting having no detectable effect. The presence of ethylene diamine tetra-acetic acid in the assay mixture resulted in a marked increase in glucuronidation and a fall in the simultaneous release of phosphate, both these effects being particularly high in the gut. Uridine diphosphate glucuronyl-transferase activity and the release of phosphate in the presence of ethylene diamine tetra-acetic acid were higher in the liver of fasted than of fed rats. Addition of saccharo-1→4-lactone, an almost specific inhibitor of β-glucuronidase, was followed by an increase of glucuronidation in the liver but not in the kidney and intestinal homogenate and there was no detectable change in the release of phosphate. Activation was observable whether ethylene diamine tetra-acetic acid was present or not, but was less marked in the fasted liver. The role of uridine diphosphate glucose dehydrogenase, uridine diphosphate glucuronyltransferase, uridine diphosphate glucuronic acid pyrophosphatase and β-glucuronidase in the metabolism of glucuronic acid and in the glucuronide production of fed and fasted animals is discussed.

SINCE the work of Dutton and Storey,[6] glucuronic acid is known to be formed from glucose via uridine diphosphate glucose.* This compound is dehydrogenated to UDPGA by a NAD-linked enzyme.[39, 40] Glucuronic acid can then either be transferred to an acceptor to form glucuronides or acid aminopolysaccharides, or it can be metabolized either to ascorbic acid or xylulose. The latter substance is further metabolized in the pentose phosphate shunt, thus completing another route of glucose metabolism, the glucuronic acid pathway (for a review see Burns 1960[2]).

Fasting animals have long been known to be able to produce glucuronic acid both *in vitro* and *in vivo* experiments but in smaller amounts than well-fed animals.[23, 24, 34, 46] No report appears to exist, however, concerning enzyme levels in the glucuronic acid

* Abbreviations used are: UDPG = uridine diphosphate glucose; UDPGA = uridine diphosphate glucuronic acid; UDP = uridine diphosphate; UMP = uridine monophosphate; NAD = nicotinamide-adenine dinucleotide; EDTA = disodium salt of ethylene diamine tetra-acetic acid; GA-1-P = glucuronic acid-1-phosphate; UDPG-DH = uridine diphosphate glucose dehydrogenase; UDP-GT = uridine diphosphate glucuronyltransferase.

pathway in tissues of fasting animals. Systematic quantitative studies in the normal state have only been made for the liver,[13, 44, 45] although glucuronidation also occurs on a large scale in the kidney and intestine.[10, 16, 23, 35, 38] The present study deals with UDPG-DH, UDP-GT (including the effect of EDTA and saccharolactone on its activity), β-glucuronidase and the release of phosphate from UDPGA in the liver, kidney and intestine of fed and fasted rats. EDTA has been shown to inhibit a pyrophosphatase splitting UDPGA in normal rat liver microsomes, hence increasing glucuronidation.[32] A similar activation has been demonstrated by us in the particle-containing supernatant of the liver, kidney and intestine of the rat.[25]

MATERIALS AND METHODS

Experimental Animals

Seven male rats of the Wistar strain with an average body weight of 280 g were subjected to three days fasting. Coprophagy was avoided, and water was given *ad libitum*. After the fasting period the rats were weighed and killed by decapitation. Seven fed controls were included in the series.

Tissue Preparation

The liver, kidneys and a 10 cm long piece of the small intestine, measured from its junction with the stomach, were rapidly removed after decapitation, cooled on ice, weighed and homogenized for 1 min with 2 volumes 0·15 M KCl in the Potter–Elvehjem apparatus. A sample of this homogenate was used for dry weight determination. The rest was centrifuged for 10 min at 2000 × *g* at 0 °C. The supernatant obtained was used for the following procedures.

UDPG dehydrogenase assay. An acetone powder was prepared from the supernatant by precipitating with a ten-fold amount of acetone. The powder was extracted with water and centrifuged. The clear supernatant was incubated with NAD and UDPG according to Strominger *et al.*[41] in a final volume of 1 ml UDPG was obtained from the Sigma Chemical Co., and NAD from the C. F. Boehringer & Soehne GmbH. Mannheim.

UDP glucuronyltransferase assay. p-Nitrophenol was used as substrate according to a modification of earlier methods.[14, 32] The usual assay mixture was 0·5 mM for *p*-nitrophenol (in 0·5 M triethanolamine buffer pH 7·5), 0·5 mM for UDPGA (Sigma Chemical Co. NH_4 . 90% purity) and contained 0·05 ml of the supernatant (equivalent to 16·7 mg wet tissue) in a final volume of 0·1 ml EDTA and saccharo-1→4-lactone were added where indicated, final respective concentrations being 20 mM and 1·24 mM. The blanks contained no UDPGA. The tubes, set up in duplicate, were incubated with shaking in a water bath at 37 °C for 30 min. The reaction was stopped by addition of 0·9 ml 0·18 M trichloracetic acid (TCA). The mixture was centrifuged and 0·5 ml of the supernatant was added to 0·5 ml of 0·8 M KOH. The absorbancy was measured in cells of 1 ml at 400 mμ in a Beckman model B spectrophotometer.

An aliquot of the TCA-supernatant was used for the measurement of the inorganic phosphate released from UDPGA during incubation. The modified method of Fiske and Subbarow[8, 27] was used for this purpose, the final volume being reduced to 1 ml.

Protein. Both from the 2000-*g* supernatant and from the water extract of the acetone powder, protein was measured by Folin's procedure [18]with human serum albumin (Cohn Fraction V) as standard.

β-Glucuronidase assay. The liver supernatant was diluted 1:40 and that of the kidney and intestine 1:20 with 0·15 M KCl for measurement of β-glucuronidase, which was performed according to Fishman.[7] The phenolphthalein mono-β glucuronic acid was obtained from the Sigma Chemical Co.

RESULTS

The organs of the fasted animals were smaller than those of the fed ones (Table 1). This reduction was relatively higher for the liver and intestine than for the total body weight. Since no change was detectable in the dry weights or protein contents of the

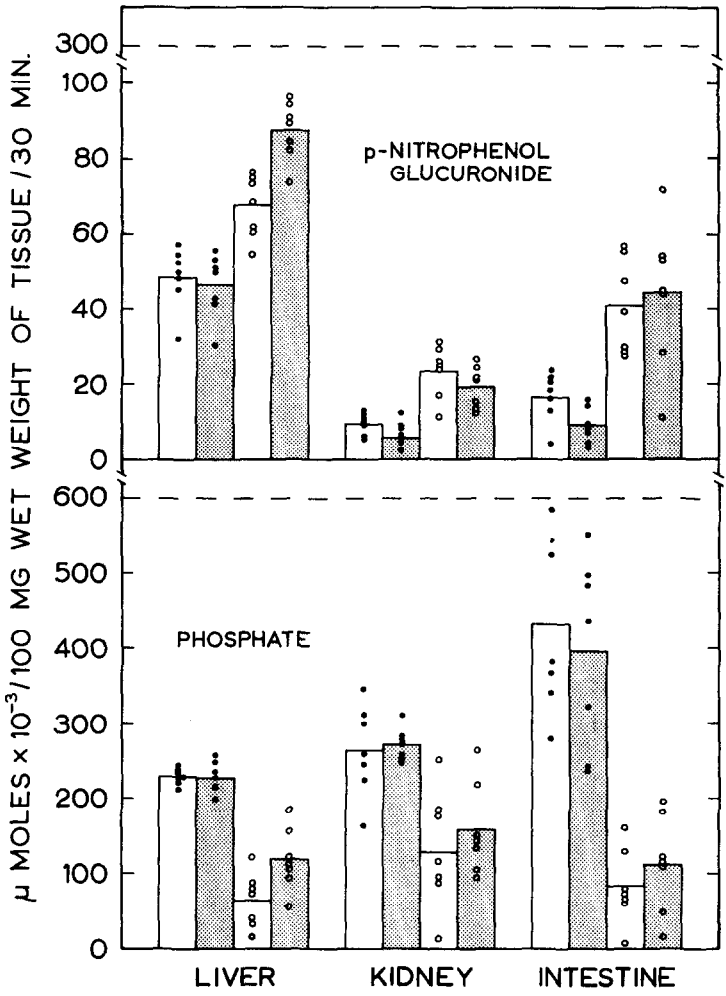


FIG. 1. Glucuronidation of *p*-nitrophenol and the simultaneous release of inorganic phosphate from UDPGA by various tissues of fed (white columns) and fasted (shaded columns) rats in the absence (●) and presence (○) of EDTA. Broken lines indicate the theoretical maxima.

TABLE 1. BODY AND TISSUE WEIGHTS (g), DRY WEIGHTS (MG/100 MG OF WET WEIGHT), AND PROTEIN OF 2000-*g* SUPERNATANT AND WATER EXTRACT OF ACETONE POWDER (MG/100 MG OF WET WEIGHT) IN FED AND FASTED RATS. MEAN ± STANDARD ERROR.

Group	Body Weight	Liver				Kidney				Intestine			
		TTW	DW	PS	PW	TTW	DW	PS	PW	TTW	DW	PS	PW
Fed	283	10·1	29·2	16·5	4·3	2·10	20·8	11·4	3·6	2·29	17·8	9·1	4·5
	±8	±0·6	±1·0	±0·6	±0·1	±0·11	±0·3	±0·3	±0·2	±0·15	±0·6	±0·4	±0·2
Fasted	229	6·5	28·5	17·2	4·4	1·75	20·8	11·7	3·7	1·63	17·4	9·1	4·7
	±8	±0·3	±0·7	±0·6	±0·1	±0·06	±0·5	±0·2	±0·1	±0·07	±1·1	±0·5	±0·2

TTW = total tissue weight, DW = dry weight, PS = protein of 2000 -*g* supernatant, PW = protein of water extract of acetone powder. Underlining indicates that the difference between fed and fasted animals is significant at the five per cent level at least.

TABLE 2. URIDINE DIPHOSPHATE GLUCOSE DEHYDROGENASE ACTIVITY IN LIVER, KIDNEY AND INTESTINE OF FED AND FASTED ANIMALS. UNITS/100 MG OF WET WEIGHT ± STANDARD ERROR. UNDERLINING INDICATES THE SIGNIFICANCE OF DIFFERENCE BETWEEN TWO GROUPS ($P < 0{\cdot}05$).

Group	Liver	Kidney	Intestine
Fed	108·7 ± 5·4	29·6 ± 4·7	44·9 ± 8·3
Fasted	86·9 ± 5·3	23·8 ± 3·2	37·0 ± 6·0

liver, kidney or intestine as a consequence of fasting ,all the values below are expressed per wet weight of tissue.

UDPG Dehydrogenase

The liver contained the highest activity of this enzyme. In the organs of the fasted animals the levels tended to be lower than in the controls, although the difference was only significant in the liver (Table 2). The fall in the level of this enzyme together with reduced total weights of the tissue, presumably results in a marked reduction of total UDPGA production in the intact animal.

UDP Glucuronyltransferase

In the system used, glucuronidation was normally far higher in the liver than in the intestine and even more so than in the kidney (Fig. 1). The conjugation was not affected by three days' fasting in the liver and kidney, whereas in the intestine this treatment appeared to decrease glucuronidation ($P < 0{\cdot}05$).

Addition of EDTA (Table 3) to the incubation mixture caused increased glucuronidation in every case. This activation was normally 2·5-fold in the intestine and kidney and 1·4-fold in the liver. The UDPGA concentration used gave an almost complete saturation of UDP-GT particularly in the liver and kidney. The effect of EDTA was higher (1·9-fold activation) in the livers of fasted than of fed animals resulting in higher final glucuronidation in the former ($P < 0{\cdot}05$) as demonstrated in Fig. 1. The low rate of intestinal conjugation in the fasted rats increased in the presence of EDTA up to the level of the fed animals.

Addition of saccharolactone, an almost specific inhibitor of β-glucuronidase, to the incubation mixture was shown to increase the glucuronidation in the preliminary experiments.[25] Additional results are presented in Table 3. This substance caused an activation in the liver (1·4-fold) but not in the other tissues of the fed animals. In the liver of the fasted rats the saccharolactone effect, although present, was significantly lower than in the non-fasted animals. In the fasted rats a small increase up to the control level was also detectable in the intestine. In the presence of EDTA saccharolactone did not change the rate of glucuronidation from that seen with EDTA alone in the kidney and intestine, whereas in the liver a significant increase was recorded. The additional effect of saccharolactone was again higher in the liver of the fed than of the fasted animals, the combined increment being only a little higher in the latter.

Release of Inorganic Phosphate

This was determined for the purpose of measuring the activity of UDPGA pyrophosphatase. The release of phosphate from UDPGA is dependent not solely on this activity but also on enzymes splitting GA-1-P, UMP and UDP, the presence of which has been demonstrated in the rat organism.[4, 9, 31, 32, 39] Our results are given in Fig. 1 and in Table 4. The release of phosphate was highest in the intestine, averaging, in the system used, 72 per cent of the phosphate added to the incubation medium as UDPGA. In the kidney and particularly in the liver the values are below 50 per cent of the theoretical maximum, indicating that the level of phosphatases is lower in these organs than in the intestine. The release of inorganic phosphate was not affected by fasting.

TABLE 3. EFFECT OF VARIOUS ADDITIONS ON GLUCURONIDATION

Absolute (mean and standard error as μmole $\times$ 10^{-3}/100 mg wet weight of tissue/30 min.) and relative (average as per cent) changes of *p*-nitrophenol glucuronidation caused by addition of EDTA and saccharolactone into assay mixture (see Methods) of 2000-*g* supernatant of liver, kidney and intestinal homogenate of fed and fasted rats in the presence of UDPGA. Relative values given indicate that the change is significant at the five per cent level at least. Effect of fasting ($P < 0.05$) indicated by underlining.

Tissue	FED							FASTED						
	Control	EDTA		Saccharo-lactone		EDTA + saccha-rolactone		Control	EDTA		Saccharo-lactone		EDTA + saccha-rolactone	
	μmole $\times 10^{-3}$	μmole $\times 10^{-3}$	%	μmole $\times 10^{-3}$	%	μmole $\times 10^{-3}$	%	μmole $\times 10^{-3}$	μmole $\times 10^{-3}$	%	μmole $\times 10^{-3}$	%	μmole $\times 10^{-3}$	%
Liver	48·5	+19·3	40	+21·1	43	+34·6	71	46·6	+40·8	87	+8·8	18	+48·8	101
	±3·1	±2·8		±2·8		±5·0		±3·3	±3·8		±2·1		±3·2	
Kidney	9·6	+14·1	147	−0·4	—	+15·2	159	6·9	+12·5	181	−1·0	—	+12·9	187
	±1·1	±2·4		±3·9		±3·1		±1·6	±2·1		±3·1		±3·5	
Intestine	16·6	±24·5	149	0	—	+31·3	188	9·3	+34·9	373	+5·3	57	+36·5	390
	±2·4	±5·8		±6·4		±11·9		±1·7	±7·2		±2·2		±5·4	

TABLE 4. EFFECT OF VARIOUS ADDITIONS ON THE RELEASE OF PHOSPHATE

Corresponding changes in the liberation of inorganic phosphate from UDPGA in the conditions described in Table 3, with the same designations.

Tissue	FED							FASTED						
	Control	EDTA		Saccharo-lactone		EDTA + saccha-rolactone		Control	EDTA		Saccharo-lactone		EDTA + saccha-rolactone	
	μmole × 10^{-3}	μmole × 10^{-3}	%	μmole × 10^{-3}	%	μmole × 10^{-3}	%	μmole × 10^{-3}	μmole × 10^{-3}	%	μmole × 10^{-3}	%	μmole × 10^{-3}	%
Liver	261·1	−198	71	−38	—	−168	64	258·0	−142	55	+14	—	−135	52
	±8·4	±24		±38		±33		±15·2	±12		±44		±35	
Kidney	265·1	−134	50	−2	—	−121	46	271·7	−112	41	−29	—	−144	53
	±23·9	±10		±42		±31		±6·5	±20		±40		±50	
Intestine	432·4	−350	81	−72	—	−387	89	395·6	−282	72	−6	—	−312	79
	±44·5	±45		±53		±86		±49·6	±47		±67		±64	

Addition of EDTA markedly diminished the phosphate released from UDPGA in all tissues, especially in the intestine. The changes caused by EDTA were the same in both groups. In the livers of the fasted rats the release of phosphate in the presence of EDTA was somewhat higher than in the fed animals ($P < 0{\cdot}05$). Saccharolactone in the assay mixture resulted in no detectable change in the phosphate values. The increased rate of glucuronidation caused by this substance in the liver is thus mainly due to inhibition of the breakdown of the glucuronide formed during incubation.

TABLE 5. β-GLUCURONIDASE ACTIVITY IN LIVER, KIDNEY AND INTESTINE OF FED AND FASTED ANIMALS. FISHMAN UNITS/100 MG OF WET WEIGHT ± STANDARD ERROR. UNDERLINING INDICATES THE SIGNIFICANCE OF DIFFERENCE BETWEEN TWO GROUPS ($P < 0{\cdot}05$).

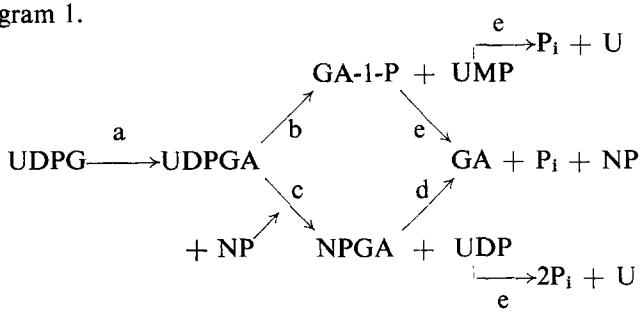
Group	Liver	Kidney	Intestine
Fed	890 ± 22·7	251 ± 17·8	260 ± 18·2
Fasted	1102 ± 55·5	293 ± 22·0	417 ± 32·0

β-Glucuronidase

The activity of this enzyme was markedly higher in the liver and intestine of fasted than of fed animals (Table 5). Addition of EDTA to some assay mixtures caused a notable inhibition of β-glucuronidase activity not only at pH 5·0 in acetate buffer but also at pH 7·5 in triethanolamine buffer, at which the glucuronidation experiments were performed. At the latter pH value the activity was about one-tenth of the optimal one. Endogenous β-glucuronidase thus seems to some extent to be able to destroy the glucuronide formed during incubation. This breakdown is obviously partly prevented by EDTA.

DISCUSSION

The enzymatic reactions responsible for the results in the present study are presented in Diagram 1.



a = uridine diphosphate glucose dehydrogenase
b = uridine diphosphate glucuronic acid pyrophosphatase
c = uridine diphosphate glucuronyltransferase
d = β-glucuronidase
e = phosphatases
NP = *p*-nitrophenol; NPGA = *p*-nitrophenol glucuronide; GA-1-P = glucuronic acid-1-phosphate; GA = glucuronic acid; UMP = uridine monophosphate; U = uridine; P_i = inorganic phosphate.

Diagram 1. Modified from those presented by Pogell and Leloir[32] and Hollman and Touster.[13]

Glucuronide production seems to be accelerated not only by the presence but also by the formation of glycogen, which since the discovery of Leloir and Gardine[20] is believed to occur mainly via UDPG. The administration of glucose and fructose to either non-fasted humans or rabbits results in enhanced glucuronide production.[1, 12, 28, 42, 43] Addition of glucose to the incubation medium of tissue slices of both normal and fasted animals is also followed by increased glucuronidation.[23, 37] During fasting glucose is synthesized from fats and proteins by the liver at an accelerated rate. The conversion to glycogen is probably bypassed. The lack of UDPG is apparent, and in turn may result in the decrease of UDPG-DH activity demonstrated in the present study. A decrease of a number of other liver enzymes occurs during fasting, presumably owing to disturbed protein synthesis.[13, 36]

Saturation of UDP-GT with substrate is difficult to achieve, especially in the intestine, owing to rapid destruction of UDPGA by pyrophosphatase.[32, 37] EDTA inhibits this latter enzyme, at least in rat liver microsomes, with resultant better saturation of UDP-GT and hence enhanced glucuronidation.[32] Our EDTA experiments (decrease of phosphate release from UDPGA with simultaneous increase of conjugation) suggest that a similar inhibition occurs also in the kidney and intestine. Glucuronidation in the presence of EDTA (EDTA + saccharolactone for the liver) in our system probably reflects maximal UDP-GT activity. EDTA has, however, an inhibitory effect on this enzyme[32] and also, as shown in the present study, on β-glucuronidase, the greater effect of EDTA on glucuronidation in the liver of fasted than of fed animals which actually suggests an increase of UDP-GT activity. Whether the enhancement of UDP-GT activity in fasting is detectable with other glucuronate acceptors remains unsolved. The heterogeneity of this enzyme and the different rate of glucuronidation of bilirubin, phenolphthalein and *o*-aminophenol suggest that the case is not necessarily so.[3, 15, 19]

The release of inorganic phosphate from UDPGA and the change in this process caused by EDTA was unaffected by fasting, suggesting that the pyrophosphatase level is unaltered in this state. That some phosphate was liberated in the presence of EDTA may imply incomplete inhibition of pyrophosphatase and/or hydrolysis of the UDP formed during glucuronidation (see Fig. 2). The amount of inorganic phosphate detected in EDTA experiments was equivalent in the intestine, higher in the kidney and lower in the liver than the respective amounts in the UDP expected to be formed by UDP-GT. Increase of the latter enzyme in fasting results in enhanced UDP production. It appears that hydrolysis of this nucleotide may explain the high phosphate release found to occur in the presence of EDTA in the liver of fasted animals.

Fasting results in elevation of β-glucuronidase activity in the liver[36] and in the lymphatic tissue.[29, 30] The present study revealed that this also occurs in the intestine. The significance of β-glucuronidase in the metabolism of glucuronic acid is not known. Its role in the transference of glucuronic acid *in vivo* appears to be negligible.[5] The glucuronidation and β-glucuronidase activities correlate poorly with each other, although a fairly close parallelism exists between them in the stomach after irradiation.[11] Feeding of saccharolactone, an inhibitor of β-glucuronidase, brings about no change in the normal urinary ascorbate level[13] but has a protecting action against gastric ulcer induced by administration of cincophen.[11] Saccharolactone in the incubation medium of mouse liver slices or suspension is without effect on *o*-aminophenol glucuronidation[16, 17, 19, 21]. In our earlier[25] and present studies, saccharolactone

invariably caused increased glucuronidation even in the presence of EDTA in the liver. Unaltered release of phosphate from UDPGA suggests that this activation is due to inhibition of β-glucuronidase. That the saccharolactone effect was less marked in the liver of fasted rats may result from its high β-glucuronidase activity, this not being inhibited equally with the controls by the same amount of inhibitor.

The metabolism of glucuronic acid has been inferred to proceed from UDPGA on a larger scale via UDP-GT than via the UDPGA pyrophosphatase pathway.[13,32,47,48] A labile glucuronide[32] would then be a precursor of free glucuronic acid, β-glucuronidase playing a minor role in its splitting.[13] There is some evidence, however, that β-glucuronidase is of greater significance in this route.[15, 32] The present paper provides further evidence of the importance of β-glucuronidase in the UPD-GT pathway, at least in the liver, where the activity of the GA-1-P phosphatase, necessary for the pyrophosphatase pathway, is low.[4] The latter may be the more important route in the kidney and intestine. This is indicated by the high pyrophosphatase activity (high release of phosphate), relatively low UDP-GT activity even in the presence of EDTA, low β-glucuronidase activity and absence of saccharolactone effect.

Increase of UDP-GT (as indicated by EDTA and EDTA saccharolactone experiments) and β-glucuronidase without change in UDPGA pyrophosphatase (unchanged release of phosphate) would imply at least a relative enhancement of glucuronic acid metabolism via the UDP-GT route in the liver during fasting. Lack of UDPGA with greatly reduced liver weight may explain the decrease of glucuronate and ascorbate[26, 33] excretion into the urine of intact fasted animals.

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