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Stanton Segal, Joseph B. Foley

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Research Article

THE METABOLIC FATE OF C^{14} LABELED PENTOSES IN MAN

By STANTON SEGAL AND JOSEPH B. FOLEY

(From the Clinical Endocrinology Branch, National Institute of Arthritis and Metabolic Diseases, National Institutes of Health, Bethesda, Md.)

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The role of pentoses in intermediary metabolism has received much attention within the past few years. The phosphate esters of D-ribose, D-ribulose and D-xylulose have been identified as key intermediates in the pentose phosphate pathway of glucose metabolism in both plant and mammalian tissue (1). More recently, another alternate pathway of glucose dissimilation, the uronic acid pathway, has been described in which L-xylulose, xylitol and D-xylulose are intermediates (2).

Several pentoses have been identified in normal urine: the aldopentoses xylose, arabinose and ribose (3, 4) and the ketopentoses D-ribulose and L-xylulose (5). Large quantities of the latter sugar are excreted in the disease essential pentosuria (6, 7), which appears to be due to a defect in the further conversion of L-xylulose to the sugar alcohol xylitol (8). In addition to L-xylulose Touster and Harwell (9) have also reported the isolation of L-arabitol from the urine of pentosuric subjects.

Because of increasing awareness of the biological importance of the pentoses, certain aspects of pentose metabolism in man have been investigated in this laboratory. Previous reports have described the physiological disposition of large quantities of infused pentoses (10), the effect of insulin on blood levels of infused pentoses (11) as well as studies of the metabolism of D-ribose (12). This paper reports some observations on the fate of C^{14} labeled D-xylose, D-lyxose, D-arabinose and L-arabinose administered in trace quantities intravenously to normal human subjects. Some of the previously published data (12) on C^{14} ribose metabolism are included for purposes of comparison.

METHODS

Five experiments were performed in four normal male volunteers aged 18 to 21. Each subject had been on a 250 Gm. carbohydrate diet for at least three days and was fasted overnight prior to study.

Five μc. of each sugar dissolved in 250 ml. of normal saline were infused over a 15 minute period. Expired air samples were collected for 4 minutes at 15 to 60 minute intervals for 6 hours. Urine samples were collected at hourly intervals for 6 hours and pooled thereafter till 24 hours had elapsed from the start of the experiment. A urine sample was obtained at the twenty-fifth hour to determine if C^{14} was still being excreted. Urine was preserved by freezing at $-20°$ C. Blood was collected at 20 minute intervals.

Methods for the collection and C^{14} counting of expired CO_2, for preparation and radioactive assay of blood samples, and C^{14} counting and chromatography of labeled compounds in urine have previously been described (12).

The C^{14} pentoses D-xylose, D-lyxose, D-arabinose, L-arabinose and D-ribose (Figure 1) with specific activity of 0.67, 1.65, 1.65, 1.11, 2.6 and 2.01 μc. per mg., respec-

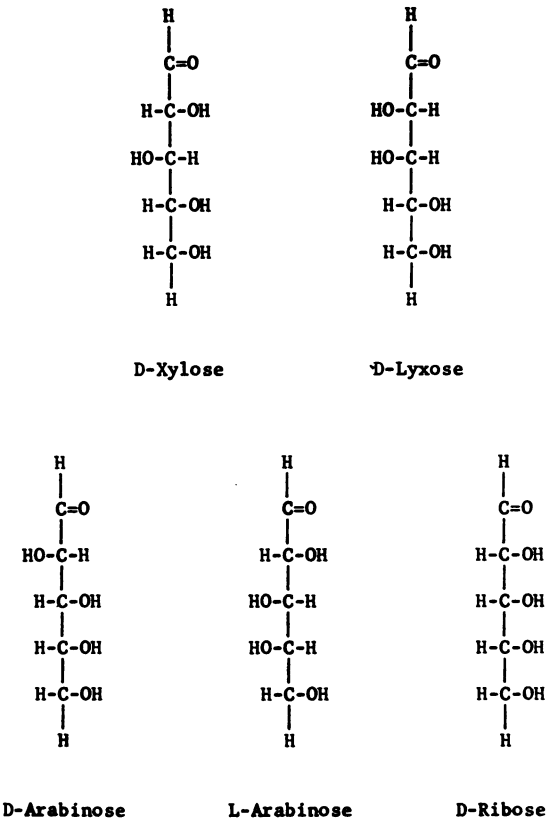

FIG. 1. STRUCTURAL FORMULAE OF PENTOSES STUDIED

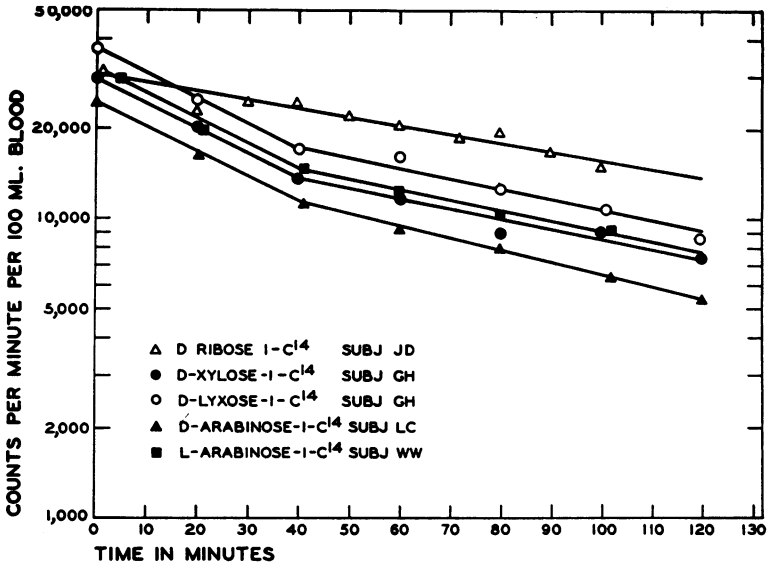

FIG. 2. C^{14} DISAPPEARANCE FROM BLOOD AFTER 5 μc. INTRAVENOUSLY

tively, were all labeled in the first carbon. They were obtained from Dr. H. Isbell of the National Bureau of Standards. The authenticity of these sugars was established by chromatographic comparison with known pure unlabeled pentoses.

RESULTS

The disappearance of C^{14} from blood

The C^{14} blood levels measured following infusion of the various pentoses are plotted on semilogarithmic coordinates in Figure 2. The straight lines obtained indicate that the removal of C^{14} occurs according to first order kinetics. During the first 30 minutes, there appears to be a deviation from the linear logarithmic curve finally established, a finding which probably represents a temporary distribution gradient between blood and tissues. One cannot exclude the possibility, however, that the initial curve represents pentose disappearance while that established after 30 minutes represents the composite disappearance curve of C^{14} pentose and C^{14} metabolites which have entered the blood. After infusion of large quantities of pentose, the disappearance curve of pentose from blood constructed from chemical analysis reveals a similar deviation during the first 30 minutes from the final linear curve (10).

A rate constant for disappearance of C^{14} from blood may be calculated from a knowledge of the biological half-time. In Table I are given the proportional rate constants expressed as K^1, the per cent C^{14} disappearing per minute for the linear curves established after 30 minutes. The rates of removal have a narrow range of 0.68 to 0.92 per cent per minute.

TABLE I

Disposition of C^{14} after infusion of labeled pentose

Subject	Sugar	% Dose expired as $C^{14}O_2$ in 6 hrs.	% Retained* C^{14} expired as $C^{14}O_2$ in 6 hrs.	Urinary C^{14} in 24 hrs.	Disappearance of C^{14} from blood
				% dose	*%/min.* (K^1)
G. H.	D-xylose	16	23	35	0.77
G. H.	D-lyxose	14	38	72	0.81
L. C.	D-arabinose	19	38	57	0.92
W. W.	L-arabinose	0.8	4	85	0.77
J. D.	D-ribose	48	50	10†	0.68

* Retained activity equals dose minus six hour urinary excretion.
† Five hour excretion; no activity detected after this time by methods employed.

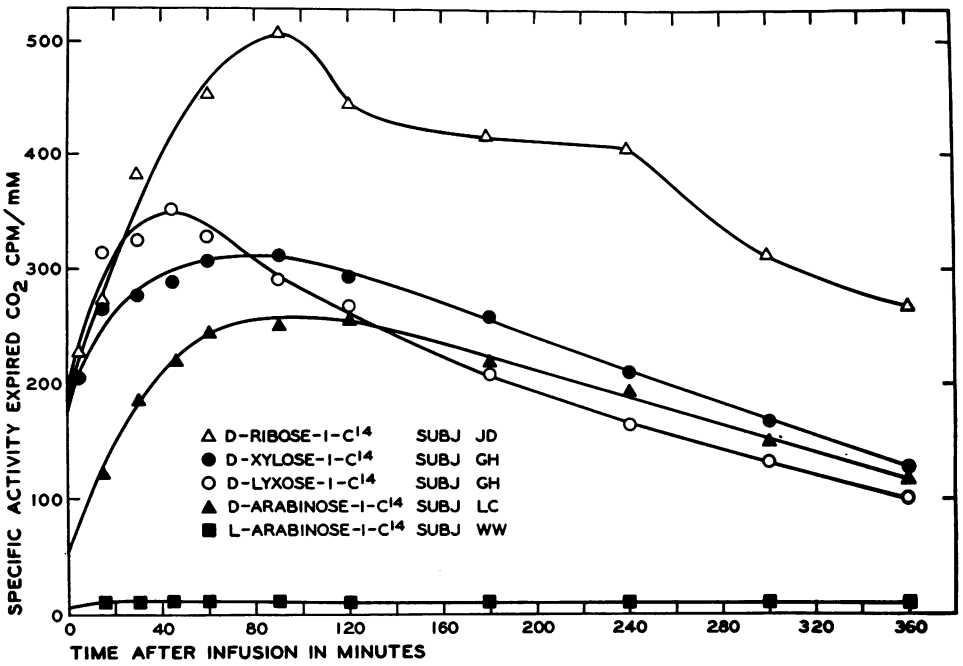

FIG. 3. EXPIRED $C^{14}O_2$ AFTER 5 μC. C^{14} PENTOSE INTRAVENOUSLY

The rate constants for the disappearance from blood of the C^{14} of D-xylose, D-lyxose and D- and L-arabinose are very similar to those calculated from the curves of disappearance of pentose determined chemically, following infusion of large quantities of sugar. These ranged from 0.77 to 1.58 per cent per minute (10). However, ribose chemically determined has been shown to be cleared from blood with rate constants much larger than the 0.68 recorded for C^{14}. For example, 3 Gm. of infused ribose was removed from blood at 11 per cent per minute (12).

Expired $C^{14}O_2$ after administration of C^{14}pentose

The specific activity of expired $C^{14}O_2$ is shown in Figure 3. Very little radioactivity was found in CO_2 following L-arabinose injection though significant amounts of $C^{14}O_2$ were derived from the first carbon of the other sugars. Quantitative data for C^{14} excretion via CO_2 are shown in Table I.

In six hours only 0.8 per cent of the dose of C^{14} L-arabinose appeared via this route whereas 48 per cent of the injected ribose radioactivity could be found. The $C^{14}O_2$ derived from D-xylose, D-lyxose and D-arabinose ranged from 14 to 19 per cent of the dose. Since much C^{14} appears in urine perhaps a more accurate appraisal of $C^{14}O_2$ derived from the pentose can be had if one calculates the per cent of retained C^{14} (the dose corrected for the six hour urinary excretion of C^{14}) in expired air. This is shown in Table I. It may be observed that a considerable portion of the first carbon of D-lyxose, D-xylose and D-arabinose actually present in the body is converted to CO_2.

Certain observations shown in Figure 3 are worthy of note. D-xylose-1-C^{14} and D-lyxose-1-C^{14} were administered to the same subject. Comparing the $C^{14}O_2$ excretion curves one finds that the dissimilation of D-lyxose with elimination of part of carbon one as $C^{14}O_2$ takes place at a faster rate and reaches an earlier peak than D-xylose. These two sugars differ only by the stereoconfiguration of carbon two (Figure 1). It is also apparent that D-ribose C^{14} differs from the other pentoses in the extent of oxidation of carbon one to CO_2 and the seemingly different mechanism is reflected in the shape of the ribose curve. Interpretation of the latter has received comment previously (12). The more extensive metabolism of ribose C^{14} as compared to the other pentoses may be related to its extensive conversion to D-glucose.

Urinary excretion of C^{14}

The per cent of the C^{14} dose appearing in urine for the 24 hour period after infusion is tabulated in Table I and shown graphically in Figure 4. The urinary excretion of C^{14} ranged from a total of 10 per cent for ribose to 85 per cent for L-arabinose. The low urinary excretion of ribose C^{14} appears to reflect the large amount of conversion to CO_2, the high renal excretion of C^{14} from L-arabinose reflecting the minute conversion to CO_2. Although the dissimilation of the other pentoses D-xylose, D-lyxose and D-arabinose occurred with a similar quantity of $C^{14}O_2$ production there are marked differences in the urinary excretion of C^{14} (Table I).

The cumulative urinary excretion curves of C^{14} indicate a similarly rapid initial rate of egress of C^{14} derived from D-lyxose and D- and L-arabinose. The early excretion rate of C^{14} from xylose and ribose is much slower. The C^{14} excretion from ribose reached a plateau after a few hours and that from xylose within 24 hours. This seems not to be the case for the other sugars for their curves suggest a continued C^{14} excretion beyond 25 hours.

In an attempt to determine whether the urinary C^{14} activity represented unaltered sugar, urine was chromatographed on paper and the C^{14} residing in bands corresponding to the sugar was eluted and counted. The results are shown in Table II. In all cases except for ribose the C^{14} in urine is only in part accounted for by unmetabolized sugar in the two hour postinfusion period studied. The quantity excreted as pentose appeared to decrease with time. Most striking is the finding that only 7 per cent of the C^{14} in the second hour urine of the subject receiving D-arabinose could be accounted for as the original sugar, whereas 64 per cent could be so detected during the first hour. These findings suggest that C^{14} containing material other than the injected sugar is present in the urine. The nature of this metabolite or metabolities remains to be identified.

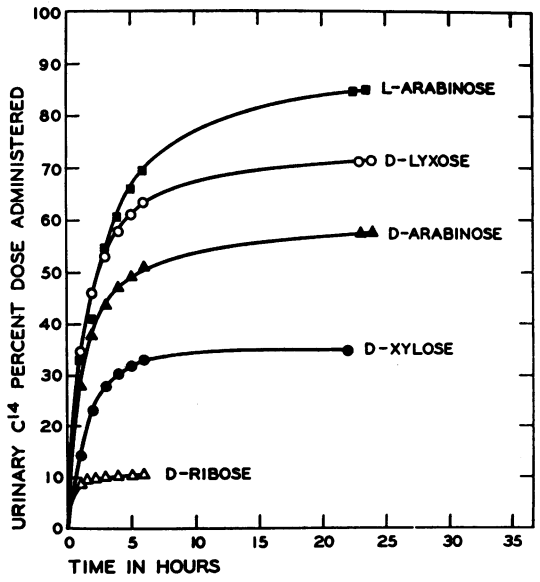

FIG. 4. URINARY C^{14} EXCRETION AFTER C^{14} PENTOSE ADMINISTRATION

TABLE II

Paper chromatography of C^{14} in urine of subjects receiving C^{14} pentose

Sugar	Solvent	% C^{14} in urine found in pentose area: 1st hour	2nd hour
D-xylose	MEK HAC H_2O*	60	61†
	BuOH HAC H_2O‡		59
D-lyxose	MEK HAC H_2O	70	51
D-arabinose	MEK HAC H_2O	64	7
	BuOH HAC H_2O		7§
L-arabinose	MEK HAC H_2O	51‖	25
D-ribose	MEK HAC H_2O	92	

* Methylethyl ketone, acetic acid, water 6:1:1.
† Recovery of the infused C^{14} pentose co-chromatographed with this urine was 94 per cent.
‡ Butanol, acetic acid, water 4:1:5.
§ Recovery of the infused C^{14} pentose co-chromatographed with this urine was 102 per cent.
‖ Recovery of the infused C^{14} pentose co-chromatographed with this urine was 114 per cent.

DISCUSSION

Previous reports from this laboratory (10, 12) described the disappearance from blood and the urinary excretion of large quantities of D-xylose, D-lyxose, D- and L-arabinose and D-ribose given intravenously. The disposition of the C^{14} from the radioactive forms of these sugars given in tracer quantities permits some comparisons. In the present studies the disappearance of C^{14} from blood was observed to obey first order kinetics. The rate constants for all but ribose are very similar to those obtained by determination of orcinol reacting substance during the load experiments. As has been pointed out (12) the curve for the removal of ribose from blood constructed

from the orcinol assay differs considerably from that based on C^{14} counting data, a finding which appears to be explained by the rapid conversion of the C^{14} labeled carbon of ribose to blood glucose and other C^{14} labeled material (12).

Chromatographic studies suggesting that C^{14} labeled material other than pentose is present in urine indicates that the C^{14} disappearance curve from blood may represent not only pentose but may be a composite curve for disappearance of other C^{14} labeled substances derived from pentose. Since the orcinol reaction is not specific for pentoses, the curves determined after the load infusions (10) may also in part represent metabolites of the infused pentose. It may be, however, that the C^{14} in blood represents only a small quantity of nonpentose material but that the renal clearance of this unknown substance is greater than that of the pentose itself or that the nonpentose C^{14} is formed in the kidney and excreted as such. Under such circumstances the C^{14} disappearance from blood would be an accurate picture of the removal of pentose.

Certain differences between the urinary excretion of pentose as determined chemically after infusion of 5 to 20 Gm. amounts and the C^{14} excretion after the tracer dose of radioactive sugar can be pointed out. In the former experiments, the urinary excretion of xylose, lyxose and both arabinoses ranged from 27 to 60 per cent of the dose and averaged 44 per cent, no significant difference being observed between these pentoses (10). Ribose measured chemically in urine revealed an excretion of about 20 per cent of the dose but this figure depended on the amount administered. In the present studies 10 per cent of the ribose dose was excreted in the urine. The C^{14} excretion of the remaining pentoses varied with each sugar (Figure 4) and the per cent excreted via this route was over 60 per cent for D-lyxose and L-arabinose, being nearly quantitative for the latter pentose. Another dissimilarity between the results obtained chemically and C^{14} analyzed experiments may be observed. When 20 Gm. of D-xylose and D-lyxose were given to the same individual the urinary excretion of orcinol reactive substance was essentially the same for both sugars (10). When this comparison was made with C^{14} sugar there was a marked difference in C^{14} urinary excretion (Subject G. H.).

Chromatography of the urine in the studies reported here has revealed evidence for radioactive material other than the infused pentose. If one corrects the urinary C^{14} excretion for the actual pentose present in the first hour and the remainder of the C^{14} for the actual pentose determined in the second hour, it may be estimated that only 21 per cent of the dose of D-xylose, 20 per cent of D-arabinose, 42 per cent of D-lyxose and 33 per cent of L-arabinose were excreted as unaltered C^{14} labeled pentose.

The dissimilation of D-xylose, D-lyxose, D-arabinose and D-ribose in man has been found to occur with elimination of a fraction of carbon one as $C^{14}O_2$. Since these sugars have been given intravenously with almost immediate production of $C^{14}O_2$ it is highly unlikely that this metabolism is a result of intestinal bacterial activity. The catabolism of L-arabinose results in negligible amounts of $C^{14}O_2$. This observation based on an experiment in one individual raises the possibility that Subject W. W. given the L-arabinose-1-C^{14} did not metabolize L-arabinose to CO_2 because of an enzymatic deficiency which is not present in other humans. This seems unlikely in view of his ability to handle 20 Gm. infusions of L-arabinose in a manner similar to other normal subjects.

Few studies of catabolism of C^{14} labeled pentoses to $C^{14}O_2$ in other animals have been reported. Bloom (13) has shown $C^{14}O_2$ production from ribose in the rat. Bloom and Stetten, however, found that D-arabinose-1-C^{14} and D-arabinose-5-C^{14} did not give rise to significant yields of $C^{14}O_2$ when incubated with rat liver slices or when administered to intact rats or rabbits (14). Since D-arabinose-1-C^{14} gives rise to significant amounts of $C^{14}O_2$ in man it appears that man is different from these animals in this regard.

The total 24 hour recovery of the adminstered C^{14} may be estimated from the urinary recovery data, the amount of expired $C^{14}O_2$ and calculation of label in the bicarbonate pool at the end of six hours (15). This latter figure is about 5 per cent of the dose for D-lyxose, D-xylose and D-arabinose, 10 per cent for ribose and zero for L-arabinose. C^{14} of lyxose was almost quantitatively accounted for at about 90 per cent of the dose. Eighty-five per cent of the L-arabinose label was excreted solely in urine. From the C^{14} excretion curve for L-arabinose it appears that further label

was excreted after 24 hours. Eighty per cent of D-arabinose C^{14} and 68 per cent of ribose could be accounted for. This is a low estimate for ribose since there was continued $C^{14}O_2$ excretion beyond six hours and since other studies (12, 16) reveal a significant portion of the ribose C^{14} to be in the glucose pool. Only about 55 per cent of xylose C^{14} could be accounted for. The fate of the remaining label is unknown.

Whether the pentoses are in some part converted to glucose is a question of general interest. After infusion of D-xylose and L-arabinose in man a rise in blood glucose has been observed (10). Little or no change occurs upon infusion of D-arabinose or D-lyxose, whereas D-ribose has been observed to cause marked decreases in blood glucose level (17). Despite the hypoglycemia induced by ribose, D-ribose-1-C^{14} has been shown to be converted to blood (16) and urinary glucose (12) via the pentose phosphate pathway. From the results reported here it appears that though the blood glucose level may rise after L-arabinose infusion, this is not due to conversion to glucose for nearly all of the label is renally excreted and none appears as $C^{14}O_2$. No direct evidence is at hand to state whether conversion to glucose is a fate of D-xylose, D-arabinose or D-lyxose in man. Comparing the $C^{14}O_2$ excretion of these sugars with that of D-ribose which is known to be glucogenic one might suspect that these sugars are converted to glucose minimally or not at all. In the intact mouse Hiatt (18) has observed slight conversion of xylose-1-C^{14} to glycogen via the pentose phosphate scheme but virtually no conversion of D- or L-arabinose.

It is possible that D-xylose, D-arabinose and D-lyxose might enter the pentose phosphate pathway. In bacteria, for example, a xylose isomerase has been isolated (19) which converts this sugar to D-xylulose which may be phosphorylated to xylulose 5-phosphate (20). It has also been demonstrated in microorganisms that D-lyxose may be isomerized to D-xylulose (21). From the $C^{14}O_2$ excretion curves presented, if these sugars were converted to D-xylulose and thence entered the pentose phosphate pathway the early peak labeling of $C^{14}O_2$ shown for D-lyxose would indicate that the lyxose isomerase enzyme was more efficient than the xylose isomerase. In *E. coli* D-arabinose has been shown to form D-ribulose (22), which after phosphorphorylation could enter the path to glucose formation.

Pathways to $C^{14}O_2$ from pentose other than via conversion to glucose exist for pentoses in bacteria. D-Xylulose may undergo phosphorolysis to acetyl phosphate and triose phosphate (23). D-Arabinose may be catabolized by the following scheme (24): D-arabinose→D-arabonic acid→2 keto, 3-deoxyarabonic acid→pyruvic acid plus glycolic acid.

From our data L-arabinose appears to be catabolized but to products other than CO_2. In bacteria, the pathway L-arabinose→L-arabinolactone →L-arabonic acid→α ketoglutarate has been described (25). Since α ketoglutarate gives rise to CO_2, if this pathway were operative in man the end product would be one prior to the keto acid. Apparently, the sequence L-arabinose→L-ribulose →D-xylulose-5-phosphate reported in bacteria (26, 27) is absent in man for this would give rise to $C^{14}O_2$ via the pentose phosphate pathway. Another alternative for the fate of L-arabinose may be conversion to L-arabitol, a substance which has been isolated from the urine of pentosuric individuals (9).

SUMMARY

Carbon 14 labeled pentoses have been infused intravenously to normal human subjects. The disposition of the label has been followed in blood, urine and expired air. It has been found that D-xylose, D-lyxose, D-arabinose and D-ribose are catabolized to some extent to $C^{14}O_2$. Much of the label appears in the urine in a form that is not the infused sugar. L-Arabinose gives rise to negligible amounts of $C^{14}O_2$. The possible pathways of metabolism of these sugars are described.

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