

**IN VIVO ASSAY FOR SOMATIC POINT MUTATIONS INDUCED BY
GENOTOXIC CARCINOGENS: INCORPORATION OF
[³⁵S]METHIONINE INTO A RAT LIVER CYTOCHROME *b*₅
NORMALLY LACKING SULPHUR-CONTAINING AMINO ACIDS**

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SUMMARY

The trypsin fragments of rat liver microsomal cytochrome *b*₅ (Tb₅) lack both methionine (met) and cysteine (cys), i.e., the sulphur-containing amino acids. Tb₅ should therefore contain no ³⁵S-radioactivity after isolation from animals treated with [³⁵S]met or [³⁵S]cys. If, however, the nucleic acids coding for this polypeptide have been damaged by a genotoxic carcinogen, a mis-coding could result in an incorporation of met or cys into the polypeptide so that Tb₅ could now be ³⁵S-radiolabelled. Two experiments are described, the first one where a toxic regimen of *N*-nitrosomorpholine (NNM) to rats resulted in a significant increase of ³⁵S-radioactivity in the Tb₅ of liver microsomes, and a second experiment with a non-toxic regimen of *N,N*-diethylnitrosamine (DENA), where no increase was observable.

Key words: Mammalian mutagenicity test - Point mutation - Protein coding - Cytochrome *b*₅ - Amino acid composition - Rat liver microsomes

INTRODUCTION

It is now widely accepted that mammalian mutagens are carcinogens, and a number of short-term tests for carcinogenicity base upon the determination

Abbreviations: Tb₅, trypsin fragment of cytochrome *b*₅; met, L-methionine; cys, L-cysteine; ile, L-isoleucine; NNM, *N*-nitrosomorpholine; DENA, *N,N*-diethylnitrosamine; O⁶eG, O⁶-ethyl-guanine.

of a mutagenicity of a test compound [1]. Very recently it has been reported that a single point mutation of guanosine to thymidine was the only genetic change required to activate the oncogene in T24 [2] and EJ [3] human bladder carcinoma cells. These findings exemplify that an assay for point mutations might represent a valid approach to the testing of potential carcinogens.

Many tests for mutagenicity are performed *in vitro* although it is evident from a number of reports that the activation of mutagens to the ultimate genotoxic form and the subsequent inactivation to detoxified compounds does not reflect the *in vivo* situation [4]. Therefore, there is a need for *in vivo* tests for mutagenicity, and we report here on a novel application of the amino acid substitution assay suggested by Popp et al. [5].

The trypsin fragments of rat microsomal Tb_5 lack both met and cys, i.e., all sulphur-containing amino acids [6]. Tb_5 isolated from rat liver microsomes should therefore contain no ^{35}S -radioactivity if the animal has been administered [^{35}S]met or [^{35}S]cys. If, however, the gene coding for this polypeptide is damaged by a mutagen, a point mutation could result from miscoding so that the daughter cell gene could code for met or cys. Consequently, mRNA transcribed from a damaged gene could contain a substituted base and lead to the incorporation of met or cys into the polypeptide chain so that resulting Tb_5 now would exhibit ^{35}S -radioactivity. Similarly, mRNA transcribed from a normal gene could also be damaged by the mutagen and contribute to the level of met or cys in Tb_5 .

We describe the results of two experiments, the first one where a toxic regimen of NNM resulted in a significant increase of ^{35}S -radioactivity in the Tb_5 of rat liver microsomes. In a second experiment with a non-toxic regimen of DENA, the increase in ^{35}S -radioactivity was no longer detectable.

MATERIALS AND METHODS

Animals and chemicals

Male rats [ZUR:SIV-Z, Sprague-Dawley derived] were used. They were held three per Macrolon cage on laboratory chow No. 890 (Nafag AG, Gossau, CH) and tap water *ad libitum*. L-[^{35}S]met of an original specific activity of 1061 and 1000 Ci/mmol and L-[4,5- ^3H (N)]ile (84 Ci/mmol) were purchased from New England Nuclear. NNM and DENA were from Fluka AG, Buchs, CH. Aminosol, a 10% hydrolysate of casein, excip. *ad infundib.* was from Vitrum AB, Stockholm, Sweden. All other reagents were of the highest purity available from Merck, Darmstadt, F.R.G.

Liquid scintillation counting

Samples were counted in Insta-Gel cocktail (Packard) in low background glass vials (Packard) in a Packard Tri Carb 460 CD scintillation counter.

Experiment 1. Three 5-6-week-old rats (rat nos. 4, 5 and 6) were treated for 16 weeks with 100 mg/l NNM in the drinking water. After 7 weeks without treatment, the animals received a single oral dose of 53.1, 99.7 and

139.6 mg/kg NNM, respectively. Eight hours thereafter, food was withdrawn in order to synchronize subsequent protein synthesis which was initialized 24 h after the carcinogen treatment with the administration of 5.7 mCi/kg [^{35}S]met and 27 $\mu\text{Ci/kg}$ [^3H]ile in 6.2 ml/kg of an aqueous solution of amino acids (aminosol 10%) and sucrose (16%). After 24 h, the animals were killed and the livers were processed immediately. Three control rats (nos. 1, 2 and 3) received tap water in the place of all carcinogen treatments.

Experiment 2. Three 5-6-week-old rats were treated for 6 weeks with 20 mg/l DENA in the drinking water. After 2 days without treatment, the animals received a single oral dose of 37.7, 36.1 and 38.7 mg/kg DENA. Eight hours thereafter, food was withdrawn in order to synchronize subsequent protein synthesis which was initialized 24 h after the carcinogen treatment with the administration of 7.66 mCi/kg [^{35}S]met and 26.9 $\mu\text{Ci/kg}$ [^3H]ile in 6.26 ml/kg of an aqueous solution of amino acids (aminosol 10%) and sucrose (16%). After 24 h, the animals were killed and the livers were processed immediately. Three control rats received tap water in the place of all carcinogen treatments.

Isolation of the trypsin fragments of liver microsomal cytochrome b_5

A liver homogenate was prepared according to Omura et al. [7]. The incorporation of ^{35}S -radioactivity into whole liver protein amounted to 12.5% and 11.3% of the administered label. The microsomal fraction [7] exhibited a specific radioactivity of $5.86 \cdot 10^6$ and $4.0 \cdot 10^6$ dpm [^{35}S]/mg microsomal protein (Expt. 1 and 2, respectively). The subsequent isolation of Tb_5 also followed an established method [7], modified as follows:

A Sephadex G 100 SF (Pharmacia) column (2.5 \times 35 cm) was used and eluted with 50 mM NaCl/50 mM Tris-HCl (pH 7.5). Ion exchange chromatography was performed on a DEAE Sephacel (Pharmacia) column (1.5 \times 6 cm), with a linear salt gradient of 100 mM NaCl/50 mM Tris-HCl to 130 mM NaCl/50 mM Tris-HCl (pH 7.5) with a total volume of 250 ml. Fractions exhibiting an absorbance ratio 413/280 nm of more than one were pooled, diluted with an equal volume of water, loaded on a second DEAE Sephacel column and eluted as before. Further purification and separation of different tryptic peptides was obtained with repeated hydroxylapatite (Bio-Rad) column chromatography as described [6] but with the following modifications: DEAE Sephacel eluent fractions exhibiting an absorbance ratio of >3 were concentrated and desalted on a Amicon M52 ultrafiltration system with a Diaflo YM5 membrane. The concentrate was loaded on a hydroxylapatite column (1.5 \times 6.5 cm) and eluted with a linear phosphate gradient from 5 to 100 mM (pH 6.7) total volume 250 ml. The second hydroxylapatite column (1.5 \times 9 cm) was eluted with a 50-100 mM phosphate gradient for the fragment eluting with 75 mM phosphate and with a 35-85 mM phosphate gradient for the fragment eluting with 58 mM phosphate. Total volume was 200 ml. Fractions containing Tb_5 were pooled and ultrafiltered as described above. The amount of Tb_5 isolated was determined on the basis of the optical density at 413 nm ($\epsilon_{413\text{ nm}} = 117 \text{ mM}^{-1} \text{ cm}^{-1}$). The purity was characterized by

the ratio of the optical densities at 413 and 280 nm. Sodium dodecylsulphate polyacrylamide gel electrophoresis in a 3-mm slab was the final purification step. A 4% stacking gel and a 15% separation gel was used. Tb_5 was loaded in a 3-cm wide slot and located with Coomassie Blue R Brilliant.

For liquid scintillation counting of the bands, the gel was digested as follows: In experiment 1, gel slices (3 mm \times 3 cm \times 1 cm) were cut into small pieces and swollen for 24 h at 50°C with 1.2 ml water and 0.6 ml soluene-350 tissue solubilizer (Packard) in a Toluene/Triton X-100 based scintillation liquid. In Expt. 2, the stained gel slices were incubated with 80 mg 60% perchloric acid and 800 μ l 30% hydrogen peroxide and digested at 70°C in a shaking water bath for 14 h in glass tubes closed with teflon-lined screw caps. The resulting clear solution was partially neutralized with about 30 mg 27% sodium hydroxide to a pH of 2–3. After addition of 8 ml Insta-Gel and 1 ml water, the mixture was shaken vigorously to yield a homogeneous sample for liquid scintillation counting. A slice of gel taken from the side of each slab was also digested and the radioactivity count was used as a background value for the samples run in each slab. The background in the tritium channel ranged from 21.4 to 24.6 cpm, and 26.0 to 32.7 cpm in Expt. 1 and 2, respectively, the ^{35}S -values were 18.3–18.5 and 11.1–11.8 cpm, respectively. Slices cut from the sample slots above or below the stained protein band did not contain any measurable radioactivity.

RESULTS

Figures 1 and 2 summarize yield and purity of Tb_5 during the isolation procedure in Expts. 1 and 2, respectively. In Expt. 1, it is conspicuous that the carcinogen-treated animals yielded a much lower amount of total microsomal protein and Tb_5 . The specific radioactivities of microsomal protein and Tb_5 ($[^3H]ile$) were, however not different (Table D). This means that the rate of cytochrome b_5 synthesis was not affected by the carcinogen treatment.

Tb_5 contains the heme moiety of cytochrome b_5 , which absorbs light at 413 nm in the oxidized state. The UV absorbance at 280 nm is general for protein. The absorbance ratio 413 nm/280 nm can therefore be taken as a criterion for purity and literature values of 6.35 [6] are given for their purest Tb_5 preparations. Our samples reached these literature values already after hydroxylapatite chromatography. For our purpose, it was of prime importance to minimize contaminations by proteins containing $[^{35}S]met$. Therefore, an additional purification step by sodium dodecylsulphate polyacrylamide gel electrophoresis was performed. It was not surprising that no contaminating protein band was visible on the gel, although up to 12 nmol (about 120 μ g) Tb_5 were applied per slot.

Trypsin digestion of cytochrome b_5 is known to yield two fragments containing 82 and 88 amino acids and it has been shown that the longer fragment can be converted to the smaller upon more extensive incubation with trypsin [6]. A separation is achieved only by phosphate gradient elution from hydroxylapatite with ionic strengths of 58 mM and 75 mM phosphate

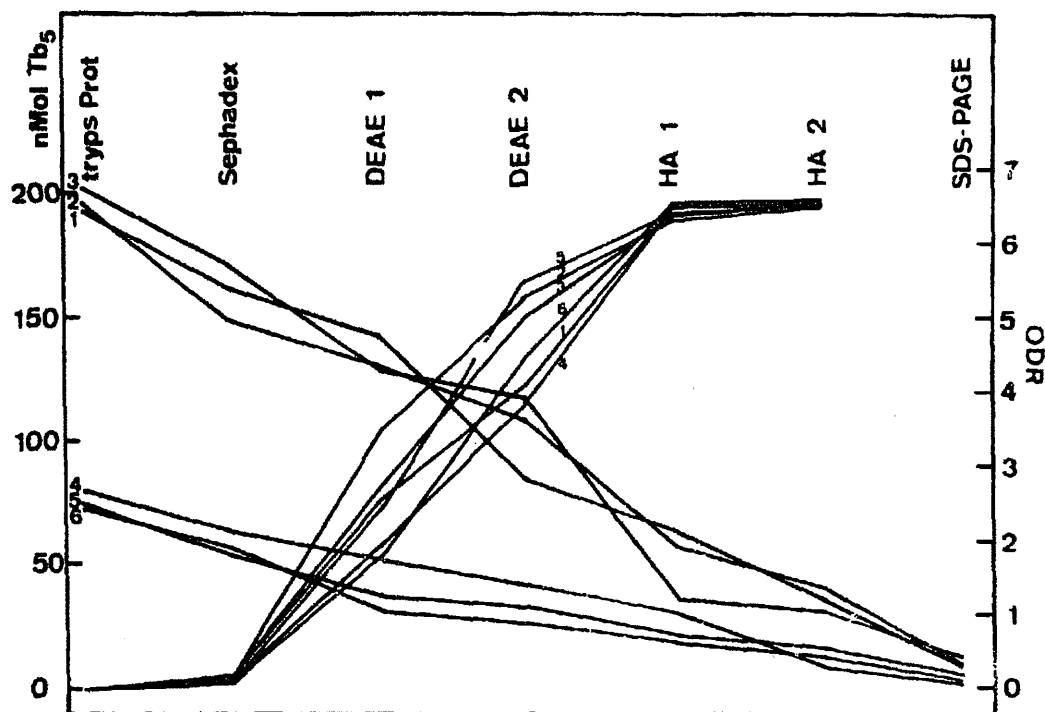


Fig. 1. Yield (left-hand ordinate) and purity (right-hand ordinate) of the trypsin fragment of cytochrome b_5 (Tb_5) isolated from rat liver microsomes without (1-3) and with (4-6) pretreatment of the animals with NNM (Expt. 1; see Materials and Methods for treatment schedule). ODR, ratio of optical densities at 413 and 280 nm.

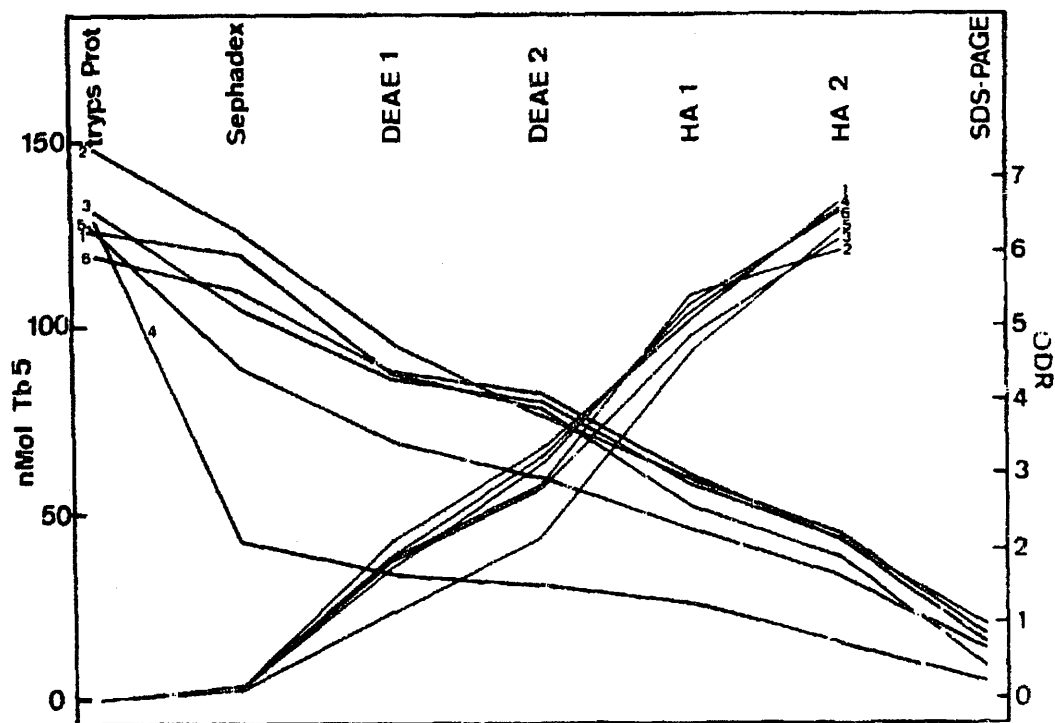


Fig. 2. Yield (left-hand ordinate, sum of both fragments) and purity (right-hand ordinate, average of both fragments) of the trypsin fragments of cytochrome b_5 (Tb_5) isolated from rat liver microsomes without (1-3) and with (4-6) pretreatment of the animals with DENA (Expt. 2; see Materials and Methods for treatment schedule). ODR, ratio of optical densities at 413 and 280 nm.

TABLE I

INCORPORATION OF [³⁵S]MET AND [³H]ILE INTO THE TRYPSIN FRAGMENTS OF RAT LIVER MICROSOMAL CYTOCHROME *b₅*, WITH OR WITHOUT PRETREATMENT WITH THE HEPATO-CARCINOGEN NNM

Animal no.	Treatment					
	Controls			Carcinogen-treated		
	1	2	3	4	5	6
[³⁵ S]met administered (mCi/kg)	5.59	5.63	5.75	5.67	5.83	5.83
[³ H]ile administered (μCi/kg)	26.4	26.6	27.3	26.9	27.7	27.6
Body wt. at time of sacrifice (g)	568	564	550	565	614	619
Liver wt. (g)	21.9	20.1	20.6	21.4	16.3	15.9
Tb ₅ yield in microsomes (nmol/mg protein)	0.941	0.991	0.878	0.569	0.577	0.576
³ H-spec. act. (dpm/nMol Tb ₅ /μCi ile/kg)	0.613	0.579	0.641	0.575	0.645	0.626
Amount of Tb ₅ in scintillation vial (nmol) ^a	7.95	10.8	11.5	0.89	5.03	2.70
Total count ³ H-channel (cpm, 4 · 40 min counting)	55.1	62.8	66.4	28.1	39.0	32.7
Total count ³⁵ S-channel (cpm, 4 · 40 min counting)	19.80	19.65	19.54	18.92	19.58	19.42
Spec. act. Tb ₅ [³ H] (dpm/nmol) ^b	16.5	15.4	17.5	15.5	17.9	17.3
Spec. act. Tb ₅ [³⁵ S]channel (dpm/nmol) ^b	0.32	0.21	0.20	0.99	0.47	0.77
Ratio ³⁵ S/ ³ H (dpm/dpm) ^b	0.019	0.014	0.012	0.064	0.026	0.045
Mean ± S.D.		0.015 ± 0.004			0.045 ± 0.019	
<i>P</i> for the hypothesis of equality (<i>t</i> -test) (one-sided and on the basis of the logarithms of the single values)		< 0.02				

^aCalculated on the basis of the ³H counts and the specific activity as determined after hydroxylapatite column 2.

^bCounting efficiency about 22.3% for ³H, 52.9% for ³⁵S.

for the elution of the longer and shorter fragment, respectively. The relative amounts of the two fragments strongly varied. In Expt. 1, rat no. 1 yielded almost exclusively the longer fragment whereas all the other isolations resulted in much shorter fragments. In Expt. 2, both fragments could be isolated in substantial amounts. They could be processed separately after the first hydroxylapatite column. Since both fragments are equally suited for our assay – they do not differ with respect to the presence of 4 ile and the lack of sulphur-containing amino acids [6] – mutual contaminations do not interfere with the interpretation of the ³⁵S/³H-activity ratio.

[³H]ile was administered simultaneously with [³⁵S]met in order to have an internal label in the newly synthesized protein. For a comparison of the ³⁵S-radioactivity of Tb₅ isolated from treated versus control animals it was

TABLE II

INCORPORATION OF [^{35}S]MET AND [^3H]ILE INTO THE TRYPSIN FRAGMENTS OF RAT LIVER MICROSOMAL CYTOCHROME b_5 , WITH OR WITHOUT PRETREATMENT WITH THE HEPATO-CARCINOGEN DENA

Animal no.	Treatment					
	Controls			Carcinogen-treated		
	1	2	3	4	5	6
[^{35}S]met administered (mCi/kg)	7.13	6.95	7.49	8.03	7.95	8.62
[^3H]ile administered ($\mu\text{Ci/kg}$)	25.1	24.4	26.3	28.2	28.0	30.3
Body wt. at time of sacrifice (g)	369	354	333	317	323	310
Liver wt. (g)	17.1	14.4	12.9	12.2	10.8	10.5
Tb ₅ yield in microsomes (nmol/mg protein)	0.749	0.689	1.112	0.790	0.926	0.812
[^3H]spec. act. (dpm/nmol Tb ₅ / μCi ile/kg)	0.501	0.322	0.401	0.496	0.350	0.228
Amount of Tb ₅ in vial (nmol) ^a	5.62	7.97	11.16	- ^b	6.18	12.26
Total count ^3H -channel (cpm, 4·40 min counting)	43.1	55.8	39.5	47.5	49.2	48.1
Total count ^{35}S -channel (cpm, 4·40 min counting)	16.1	15.17	18.17	-	14.93	15.15
Spec. act. Tb ₅	12.6	7.9	10.6	14.0	9.8	6.9
[^3H] (dpm/nmol) ^{c,d}						
Spec. act. Tb ₅	1.37	0.65	0.88	-	0.96	0.42
[^{35}S]chann. (dpm/nmol) ^e	1.69	1.57	1.11	2.82	0.94	1.12
Ratio $^{35}\text{S}/^3\text{H}$ (dpm/dpm) ^f	0.109	0.083	0.083	-	0.098	0.061
Means \pm S.D.		0.119 \pm 0.044			0.124 \pm 0.057	
<i>P</i> for the hypothesis of equality (<i>t</i> -test)		0.89				

^aFirst line: Tb₅ fragment of 82 amino acids; second line: Tb₅ fragment of 88 amino acids. Amount calculated on the basis of the ^3H counts and the specific activity as determined after hydroxylapatite column 2.

^bShorter fragment lost because of malfunctioning of fraction collector.

^cCounting efficiency 28.9% for ^3H , 64.8% for ^{35}S .

^dThe specific activity was determined after hydroxylapatite 2 and was about the same for both trypsin fragments of cytochrome b_5 .

^eThe difference between the two fragments is probably due to differences in protein contaminations

therefore not necessary to have equal amounts of Tb₅ in the scintillation vial or equal rates of protein synthesis in the liver, but it was possible to compare the ratio $^{35}\text{S}/^3\text{H}$ which was expected to be higher if a mutation leading to the incorporation of met had occurred after carcinogen treatment.

Table I compiles the experimental details for Expt. 1. The $^{35}\text{S}/^3\text{H}$ ratios were indeed higher in the carcinogen-treated rats. Statistical analysis of the data with a one-sided Student's *t*-test revealed a level of significance of <0.02 . Tb₅ isolated from control animals did also contain a minute ^{35}S -radioactivity, probably due to traces of contaminating protein. We cannot, therefore, exclude, the theoretical possibility that the difference in the $^{35}\text{S}/^3\text{H}$

ratio between carcinogen-treated and control animals could also be based upon higher protein contaminations in the Tb_5 isolated from the treated group. However, we cannot find any scientific basis for such a hypothesis, and therefore consider this possibility much less likely than the generation of sulphur-containing Tb_5 variants.

It was the aim of this first experiment to find out whether the assay works at all. We therefore chose a very drastic carcinogen regimen. The treated rats had a severely damaged liver. The livers had necroses and histological examination revealed preneoplastic and neoplastic foci. Thanks to the fact that the rate of protein biosynthesis was not different in the two groups, this observation should not confound our analysis of the data.

On the basis of the promising results with this first experiment, a second experiment was carried out with the following modifications: Instead of NNM, the ethylating agent DENA was used because this carcinogen is known to give rise to a relatively large proportion of O^6 -guanine ethylations [8] known for their miscoding properties [9]. The doses used were much lower so as not to induce any liver damage [10]. And thirdly, the isolation of Tb_5 was speeded up so that 62% of the initial ^{35}S specific activity was available at the time of the measurement of the radioactivity on Tb_5 . Table II summarizes the results. The $^{35}S/^3H$ ratio is only marginally higher in the treated group. The nucleic acid damage produced in the second experiment therefore did not result in any observable increase in misincorporations.

DISCUSSION

A number of different events can lead to the incorporation of the 'wrong' amino acid methionine into newly synthesized Tb_5 . DNA damaged by the carcinogen could either lead to a mutated daughter cell, now expressing a met in Tb_5 or be transcribed to a mutated mRNA molecule coding for met. Furthermore, the alkylation of mRNA by the carcinogen could lead to a miscoding during translation and incorporation of met into the protein. In addition, there are a number of possibilities arising from an alkylation of the anticodon of the tRNA or of the enzymes involved in replication, transcription, or translation, i.e. also from non-mutational events.

The carcinogen treatment of the animals was both chronic and acute in order to increase the possibilities for an incorporation of met. The long-term treatment of the rats with the carcinogen was meant to generate mutated cells containing a DNA coding for a met in Tb_5 . Such a cell can be viable and produce functional cytochrome b_5 as is nicely shown in man, where the amino acid 74 is methionine, whereas all animal species examined have leucine at this position [11]. This replacement of a neutral amino acid by another neutral one would leave the chromatographic properties of the polypeptide largely unchanged so that the altered Tb_5 chain would not be lost during the isolation procedure.

An acute dose of the carcinogen was given 24 h before the administration of the radiolabelled amino acids. This treatment was meant to acutely

alkylate DNA and RNA so that DNA-mRNA and mRNA-tRNA miscodings could occur.

It is tempting to speculate whether the limit of detection in our experiments was anywhere near the frequency of miscodings to be expected from the carcinogen treatments. We do not see a way for a reliable approximation of the met yield to be expected from DNA-DNA mutations. The consequence of DNA-mRNA and mRNA-tRNA miscodings arising after the acute carcinogen treatment in Expt. 2 should be better amenable to an estimation: the single dose of 38 mg/kg DENA has resulted in about 38 ethylations per 10^6 DNA nucleotides, as can be calculated from the appropriate binding data [12]. Of the total number of ethylations, only about 9% are at the O^6 of guanine, resulting in a critical premutagenic lesion [8]. Taking into account that guanine represents 22% of all DNA bases in the rat, the chance for a guanine to become an O^6 -ethylguanine (O^6 eG) therefore was: $38 \cdot 0.09 \cdot 10^{-6} / 0.22 = 1.56 \cdot 10^{-5}$. Only a fraction of all possible amino acid substitutions by DNA-mRNA and mRNA-tRNA miscodings leads to the incorporation of met. For instance, O^6 -ethylation of a guanine of a DNA codon for threonine (TO^6 eGC) could result in an AUG triplet in the mRNA synthesized. This AUG triplet would result in a met incorporated into the polypeptide chain. Taking into account the number of threonine residues in Tb_5 (=6), the multiplicity of codons for thr (=4), and the chance for a guanine to become an O^6 eG ($=1.56 \cdot 10^{-5}$), the estimated frequency of this event is about $6 \cdot 1/4 \cdot 1.56 \cdot 10^{-5} = 2 \cdot 10^{-5}$ per Tb_5 gene. Similar calculations with ile-, arg- and leu-met replacements will result in a total frequency of about $3 \cdot 10^{-5}$ so that about one out of 33000 newly synthesized Tb_5 molecules is expected to contain a met. This frequency would result in 0.08 dpm ^{35}S -radioactivity in a vial containing 6 nmol Tb_5 isolated from a carcinogen-treated animal. This value was estimated on the basis of the experimentally found [3H]ile activity in Tb_5 (10.3 dpm/nmol), the amount of Tb_5 isolated, the ratio of the frequencies for met and ile (4 per Tb_5 molecule), respectively, taking into account that 280 times more [^{35}S]met radioactivity has been administered and correcting for the decay of ^{35}S -radioactivity during the isolation procedure. The calculation therefore was: $10.3 \cdot 6 \cdot 1/4 \cdot 280 \cdot 0.65 / 33000 = 0.08$ dpm.

Our experimental limit of detection is primarily dependent on the amount of Tb_5 synthesized during the presence of radiolabelled precursor amino acid and put into the scintillation vial, on the amount of ^{35}S -radioactivity administered, and on the purity of the isolated Tb_5 . In the second experiment, this experimental limit was about 45 times above our theoretical limit, because only an increase of 3.6 dpm ^{35}S -activity would have led to a significantly different population of $^{35}S/^3H$ values compared with the controls. It must, however, be added that a large number of possibilities that can also lead to an incorporation of met into Tb_5 cannot be estimated quantitatively so that only the experiment could answer the question about the limit of detection. One important mechanism by which the ^{35}S -radioactivity could be effectively increased, is the possibility that one of the mutated cells might

have grown into a group of mutated progeny or even into a hyperplastic nodule or a neoplastic lesion. This could be one of the reasons why the respective effect was observable only in the high-dose Expt. 1.

Changes in amino acid composition of peptides as a result of treatment of animals with chemical mutagens are recently being studied more often, primarily with protein mapping on two-dimensional gel electrophoresis [13,14]. We are, however, not aware of any breakthrough in the risk assessment of exposure to chemical carcinogens on the basis of this type of approach. The assay described here has a number of general advantages over the mapping procedures: (i) Organ specificity of mutagens can be followed directly with the assay described here because cytochrome b_5 is present in most tissues [15]. (ii) The determination of the number of point mutations induced ~~can~~ allow a quantification of the mutagenic and probably also carcinogenic potency of a genotoxic chemical. So far, only in vivo assay of a DNA damage seems to produce a reasonably good correlation with carcinogenic potency [16], but does not fully take into consideration DNA repair or the mutagenicity of the many types of DNA damage. With the present somatic mutation assay, these two important modulatory influences are included in part so that a correlation with carcinogenic potency should be much improved. In addition, there is no need for radiolabelled test compound as is the case with DNA binding.

In conclusion, this assay system could show a way for the detection of somatic point mutations in vivo. With the yield of pure Tb_5 as obtained in these experiments, only high dose exposure to a carcinogen was detectable. In order to lower the sensitivity of this assay, it seems worthwhile to improve the isolation procedure for Tb_5 or to search for other proteins with amino acid compositions that could lend to this type of assay.

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