gens. In general, data on relative carcinogenic potencies is not easy to obtain because animal tests have not been designed to yield such information, and results may vary considerably with experimental method. However, Russel and Meselson[3] have recently compiled sufficient data to arrange 10 chemicals in order of carcinogenic potency and it is of interest to compare them for Z* value. In terms of carcinogenic potency aflatoxin > sterigmatocystin > benzo-(a)pyrene > 1,3-propanesultone > dibenz(a, h)anthracene = 4-aminobiphenyl > β-napthylanine > benzidine > MOCA > MMS. However, in order of Z* value (a low Z* value assumed to predict greater carcinogenicity), MOCA > 4-aminobiphenyl > benzidine > β-napthylamine > dibenz(a, h)anthracene > Benzo(a)pyrene > MMS > 1,3-propanesulfone > aflatoxin > sterigmatocystin. If there is any trend here at all, it would seem to run in the direction *opposite* to that proposed by Veljkovic and Lalovic.

Another point against quasi-valence number as a predictor of carcinogenicity is its insensitivity to isomerism. Recently, however, McCann and Ames have presented a series of examples of isomers which vary greatly in carcinogenic potency[4]. Thus, quasi-valence number cannot distinguish between 2-acetylaminofluorene which is carcinogenic and 4-acetylaminofluorene which is not. In the same way there are great differences in carcinogenic potency between 2-aminoanthracene and 1-aminoanthracene, β-napthlamine and α-napthylamine and 4-aminobiphenyl and 2-aminobiphenyl, yet each pair possesses identical Z* values.

Activation. Many carcinogens are not capable of damage until activated to a more reactive form. This activation may involve an increase in carcinogenic potency of an order of magnitude or more. A theory with a substantive basis in fact should reflect this, the ultimate carcinogen appearing as much more potent than the procarcinogen. Veljkovic and Lalovic, however, report a change of not more than 10%. For instance, the active form of 2-acetyl-aminofluorene is thought to be acetylaminofluorene-N-sulfate[5], yet the Z* value for this metabolite is 3.24, in the range of non-carcinogens. Similarly, Benzo(a)pyrene has a lower Z* value than its 7,8-dihydrodiol-9,10-epoxide even though the latter is thought to be the active form[6]. Finally, cycasin is activated to its carcinogenic metabolite methylazoxymethanol by gut flora[7]. Cycasin itself is not carcinogenic as indicated by its total lack of effect in gnotobiotic mice[7]. Both cycasin and methylazoxymethanol, however, have very similar Z* values.

Conclusions. The use of average quasi-valence number does make sense because a common characteristic of all carcinogens is their electrophilic nature[8] and a small number of valence electrons in an organic molecule might in some cases tend to make that molecule electrophilic. In general, it is impossible to go beyond this simple statement in predicting the carcinogenic activity of a molecule[8], although *within a closely related chemical group* there may be structural correlates. Certainly, the inverse of such a statement, namely that all electrophilic molecules are carcinogenic, is not true. Since the Z* value is essentially a quantification of this criterion, attempts to use it for prediction of carcinogenicity ought to be viewed with disfavor, at least until the correlation can be shown to hold over a broad range of chemicals. As we have illustrated, this will be difficult to do.

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Simple theoretical criterion of chemical carcinogenicity – a refutation

D.H. Rosenblatt and J.C. Dacre

U.S. Army Medical Bioengineering Research & Development Laboratory, Environmental Protection Research Division, Fort Detrick, Frederick (Maryland 21701, USA), 14 November 1978

Summary. The quasi-valence number criterion for chemical carcinogenicity has been shown, through several examples, to be untenable.

The article by Veljković and Lalović, which appeared in 1977 in this journal[1], is the cause of considerable concern. It claims to predict carcinogenicity and especially noncarcinogenicity ('In the case of noncarcinogenicity the quasi-valence number is necessary and sufficient criterion.') on the basis of the easily calculated 'quasi-valence number',

$$Z^* = \sum_{i=1}^{m} N_i Z_i \Big/ \sum_{i=1}^{m} N_i,$$

where N_i is the number of atoms of the i-th type in the given molecule, Z_i is the number of valence electrons in the atom of the i-th type, and m is the number of chemical elements in the molecule; except that for halogen elements $Z = 1$ instead of 7. This publication may have aroused high hopes among many of the readers of this journal for a meaningful reduction in the expense of establishing the hazard or safety of the large number of organic compounds

to which human beings are exposed. A brief examination of the table presented by Veljković and Lalović, and of additional appropriate examples, strongly suggests that the authors may have deceived themselves, and that they have certainly misled the scientific community.

Thus, they listed dimethyl sulphate as a noncarcinogen, when it has a well-known reputation for carcinogenicity[2]; it is certainly as potent as urethane[3], which appeared in the authord list of carcinogens. Calculation of Z* values, shown in parentheses, would place the following organics below $Z^* = 3.20$ and thus in the class of carcinogens: Ethane (1.75), butane (1.86), acetic acid (3.00), and ethanol (2.22). On the other hand, the known carcinogen N-nitroso-N-methylurea[4] (3.33), would be classed as a noncarcinogen. If one considers covalent inorganic compounds, in view of the authors' inclusion of hydrazine (2.33), then water (2.67) and ammonia (2.00) must appear among the list of carcinogens.

Finally, it appears that Veljković and Lalović[1] arbitrarily assigned $Z = 1$ to halogen elements; no rationale was presented for this. It would appear that the a priori knowledge that many organohalogen compounds are carcinogens prompted this modification to the basic concept, rather than any insight arising from accepted physical theory.

Our examination of the 'quasi-valence number' criterion for defining chemical carcinogenicity strongly indicates that it is untenable and should therefore be discarded. Nothing in succeeding papers of Veljković, in particular that dealing with the cytostatic activity of organic compounds[5], provides a reason to alter this assessment.

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PRO EXPERIMENTIS

A novel method in enzyme immunoassay: Maleimide derivative of hapten for enzyme coupling

A. Castro and N. Monji

Hormone Research Laboratory, Department of Pathology, University of Miami School of Medicine, Miami (Florida 33152, USA), 20 October 1978

Summary. Meta-maleimidobenzoyl derivative of L-thyroxine methyl ester (MBTM) was synthesized and coupled to β-galactosidase at molar ratio of over 5 to 1. More than 97% of the enzyme was found to be labeled with MBTM. A thyroxine enzyme immunoassay was carried out with sensitivity in the 0–10 µg/100 ml range.

Maleimide derivatives have been used for coupling enzymes to proteins, such as immunoglobulins with N,N-o-phenylenedimaleimide[1,2] and insulin with m-maleimidobenzoyl-N-hydroxysuccinamide ester[3].

Coupling of haptens to the enzyme, on the other hand, is often identical to the preparation of hapten-protein conjugates for immunization. Such coupling involves either amino or carboxyl groups of the enzyme, resulting in either low efficiency of coupling[4] or reduction of enzyme activity[5]. In order to avoid such disadvantages, we report here the synthesis of m-maleimidobenzoyl derivative of hapten for coupling to sulfhydryl groups of the enzyme. A high efficiency of binding to the enzyme, high yields, and minimum loss of both enzyme activity and immunoreactivity were found.

Preparation of m-maleimidobenzoyl derivative of thyroxine methyl ester for development of thyroxine enzyme immunoassay is described (figure 1). β-galactosidase was used for several reasons: 1. it can be obtained in highly purified form, 2. it has a high catalytic number, 3. conjugation to sulfhydryl groups of the enzyme does not reduce enzyme activity, 4. the enzyme and its conjugates are stable up to 1 year when stored at 4 °C, and 5. it is not present in human or animal biological fluids.

Chemicals. L-thyroxine and o-nitrophenyl-β-D-galactoside were obtained from Sigma Chemical Co., β-galactosidase from *E. coli* from Boehringer Mannheim Biochemicals, and meta-aminobenzoic acid, benzaldehyde, and maleic anhydride from Aldrich Chemical Co.

Antiserum. Antiserum to thyroxine was produced in rabbits by injection of thyroxine conjugated to bovine serum albumin prepared by the method of Gharib et al.[6]. Cross reactivity with triiodothyroxine was minimal. Goat anti-rabbit immunoglobulin antibody was obtained from Calbiochem.

Synthesis of m-maleimidobenzoic acid (MBA). Meta-carboxymaleanilic acid was first prepared from meta-aminobenzoic acid and maleic anhydride following the method of Parola[7]. It was then cyclized with acetic anhydride to give MBA following the procedure of Searle[8].

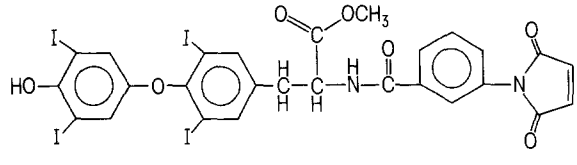

Fig. 1. Synthesis of m-maleimidobenzoyl derivative of L-thyroxine methyl ester. MBA (200 mg) was dissolved in 3 ml of thionyl chloride ($SOCl_2$) and refluxed for 30 min. Excess $SOCl_2$ was then evaporated under diminished pressure. The m-maleimidobenzoyl chloride (MBC) was kept overnight in a vacuum desiccator. The dried MBC was dissolved in 10 ml of tetrahydrofuran (THF) and added dropwise to a stirred THF solution containing L-thyroxine methyl ester (400 mg) and a slurry of sodium carbonate (400 mg). The reaction mixture was refluxed for 30 min. At this point linkage was complete and m-maleimidobenzoyl L-thyroxine methyl ester produced was examined by TLC using Eastman chromatogram 13179 as an eluting plate and ethyl acetate as eluting solvent. The reaction mixture was then filtered and the solvent removed under diminished pressure to yield crude pale-yellow product. The MBTM was purified by silica gel column chromatography (1.5 × 30 cm) using chloroform as eluting solvent. The isolated white powder of MBTM gave a single spot on TLC, $R_f = 0.56$, using ethyl acetate as solvent. The presence of maleimide group in the isolated product was confirmed by IR and by its ability to react with cysteine using the method of Grassetti and Murray[10]. Melting points 137–141 °C.