3β-HSD activity and AsA content, while DHA is significantly elevated in both testes after unilateral AMD injection. The enzyme Δ^5-3β-HSD is present in both the tubular and Leydig cells, as was observed previously[6,16]. Testicular Δ^5-3β-HSD activity in toad is known to be stimulated by AsA and DHA[4,6]. AsA is converted to DHA in the testis of the toad[17] and DHA possibly stimulates Δ^5-3β-HSD activity by oxidizing reduced DPN[6]. Thus the oxidized form of AsA (DHA) is reduced to AsA reversibly by steroid dehydrogenase. It seems reasonable to speculate that the increase in DHA and decrease in AsA in the testes after AMD injection may be due to decreased Δ^5-3β-HSD activity. On the other hand, testicular steroid hormones, related to the enzyme Δ^5-3β-HSD, stimulate the synthesis of ascorbic acid in the kidney of toad[18]. So the fall of total ascorbic acid is possibly the result of less ascorbic acid synthesis due to the inhibition of Δ^5-3β-HSD activity in AMD-treated toads.

The mechanism by which AMD decreases Δ^5-3β-HSD activity in the toad testis has not been established. AMD is known to inhibit DNA-dependent RNA synthesis[19,20]. This antibiotic also reduces the basal rate of progesterone synthesis and luteinizing hormone-induced progesterone secretion in the follicles[12]. Savard et al.[21] have reported that AMD, as well as puromycin, inhibits steroidgenesis in the bovine corpora lutea. The present investigation shows that AMD possibly decreases Δ^5-3β-HSD activity by inhibiting protein synthesis in the toad testis, and the effect of AMD, unlike in the hamster[22], is not confined to the unilateral site of injection. This could be explained by the fact that the blood-testis barrier responsible for permeability restrictions in the higher vertebrates[23] is not well developed in toad.

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2 Acknowledgments. Thanks are due to Prof. C. Deb, Department of Physiology, Calcutta University, for his suggestions and constant encouragment. Thanks are also extended to Mr R.K. Bhattacharya, Microphotographer, Department of Physiology, for his kind cooperation.

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DISPUTANDUM

Comments on the significance of the quasi-valence number for chemical carcinogenesis

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Summary. A quasi-valence number of less than 3.20 was reported to be significant in correlating carcinogenicity. This criterion has no meaningful relationship since such a large proportion of organic compounds fall in this group that it provides no selectivity.

The recent correlation of the quasi-valence number with carcinogenicity appeared to provide a convenient and simple method of identifying those compounds which might be chemical carcinogens[2]. This concept, to be significant, would have to provide a structural screen which would eliminate a large number of structures which are not carcinogenic while identifying most of the structures of active compounds. The quasi-valence number in our opinion does not meet either of these important requirements. The quasi-valence number Z* is defined by the equation:

$$Z^* = \frac{\sum_{i=1}^{m} N_i Z_i}{\sum_{i=1}^{m} N_i}$$

N, number of atoms of *i*th type, Z, number of valence electrons on atoms of the *i*th type (halogens = 1).

The maximal limit of 3.20 for Z* does not exclude a significant number of organic structures which are not carcinogens. All saturated compounds of the general formula C_nX_{2n+2} where X is univalent (hydrogen or halogen) will fall in the category of potential carcinogenic compounds. Any unsaturated or cyclic organic compound with greater than (0.35)X for every carbon will have Z* values below 3.2 and may be presumed to be carcinogenic. Saturated compounds which would give the largest Z* values are the oxygenated compounds such as sugars which contain large numbers of atoms with many valence electrons. The structures $C_nX_{2n+2}M_n$ and $C_nX_{2n}M_n$ where X is univalent and M is oxygen or sulfur all fall in the category of potential carcinogenic compounds. Only if more than one-third of the oxygens are in the carbonyl form ($C_nX_{2n-2}M_n$) or if the hydroxyl groups are converted to methanesulfonate esters, do compounds fall in the non-carcinogenic category with $Z^* > 3.2$. Ironically, some of these compounds, mannitol myleran, for example, are carcinogens.

It is difficult to estimate the fraction of organic compounds which have Z* values below 3.2, but 50% would be a lower limit. Thus application of the quasi-valence criterion to a structure would be no more effective than dividing all organic compounds into 2, approximately equal, groups.

The quasi-valence number is no more effective in predicting non-carcinogenicity. The number of carcinogens containing multiple nitrogen and/or oxygen and sulfur is very great. 2 compounds listed by the authors as non-carcinogenic ($Z^* > 3.20$), dimethyl sulfate and 6-mercaptopurine ($Z^* = 3.38$ and 3.57, respectively), are in fact carcinogens. Other reported carcinogens which are above the carcinogenic limit of the quasi-valence number include aflatoxin ($Z^* = 3.31$), nitrosoimidazolidone ($Z^* = 3.38$), 1,3-propanesultone ($Z^* = 3.23$), N-methyl-N'-nitro-N-nitrosoguanidine ($Z^* = 3.73$), maleic hydrazide ($Z^* = 3.50$), N-nitrososarcosine ($Z^* = 3.29$), N-nitrosodiacetonitrile ($Z^* = 3.54$), N-methyl-N-nitrosourea ($Z^* = 3.33$), N-methyl-nitrosobiuret ($Z^* = 3.50$), and N,N'-dinitrosomethyloxamide ($Z^* = 3.67$). In addition, the most potent of the carcinogenic 2-nitrofuryl derivatives, N-4-(5-nitro-2-furyl)-2-thiazolyl-formamide ($Z^* = 3.90$) along with its non-carcinogenic derivatives, have Z* values above 3.20. These are but a few of the carcinogenic compounds that have quasi-valence numbers greater than 3.20. These data show that this property has too many 'exceptional carcinogens' to provide a useful screen for carcinogens.

On the basis of these examples, one must assume that the quasi-valence number fails to correlate the currently available data, for the maximum value of 3.20 does not exclude a reasonable number of non-carcinogens. Equally significant is the large number of carcinogenic compounds which have Z* values greater than 3.20. From a logical point of view it would be expected on the basis of functional groups that carcinogenicity would be associated with larger quasi-valence numbers.

1 Acknowledgment. The authors express appreciation to the American Cancer Society (CH-57), the National Institute of Environmental Health Sciences (1-RO1-ES01975-01 PTHB) and the Robert A. Welch Foundation (B-702) for financial assistance of projects related to this paper.

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PRO EXPERIMENTIS

A rapid and simple method for the isolation of pure eosinophilic leukocytes from horse blood

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Summary. An improved and short method is described for the isolation of intact eosinophilic leukocytes from horse blood with high yield (1–1.5 g/20 l). Viability and purity of the preparations were verified by light and electron microscopy and by the trypan blue exclusion test. Isolated eosinophils were 98–100% pure, intact and viable, and they could be shown to phagocytise immune-complexes.

Blood eosinophilia is frequently observed during allergic disorders and parasitic infestations, and it has led to a great number of clinical and histological studies. Less work has been devoted to the study of the biological functions, the biochemical structure and the metabolism of these cells, because of the difficulties in isolating pure and intact eosinophils in sufficient quantities. Recent isolation methods[2-4] have used human blood with high eosinophilia or peritoneal eosinophil-rich exudates from experimental animals.

The method described here uses normal blood, it is rapid, easier and more effective than previously reported[5,6]. It allows the isolation within 5–6 h of 1–1.5 g of pure eosinophils from 20 l of horse blood. The quantity, purity and viability of the cell preparations isolated with this method allows the study of biochemical and functional properties of the horse eosinophils. The results of these studies will be presented in following report.

Material and methods. 20 l of horse blood are collected directly from the jugular veins into a plastic container charged with 1.2 l of a Na_2-EDTA solution (2.5% w/v) adjusted to pH 7.4. After spontaneous sedimentation of the erythrocytes at 4 °C for 45 min, the leukocyte-rich supernatant is collected, together with the upper quarter of the erythrocyte sediment, by aspiration. The remaining erythrocyte layer is discarded. The cell-plasma-suspension is centrifuged at 4 °C (15 min 3500 × g).

The eosinophils penetrate during the centrifugation into the erythrocyte layer and are separated in this way from the other leukocytes, forming a compact layer over the erythrocyte sediment. The supernatant and the compact leukocyte layer are carefully aspirated. The erythrocyte layer, containing the eosinophils is then suspended in an equal volume of a polyvinylpyrrolidone solution which was prepared by mixing 2 vol. of a solution, containing 30 g of polyvinylpyrrolidone K-60 (Fluka), and 10 g of NaCl/l, with 1 vol. of clear centrifuged horse plasma (15 min 6000 × g). The cell suspension is transferred to a graduated cylinder. After sedimentation of the erythrocytes (45 min at room temperature), the supernatant containing the eosinophils and some erythrocytes is sucked off. To the remaining erythrocyte layer, an equal volume of the same polyvinylpyrrolidone solution is again added and the sedimentation and removal of the supernatant is repeated. The 2nd sedimentation increases the eosinophil yield by about 10%. The pooled supernatants are centrifuged (15 min 1000 × g at 4 °C). The supernatant is removed and the cell residue resuspended in 50 ml of centrifuged, clear horse plasma. To eliminate the remaining erythrocytes by heamolysis, this cell suspension is mixed with 100 ml of distilled water. After 50 sec, the haemolysis is stopped by addition of 100 ml of a 2% NaCl solution (w/v) and the eosinophils are centrifuged (15 min 1000 × g at 4 °C). For complete separation of the haemoglobin and the erythrocyte ghosts, the cells are suspended in a 1% NaCl solution, containing 0.2% of albumin, and centrifuged at 4 °C (10 min, 300 × g). The cell sediment, suspended in a small volume of the same solution is then superposed over a 10% albumin solution,