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,

containing 1% of NaCl and centrifuged (10 min 300 × g at 4 °C). Cell debris and traces of haemoglobin remain in the supernatant NaCl solution, the eosinophils pass through the albumin solution and form a yellowish sediment. The yield is 1–1.5 g. If necessary, the albumin is washed out by 1 or 2 centrifugations of the cells resuspended in 1% NaCl solution.

The purity of the cell preparation is controlled by light microscopy of stained cell smears (May-Grünwald-Giemsa staining) and by electron microscopy, and the viability of the eosinophils was assessed by the trypan blue exclusion test.

Results. Fig. 1, representing a stained cell film, shows the high purity of the eosinophil preparations, which was estimated to be 98–100% as assessed by differential leucocyte counting. When a small amount of contamination was found, it was due to the presence of neutrophilic granulocytes. Electron microscopic examination of the cell preparations (figure 2) demonstrate the preservation of the cell structures, especially the presence of pseudopodia, which indicates active cell movements. The viability of the eosinophils was further confirmed by the results of the trypan blue exclusion test: only 1–2% of the freshly isolated cells absorb the dye. If the cell preparation was stored at 4 °C, 20% of the cells show dye absorption after 24 h and 75–85% after 1 week. The freshly isolated eosinophils phagocyte radiocatively labelled immune-complexes and during this phagocytosis oxygen consumption increases[7]. These facts suggest that the cell preparation is viable; 1–1.5 g of the preparation contain $2–3.5 \times 10^9$ cells or 65–85% of the eosinophils originally present in 20 l of normal horse blood.

Discussion. The method described above is rapid and easy. It can be used with modifications, which will be the subject of another report, for the isolation of human eosinophils. From swine and cattle blood, however, only eosinophil preparations of lesser purity (40–70%) have so far been obtained. Special care has to be taken for a really complete removal of the leukocyte layer from the erythrocyte sediment containing the eosinophils in order to obtain the highest purity. No white-grayish striae of remaining leuko-

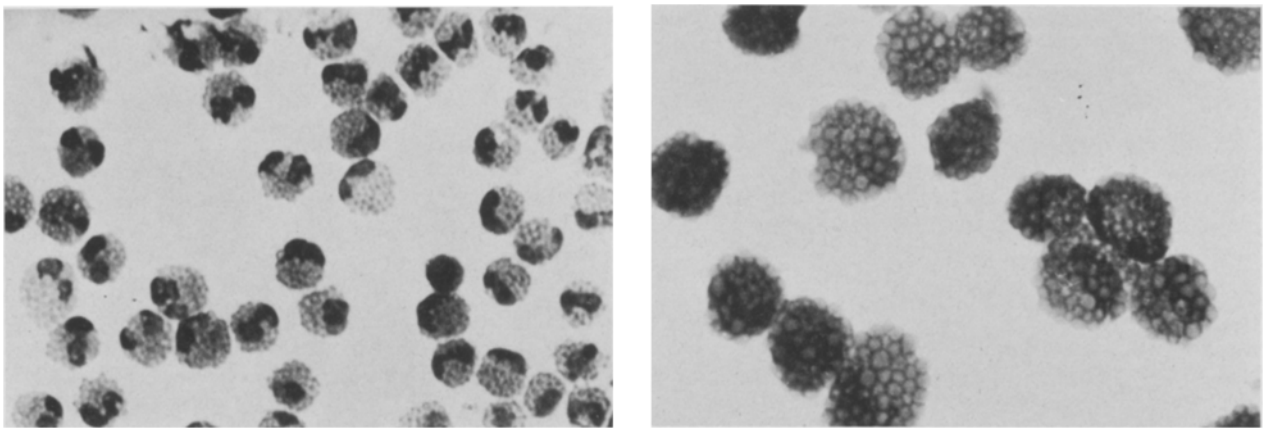

Fig. 1. Light micrograph of an eosinophilic cell preparation, May Grünwald-Giemsa staining. × 500 and × 1000.

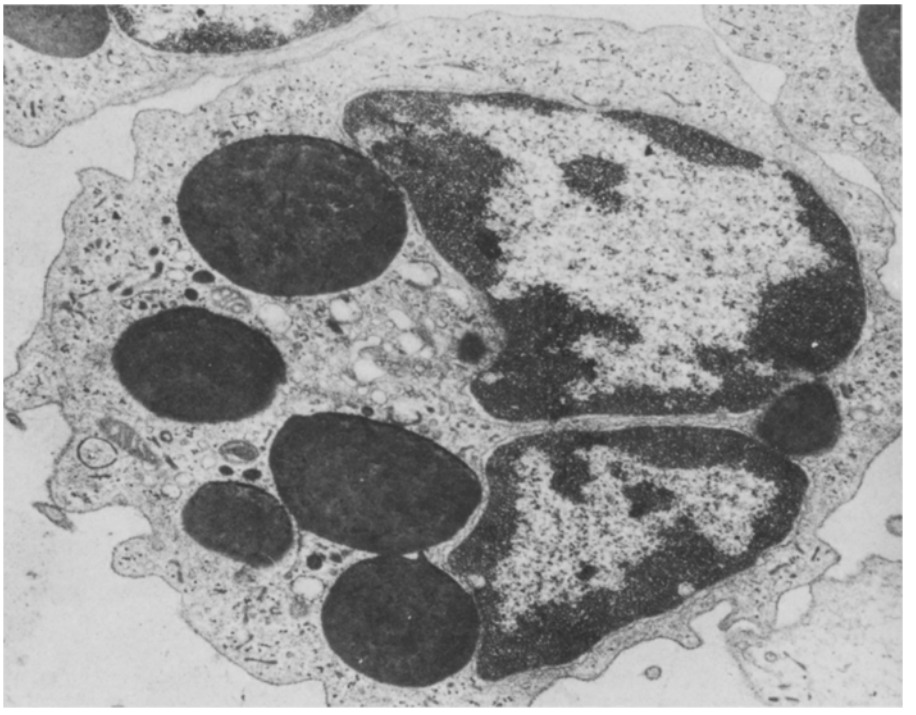

Fig. 2. Transmission electron micrograph of eosinophilic granulocytes isolated from horse blood. × 16,000.

cytes should be visible on the surface of the erythrocyte layer, and it is also very important to remove the leukocyte film adhering to the walls of the centrifuge tubes above the erythrocyte layer. The polyvinylpyrrolidone selectively increases the sedimentation rate of the erythrocytes, and therefore permits a rapid and nearly complete separation of these cells from the eosinophils. The small number of erythrocytes remaining with the eosinophils in the plasma can be removed by a short and mild haemolysis, which keeps intact the eosinophilic leukocytes. The quantity, the purity and the viability of this preparation gives rise to new possibilities for the biochemical study of eosinophils and eliminates the ambiguity of earlier results obtained with eosinophil preparations containing 20–40% neutrophils, monocytes and lymphocytes.

1 The authors are indebted to the 'Schweizerischer Nationalfonds zur Förderung der wissenschaftlichen Forschung' for financial support.

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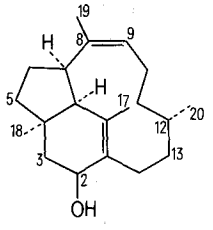
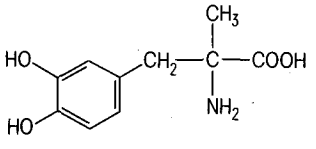
CORRIGENDA

A.V. Juorio: *The occurrence of para-octopamine in the hypothalamus of the domestic fowl: Effects of drugs on its storage and metabolism*, Experientia *34*, 1329 (1978). Lines 27 and 28 on the right column of page 1329 should correctly read: ... octopamine was 9.00±0.55 (7) and that of the guinea-pig 1.23±0.22 (9), (results in ng/g of fresh ...

G.D. Prestwich: *Isotrinervi-2β-ol. Structural isomers in the defense secretions of allopatric populations of the termite Trinervitermes gratiosus*, Experientia *34*, 682 (1978). The structure **II** on page 683 is incorrect and should be pictured as follows:



R.S. Thakur, S.C. Bagadia and M.L. Sharma: *Hypotensive activity of some dihydroxycoumarins and their congeners*, Experientia *34*, 158 (1978). The formula for L-α-methyl dopa (**II**) should correctly be:



P.K. Adhikary, J.K. Haynes, H.L. Patthey and R.S. Rhodes: *A new antisickling agent: In vitro studies of its effect on S/S erythrocytes and on hemoglobin S*, Experientia *34*, 804 (1978). Table 1 on page 805 should be substituted by the following table:

Table 1. Dose relationship of BAPB to its antisickling effect

% Normal cells* before deoxygenation	Molar concentration of BAPB	% Normal cells* after deoxygenation	% Inhibition of sickling
85** ± 2.0***	none	5** ± 3.0***	–
85 ± 3.0	10^{-3} M	62 ± 3.0	74**** ± 3.0***
82 ± 2.0	2×10^{-3} M	63 ± 1.0	77 ± 1.0
85 ± 2.0	3×10^{-3} M	68 ± 2.0	80 ± 2.0
87 ± 1.0	5×10^{-3} M	83 ± 2.0	96 ± 2.0
83 ± 2.0	10^{-2} M	79 ± 3.0	95 ± 3.0

* Normal cells are defined as those with biconcave disc shapes. The other cells are irreversibly sickled cells (ISC). ** % Normal cells in a field of 500 cells ± SD of 3 samples. *** SD between the counts of 3 samples. **** % Normal cells after eliminating ICS's from the count ± SD of 3 samples.