Arthritogenic Properties of Lipophilic, Aryl Molecules* (33949)

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Intracapsular injection of either indole or skatole (3-methylindole) into knee joints of rabbits may produce arthritis with severity depending upon the amount of injected chemical (1). The pathology of experimental arthritis somewhat resembles that of rheumatoid arthritis, e.g., enlargement of knee joint with pannus formation and marked fibrous tissue proliferation subsequent to synovitis[1]. Although it is difficult to find a direct relationship between arthritic lesions produced by indole or skatole and the enhanced tryptophan metabolism found in patients with rheumatoid arthritis (2, 3) investigation of how these compounds and related chemicals may cause joint destruction could provide some insight into the formation of lesions at the cellular level. Herein, an equation correlates joint damage with lipophilicity for 13 aryl compounds.

Materials and Methods. Aryl compounds, 0.1 mmole in 1 ml of aqueous 50% propylene glycol or 0.9% NaCl, were injected aseptically into cavities of back knee joints of male New Zealand rabbits, weighing about 1 kg. The opposite knee joints, injected with 1 ml of aqueous 50% propylene glycol or saline, were used as controls. Saline solutions of acidic compounds were neutralized with NaOH prior to injection. After 6 weeks of weekly injections, the rabbits were sacrificed on the eighth week. The right and left hind legs were removed and the knees were autopsied for pathological changes. Arthritic indices, 0, 1, 2, 3, and 4, were obtained from pathological grading of joint lesions with 4 representing the most severe injury (Fig. 1) and zero reflecting no injury. All control knee joints were grossly and microscopically normal (grade zero).

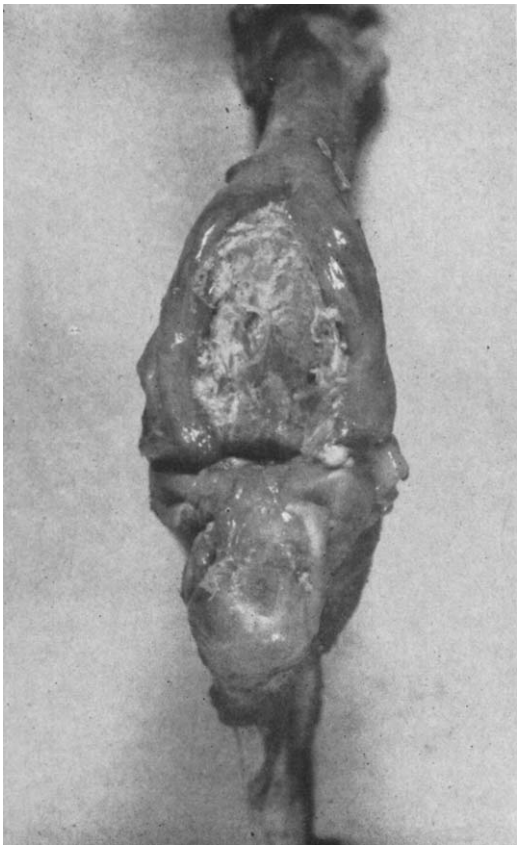

FIG. 1. Arthritic index, 4: the picture shows the gross anatomy of a rabbit knee joint damage severely by intracapsular injections of skatole. Experimental details are given in the text.

Partition coefficients (a measurement of lipophilicity) were determined from the ratio of aryl concentrations in *n*-octanol:aqueous sodium phosphate buffer, 50 m*M*, pH 7.4, phases using a procedure previously described (4). As the partition coefficient logarithm ($\ln\bar{P}$) is increased, the propensity for aryl solubilization in nonpolar or lipid solvents is also increased.

Results and Discussion. Maximum biological response (drug activity, plant growth, in-

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[1] Irene Nakoneczna, J. C. Forbes, and K. S. Rogers, unpublished data.

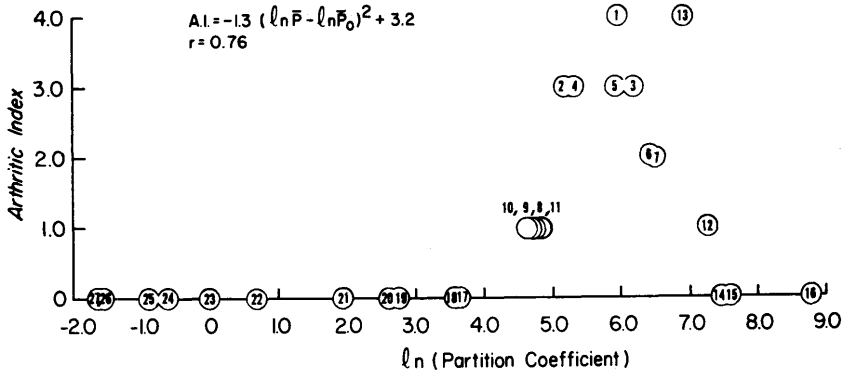

FIG. 2. Arthritogenic properties of lipophilic, aryl compounds: The degree of arthritic injury (A.I.) is compared with the logarithm of the partition coefficient (ln $\bar{P}$). The regression coefficient, r, expresses the fit of experimental data for compounds 1 through 13 to the equation at the top of the figure ($\ln\bar{P}_0 = 5.984$). Additional experimental details are given in the text. (1), 3-methylindole; (2), indole; (3), 5-methylindole; (4), 5-cyanoindole; (5), 5-nitroindole; (6), indene; (7), 1, 2-dimethylindole; (8), 5-methoxyindole; (9), anisole; (10), benzothiazole; (11), toluene; (12), biphenyl; (13), 5-bromoindole; (14), diphenylamine; (15), carbazole; (16), pyrene; (17), benzene; (18), benzoxazole; (19), benzimidazole; (20), oxindole; (21), quinoxaline; (22), kynurenic acid (23), indican; (24), nicotinamide; (25), nicotinic acid; (26), xanthurenic acid; and (27), salicylic acid.

sect toxicity, etc.) for series of related aromatic chemicals has been defined (5, 6) in terms of molecular partitioning across nonpolar:polar phases (membrane permeability) and chemical reactivity. In some instances, either molecular index correlates well with biological response. A linear correlation of biological activities with the chemicals' ln $\bar{P}$ values reflects an interaction on or within a cellular membrane after a single partitioning from a hydrophilic to a lipophilic phase. The biological response to the partitioning process may be more complex if the chemical moves across many different membranes or nonpolar:polar interfaces before it binds at the site(s) of action. Consequently, biological activity may not bear a linear relationship to $\ln\bar{P}$ and instead may reflect an optimal partitioning response. Hansch and Fujita (5) suggest that free energy values for multistep partitioning are minimized so that a sufficient amount of agent can combine effectively with the site for reaction.

Values of arthritic injury (A. I.) together with the partition coefficient logarithms (ln $\bar{P}$) are compared for 27 aromatic compounds in Fig. 2. A pathological response of joint damage for this series of compounds appears to be limited to those chemicals that have ln $\bar{P}$ values within the range of 4.5–7.4, i.e., compounds 1 through 13. Chemicals with values outside this range of partition coefficients do not produce a pathological response (compounds 14 through 27). Since pathological response does not parallel ln $\bar{P}$ values, a multipartitioning process may be involved in the production of joint damage (6). Our results (compounds 1 through 13) seem to be correlated with a linear regression equation[2] for arthritic indices and a binomial function for an approximate gaussian distribution of ln $\bar{P}$ values, Fig. 2. The regression coefficient (r, 0.76) shows a reasonable fit for experimental data to the equation, considering the arthritic indices are determined subjectively and the optimal ln $\bar{P}_0$ value, 5.984, is assumed to be that determined for 3-methylindole. This correlation indicates that multistep

[2] The regression equation might be used to calculate arthritic indices for compounds with known partition coefficients. In some instances, negative values for arthritic indices may be obtained which are meaningless and would be considered zero since normal knee joints are represented as zero.

partitioning processes may be involved in the arthritic response to intracapsular injection of aromatic compounds into the rabbit knee. Thus, the degree of joint damage may be a reflection of the aryl molecule's lipophilic capability to successfully penetrate many nonpolar:polar interfaces (membranes) so that sufficient material arrives at the toxicant site(s). Movement of a chemical that has a high ln $\bar{P}$ value compared to the optimum lipophilic value would be slowed or stopped by a polar phase; similarly, the movement of a compound with a low value for ln $\bar{P}$ would be slowed or stopped by a nonpolar phase. In either case, the concentration of chemical at the toxicant site(s) would be insufficient to cause a reaction. Correlation between arthritic response and the exponential function of ln $\bar{P}$ values is not improved by including terms for chemical reactivity (electron donation or acceptance) in the linear regression equation. This suggests that combination of the aryl molecule with a specific cellular membrane might occur for the initiation of joint damage.

Since development of our experimental arthritis[1] is similar to that found for intracapsular injection into rabbit knees of streptolysin S which ruptures lysosomes (7) and also since these aryl reagents, by virtue of their lipophilicity, hemolyze red blood cells (4) then lysosome rupture with release of hydrolytic enzymes might be involved in the arthritogenic action of indole, skatole, and related aromatic molecules. Chemicals that lyse red blood cells generally also labilize lysosomes (8).

Summary. Pathological responses to intracapsular injections of indole, skatole, and 11 aromatic compounds into rabbit knees are correlated with a binomial function for an approximate gaussian distribution of aryl partition coefficient logarithms (ln $\bar{P}$). This suggests that multistep partitioning processes may be involved in joint injury by these chemicals.

1. Forbes, J. C. and Neale, R. C., J. Lab. Clin. Med. **22**, 921 (1937).

2. Bett, I. M., Ann. Rheumatic Diseases **21**, 63 (1962).

3. Forbes, J. C. and Neale, R. C., J. Lab. Clin. Med. **20**, 1024 (1935).

4. Rogers, K. S., Proc. Soc. Exptl. Biol. Med. **130**, 1140 (1969).

5. Hansch, C., Muir, R. M., Fujita, T., Maloney, P. O., Geiger, F., and Streich, M., J. Am. Chem. Soc. **85**, 2817 (1963).

6. Hansch, C. and Fujita, T., J. Am. Chem. Soc. **86**, 1616 (1964).

7. Weissman, G., Becher, B., Wiederman, G., and Bernheimer, A. W., Am. J. Pathol. **46**, 129 (1965).

8. Weissmann, G., New Engl. J. Med. **273**, 1084 (1965).

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