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13. A NOVEL IN VITRO ASSAY FOR ANTI-INFLAMMATORY AGENTS BASED ON STABILIZATION OF ERYTHROCYTES. John H. Brown, H. Kenneth Mackey and Dominic A. Riggilo, Department of Pharmacology, Mead Johnson Research Center, Evansville, Indiana 47721.

An in vitro technique for screening non-steroidal anti-inflammatory compounds based on their ability to inhibit heat-induced hemolysis of canine erythrocytes has been demonstrated by incubating red blood cells (3.0 ml) as a 40% (v/v) suspension in M/15 sodium phosphate, pH 7.4, for 20 minutes at 53° C with non-steroidal anti-inflammatory agents (3.0 ml) dissolved in 0.9% saline, pH 7.4. These agents inhibit hemolysis by 40 to 60% at concentrations of 5.0×10^{-4} M or less. Among these are, in order of decreasing activity, lysergic acid diethylamide, phenylbutazone, flufenamic and mefenamic acids, oxyphenbutazone, indomethacin, and aspirin. An inhibition of hemolysis of 20% was found to be significantly different from zero at the 95% confidence level. With the exception of flufenamic acid versus aspirin the inhibitory response of 5.0×10^{-4} M concentrations of the compounds tested did not differ significantly. The slopes of semilogarithmic plots of per cent inhibition of hemolysis versus molarity did not differ from each other at the 95% level, indicating a similar mechanism of action for these compounds, but each slope differed significantly from zero. To determine the specificity of the test, more than thirty non-anti-inflammatory agents were tested and found inactive.