Riassunto. Allo scopo di ridurre il tempo necessario per le lunghe e laboriose operazioni richieste per uno studio quantitativo degli spettri stellari, e di aumentare la precisione delle misure, è stato progettato un microfotometro automatico digitalizzato che perfora su nastro le trasparenze e le corrispondenti posizioni ogni 2 μ lungo l'intero spettro. Nel presente lavoro vengono forniti i dettagli costruttivi dello strumento.

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A Radiometric Method for the Quantitation of Experimental Inflammation and Anti-Inflammatory Activity

Methods for the evaluation of anti-inflammatory activity may be classified into two groups: (a) those based on the production of physiological *in vivo* changes (e.g., eosinopenia) by the antiphlogistic agents, and applicable primarily to the evaluation of corticosteroids; (b) those based on the prevention or reduction of inflammation produced experimentally by a variety of noxious chemical agents or physical forces, which are applicable to the evaluation of non-hormonal as well as hormonal antiphlogistic compounds.

Artificial models for the evaluation of anti-inflammatory activity are generally based on a major component of the inflammatory process, namely the seepage of plasma into the inflamed focus. Accurate measurement of this fluid shift is a limiting factor in current assay techniques. A radiometric tracer method was developed which enables quantitation of the degree of inflammation produced by a phlogogenic agent and the extent of protection afforded by non-hormonal compounds.

Radio-iodinated (I^{131}) serum albumin (RISA), 0.1 ml, was injected into a tail vein of fasted (12 h), 24–28 g, male Swiss-Webster mice. 30 min later, 20–40 μl of blood was withdrawn from the *sinus cavernosus* by means of a micropipette, transferred immediately to a tared 10 × 75 mm Pyrex tube, allowed to coagulate and weighed. This sample was used to determine the radioactivity per μl of blood (sp. gr. 1.052). 90 min after the RISA injection, an inflammatory response was induced by a modification of the method of Kelemen[1]. An amount of 5-hydroxytryptamine creatinine sulfate, equivalent to 0.4 μg of 5-HT base, dissolved in 0.05 ml of 0.9% NaCl solution, was injected into the plantar surface of the left hind paw of the mouse; the right hind paw was injected with 0.05 ml of saline ($^1/_2$ inch, 27 gauge needle). 1 h after the injection of 5-HT, the animals were sacrificed by immersion in an acetone-dry ice mixture (approximately −70°C), and the hind paws were removed by severing at the ankle. Each paw was placed in a separate tared tube and weighed. The radio-activity of each sample was determined by means of a scintillation well-counter and a Baird-Atomic Pulse Height Analyzer at the I^{131} γ peak of 0.360 Mev. Counting times were of sufficient duration to obtain at least 2000 counts. Inflammatory response was expressed as a function of the increase in inflammatory exudate of the 5-HT injected-paw as opposed to the saline-injected paw. Volume of 5-HT inflammatory exudate per g of tissue (μl/g) = [activity per g of *left* paw (cpm/g) − activity per g of *right* paw (cpm/g)] ÷ [activity per μl of blood (cpm/μl)].

Preliminary studies revealed that the inflammatory response to varying doses of 5-HT (10^{-3} to 10 μg calculated as the base) resulted in a sigmoid curve ($P < 0.01$); the linear portion of the curve fell between 0.1 and 0.8 μg. The response to 0.1 μg of 5-HT did not differ significantly from saline, but doses of 0.2, 0.4, and 0.8 μg were significantly phlogogenic, and differed between themselves ($P < 0.05$ in each case). The 0.4 μg dose of 5-HT was selected for use in subsequent studies, since it produced a significant increase in plasma exudate with the least variation and cor-

Tab. I. Inflammatory response, expressed as μl of plasma exudate per g of tissue, induced by 5-hydroxytryptamine in the mouse hind paw. Radio-iodinated (I^{131}) serum albumin (0.1 ml i.v.) was used to trace the extent of plasma exudate

Section A	Effect in mice sacrificed 1 h after injection of various doses of 5-HT in 0.05 ml of saline	
5-hydroxytryptamine μg	No. of mice	Plasma exudate (μl/g) mean ± S.E.
0	20	8.7 ± 7.4
0.1	22	17.2 ± 10.6
0.2	20	79.6 ± 13.0
0.4	18	107.2 ± 11.8
0.8	18	152.4 ± 17.2
Section B	**Effect in mice sacrificed at various time intervals after injection of 0.4 μg of 5-HT in 0.05 ml of saline**	
Time interval between injection of 5-HT and removal of paw min	No. of mice	Plasma exudate (μl/g) mean ± S.E.
30	18	163.7 ± 9.8
60	19	127.1 ± 13.4
90	17	87.1 ± 8.2
120	18	92.6 ± 8.1
150	18	89.5 ± 10.5

Table II. Reduction by sodium salicylate of inflammatory exudate induced by 5-hydroxytryptamine in the mouse hind paw. Animals were sacrificed 6 h after administration of sodium salicylate and 1 h after injection of 0.4 μg of 5-HT in 0.05 ml of saline

Sodium salicylate mg/kg, *per os*	No. of mice	Plasma exudate (μl/g) mean ± S.E.	Mean % reduction of plasma exudate	P
0	44	125.7 ± 9.8		
50	21	99.5 ± 11.4	20.7	< 0.05
100	18	96.4 ± 12.2	23.3	< 0.05
200	18	80.6 ± 12.2	36.9	< 0.001
400	20	55.4 ± 11.6	56.0	< 0.001

[1] E. Kelemen, *Permeability in Acute Experimental Inflammatory Oedema* (Publishing House of the Hungarian Academy of Sciences, Budapest 1960).

responded to the point of maximal slope (Table I, Section A).

The inflammatory exudates (µl/g) determined at various intervals after the injection of 0.4 µg of 5-HT are reported in Table I, Section B. Significant differences existed among the inflammatory responses occurring at 30, 60, and 90 min, however, there were no differences among the inflammatory responses at the 90, 120, and 150 min intervals. The 60 min interval was selected for this assay.

Loss of activity from the blood takes place slowly following the intravenous injection of RISA. A reduction of radioactivity equivalent to $6.50 \pm 2.19\%$ ($N = 38$) occurred during the time elapsed between collection of the blood sample and removal of the hind limbs. Optimally, the blood should be collected simultaneously with removal of the limbs, 30 min after the injection of 5-HT; in which case the absolute values for plasma exudate would be correspondingly greater. However, based on the % reduction of inflammatory response relative to a control group, the index of protection afforded by antiphlogistic compounds would be unchanged. The parameters described were selected with consideration to the technical aspects of the assay involving large numbers of experimental subjects.

Sodium salicylate was selected as a model antiphlogistic agent to determine the efficacy of this method for the evaluation of non-hormonal anti-inflammatory compounds. Table II indicates the volume of plasma exudate and the % reduction in exudate obtained with 50 to 400 mg/kg of sodium salicylate administered orally, 5 h prior to the injection of 5-HT.

Significant reduction of plasma exudate was obtained with a dose of sodium salicylate as low as 50 mg/kg *per os*. Larger doses resulted in correspondingly greater inhibition of the inflammatory response to 5-HT. Other investigators[1-3] have reported that doses of 500 to 600 mg/kg administered parenterally were required to produce a significant inhibition of 5-HT induced oedema in the rat.

Current investigations of the comparative activity of various types of potential anti-inflammatory compounds will be presented in a subsequent publication[4].

Zusammenfassung. Nach Einspritzung von 5-Hydroxytryptamin in den einen Fuss einer Maus und physiologischer Salzlösung in den anderen, wurde die Radioaktivität beider Füsse verglichen. Mit radioaktivem Jod (I^{131}) behandeltes Serumalbumin wurde dazu benutzt, den Umfang des Plasmaexsudates zu bestimmen. Das Ausmass der Hemmung der durch 5-HT induzierten Entzündung ist von der Dosis der physiologischen NaCl-Lösung abhängig.

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Department of Pharmacology and Department of Radiochemistry, Philadelphia College of Pharmacy and Science, Philadelphia (Penn. USA), October 30, 1961.

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Simultaneous Staining of Heinz Bodies and Reticulocytes with New Methylene Blue *N* in the Toad *Bufo marinus* after Iproniazid Treatment

In the dog[1] and the lizard *Uromastix acanthinurus*[2] given Iproniazid ('Marsilid') it was found that the method of BRECHER[3] for counting reticulocytes clearly showed the presence of Heinz bodies[4], which yielded a method for demonstration of both reticulocytes and Heinz bodies in the same preparation. In continuation of this work we performed a corresponding investigation with an amphibian species, the toad *Bufo marinus*. As staining method the BRECHER's technique[3] was used according to the technique of THOMPSON[1]. The erythrocytes appeared to be stained a pale greenish-blue, while the reticulum was sharply outlined and deep blue. The Heinz bodies were pale to deep blue and stood out prominently against the pale-green background of the erythrocyte. As the erythrocytes of the dog[1] and *Uromastix acanthinurus*[2], these bodies were stained at least as well as by the usual methods[4-6]. As these results are in good agreement with those obtained in the dog[1] and *Uromastix acanthinurus*[2], a more general importance should no doubt be attached to it.

Zusammenfassung. Methode für gleichzeitige Koloration von Heinz-Körperchen und Reticulocyten mit Neu-Methylenblau *N* in der Kröte *Bufo marinus*.

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Histological Laboratory, Free University, Amsterdam (The Netherlands), January 17, 1962.

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STUDIORUM PROGRESSUS

Die Genomsonderung in den Mitosen der Rattenleber*

Es ist allgemein bekannt, dass sich cytologische und morphologische Untersuchungen über Chromosomengestalt und deren Veränderung am günstigsten in sich teilenden Zellen, also während des Mitoseablaufes, durchführen lassen. Als hierfür besonders geeignet erweist sich das Stadium der Metaphase, in dem die Chromosomen besonders stark kontrahiert und dadurch besonders deutlich sichtbar sind. Die Metaphasechromosomen zeichnen sich

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