

Calcium content of experimental granuloma as determined by X-ray fluorescence. Influence of non-steroid anti-inflammatory drugs

M.A. Continenza, P. Conti and G.E. Gigante

Departments of Anatomy, Pathology and Physics, Institute of Medicine, L'Aquila, Collemaggio, I-67100 L'Aquila (Italy), 5 February 1979

Summary. The deposit of calcium in experimental granulomas as determined by X-ray fluorescence is notably decreased by orally given indomethacin and diflunisal.

It is well known¹⁻³ that calcium accumulates in chronic inflammatory lesions induced by potassium permanganate. In this study we have calculated the quantity of calcium in experimental granuloma^{4,5} means of X-ray fluorescence⁶. Furthermore, we have studied the effect of non-steroid anti-inflammatory drugs⁷⁻⁹ like indomethacin and diflunisal on the calcium deposits. The use of the X-ray fluorescence technique appears to be particularly suitable for the study of the $Z > 15$ elements content in whole tissue preparations¹⁰.

Materials and methods. Male Wistar rats (190–200 g) were used in these experiments. Each animal was given 4 s.c. inoculations of KMnO_4 (0.2 ml) in the back and 4 in the abdomen^{11, 12}. Then the animals were divided into 3 groups of 10 animals each: the 1st was used as a control; the 2nd was given 5 mg/kg of indomethacin; the 3rd was given 74 mg/kg of diflunisal p.o.

After 1 week the animals were killed by cervical dislocation after ether anesthesia. The granulomas which had formed in the s.c. tissue¹³⁻¹⁵ were dissected, their maximum diameter was measured, and they were weighed. Slices less than 1 mm thick were then used for X-ray fluorescence analysis. The X-ray spectrometer consists of a solid state detector of the type Si(Li), an amplifier, a multichannel analyzer and an H_3 (titanium target) radioisotopic sealed source which emits mainly at 4.5 keV¹⁶.

The granuloma slices were placed in a specially designed sample holder. The whole energy spectrum of each sample was recorded for a present time (100 sec). Then the ratio between the area under the peak related to the $k\alpha$ transition of calcium (peak Ca) and that under the scattered radiation (peak D) was calculated. This value was calculated for each granuloma. The data obtained were statistically evaluated. The error in each determination is about 3–4%.

Results. Table 1 shows the average diameters and weights of granulomas taken from different animal groups. Both parameters are significantly lower in animals treated with anti-inflammatory drugs.

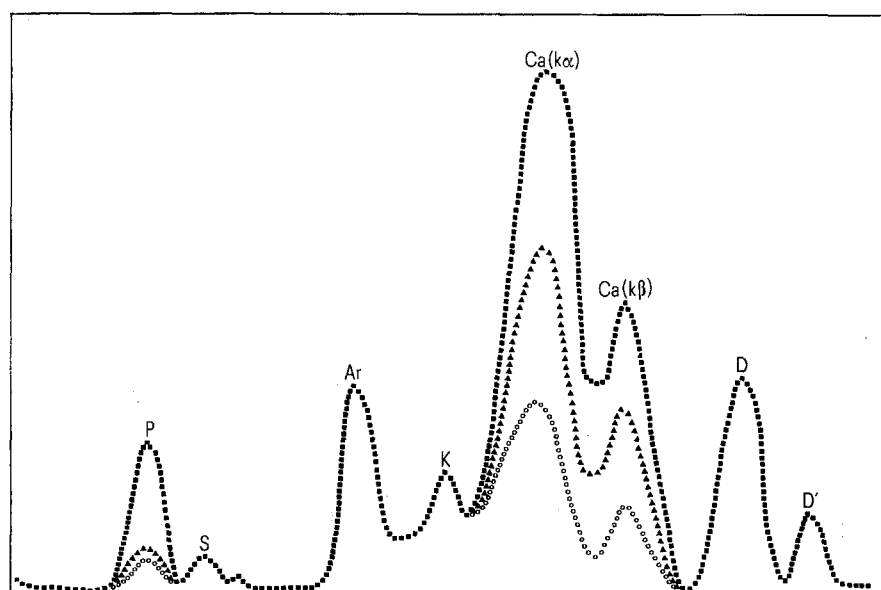
The figure shows the X-ray fluorescence spectrum of granulomas from the 3 groups of animals. At first sight, it appears that the relative height of the calcium peak is lower in treated animals than in the controls. This observation is confirmed by table 2 where the mean ratio Ca/D is shown. The amount of calcium present in granulomas appears to be strongly decreased by treatment with anti-inflammatory drugs. It is interesting to note that the phosphorus peak

Table 1. Difference in diameter and weight of potassium permanganate induced granulomas between controls and treated animals

Treatment	Diameter (mm)	Decrease (%)	Weight (g)	Decrease (%)
None	15.5 ± 3.9	–	0.40 ± 0.08	–
Indomethacin	11.9 ± 3.9	23	0.29 ± 0.1	27
Diflunisal	13.4 ± 3.7	13	0.35 ± 0.08	12.9

Table 2. Area of calcium peak relative to the diffusion peak in granulomas from different groups of animals

Treatment	Ca/D	Decrease (%)
None	8.13 ± 1.46	–
Indomethacin	3.05 ± 0.85	62.4
Diflunisal	5.45 ± 1.8	32.9



X-ray fluorescence spectra of granulomas taken from controls (—), indomethacin (---), and diflunisal (·····) treated animals. The letters indicate the fluorescence of various elements: P, phosphorus; S, sulfur; Ar, argon; K, potassium; Ca, calcium; $k\alpha$ and $k\beta$, characteristic emission of calcium; D and D', scattered radiation. The spectra are normalized with respect to the scattered radiation (peak D and D').

varies in the same way as the calcium peak, this indicating a relationship between the 2 elements.

Discussion. The results reported above show that orally given non-steroid anti-inflammatory drugs can reduce the lesion induced by potassium permanganate. In fact, both the diameter and weight of granulomas resulting from the s.c. injection of potassium permanganate are reduced in treated rats in comparison to the controls. Indomethacin appears to be more effective than diflunisal under these

experimental conditions. Interestingly, both drugs greatly reduce the calcium deposit in the granuloma. The decrease in calcium content is more pronounced than weight decrease. Furthermore, the use of X-ray fluorescence as a tool for the study of element contents in normal and pathological tissue appears promising since it gives a lot of information about many elements at the same time without loss of material. In this context a detailed distribution study of metabolically related elements like calcium and phosphorus would be of particular relevance.

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Toxicity of some insecticides for crowded and isolated nymphs of *Grylodes sigillatus* Wlk. (Orthoptera: Gryllidae)

M. Zutshi, Y. Saxena and P.P. Sharma¹

Department of Zoology, University of Rajasthan, Jaipur (India), 23 April 1979

Summary. 6 insecticides; chlordane, malathion, fenitrothion, pyrethrum, rotenone and sevin, were screened against *Grylodes sigillatus* Wlk. reared under crowded and isolated conditions. Chlordane was found to be most toxic, followed by malathion, fenitrothion, pyrethrum, rotenone and sevin. Insects reared under crowded conditions were more susceptible to insecticides than insects reared under isolated conditions.

Several entomologists have contributed a great deal to studies on the effects of population density upon fecundity, fertility, nymphal duration and adult emergence. Khan and Aziz² noted that the rate of development of male and female hoppers of *Oedaleus abruptus* under crowded conditions was slower than in individuals in isolation. Singh et al.³ recorded similar observations on the nymphal duration of *Atractomorpha crenulata* Fabr. Rizvi et al.⁴, on the contrary reported that the nymphs of *Hieroglyphus nigroripletus* developed at a faster rate under crowded conditions. Not much information is, however, available on the effects of insecticides on nymphs of *Grylodes sigillatus* when reared under crowded conditions. The only reference is that

of Hartman⁵ on the insecticidal action of carbamates on the eggs of the house cricket. In view of this the present investigation was conducted.

Materials and methods. The laboratory culture was raised from 50 adults collected in big muslin-topped glass jars (75 × 25 cm) at a temperature 28 ± 2 °C. Some moist soil and twigs were provided at the bottom of the jar to give the insects natural surroundings for laying eggs. The insects were provided daily with wheat soaked in milk and sugar as food.

For the experiments, 60 freshly hatched nymphs were placed individually in glass jars (18 × 8 cm). The open end of each glass jar was covered with a muslin cloth and tied

Effect of insecticides when nymphs were reared under isolated (i) and crowded (c) conditions

Insecticide	Concentration (%)	Mortality (%)		Survival (%)		No. fledged into adult		Male c	Female c
		i	c	i	c	Male i	Female i		
Chlordane	0.005	100	100	Nil	Nil	—	—	—	—
Malathion	0.005	80	100	20	Nil	3	3	—	—
Fenitrothion	0.005	70	90	30	10	10	8	4	2
Pyrethrum	0.005	20	40	80	60	26	22	16	20
Rotenone	0.005	10	30	90	70	29	25	20	22
Sevin	0.005	Nil	20	100	80	32	28	23	25