A NOTE ON IRRADIATED HEPARIN: SOME BIOLOGICAL AND CHEMICAL PROPERTIES

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The effect of gamma radiation and high energy electrons on the anticoagulant and antilipaemic properties of heparin has been investigated. There was a fall in both activities, but the anticoagulant was reduced more readily than the antilipaemic. After electron bombardment the anticoagulant activities of two different batches of heparin were reduced almost identically, an unexpected finding since such a high degree of reproducibility is not possible with chemical degradation.

IN the course of investigations into the effects of irradiation on the stability of a number of pharmaceutical products, it was noted by our colleague, Mr. K. L. Smith, that there was a decrease in the anticoagulant activity of heparin after electron bombardment. More recently Howe, Balazs and Laurent (1958) and Horne (1958) have briefly mentioned a similar finding, and the former workers have also shown that there is a decrease in anionic groups and an increase in reducing substances.

It therefore seemed of interest to examine more fully the effects of electron bombardment and of exposure to gamma rays on the biological properties of heparin, and in particular to observe any possible changes in the antilipaemic activity during these reactions. Zollner, Rothemund and Seitz (1954), by a chemical degradation of heparin, claimed to have reduced its anticoagulant activity to 12·5 per cent without affecting the antilipaemic activity. Houck, Morris and Lazaro (1957) were able by alkaline degradation to decrease the anticoagulant activity without altering the antilipaemic activity, but it is impossible to make any quantitative estimates from their results. A modified form of heparin with reduced anticoagulant activity but unchanged antilipaemic effects would be of interest as a potential therapeutic agent.

METHODS

Irradiation

Sodium heparin of B.P. quality, and a 25 per cent solution of sodium heparin in distilled water, were irradiated by gamma rays from a 100 curie ^{60}Co source. High energy electrons were produced by a 4MeV linear accelerator at the Atomic Energy Research Establishment, Wantage.

The gamma radiation dose rates were about 1,000 rads/min. and were measured with ferrous sulphate solution. The electron dose rates were about 3 megarads/min. and had been measured calorimetrically. Air cooling prevented the temperature from rising more than 10°.

Anticoagulant activity. This was determined *in vitro* by the sulphated whole blood method of Adams and Smith (1950).

Antilipaemic activity. We wish to emphasise that in this communication the term antilipaemic refers to the ability of heparin to liberate clearing

factor *in vivo*. A method based on the liberation of clearing factor in the rat was used. Rats were injected subcutaneously with heparin, and 1 hr. later were bled under ether anaesthesia from the carotid artery into ice-cooled oxalated tubes. Plasma was obtained by centrifugation at 1,500 r.p.m. for 30 min. at a temperature of 0 to 5°. One ml. aliquots of plasma were diluted with 1 ml. of distilled water and the temperature raised to 30°. 0·1 ml. of a 1 per cent dilution of "Ediol"* emulsion in water was then added, and the optical density immediately measured on a Hilger absorptiometer using a neutral filter and a 2 cm. cell. After incubation at 30° for 30 min. the optical density was measured again. The decrease in optical density after this incubation was taken as a measure of clearing factor activity in the plasma. By comparing the effects produced by three doses of the untreated heparin, 1·25, 2·5 and 5 mg./kg.

TABLE I

THE ANTICOAGULANT ACTIVITIES EXPRESSED AS A PERCENTAGE OF THE ORIGINAL UNTREATED MATERIAL OF A NUMBER OF HEPARIN PREPARATIONS AFTER ELECTRON BOMBARDMENT FROM A LINEAR ACCELERATOR

Irradiation dose in megarads	Heparin solution (25 per cent)		Heparin powder (5–6 per cent moisture)		Dried heparin powder (1 per cent moisture)	
	Batch 680 N	Batch 176 P	Batch 680 N	Batch 176 P	Batch 680 N	Batch 176 P
0	100	100	100	100	100	100
40	23	23	—	—	—	—
60	10	11	—	—	—	—
80	6	7	19	17	26	30
120	—	—	9	8	18	16
160	2	1	5	5	8	9

subcutaneously, with those produced by three doses of irradiated materials, an assessment of the antilipaemic activity of the latter was made; six experiments were usually carried out on each sample.

There was evidence to suggest that irradiated samples showed some instability, since a fall in anticoagulant activity was noted on prolonged storage. For this reason all samples were examined as soon as possible after irradiation, and were always stored at 0 to 4°.

Reducing power. The reducing power of all samples was estimated by the method of Hagedorn and Jensen (1923).

Metachromatic activity. This was determined by the method described by MacIntosh (1941), except that all heparin solutions were made in distilled water instead of 0·2 per cent saline.

RESULTS

In Table I are shown the results of exposing two different batches of heparin and their solutions to electron bombardment by the linear accelerator. Similar results were also obtained using ^{60}Co as the source of irradiation. The loss of activity was greatest in the heparin solutions and lowest in dried heparin which contained only 1 per cent moisture. Having established that these reactions could be readily reproduced we

* Ediol, oral fat emulsion manufactured by Schenlabs, New York.

then proceeded to examine the effects of irradiation on both antilipaemic and anticoagulant activity. Most of these experiments were conducted with a source of ^{60}Co, and the effects of this gamma radiation on the biological and chemical properties of heparin are given in Table II. Similar results could also be produced by electron bombardment.

DISCUSSION

It can be seen from Table II that the anticoagulant activity of the irradiated samples fell more rapidly than did the antilipaemic: this has been a consistent finding in all our experiments. These results indicate that the chemical pattern responsible for the antilipaemic activity of heparin is not exactly the same as that producing the anticoagulant effects. Unfortunately the fall in anticoagulant activity relative to antilipaemic

TABLE II

SOME BIOLOGICAL AND CHEMICAL PROPERTIES OF A 25 PER CENT SOLUTION OF SODIUM HEPARIN, SUBJECTED TO VARYING DOSES OF IRRADIATION FROM A SOURCE OF ^{60}CO

Irradiation dose in megarads	Anticoagulant activity*	Antilipaemic activity*	pH	Reducing power†	Metachromatic activity‡
0	100	100	6·0	1·0	114 μg.
12·7	65	93 (74–114)	5·5	6·3	120 μg.
28·0	35	62 (50–78)	4·7	9·6	121 μg.
41·0	25	40 (32–50)	4·6	12·0	152 μg.
59·9	10	19 (15–24)	3·8	16·9	188 μg.

* Expressed as percentage of the activity of the untreated material. Figures in parenthesis are approximate limits of error, P = 0·95.
† Expressed in relation to that of original material = 1.
‡ The amount of heparin in μg. required to reduce by 50 per cent the colour of 10 ml. of a 0·0025 per cent solution of toluidine blue.

activity was not as great as we had hoped, and this type of product has, therefore, no value as a therapeutic agent.

A number of other observations seem worthy of note. A fall in the biological activity was accompanied by a fall in pH and an increase in reducing power. A decrease in the metachromatic reaction also occurred but was less marked than we expected and indicates that the measurement of metachromasia bears no relationship to biological activity (Table II). Thus heparin retaining only 10 per cent of its anticoagulant activity nevertheless produced a fairly strong metachromatic reaction. These results agree with those of Wolfrom, Weisblat, Karabinos, McNealy and McLean (1943) who reported similar findings when using acid hydrolysed heparin.

Table I indicates that the biological properties of two different batches of heparin were reduced almost identically by electron bombardment. It is surprising that this type of reaction can produce such a precise and reproducible biological change in a molecule as complex as heparin, in view of the difficulty of achieving this degree of reproducibility by chemical means.

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