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Short Communications

Defining of the pyrogenic assurance level (PAL) of irradiated medical devices

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Several procedures have been proposed to determine a validated lower dose of ionizing rays, with practical and economical consequences (Tallentire, 1978; AAMI, 1983; Davis et al., 1984, Doolan et al., 1985). These methods are based on experimental determination of an intermediate sterility assurance level (SAL), like the one we have proposed in a recent work (Darbord and Laizier, 1987). The theoretical bases of the determination of the specific efficient dose take into account only the living microorganisms. But in numerous Pharmacopeia texts (i.e. the French Pharmacopeia), medical devices used for parenteral preparations have to be free of pyrogens formed by the endotoxin of the Gram-negative bacteria, and the rabbit test must be done. In fact, the ionizing rays have, only in aqueous solution, a reduced activity on the lipid part of the endotoxin, which supports the biological activities (Koppensteiner et al., 1976; Cszako et al., 1983). No inactivation of dried endotoxins was detected by these authors, using the *Limulus* gelation test.

With the generalization of good manufacturing practice, and the validation procedures described above which allow only weaker initial contaminations (1–100 bacteria per item), it is possible to evaluate the real pyrogenic risk. We have done endotoxin extractions by the Wesphal method (Rudback et al., 1976) getting 11–30 Endotoxin Units (EU) for 10^6 bacteria, evaluated by the *Limulus* chromogenic test (*Pseudomonas aeruginosa, Chromobacterium violaceum*). An item, with 100 bacteria, contains in this case $30 \times 10^2 \times 10^{-6} = 3.10^{-3}$ EU before irradiation. If a pyrogenic assurance level (PAL defined as a SAL) of 10^{-6} is kept for this interpretation, the pyrogen risk is theoretically brought by $3.10^{-3} \times 10^6 = 3000$ EU/item. In this worst possible case, the endo-

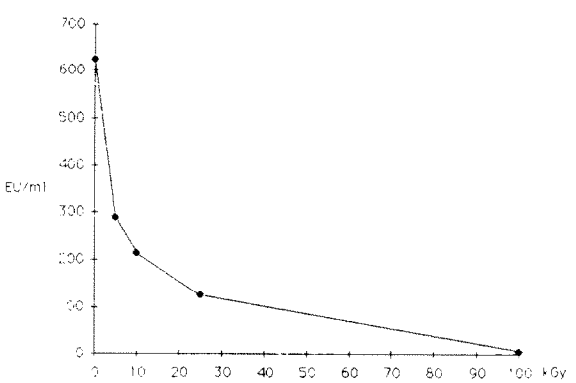

Fig. 1. Effect of gamma rays on endotoxins (*Escherichia coli* $O_{55}B_5$, dried on polystyrene).

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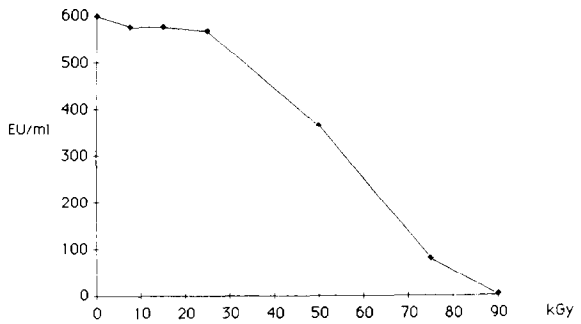

Fig. 2. Effect of electron beam on endotoxins (*Escherichia coli* $O_{55}B_5$, dried on polystyrene).

toxins injected are a quantity higher than the pyrogenic reference usually considered (10 EU/kg/h, or 700 EU/h for 70 kg), and need 3000 : 700 = a 4.5-fold inactivation.

Thus we have determined the sensitivity of endotoxins, dried on glass, polystyrene or polypropylene supports:

- by rabbit test, the pyrogenic effect of 50 EU/kg injection is lost when the endotoxins are irradiated by 25 kGy with gamma rays, or by 100 kGy with an electron beam. Non irradiated endotoxins are pyrogenic for 10 EU/kg, which proves at least a 5-fold biological inactivation when gamma rays are used (25 kGy).
- by *Limulus* chromogen test (Coatest Kabi Vitrum), the averages of several determinations are shown in Fig. 1 and Fig. 2, and show a 5-fold inactivation by gamma rays (25 kGy).

The aim of this paper is to demonstrate that when gamma rays are used, the elimination of the pyrogenic risk is validated with a PAL of 10^{-6} and we think that it is useless to carry out the prescribed assays to detect pyrogens. It is quite different when the sterilization is made by electron beams. In this case, for a parenteral use, and if the PAL of 10^{-6} is kept, we have to test for pyrogenic substances by the rabbit assay, or for bacterial endotoxins by the *Limulus* test in the finished product. The proof of an initial contamination lower than 10 bacteria/item can indicate exemption from these tests. These conclusions are applicable only when the bacterial inactivation validation is achieved.

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