A NOTE ON THE INFLUENCE OF ULTRASONIC VIBRATIONS ON PYROGEN MATERIALS IN DISTILLED WATER

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Pyrogenic distilled water and an autoclaved standard suspension of *B. subtilis* lost their pyrogenic activity when treated with ultrasonic vibrations.

No reference to the effect of ultrasonic vibrations on pyrogen materials from distilled water has appeared in the literature. It is accepted that pyrogens are bacterial residues or products of bacterial metabolism[1] containing nitrogen and that they may be either proteins or polysaccharides[2]. Studies of the effect of ultrasonic vibrations on the structure of polysaccharides and proteins[3,4] has shown that the changes of molecular structure could be induced. Therefore we have attempted to change the structure of pyrogens by this means with the object of destroying their pyrogenic activity.

Strongly reacting and pyrogen negative solutions were chosen for treatment. The pyrogen tests were made according to the requirements of the U.S.P. XV. 50 ml. batches of distilled water were placed in a heavy open glass cylinder 30 mm. in diameter and 25 cm. in length. The bottom of the cylinder was closed with a rubber membrane, 45μ thick, and the top with a glass cover. The rubber membrane was placed directly on the quartz crystal of a suitable generator. The system was placed in a water bath with constant temperature (18°) to avoid the effects of heating.

Solutions were exposed to vibrations under the following conditions:

1. Frequency: 800 kc./sec.
   Intensity: 1·5 W/cm.^{2}
   Duration: 5 and 10 min.
2. Frequency: 2 Mc./sec.
   Intensity: 2 W/cm.^{2}
   Duration: 10 min.

One group of rabbits received pyrogenic distilled water as an isotonic solution. A second group received the same solution previously treated with ultrasonic vibrations at 2 Mc./sec. Figure 1 shows the temperature changes of the two groups.

As can be seen, the control group showed a pyrogenic reaction, and the animals which received the pretreated solution did not react. The difference was found to be significant.

A frequency of 800 kc./sec. and intensity of 1·5 W/cm.^{2} for both, 5 and 10 minutes, produced a diminution in the pyrogenic reaction but it was not significant.

All animals which were injected with the treated distilled water did not show any change in temperature, appetite and general condition in the 30 days after injection.

The pH and refractive index of distilled water were measured before and after treatment. No change in refractive index was observed. The

treatment of pyrogenic distilled water caused a fall in pH from 6·75–6·80 to 6·20–6·10.

To compare the results obtained on pyrogenic distilled water with those obtained from a known pyrogenic system, a standard suspension of *B. subtilis* was added to apyrogenic distilled water and autoclaved at 120° for 30 minutes. The resulting solution gave a definite pyrogen

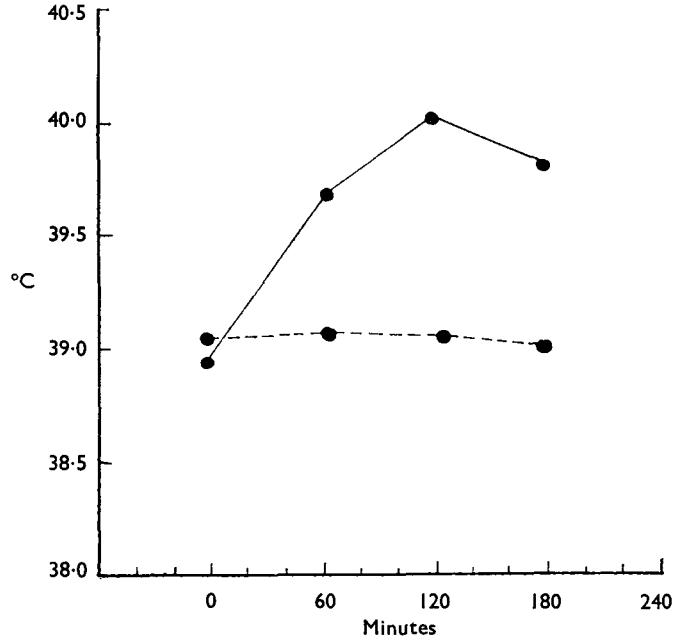

FIG. 1. Temperature changes in rabbits injected with ●—● untreated pyrogenic distilled water and ●---● distilled water treated at a frequency of 2 Mc./sec. intensity 2 W/cm.² for 10 min.

reaction. After treatment by 2 Mc./sec. the solution gave no pyrogen reaction in four groups of rabbits. 800 kc./sec. produced a reaction lower than the untreated solution. These results are parallel to those obtained with pyrogenic distilled water.

We conclude that the pyrogenic distilled water lost its pyrogenic activity when treated. Similarly, an autoclaved standard suspension of *B. subtilis* lost its pyrogenic activity.

Therefore it is possible that the vibrations have an application both in the production of apyrogenic distilled water and in the study of these ubiquitous substances. Further work is in progress and a full report will be published later.

REFERENCES

1. German, *Ann. pharm. franç.*, 1948, **6**, 464.
2. Co Tui, *Proc. Amer. pharm. Manuf. Ass.*, 1943, **40**, 51.
3. Busnel, Richard and Bousignes, *J. Chim. phys., Paris*, 1953, **50** (2).
4. Gligorijević, Puhač and Budimirović, *Acta Veterinaria, Beograd*, Vol. III, Fasc. 1.