The survival of heat resistant spores in aqueous injections after an official sterilization treatment

SIR,—Differing concentrations of spores of *Bacillus stearothermophilus* were prepared and counted as described by Cook & Gilbert (1965). Suspensions of spores in 1 ml volumes of water for injection B.P. and sodium chloride injection B.P. were heated in ampoules at 115° in an oil bath (Gilbert 1966) for between 10–120 min. The ampoules were then cooled in water at 4°, opened, and the contents transferred aseptically to tubes containing 10 ml volumes of tryptone (Oxoid) 1%, dextrose 0·5% and soluble starch 0·1% in water. Soluble starch was included in the medium to aid the recovery of surviving spores and the pH adjusted to 7·3 as recommended by Cook & Gilbert (1968). The usual indicator bromo-cresol purple, included in media to detect flat-sour organisms such as *B. stearothermophilus*, was omitted as recommended by Cook & Brown (1960). The tubes were incubated for 3 days at 50°.

TABLE 1. HEAT RESISTANCE OF *B. stearothermophilus* SPORES AT 115° IN AQUEOUS INJECTIONS

Heating medium 1 ml volumes	Concentration of spores in heating medium*	Tubes of recovery medium yielding growth after heating ampoules				
		Heating time at 115° (min)				
		10	30	60	90	120
Water for injection B.P.	10^5	—	10/10	10/10	7/10	0/10
	10^4	—	10/10	7/10	0/10	0/10
	10^2	10/10	3/10	0/10	0/10	—
Sodium chloride injection B.P.	10^1	—	10/10	2/10	0/10	0/10

* Calculated from plate counts of unheated spores.

Table 1 shows that heating at 115° for 30 min was insufficient for sterilization of water for injection or sodium chloride injection, and the time for sterilization increased with increase in spore concentration in the ampoules. The influence of spore load has also been stressed by Kelsey (1961) using spore strips impregnated with *B. stearothermophilus* spores heated in steam at 121°. *B. stearothermophilus* is a non-pathogen with a very wide growth range of about 37°–70° and is normally associated with thermophilic spoilage of low-acid canned foods.

We do not propose that the B.P. official sterilizing time: temperature relation be altered or that tests for sterility include incubation at 50°, but it should be recorded that low concentrations of spores can survive the B.P. sterilization process in aqueous injections. Spores of important pathogenic organisms e.g. *Clostridium tetani*, *Clostridium welchii*, *Bacillus anthracis*, are readily killed by moist heat at 115°.

School of Pharmacy,
University of London,
Brunswick Square, London, W.C.1.,
England.
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A. M. COOK*
R. J. GILBERT†

* Present address: Welsh School of Pharmacy, University of Wales Institute of Science and Technology, Cathays Park, Cardiff, Wales.
† Present address: Food Hygiene Laboratory, Central Public Health Laboratory, Colindale Avenue London, N.W.9, England.

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The effect of chlordiazepoxide and fluphenazine on critical flicker frequency

SIR,—The critical flicker frequency (CFF), which is the rate at which a light of increasing intermittency first appears to be flickering, has for many years been used as a sensitive test of central nervous system activity (Landis, 1954; Turner, 1968). CFF not only varies with age and in neurological disorders (Misiak, 1967) but is affected by centrally acting drugs. Earlier studies had used large doses of oral agents, but it has now been shown (Turner, 1968) that single therapeutic doses of several compounds including phenmetrazine, amphetamine, chlorpromazine and amylobarbitone may alter the CFF, and so it seems reasonable to regard measurement of CFF as a potential screening test for centrally acting drugs.

We have now compared chlordiazepoxide and fluphenazine hydrochloride which were shown recently (Rickels, Raab & others, 1968) to be effective in the management of anxiety states. Chlordiazepoxide, 10 and 20 mg, in two divided doses, 4 hr apart, and 2 mg of fluphenazine hydrochloride in a single dose were compared with placebo in a double blind trial. A latin square design of administration was used in eight young volunteers of either sex. CFF, both ascending and descending, was measured before, and at 2 hrly intervals for 8 hr after ingestion. The apparatus and technique were described by Turner (1968).

The results were submitted to an analysis of variance which revealed no significant difference between the two drugs and placebo. However, as has been consistently found with this method, a significant ($P < 0.001$) fall of CFF was observed over the day (Turner, Sneddon & Smart, 1967).

These results are in accordance with those of Ideström & Cadenius (1963) who found that while a single dose of 20 mg chlordiazepoxide did not significantly reduce CFF, a 40 mg dose did so. Austen, Gilmartin & Turner (1968) have measured the action of chlordiazepoxide (10 mg) on visuomotor co-ordination, visual field, extraocular muscle balance and colour matching ability and found no significant change. Although fluphenazine hydrochloride, 1 mg, has been shown not to alter CFF (Turner, 1966) it is now recommended in doses of 2 mg daily in the management of anxiety states and therefore requires re-evaluation.

Clinical Pharmacology Division,
Medical Professorial Unit,
St. Bartholomew's Hospital,
London, E.C.1.

NORMA A. LIND
PAUL TURNER

July 26, 1968

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