Table 1 shows the results of two experiments in which brain DOPAC concentration was measured in control rats and in spiperone-pretreated rats given the three drugs. In both experiments, spiperone caused a greater than three-fold increase in DOPAC concentration. Amfonelic acid, which alone had only a slight effect on DOPAC, greatly enhanced the spiperone-induced elevation of DOPAC. This is in agreement with the report of Shore (1976a, b), who used haloperidol instead of spiperone. Amphetamine alone markedly decreased DOPAC and attenuated rather than enhanced its spiperone-induced elevation. Brain DOPAC concentration was not significantly changed by pemoline alone in this experiment (in other experiments we have observed a slight but statistically significant decline in DOPAC at 1 h after pemoline at this dose). Pemoline significantly diminished the spiperone-induced increase in DOPAC. Both pemoline and amphetamine lowered DOPAC by 23% in spiperone-pretreated rats whereas amfonelic acid increased DOPAC by 164% in these rats. Thus pemoline resembles amphetamine rather than amfonelic acid.

Amfonelic acid and amphetamine similarly inhibit dopamine uptake into synaptosomes *in vitro* but cause opposite changes in brain DOPAC concentration *in vivo*. This difference might be explained if amphetamine acts *in vivo* primarily by inhibiting dopamine uptake across the outer neuronal membrane, whereas amfonelic acid acts *in vivo* primarily by facilitating the release of vesicular dopamine. DOPAC is primarily formed *in vivo* inside the dopamine neuron (Roffler-Tarlov, Sharman & Tegerdine, 1971). Amfonelic acid facilitates release of granule-bound dopamine, exposing it to metabolic attack by intraneuronal monoamine oxidase.

Our results indicate that pemoline does not affect brain dopamine neurons in the same way as amfonelic acid, rather its action may be qualitatively like that of amphetamine. *In vitro* pemoline is a weaker inhibitor of dopamine uptake than amphetamine, and *in vivo* it lowers brain DOPAC concentration to a lesser extent than does amphetamine.

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Reduction of pyrogens—application of molecular filtration‡

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Although heat or alkali effectively inactivate pyrogens associated with glass or production equipment, such treatment may adversely affect chemical constituents of the dosage form (Avis, 1970). Several methods have been suggested for removing pyrogens associated with the solute: recrystallization, careful heating in the presence of dilute alkali, acid or oxidizing agents (Avis, 1970); adsorption on charcoal (Ferenczi-Szirovicza & Mod, 1975) asbestos (Avis, 1970) or other materials (Hollander & Harding, 1976); anion exchange chromatography (Palmer & Whittet, 1961: Grabner, 1975) or silicic acid thin layer chromatography (Chen, Chang & others, 1975).

Recently, pyrogenic contaminants were encountered in 5-methyltetrahydrohomofolate disodium, an expensive, oxidizable, folate antagonist with a mol. wt of 517. Four bulk lots were tested for pyrogens, by the United States Pharmacopeia (U.S.P.) XIX procedure, in 24 rabbits at 100 mg kg^{-1}. All rabbits exhibited a peak increase in colonic temperature of 0·6° or greater (mean = 1·2° range, 0·6–3·1°). Therefore, these materials did not meet compendial requirements. Since we felt that published methods may not provide optimal removal of pyrogens from an unstable drug on a multi-litre scale, molecular filtration was evaluated. Briefly, solutions containing drug and antioxidant were filtered through a 293 mm, 0·22 μm membrane to remove any

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‡ After this text was submitted a similar report came to our notice (Zimmerman G., Kruger, D. and Woog M. 1976, *Drugs made in Germany* **19**, 123–128). Both reports are in general agreement.

insoluble matter. The filtrates (about 32 litres per batch) were then cycled under sterile nitrogen through a high volume molecular filtration system (Pellicon, Millipore Corporation) using a 5 ft^2, 10 000 nominal molecular weight filter (Catalogue No. PTGC 000 05) in a 1 to 1 filtrate—retentate ratio. About 4–5 h were required for molecular filtration and 97% of the initial volume was recovered in the ultrafiltrate. The solution was then sterilized by filtration through a 0·22 μm membrane, subdivided into vials and lyophilized.

Samples obtained at various steps in manufacturing were evaluated for pyrogens by the U.S.P. XIX method (Table 1). Both lots were pyrogenic after 0·22 μm filtration, but were well within compendial requirements after molecular filtration. Since rabbits can become tolerant to intravenously administered endotoxin, an additional test was conducted to rule out this factor as being responsible for the negative data. Animals previously used in the evaluation of lyophilized lot 2 were injected with a solution corresponding in composition and treatment to the 0·22 μm filtration sample. The peak increases in colonic temperature were 1·7, 1·5 and 0·8°, respectively. The effect of ultrafiltration was also monitored by the limulus lysate method using commercially available kits (Pyrogent, Mallinckrodt Chemical Company). The 0·22 μm filtrate from lot 2 was positive at 10 and 1 mg ml^{-1}, but negative at 0·1 mg ml^{-1}. After ultrafiltration the results were negative at all three concentrations. More concentrated drug levels were not examined since preliminary experiments indicated drug interference. However, the solution retained from molecular filtration was tested at log dilutions over a drug concentration of 10 to 10^{-4} mg ml^{-1}. The 10^{-4} mg ml^{-1} dilution was negative, but all the higher concentrations were positive. The results demonstrate that molecular filtration effectively reduced pyrogens to permit the products to easily conform to compendial requirements, and indicate that the hyperthermia was due to a high molecular weight endotoxin-like contaminant. The limulus lysate results suggest that molecular filtration reduced the pyrogen concentration by about three orders of magnitude. We have since used the same procedure to depyrogenate a large batch (~45 litres) of calcium leucovorin.

Table. 1. *Effect of Molecular filtration on pyrogen response.* Pyrogen tests were conducted as described in U.S.P. XIX at a dose of 100 mg kg^{-1}. Maximum peak increase in colonic temperature of each of three rabbits is reported.

Sequential treatment	USP pyrogen test (↑ °C) Lot 1	Lot 2
1. 0·22 μM filtration	0·9, 1·1, 0·8	1·5, 1·6, 0·7
2. Molecular filtration	0·0, 0·1, 0·1	0·3, 0·3, 0·0
3. Lyophilization	0·0, 0·0, 0·1	0·2, 0·4, 0·0

Any depyrogenation procedure should not affect the concentration of formulation components. In addition, the method should be rapid and effectively sequence with other formulation processes. Essentially no change (less than 2%) of drug or antioxidant was detected by high pressure liquid chromatography in the ultrafiltrate or in the sterile lyophilized product. Following molecular filtration the effluent may be directly sterilized and then subdivided into vials.

Differences in molecular weight of drug solute and endotoxin-like materials were exploited by molecular filtration to achieve effective separation on a production scale. This approach should be applicable to other low (<10 000) molecular weight compounds. Recently, Salvesen (1977) utilized molecular filtration to concentrate pyrogens in a radiopaque formulation and increase the sensitivity of the limulus lysate method.

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