Individual Rodent Observation Cages for Pharmacodynamic Studies

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A useful new plastic caging system for mice that offers the advantages of being simple to construct, inexpensive, and easy to clean and use is described. Its greatest advantage is that it provides an effective way to observe large numbers of mice in pharmacodynamic studies.

THE OBSERVATION of large numbers of mice individually has always been a problem for pharmacologists in drug evaluation programs. Steel wire mesh cages with hinged metal tops for such procedures have frequently been used. With standard laboratory cages, it has been difficult not only to observe mice during a procedure, but also to remove them at the conclusion of the work. The cages are usually expensive and difficult to clean.

Often a study, such as the determination of the acute toxicity of a compound, would require the use of as many as 100 or more such cages. Such large numbers make it almost impossible for even a highly skilled observer to note responses of the individual animal. Because mice tend to cling tightly to wire mesh materials and frequently bite into the cage during a convulsive death, removal of the animals at the end of an experiment is, at least, an inconvenient procedure. Moreover, the auditory stimulus from the noisy opening of a metal cage lid frequently induces convulsions prematurely in certain studies. A very satisfactory solution to the problem was found with multicompartmental cage units made of transparent plastic.

MATERIALS

The cages illustrated in Fig. 1 were constructed of $^1/_4$ in. clear acrylic plastic, each compartment measuring 4 × 4 × 4 in. Because of the frequent use of groups of 10 mice, the cages were assembled with 10 compartments per unit, making an overall size of $21^1/_2 \times 10^3/_4 \times 4^1/_4$ in. For observational convenience, the cage units were hung on an upright plywood backboard with conventional hangers available at hardware stores. A single brass spring-return hinge was used for each door with a $^1/_4$-in. air space at each side. The air space also provided for convenient opening of the doors. In our laboratories, doors have been constructed to open either from the top or from the bottom, according to individual preference. The details of construction are illustrated in Fig. 2. It should be noted that the plywood board on which the units are hung forms the back of the cage. This feature simplifies both construction and cleaning. The cleaning difficulties encountered with the individual cages have been reduced to a simple removal of a plastic unit from the hanger which is washed with hot water from a hose. To prevent the formation of rust, brass screws and hinges were used. Prior to assembly, the spring hinges were sprayed with a clear protective plastic coating as a rust-preventive measure; replacement has seldom been necessary. Some possible modifications include the construction of larger units for larger rodents and installation of feeding and watering devices for experiments where longer periods of caging are desired.

Fig. 1.—Typical arrangement of cage units in use.

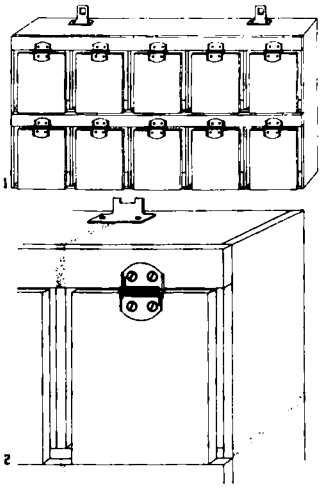

Fig. 2—Line drawing of cages showing construction details.

Among the experiments suitable for this arrangement are anticonvulsant studies using chemoshock techniques, routine toxicologic studies, barbiturate sleeping time determinations, and other experiments requiring temporary storage of mice between drug treatment and another procedure.

Wax pencils have been convenient for marking the occurrence of convulsions or other pertinent events upon the cage door as the event occurs. For example, by sitting in front of a group of these units, one investigator can conveniently observe and mark the occurrence of convulsions in as many as 50 or more mice. Other apparent applications include the intravenous injection of mice accomplished by pulling the tail of the animal through the side space of the door. We have not observed an alteration of drug toxicity due to aggregation of mice as occurs after treatment with *d*-amphetamine, since the mice have no physical contact with one another.

Although the expense of the units could be reduced by using a $^1/_8$-in. thick plastic, we have found the $^1/_4$-in. stock very sturdy and convenient to handle.

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Each unit weighs about 8 lb. and can be constructed in most laboratory shops for a cost of less than $12. for materials. The only similar cages that we have found in use were those reported by Moos, *et al.*(1), where plastic cages for solitary housing of mice for irradiation and longevity experiments were described. A photograph of the present cages, but no description of the units, appeared previously in a report by Burch (2). The caging units have been in use in our laboratory for about 3 years and have become an indispensable laboratory item.

REFERENCES

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Circular Thin-Layer Chromatography of Tetracyclines

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A simple circular thin-layer chromatographic method is described for the separation of hydrochlorides of tetracycline, oxytetracycline, and chlortetracycline employing three sequestering agents—viz., disodium ethylenediaminetetraacetate, tartaric acid, and oxalic acid. Better resolution has been achieved by the use of disodium ethylenediaminetetraacetate in the stationary phase and *n*-butanol saturated with water as the mobile phase.

ALTHOUGH PAPER PARTITION chromatography of tetracyclines has been reported by several workers (1–10), thin-layer chromatography (TLC) of these antibiotics has only been recently investigated. Nicolaus, Coronelli, and Binaghi (11) have studied the chromatography of several tetracyclines with various solvent mixtures by ascending TLC. They were, however, unsuccessful in resolving more than two tetracyclines by a single solvent system on one chromatogram.

The property of tetracyclines of forming chelate complexes with metallic ions suggests that such complexation might occur between these antibiotics and the metallic ions of the adsorbent and/or calcium sulfate added as the binder. In such a case, sequestering agents possibly could effect the separation of these compounds. The use of disodium ethylenediaminetetraacetate (EDTA) and oxalic acid has been promising in the chromatography of certain naturally occurring polyphenolic compounds that showed strong chelating property (12). These sequestering agents might also be employed in the resolution of tetracyclines by TLC.

This report presents the results of preliminary work in which three sequestering agents—*viz.*, EDTA, tartaric acid, and oxalic acid were used—and the separation of hydrochlorides of tetracycline, oxytetracycline, and chlortetracycline was studied. For the development of chromatograms the circular TLC technique of Bryant (13) was modified. The separation of the three antibiotics was achieved by employing all the three sequestering agents, although better separation was accomplished with EDTA.

EXPERIMENTAL

Apparatus.—The Desaga-Brinkmann apparatus for TLC (applicator model "S II") distributed by Brinkmann Instruments, Inc., Great Neck, Long Island, N. Y., was used for the preparation of plates. Glass plates (20 × 20 cm.) with 1/8 in. hole drilled at the center of each were utilized. Pyrex Petri dishes (diameter—14 cm., height—2.5 cm.) were employed as the solvent containers for the development of chromatograms. The adsorbent used was silica gel G (made according to Stahl by Merck, A. G., Darmstadt, Germany, and distributed by Brinkmann).

Preparation of Plates.—*Technique A.*—A slurry of 30 Gm. of silica gel G and 60 ml. of water was made in a dry mortar. This was poured into the applicator and coated over five plates which were then dried at room temperature for 15 minutes. The plates were further activated in an oven at 105–110° for 1 hour and stored in a vacuum desiccator. The adsorbent layer was kept constant at standard 250 μ thickness.

Technique B.—Nine grams of EDTA was dissolved in 60 ml. of water and, using 30 Gm. of silica

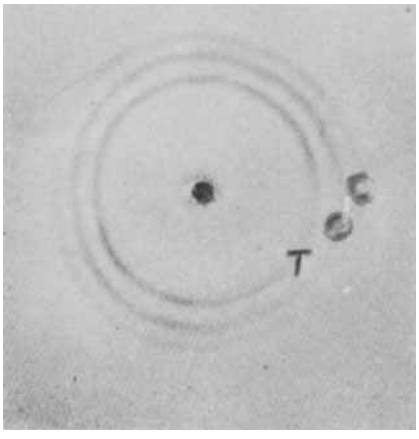

Fig. 1.—Separation of tetracycline (*T*), oxytetracycline (*O*), and chlortetracycline (*C*) hydrochlorides on silica gel G plate admixed with disodium ethylenediaminetetraacetate, using *n*-butanol saturated with water as the solvent.

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