

nary ammonium compounds containing aromatic or alkyl-phenyl radicals: Zephiran [alkyl (C_8 to C_{18}) dimethyl benzyl ammonium chloride]; Triton K-12 (chiefly lauryl dimethyl benzyl ammonium chloride); Triton K-60 (chiefly cetyl dimethyl benzyl ammonium chloride); Hydrocide (alkyl hydroxy benzyl dimethyl ammonium phosphate); (b) *quaternary ammonium compounds containing only aliphatic groups:* Dupont Retarder LA (stearyl trimethyl ammonium bromide); Damol [$(CH_3)_2(C_{12}H_{25})N(Br)-CH_2-CHOH-CH_2-(Br)N(C_{12}H_{25})(CH_3)_2$]; Emulsol-605 [$C_{11}H_{23}-COO-C_2H_4-NH-CO-CH_2-N(CH_3)_3Cl$];⁸ (c) *quaternary ammonium salts containing heterocyclic nitrogen:* CēPryn chloride⁹ (cetyl pyridinium chloride); Emulsol-660 B (lauryl pyridinium iodide); (d) *non-quaternary compounds:* only one such detergent was available for our studies, Emulsol-606, which is the lauryl ester of glycine hydrochloride.⁸ It was found that all of these compounds inhibited the metabolism of the organisms almost completely at a concentration of 1:3000. Most of the compounds were equally effective at 1:30,000. In a few cases a marked effect on bacterial metabolism was noted at concentrations as low as 1:60,000. *These cationic detergents inhibited the metabolism of both gram-positive and gram-negative microorganisms to the same degree.*

The effects of the following *anionic* detergents were studied: sodium cetyl sulfate; Duponol LS (sodium oleyl sulfate); Igepon A ($R-COO-CH_2-CH_2SO_3Na$) and Igepon T [$R-CO-N(CH_3)-(CH_2)_2SO_3Na$]; Tergitol 7 (sodium alkyl sulfate, alkyl = 3,9 diethyltridecanol-6); Drene (triethanolamine lauryl sulfate); Triton W-30 (sodium salt of alkyl phenoxy ethyl sulfonate); Triton 720 (sodium salt of alkyl phenoxy dialkoxy sulfate); Nopecocastor V (sulfonated castor oil); sodium taurocholate. In contrast to the results

with the cationic compounds, it was found that few of the anionic wetting agents inhibited the metabolism of either gram-positive or negative organisms appreciably at a concentration of 1:30,000. Only one, Tergitol 7, was able to inhibit completely the six gram-positive organisms at a dilution of 1:3000. At this concentration, the other anionic compounds inhibited some, but not all of the *gram-positive* bacteria. There was seldom any significant effect by these compounds on the metabolism of *gram-negative* organisms at the 1:3000 dilution.

It may be concluded that the *cationic* type of detergent is a more general inhibitor of bacterial metabolism than the *anionic*.

Variations in the pH of the buffer medium caused striking differences in inhibitory action. It was found that the effect of the cationic compounds increased progressively as the pH was shifted toward the alkaline side, whereas inhibition by the anionic types increased with a shift toward the acid side.

A series of pure alkyl sulfates ranging from C_8 to C_{18} was studied on some gram-positive organisms. It was found that the C_{12} and C_{14} (lauryl and myristyl) compounds gave the maximum inhibitory effects.

We have found that Damol, Emulsol-605 and Emulsol-606 possess a reasonably low toxicity for mice by intraperitoneal injection, and that they produce little or no irritation in the rabbit eye at concentrations of 1:500 to 1:1000. Of these, the lauryl ester of glycine hydrochloride (Emulsol-606) is the least toxic. The protective action of these compounds towards experimentally-induced infections is being studied.

The experiments described here will be published in full elsewhere.

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SCIENTIFIC APPARATUS AND LABORATORY METHODS

ETHYL METHACRYLATE AS A MOUNTING MEDIUM FOR EMBRYOLOGICAL SPECIMENS

A SUITABLE method of mounting various embryological specimens such as small mammalian embryos, older chick embryos and amphibian eggs and embryos has always been a problem for the teacher of embryology. These objects were too large for balsam mounts; if they could be mounted in balsam, they dried very

⁸ We are indebted to Messrs. A. K. Epstein and B. R. Harris, chemists of the Emulsol Corporation, for suggesting that bactericidal compounds of the type 605 and 606 would possess low local and systemic toxicity.

⁹ The toxicity and germicidal action of cetyl pyridinium chloride have been investigated by R. S. Shelton *et al.* (Abstracts 99th Meeting of the American Chemical Society, April, 1940).

slowly and were very easily broken. The only satisfactory method has been to study these objects in dishes of a preservative such as alcohol. Experiments carried out recently in this laboratory have shown that permanent preparations of splendid optical qualities can be made by embedding these objects in one of the clear plastics, ethyl methacrylate. The manner in which this is accomplished is described below.

The unpolymerized ethyl methacrylate monomer can be obtained from the Rohm and Haas Chemical Co., Philadelphia, Pa., at a cost of about \$6.00 per gallon. This material is shipped with an inhibitor, hydroquinone, which must be removed before polymerization. This inhibitor is removed by washing a sample of the monomer (300 cc samples are used in this laboratory) four or five times with a 5 per cent. KOH solution.

Excess KOH is removed by several washings with tap water. The sample is dehydrated by allowing it to stand over anhydrous sodium sulphate for twelve hours. When filtered the material is ready for polymerization.

Polymerization is effected by a catalyst, benzoyl peroxide. A stock solution of the catalyst is made by dissolving 5 grams of benzoyl peroxide in 100 cc of the inhibitor-free monomer. This solution should be kept in a refrigerator. When the sample is ready for polymerization, this solution is added in the ratio of 1 part to 10 parts of the inhibitor-free monomer. The sample, now measuring 330 cc is placed in a well corked 500 cc Erlenmeyer flask. The flask is placed in a water bath at a constant temperature of 85° C. The flask should be continually agitated while in the water bath. Every two or three minutes the flask should be removed from the water bath, the cork removed to admit air, replaced and the flask well shaken. This treatment allows for a dissipation of the heat generated in the polymerization reaction. If this procedure is not carefully followed, the reaction will get out of control, a rapid boiling and hardening of the material will result in a loss of the entire sample. After 20 to 30 minutes of heating and shaking, the sample will be partially polymerized and will have a viscosity about like that of molasses. At this point, the flask should be well corked and the partially polymerized material placed in the refrigerator until needed.

Small, thin-walled glass preparation dishes holding about 25 cc make very good molds for these preparations. A dish of this type is filled to a depth of about $\frac{1}{4}$ inch with the partially polymerized material prepared above. The dish is well covered and placed in an oven at 50° C. for a period of 24 hours. This final heating results in a complete polymerization of the material and forms a solid base on which the object may be mounted.

The embryo to be mounted, for example, a 4-day chick embryo, is prepared in the same manner as for balsam mounting. The embryo is stained with borax-carmines or alum-cochineal, dehydrated with a series of alcohols and cleared in xylene. From the xylene it is placed in a small open dish of the partially polymerized material for a period of 30 minutes to allow for an evaporation of the xylene. From this medium it is transferred to the dish containing the polymerized base and well covered with the partially polymerized monomer. The dish is covered, returned to the oven at 50° C. and kept there until the preparation is thoroughly hardened. When completely hardened, the specimen is chilled with ice water or solid carbon dioxide, which loosens the cast from the glass mold. No satisfactory way of getting the cast from the mold has been found and, in most cases, the glass mold must be broken.

Preparations made in this way have many distinct advantages. When studied under the dissecting microscope or the lower magnifications of the compound microscope, the optical qualities of such a preparation are as good, or better, than those of balsam-glass preparations. The embryo may readily be studied from either side and the mount is unbreakable. This plastic material is more susceptible to scratching than is glass, but with reasonable care, this is not a serious objection to the method.

If it is desirable to make these mounts in the nature of microscope slides, this may easily be done. Polished sheet Plexiglas (methyl methacrylate) 0.08 inch in thickness and cut to standard microscope slide size, 3 × 1 inches, can be purchased from the Rohm and Haas Chemical Co., Philadelphia, Pa. Rings for making the cells in which the embryos will be mounted on the slide can be made in the following way. Polished Plexiglas rod, $\frac{3}{8}$ inch in diameter can be obtained from the company mentioned above. Using a lathe, this rod is converted into a tube with a bore of approximately $\frac{5}{8}$ inch. Using the cutting tool of the lathe, this tube is now cut into rings of various thicknesses, $\frac{1}{4}$, $\frac{3}{16}$ and $\frac{1}{8}$ inches, dependent on the size of the object to be mounted. These rings are fastened to the Plexiglas slide with a cement, Acryloid B-7, which is a 20 per cent. solution of polymerized methyl methacrylate in ethylene dichloride. The object to be mounted is prepared in the same way as for mounting in a disc. It is transferred to the cell just described, well covered with the thick, partially polymerized ethyl methacrylate and placed in the oven at 50° C. to complete polymerization. There is some loss in volume during the polymerization process and fresh monomer must be added as the hardening process takes place.

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