METHODS

Non-Wettable Surfaces

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THE IMPORTANCE of "non-wetting" surfaces for the in vitro handling of blood is now generally accepted. The harmful influence of glass, rubber and metal surfaces had been overlooked from the time of the pre-anticoagulant blood transfusion era until the introduction of silicones as protective surfaces by Jacques, Fidlar, Feldsted and Macdonald[1] in 1945. Since that time there has been increasing recognition of the importance of the application of non-wetting surfaces to obtain blood cells and plasma proteins in their natural state.[2] With the use of untreated glass, the most readily discernable harmful effect is platelet disintegration with consequent activation of the clotting mechanism. Equally severe damage occurs to leukocytes. These changes consist not only of decrease in ameboid motility and phagocytic index, but in respiration as well. Martin[3] was able to show a decreased oxygen consumption after a single pipeting of cells into nonsiliconed glass. To date, there is no evidence that red cells are harmed by wettable surfaces, but this might be predicted from the fact that erythrocytes are preferentially wet by oil as opposed to leukocytes which are preferentially wet by water. It is probable that the lipoproteins as well as other plasma proteins are also injured by wettable surfaces, but evidence for this is incomplete.

With the simplification of methods for the elimination of wettable surfaces, there is little reason for failure to use such protective environments. A few principles should be borne in mind in selecting the proper surface for the proper container. Glass can easily be rendered non-wettable by treatment with methyl chlorosilane or liquid silicone. The methyl silicone polymers have a high degree of water repellency, exceeding even that of paraffin. Moreover, the resulting film is chemically attached to the surface of the glass and thus is susceptible to sterilization with high pressure steam.

The problem of rendering stainless steel and other metal surfaces non-wettable is distinctly different. Methyl chlorosilane will not react chemically with a polished metal surface, nor will silicone oil adhere permanently to it. At least two other types of agents are available, however, which will satisfactorily render metal non-wettable. Arquad 2-C* (an organic amine) and the silicone resin lacquers form firm adhesion to metals at high temperatures and may be applied easily to most types of metal except polished aluminum which is itself prac-

This work has been supported by funds of Harvard University and the Eugene Higgins Trust, by grants from the Rockefeller Foundation, the Atomic Energy Commission, the National Institutes of Health, and by contributions from industry.

Submitted March 15, 1952; accepted for publication June 2, 1952.

This is Number II in the series on The Separation, Characterization and Preservation of the Formed Elements of the Blood; it is also Number 103 in the series on The Preparation and Properties of Serum and Plasma Proteins.

* Available from Armour & Co.

tically non-wetting. Finely divided Teflon in a proper dispersing agent also can be applied as a spray to metal surfaces, sintered in place at high temperatures.

Silicone

The chemistry of the silicone technics has been adequately described previously by Rochow, Jaques and Brown.[4] It should be borne in mind that although the silicone presents a non-wetting surface, its actual deposition on glass comes about from the reaction between methyl chlorosilane and the thin film of water vapor on the surface of glass, and hence is influenced by the presence of that film. Liquid silicone (Dri-Film 9987)* is applied by actual immersion of the surface to be siliconed followed by subsequent rinsing to complete the hydrolysis and the removal of hydrochloric acid. This method has been largely superseded by the vapor silicone process due to its ease of application.

Vapor Silicone

This vapor treating material (Dri-Film 9977)† lends itself readily to the siliconization of large amounts of equipment in a minimum of time. All glassware must be properly washed with soap and water 24 hours before application of the silicone.† After washing, the glassware is thoroughly rinsed in warm tap water followed by hot pyrogen-free distilled water. It is then placed in a hot air oven and dried for 2 hours at 120 C. Following this, it is allowed to stand overnight at room temperature in order to equilibrate with the moisture in the atmosphere. This overnight equilibration is absolutely essential as the vapor will not react with or adhere to a completely dry surface. The glassware is put in cardboard cartons, or other similar containers and placed under a chemical hood. About 30 cc. of Dri-Film (No. 9977) is placed in a gas washing bottle and air is bubbled slowly through it. The resultant vapor is directed through a rubber tube and glass nozzle into each of the cartons containing glassware. The tube should only be held in each carton long enough for the operator to count slowly to six.‡ The gas bottle is then disconnected and a stream of compressed air is directed into each carton for the same period of time in order to displace the extra methyl chlorosilane vapor. The glassware is then removed from the cartons, rinsed three times with hot pyrogen-free distilled water, to free it of the hydrochloric acid which has been generated. It is now ready for sterilization or immediate use as desired.

Although this surface is theoretically sufficient for two or three experiments, experience has shown that it is preferable to remove the silicone layer after each use. With technics so simple for application, there is little reason for not stripping off the silicone each time; this assures added protection against pyrogens and other contaminates. Removal is accomplished by exposing the glassware to 10 per cent sodium hydroxide solution for at least two hours followed by thorough washing and rinsing.

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* Available from General Electric Company, Chemical Division.

† Detergent should not be used on glassware which will come in contact with leukocytes. It is extremely difficult to remove the leukotoxic effects even by prolonged water rinses.

‡ Serological pipets or other containers with a very narrow internal diameter should be actually attached to the gas nozzle for 6 seconds.

Liquid Silicone

The method of preparation of glassware is similar to that described above. The method of application is adequately described elsewhere.[5]

Arquad*

This material, dicoco dimethyl ammonium chloride, is ideally adapted for the application of non-wettable surfaces to needles and other stainless steel parts having a narrow internal diameter or shape which is inaccessible to painting or lacquer silicone. Needles are prepared by routine cleaning methods and then boiled in a 1 per cent solution of Arquad for 2 minutes. They are then rinsed, allowed to dry and may be autoclaved as desired. The surface should be applied after each use. Arquad is removed simply by treatment with warm soap and water.

Lacquer Silicone

Lacquer silicones, which are silicone-resins and quite different from the dri-film chlorosilane preparations, were originally developed for the baking industry in order to render oven pans permanently "greased." A single application is theoretically sufficient for the life of the pan, but has thus far been limited to about 150 consecutive exposures. This surface is ideally suited to stainless steel parts† that are sufficiently open for easy application and thorough washing between use in order to free the non-wettable surfaces from pyrogens. Two commercial types are available: Dri-Bake‡ and Pan-Glaze.§ The materials to be treated are prepared by dipping or brushing with the resin solution and are allowed to drain. They should be placed in a vertical position to allow complete drainage. They are left in this position for 30 minutes, allowing the resin to air dry so that it will not blister when it is permanently baked on. The air dried pieces are then baked in an oven at 230 C. for three hours, after which they are ready for use. Following 150 or more exposures to blood, the previous coating may be removed by treatment with strong alkali. These baked-on surfaces are hard and durable. Althought they can be scratched, they are not ordinarily damaged by the usual laboratory manipulations.

Teflon¶

This material was originally available as a solid, non-wettable plastic from which test tubes, centrifuge cups and other vessels could be machined. It has physical properties of hardness and resilience which lend themselves well to machining, but its high cost and scarcity make its routine use impractical. More recently it has become possible to apply this same plastic as finely divided particles, which may be suspended in an appropriate dispersion agent and then poured or sprayed over the metal surface. The resultant surface, after drying and heating, may be polished and remain as a semi-permanent coating. Experience

* Available from Armour & Co.

† Metal ion-exchange columns, heat exchangers and metal centrifuge cups.

‡ General Electric No. 81202 Silicone-Resin.

§ Dow-Corning Company.

¶ Available from E. I. Dupont Co.

with this material to date reveals that it has satisfactory non-wettable properties but the questions of pyrogenicity and permanence of coating remain to be tested.

SUMMARY

Types of non-wettable surfaces and their means of application are outlined and discussed.

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