## SOME CRYSTALLINE CONSTITUENTS OF THE NON-SAPONIFIABLE FRACTION OF BONE MARROW

WE were interested in the isolation of pure substances from yellow bone marrow in the hope that we might find one or more especially effective in the formation, maturing and release of white blood cells, especially the granulocytes. Others have reported that it is the non-saponifiable fraction of bone marrow fat which is effective in the treatment of agranulocytosis. Consequently, we centered our efforts on this fraction, amounting to a few tenths of one per cent. of the entire marrow.

Since the greater part of the marrow in the large bones of beef is fat, we first had this fat extracted by suitable solvents and then saponified it, extracting the valuable non-saponifiable portion from the soap. Our second method called for direct saponification of the entire marrow, a process that probably broke down certain protein structures in the aqueous fraction of the marrow with release of more fat or lipid than is possible with solvents alone. There are advantages in both methods.

By careful fractional crystallization of the non-saponifiable fraction from suitable solvents we isolated four crystalline substances of high purity and a few others which, if not quite pure, are mixtures of closely related compounds not readily separated by solvent fractionation. All these substances were obtained by the second method mentioned above, that is, saponification of the entire bone marrow. Of course, the proteins and water solubles of the aqueous-type fraction were eliminated by the solvents used in extraction.

In addition to the four colorless crystalline products reported below we secured a number of highly colored oily or semi-solid fractions set aside for further study. The four listed below contained oxygen and were carbinols.

CRYSTALLINE PRODUCTS

(1) Carbon 73.6 per cent., hydrogen 13.4 per cent.; melting point 66°–67° C. The benzoate melted at 35°–36° and the acetate at 34°–35°. Molecular weight by Rieche micro method (usually found 5–10 per cent. low on known pure substances) was 296.

(2) Carbon 83.8 per cent., hydrogen 12.2 per cent.; melting point 147°. Benzoate melted at 144°–144.5°, acetate at 113.5°–114°. The sterol recovered by saponification of the benzoate melted at 147°. Gave good Liebermann-Burchard test for sterols. Mixed melting point test with cholesterol confirmed identity of this substance with cholesterol.

(3) Carbon 76.9 per cent., hydrogen 13.0 per cent.; melting point 61°–63°. The benzoate melted at 137°–138° with preliminary softening and the acetate at 37°–38°. Molecular weight by Rieche method, 268.

(4) Carbon 82.2 per cent., hydrogen 12.1 per cent., for substance contaminated with cholesterol. Melting point after removal of cholesterol was 124°, not sharp. The benzoate of the purified substance melted at 123°–125°. A sterol, forming a digitonide.

This report is wholly preliminary. Further separations and purifications of marrow substances, in addition to biological testing, are in process and will be reported later in greater detail.

We are greatly indebted to the Abbott Laboratories of North Chicago for preliminary processing of the bone marrow which was generously supplied by Swift and Company, and to Dr. H. K. Alber, of the Biochemical Research Foundation of Philadelphia, for his micro determinations of carbon, hydrogen and molecular weights.

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# SCIENTIFIC APPARATUS AND LABORATORY METHODS

## LATEX EMULSIONS IN HUMAN VASCULAR PREPARATIONS

FOR generations vessels and ducts have been filled with materials to make them stand out in anatomic preparations. Many different substances and combinations have been used. The older anatomists introduced many masses which are still useful. For example, in Paris one sees the preparateur injecting melted tallow colored with vermilion. In this country in human anatomy the use of a starch paste prepared by mixing lump starch and color, either red lead or a red lead substitute, with cold water is a common practice. Teichmann's mass was preferred for many years and is still used occasionally. This mass is essentially a heavy oil paint. The late Professor C. R. Bardeen, at the University of Wisconsin, used a shellac which was colored with Prussian blue pigment, in the arteries of human cadavera. This violation of the classic color for arteries resulted in giving the students an opportunity to see small arterioles in beautiful contrast to the surrounding reddish tissues. For many years I have used, both here and formerly at the University of Cincinnati, a variation of this mass, a material prepared for the electrical industry. This material (Ajax —insulating varnish, black air drying No. 26, Sherwin-Williams) I demonstrated at the meetings of the American Association of Anatomists at Cleveland. It has been equally as satisfactory as the mass that I learned to use in Dr. Bardeen's laboratories. It has the advantage of being ready prepared and cheap.

For corrosion preparations the completely satisfactory mass has not yet been found. Substances satisfactory for smaller structures are unsatisfactory for

large cavities and vessels. Some of the newer materials produced by synthetic chemistry partially bridge this.[1]

With the continuing chemical advances new substances will be available; one of these seems to be latex emulsion. In 1936 I first became acquainted with the properties of latex emulsion through Dr. George P. Phillips, of Boston. Dr. Phillips told me something of the working properties of the material and advised me as to where it might be obtained and also stated that the firm was very cooperative in supplying small amounts of the material to the dental and to the medical profession. Since that time I have used the material in its various forms, colored and uncolored, in the preparation of prostheses and in various laboratory procedures.

Latex emulsion (Vultex[2] is the preparation that I have used) is a slightly ammoniated water emulsion of rubber. When freshly received, the clear material has a milk-white appearance and is of the consistency of cream. In my experience the emulsion may be "cracked" in three ways; simple drying reduces the emulsion to the consistency of rubber found in latex rubber gloves. The emulsion is cracked by acidulating it with any weak acid. It retains its milk-white color until the water has thoroughly dried out. The emulsion can be made into rubber by pouring it into plaster of Paris moulds. These moulds should not be previously treated in any way which would destroy their porosity. The porosity of the mould absorbs the water from the emulsion, leaving the rubber behind. It takes several days or even a week or two for this remaining material to assume its final character.

Considering my interest in the injection of vessels and in corrosion preparations I am surprised that I did not try latex until this present year. We have now in the laboratory several human cadavera in which the arteries and the veins have been injected with colored latex emulsion. We have also used this material for three color injections of the kidney. Our work on the nasolacrimal apparatus has not been satisfactory because of the difficulty in causing the material to flow through small caliber cannulae. The elasticity of the material makes it seem ideal for the routine injection of human cadaveric material. It is not suitable for corrosion preparations where one expects to retain the size of the vessel. Corrosion is accomplished as usual with concentrated hydrochloric acid. In my work with latex in plaster moulds I have found a shrinkage of about one in six, so that when sizes must be duplicated one has to expand the mould to allow for this shrinkage.

Our procedure for human material is as follows. We use air pressure and an injecting bottle for the latex emulsion much as one would use for the injection of a gun-cotton mass. We rinse the system with weak ammonia water before adding the latex emulsion. We make all connections between the bottle and the structure being injected as short as possible to simplify cleaning. If the material has become somewhat thickened from standing we dilute it slightly with weak ammonia water. We find that about one liter suffices for the arterial injection of the average cadaver. This amount compares with what is used with the other colored masses in the human cadaver. The amount is increased to advantage in many instances. The material is injected from 12 to 24 hours after the intra-arterial embalming is completed. For special venous injections we sometimes make the latex injection before embalming, so that the vessels are not filled with the blood from the capillaries. In work with the human cadavera we have not found it satisfactory to routinely practice the washing out of the venous system. Occasionally we open a vein to allow for some lessening of blood on the venous side.

In working with latex and in preparing to describe its use I made inquiry concerning a material used by a biologic supply firm.[3] The nature of this material was not disclosed, but its description by letter seemed to fit the material that I was using. A reply to a direct question confirmed the fact that this material was indeed a latex emulsion. Examination of a purchased specimen also showed this. There has recently appeared in the house organ[4] of another firm an account of the use of latex emulsions. I can not agree with the statement there made about the expansion of the material. This expansion, if it occurs at all, is very transient and is immediately followed by shrinkage.

The material in question can be obtained from the Vultex Chemical Company, 666 Main Street, Cambridge, Massachusetts. The specific materials that I am at present using for colored injections are as follows: F-934 Red No. 244, F-934 Blue No. 9B, and F-934 Yellow No. 154. Previously the material was furnished in glass or in tin screw-top containers and now for two or three years in iron buckets. In the course of time, especially after opening the material, it begins to rust the bucket, therefore, we transfer the material to glass containers in the laboratory.

Using Abernathy's[5] classification this material would have to be a coarse mass. It will never be suitable for such fine vessels as one may inject with preparations of vermilion nor will it be suitable for formal museum corrosion preparations. I believe it will be invaluable

[1] O. V. Batson, SCIENCE, 81: 2108, 519–20, March 24, 1935.

[2] Trade name used by the Vultex Chemical Company for their material prepared under the Schidrowitz patents.

[3] Ward's Natural Science Establishment, Rochester, N. Y.

[4] *Turtox News*, General Biological Supply Company, Chicago, Ill.

[5] Abernathy: *Phil. Trans. Roy. Soc.*, London (for 1798), 18: 287, 1809.

for routine preparation of human cadaveric material and will lead to a better concept of structures in many parts of the body. We have adopted it as our routine mass.

It's a pleasure to again acknowledge my indebtedness to Dr. Phillips for having first introduced me to this material, and I should also like to express my thanks for the great consideration given numerous queries and small orders by the various members of the firm of the Vultex Chemical Company.

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## LIQUID LATEX AS AN INJECTION MASS FOR BLOOD-VESSELS

NUMEROUS substances have been used in injecting the circulatory systems of laboratory specimens to enable students to trace the course of blood-vessels with greater ease. Gelatin and corn-starch masses in various colors have been used for many years, but both of these have serious faults. Gelatin tends to stain tissue by "jumping" the capillaries and has the added disadvantage of becoming excessively brittle in formaldehyde. Starch mass does not set well if used too thin, and when made thick enough to prevent the running of the mass when a blood-vessel is accidentally cut by the student, it will not fill the smaller vessels.

Recently, plastics have been used with some degree of success, but the polymerization to the solid substance after injection presents such formidable obstacles that it is not yet practical to use for laboratory specimens.

Mr. William Kruse, of Ward's Natural Science Establishment, first suggested the use of latex as an injection mass in March, 1939. Since that time experiments have proven that latex is the perfect substance for this purpose. It will enter the smallest vessels without staining tissue; it may be diluted with water to give the proper consistency; it is used cold, and solidifies to form a tough, flexible solid which forms a perfect cast of the circulatory system. Latex will replace all other substances previously used for filling blood-vessels, and in addition has untold possibilities for use in research on the circulatory, respiratory and excretory systems.

Latex solution of heavy consistency and high pH value, colored with fast, soluble dyes, has proven most practical in this work. The latex may be thinned to any desired consistency by adding distilled $H_2O$. In larger vessels and ducts the mass should be thicker than for use in smaller cavities and thinner when it is desired to fill blood-vessels to their smallest branches. Syringes with glass cylinders and rubber pistons must be used since it was found that contact with the lubricants used for smooth operation of an all-metal syringe set the mass around the piston, causing it to stick. All-glass syringes were unsatisfactory because rubber solution filled the tiny cavities in the ground-glass piston and set under pressure, making the piston immovable.

The material is injected in the ordinary way through metal hypodermic needles inserted into the cavity it is desired to fill. It sets into a tough, flexible solid almost immediately in animals that have been previously embalmed with solutions of phenol or phenol derivatives or preserved in formaldehyde. When injected into larger spaces in freshly killed animals it is difficult to set. When freshly killed animals are used they must be fixed immediately either in alcohol, embalming fluids containing phenol or phenol compounds or in solutions of 5 to 8 per cent. formalin containing 1 or 2 per cent. glacial acetic acid. If the latter fixative is used it must be injected internally so that it will come into close contact with injected vessels and organs and the animals should also be immersed in the fixative. To prevent the latex from escaping when the needle is withdrawn, a drop of 1 per cent. glacial acetic acid or 95 per cent. alcohol may be applied at the spot where the needle was inserted. A clamp or tie should be used on larger vessels.

Dr. Oscar V. Batson, in the current issue of SCIENCE, describes the use of an emulsion of latex sold under the trade name Vultex. He states that he has experienced difficulty in causing the material to flow into the very finest vessels and further expresses the opinion that latex emulsion will never be suitable for the injection of fine vessels.

Dr. Batson undoubtedly refers to vessels of almost capillary size. We have found that our material, which is a rubber solution in contrast to an emulsion, will pass through capillaries if diluted sufficiently and can be used with the finest of cannulae.

D. L. GAMBLE
WARD'S NATURAL SCIENCE ESTABLISHMENT, INC.
THE FRANK A. WARD FOUNDATION OF NATURAL SCIENCE OF THE UNIVERSITY OF ROCHESTER

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