

ON THE ARSENIOUS ACID - GLYCERIN - GELATIN
("ARSENIUS JELLY") METHOD OF PRESERV-
ING AND MOUNTING PATHOLOGICAL SPECIMENS
WITH THEIR NATURAL COLOURS, AND ON THE
USE OF NEW FORMS OF RECEPTACLES FOR
KEEPING MUSEUM SPECIMENS.¹

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(PLATE XXXVII.)

It is a matter of importance not only from an educational point of view, but also in connection with the keeping of accurate scientific records, to be able to preserve pathological specimens with their natural colours, or something approximating to them. I began to pay attention to this subject when assisting Dr. Hamilton in the Pathological Department of the Royal Infirmary of Edinburgh in 1882. We experimented with various preservative fluids, such as: (a) mixtures of glycerin, alcohol, water, and phenol; (b) watery solutions of boracic acid; (c) watery solutions of arsenious acid. Professor Hamilton gave the preference to a carbolised glycerin fluid and a saturated solution of boracic acid,² but I preferred a saturated solution of arsenious acid, to which glycerin was added in the proportion of 2 to 3. In further experiments made between the years 1884 and 1887, at St. George's Hospital, I found that good results could be obtained with bichromate of potash and with biniodide of mercury, and after 1893 I experimented also with solutions of formaldehyde. I was able to obtain good results with each of these methods, but I finally found that the simplest and most effective was the one which I have adopted in current work since 1897, and which consists in fixing and hardening the tissues with a 4 to 8 per cent. solution of

¹ [Received November 7, 1913.] A collection of tuberculous guinea-pigs and other pathological specimens and of fifty photographic enlargements of specimens prepared by this method was exhibited in the Museum Section at the International Congress of Medicine held in London, August 1913.

² Hamilton, "Text-Book of Pathology," 1889, vol. i. p. 34.

formaldehyde, after which the specimen is transferred to alcohol and then to a saturated watery solution of arsenious acid mixed with glycerin. In this fluid specimens can be preserved in jars, but I prefer to transfer them to arsenious acid glycerin jelly, which allows the specimens to be mounted under glass plates and has a beneficial effect on the preservation of colour.¹ When the Kaiserling method was introduced I made some comparative experiments in order to ascertain whether it yielded better results than my method, and I dealt in the same way with several of the modifications of the Kaiserling method; but I found that arsenious acid jelly yielded results at least equal to, and generally better than, those obtained by other methods, and that, as regards subsequent mounting and preservation, my original method had distinct advantages.

Although I have on many occasions since 1887 exhibited specimens prepared by my method,² I thought it best to wait until I had overcome certain difficulties in mounting before giving a detailed account. This was necessary because mounting media composed chiefly of gelatin and glycerin undergo slow changes, causing exudation of glycerin and reduction in the general bulk of the mounting medium. The effects of this contraction may not be apparent for years, and it was only after keeping specimens for some ten years or more in media containing various proportions of ingredients, that I felt satisfied with the reliability of the process which I now recommend.

The fixing of tissues and the preservation of colour are easy compared with the finding of a suitable and permanent mounting medium. The fixing agents which have given me the best results are a 2 per cent. watery solution of bichromate of potash and a 4 to 8 per cent. watery solution of formaldehyde. To both these fixing and hardening reagents sulphate of soda may be added, so as to bring the specific gravity of the solution up to from 1024 to 1028. These solutions allow one to give to the object the consistency suitable for cutting it into slices or for the carrying out of necessary dissections; they do not affect the transparency of membranes, of colloid, or of mucous products; they do not alter, materially, the appearances of fat, muscular tissue, fibrous tissue, bone, etc. With hæmoglobin they both produce insoluble compounds which have a colour resembling closely the normal colour of that product after passage of the fixed tissues through alcohol and embedding in "arsenious jelly."

The bichromate method has a serious disadvantage, owing to the fact that when the chrome salt is not entirely removed by long washing after hardening, a green discoloration is gradually produced.

¹ Even spirit specimens sufficiently old to have lost colour can be made to regain some differential colouring when fresh sections are treated by arsenious acid glycerin and mounted in arsenious jelly according to my method.

² Many of these specimens were exhibited at meetings of the Pathological Society of London between the years 1888 and 1891.

This difficulty is easily overcome when very thin slices of organs are treated, and I have kept sections prepared by the bichromate method twenty-five years ago which have not altered in appearance. In the case of whole organs or thick sections, formalin is better; it is also applicable to thin sections.

The method which I now generally adopt is as follows:—

A.—FIXING SOLUTION.

Formal (commercial) 40 per cent. solution	100 c.c.
Water	900 „
To which may be added—		
Sulphate of soda	20 grms.

The specimens are left in this solution for from three days to two weeks, according to thickness. A piece of tissue half an inch thick is sufficiently fixed and hardened in two days.

(For a time I used a larger proportion of formaldehyde, 5 per cent. and even 8 per cent., but I found that for most purposes a 4 per cent. solution was sufficient.)

B.—ALCOHOL (80 to 90 per cent.).

The specimens are left in this fluid until the colour has returned as nearly as possible to its original tint. A few hours are generally sufficient to obtain a satisfactory and permanent superficial effect.

C.—ARSENIOUS ACID GLYCERIN FLUID.

Arsenious acid solution (made by boiling an excess of arsenious acid in water for two hours, and allowing the fluid to stand for twelve hours)	400 c.c.
Pure glycerin	600 „

The specimens are transferred from the alcohol to this solution, where they must be kept for one or two days at least. They may without serious disadvantage remain in it for two or three weeks, if the solution does not cause marked shrinking.

D.—ARSENIOUS ACID GLYCERIN JELLY.

(Formula for about 8 litres.)

1. Coignet's gelatin (Gold label) 425 grms.
 Arsenious acid, saturated watery solution (see C) 1500 c.c.
 The dry gelatin, thoroughly cleansed, is added to the hot arsenious acid solution, in which it should be entirely dissolved in less than half an hour.
2. Hot arsenious acid jelly (see 1) 1925 c.c.¹ (say 2000)
 Pure glycerin (hot) 5760 „ (say 5800)

The two are mixed together and allowed to cool to about 20° C, then the white of *six* eggs and their broken shells are added and mixed thoroughly with the mass. The mixture is brought again to nearly boiling-point to coagulate the albumin, and is maintained at this temperature for two hours.

The hot fluid is strained through flannel and then filtered through filter

¹ Out of a series of mixtures the one containing exactly the amounts given in the formula gave the best results as regards transparency, stability, and resistance to heat, but the round figures indicated in the brackets are sufficiently near for practical purposes.

paper, the temperature of the fluid being kept at about 50° C. This takes from one to three days.

At the present price of glycerin and formaldehyde, 1 litre of this medium costs 3s. 6d.

This medium is absolutely transparent, and when set does not melt at the highest summer temperature.

This arsenious acid glycerin gelatin (or shortly "arsenious jelly") does not act as an ordinary mounting medium only, but has the property of preserving colour better than the potassium acetate glycerin fluid or other preserving fluids used in the Kaiserling and allied methods. It can be used to mount specimens in jars, but my chief object in devising this solid medium was to find a method by which specimens, and more especially flat sections, could be fixed to glass plates so as to do away with the distortion produced by the uneven walls of museum jars. The use of glass plates has also the great advantage of diminishing cost and reducing the space occupied by specimens.

Mounting of Specimens on Glass Plates.

For many years the method which I used consisted in mounting sections, or parts of organs, not exceeding a quarter to half an inch in thickness, in arsenious acid glycerin jelly between two glass plates, a paper or a glass border being used during mounting to hold the jelly. The excess of gelatin having been removed, the edges were covered with adhesive indiarubber plaster, over which a continuous layer of lead foil was glued. The preparation was then finished by gluing a cloth band over the lead foil or by mounting in a wooden frame.

Very thin sections mounted in this way are very permanent. Some in my collection are over twenty-five years old, but thick sections are frequently a source of trouble after a few years, owing to the contraction of the gelatin and the penetration of air under the glass. To avoid this difficulty I finally abandoned the idea of enclosing the specimen and medium in an air-tight space between fixed plates. I now mount the specimen against the glass plate through which it is to be seen. It is embedded in jelly, which is covered at the back with a second glass plate smaller than the front plate. The back plate is also used to apply a backing of suitable colour, generally white, grey, or black. Nothing is done to close the sides, which are protected by resting the front plate upon the rim of a box made of plaster of Paris, glass or metal.

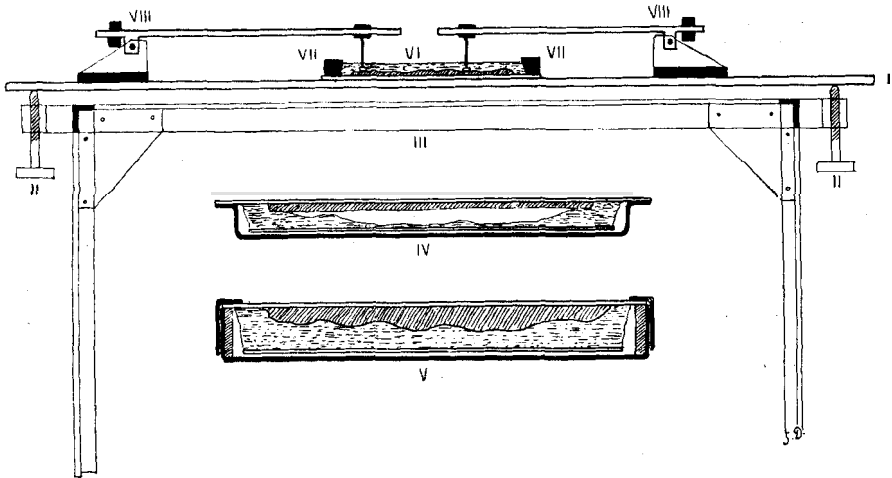
The little glycerin which oozes out of the medium is absorbed by some porous paper, plaster of Paris or felt, which is placed between the back plate and the bottom of the box; this padding also supports the back plate. Under these conditions the back plate follows the gelatin when it shrinks, and air does not penetrate under the glass.

Specimens of almost any size can be prepared by this method.

(The longest I have mounted up to the present is 2 ft. 8 in. in length.)

As to stability, I have specimens mounted seventeen years ago which are still perfect, and have retained their colour without any appreciable change. They have been exposed to light for long periods.

To facilitate manipulations and reduce the amount of time needed to mount specimens by this method I have devised certain appliances which I will describe.



Apparatus used to mount pathological specimens according to the Delépine method—

- (I) Glass table seen in section (with far side in faint lines). (II) Levelling screws (third not seen). (There are three more supporting screws.) (III) Iron framework of levelling table. (IV) Sections of preparation mounted in aluminium dish with flange (arsenious jelly between two glass plates indicated by faint horizontal shading). (V) Section of preparation mounted in aluminium box. (VI) Section of specimen in process of mounting. The specimen is laid on the glass plate, through which it is to be seen, and it is covered with arsenious jelly. (VII) Heavy steel bars used to hold the jelly while it is setting. (VIII) Counter-balanced levers used to press the specimen against the glass plate by means of rods sliding on the levers.

Levelling table.—A large glass table (with levelling screws) with tall legs to allow of the inspection of the specimen from below whilst it is being mounted.

*Heavy steel bars*¹ of suitable shape and thickness to form the sides of a mould for the gelatin medium.

The inner side and one end of each of these bars are polished, and cut at such an angle that they come in perfect apposition with the ends of other bars; the gelatin sets rapidly on coming in contact with the cold metal.

Moulds for making plaster of Paris boxes, which need not be described.

Aluminium dishes and boxes.—In order to save the time required for the preparation of plaster of Paris dishes I had various kinds of earthenware and glass dishes made, but these were heavy, clumsy, and expensive. Finally, I adopted a form of aluminium dish which has answered the purpose very well. The first type has a flat flange to which the top glass plate is fixed by suitable

¹ The table, steel bars, and levers are made by Mr. Charles W. Cook, University Works, Bridge Street, Manchester.

binding. To simplify still further the work of mounting, I have had boxes made to the lid of which the front glass is fixed, the box is closed like an ordinary box. Whether plaster of Paris or metal cases are used, the gelatin must not come in contact with the sides of the dishes. The tools necessary to make these boxes have proved fairly expensive, but the boxes themselves can be obtained at a comparatively small cost.¹

¹ These are made specially for me by Messrs. W. M. Still & Co. Ltd., Charles Street, Hatton Garden, London, who at present charge the following prices:—

The smallest size trays at present made ($6 \times 9\frac{1}{2}$ inches) cost £1, 3s. per dozen. The next size ($9\frac{1}{2} \times 12$ inches), £1, 11s. per dozen. The smallest size boxes ($6 \times 9\frac{1}{2}$ inches) cost £1, 6s., and the next size ($9\frac{1}{2} \times 12$ inches) £1, 16s. per dozen, but these save some labour in mounting. When the first cost of tools has been covered it is probable that some reduction in price may be obtained.

DESCRIPTION OF PLATE XXXVII.

Photograph of a transverse section of the lower lobe of the lung of a pig, and of the parietal pleura of part of the same lobe. Taken direct from a mounted specimen five years old. (Two-thirds of natural size.)

Caseous tubercles of various sizes, grey tubercles visible in several places under the pleura. The specimen had been freely exposed to daylight for about two years. The photographs give a fair idea of the colours, but not of the transparency of the pleura.

