KONINKLIJKE AKADEMIE VAN WETENSCHAPPEN TE AMSTERDAM.

PROCEEDINGS OF THE MEETING

of Saturday February 24, 1900.

(Translated from: Verslag van de gewone vergadering der Wis- en Natuurkundige Afdeeling van Zaterdag 24 Februari 1900 Dl. VIII).

The following papers were read:

Anatomy. — Prof. J. W. VAN WIJHE describes: "*A simple and rapid method for preparing neutral Pikro-carmine*".

By many it will no doubt be deemed unnecessary trouble to add another to the manifold prescriptions for the preparation of pikro-carmine. Most investigators who use it, will be content with one of the well-known methods of preparation which they have been in the habit of following, as was also the case with me, until, about a year ago, the stain disappointed me.

This happened during the study of young embryonic tissue, which had been blackened by osmic acid and had afterwards been bleached.

The ordinary means to tinge nuclei: haematoxylin, alum-carmine and different aniline-stains produced a diffuse colouring, whereas only after having been for about fourteen days in pikro-carmine the nuclei became visible.

Then however the protoplasm of the cells had disappeared; it could not be otherwise than dissolved in the alkaline pikro-carmine, and it seemed only natural rather to take a neutral solution of this tincture. I tried different prescriptions, but I was not successful in finding a neutral solution: a moist red litmuspaper hung in the bottle above the liquid, was tinged blue after a few hours.

PAUL MAYER in his article: "Ueber Pikrocarmin" [1]) says not to believe that: "Carmin in einer ganz neutralen Flüssigkeit, die noch dazu eine relativ grosse Menge pikrinsauren Salzes enthält, gelöst bleiben kann" (l. c., p. 19). He examined pikro-carmine from the anatomical laboratory at Munich and from the Collège de France, moreover liquid and solid samples of GRÜBLER and different solid samples of MERCK.

The pikro-carmine is a solution of two solids: picrate of ammonium and ammoniumcarmine — the discoverer RANVIER believed it to be a chemical combination, but this is an assertio gratuita — and now it is (leaving the alkaline reaction out of the question), a deficiency of most prescriptions that they cannot specify the relative proportion of these elements and leave it to the inconstancy of chance.

This is the case with all prescriptions in which bacteria from the air are called to aid, according to the method followed in the Collège de France [2]), moreover the preparation then lasts several months, and, as experience has taught me, there is considerable danger of obtaining a totally useless product.

Because of the difficulties just mentioned and others besides, PAUL MAYER says at the end of his article (l. c., p. 28): „Das Facit wäre also: das Pikro-carmin gehört zu den Färbmitteln, die eine bewegte Vergangenheit hinter sich haben, und von denen man möglichst wenig Aufhebens mehr machen sollte."

Pikro-carmine can however not yet be considered out of date as a stain in microscopical technics, and I have been successful in preparing in a simple way a liquid, which may practically be called neutral, at the same time containing fully known quantities of picrate of ammonium and ammoniumcarmine. The method can

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[1]) PAUL MAYER, "Ueber Pikrocarmin", Zeitschrift für wissenschaftliche Mikroskopie und mikroskopische Technik. Bd. 14, 1897.

[2]) See A. BOLLES LEE, The Microtomist's Vademecum, fourth Ed. 1896, p. 153.

partly be considered as a modification and simplification of HOYER's prescription [1]) running thus:

Take 25 cc. [2]) of an old strong [3]) solution of carmine in ammonia and pour it carefully in 100 cc. of strong alcohol (of circa 96 pCt.), a voluminous precipitate of ammoniumcarmine now forms itself. Filter after half an hour or longer, rinse the precipitate on the filtrum with 100 cc. strong alcohol and dry it for 24 hours in a thermostate of 40—45° C. [4]).

If the solution was old enough a dark red, nearly black crumbled mass is obtained, which is easily rubbed down to a powder thoroughly and clearly soluble in distilled water or in picrate of ammonium of whatever strength. If picric acid is added to the solution, a precipitate is immediately formed; the picrate of ammonium may therefore not contain free picric acid which was the case with a certain quantity which I received from MERCK [5]).

The relative proportion of ammoniumcarmine and picrate of ammonium, which, as a rule, seemed to me most favourable for staining was as 1 : 2. In order to obtain a liquid, which would at the same time fix the tissues to some extent (as pikrocarmine is expected to bring about), I took a 1 pCt. solution of picrate of ammonium, — i.e. a nearly concentrated solution [6]) and added thereto $^1/_2$ pCt. ammoniumcarmine.

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[1]) HOYER, Beitrage zur histologischen Technik, Biologisches Centralblatt, Bd. 2, 1882. Following the somewhat lengthy prescription of HOYER, I was not successful in obtaining a powder quite soluble in water. Besides a good deal of carmine is lost.

The "Pikrocarmin nach HOYER" of GRÜBLER must, according to the List of Prices, be dissolved with ammonia, and could therefore not be used for my purpose.

[2]) These and other quantities are of course taken ad libitum, the statement is for the convenience of those who may want to follow the prescription.

[3]) At first I dissolved 30 gr. carmine in 100 cc. not diluted ammonia of circa 10 pCt. Afterwards I found out that the carmine dissolves better in ammonia diluted with the double quantity of distilled water.

[4]) The filtered liquid is thrown away. After evaporation a tough red substance is obtained, which, when thoroughly dried forms a coherent, hard mass, soluble in alcohol as well as in water. With alcohol the watery solution gives no precipitate.

In the same manner it appears that the solution of *carminic acid* in ammonia consists of two kinds of ammoniumcarmine, one of which can be precipitated by strong alcohol, but the other cannot.

[5]) A sample, which I received from GRUBLER was excellent on the contrary. Picrate of ammonium can easily be prepared. For instance 9 gr. picric-acid are dissolved in 100 cc. alcohol of circa 96 pCt. adding 15 cc. ammonia and evaporating on the thermostate at about 60°.

[6]) In winter crystals are formed in the 1 pCt. solution of picrate of ammonium. To prevent the stain from crystallising in winter, it can be diluted with half the quantity of distilled water.

The solution was not neutral however, although both ingredients were perfectly dry. Although they were dried for a whole week in a temperature of 45°, a moist red litmuspaper hung in the bottle above the liquid, turned blue after some time. Probably free ammonia clung to the dry powders. To get rid of it a solution was boiled in a glass receiver for some time, until a red litmuspaper did not turn blue in the vapour. This was the case after boiling from a quarter to half an hour. After cooling down, the liquid looked slightly unclear, which was easily amended by filtering. The loss of volume was restored with distilled water.

The tincture was now ready; to keep it free of mould, it was needful to add an antiseptic; 1 pCt. chloral, recommended by HOYER, proved efficient.

This pikrocarmine [1]) is practically neutral, for a moist red litmuspaper hung in the closed bottle above the surface of the liquid, was not yet tinged blue after four months.

The tincture [2]) contains $^1/_2$ pCt. ammoniumcarmine and 1 pCt. picrate of ammonium, for the loss of weight in consequence of the unclearness after boiling, is so insignificant, that it cannot be taken into consideration. The preparation is finished in two days; should time be short it could even be done in one day; in which case the drying is left undone, and after a preliminary experiment, the quantity can be calculated which must be taken from the moist precipitate. The insignificant quantity of alcohol, which it contains, is of no consequence, and is moreover dispersed by boiling.

A difficulty with this method is that an *old* carmine solution in ammonia must be used. Mine was two years old. Fresh solutions, and such as well which were half a year old, produced, instead of a black, a more or less clear red ammoniumcarmine powder, which was only partly and unclearly soluble in water.

The carmine-solution must therefore "ripen", how long, I have not been able to ascertain; but two years is not too much. The question now is wherein this ripening consists and if it cannot take place at once. It is well-known that a ripe solution (from which the superfluous ammonia has been allowed to evaporate as much as possible), stains the tissues better than a fresh one, and this is very generally — amongst others by GIERKE [3]) — ascribed to the for-

[1]) To be obtained at the address of Dr. G. GRÜBLER, Leipzig.

[2]) One drop of the tincture on the filtering-paper gives, after being dried, a brown-yellow stain with red edge. This edge is much broader with the boiled, than with the unboiled liquid.

[3]) H. GIERKE, Färberei zu mikroskopischen Zwecken, 1885, p. 14 and 15.

mation of ammonium-carbonate, the carbonic acid being resorbed from the air. There is no doubt that this resorption takes place; and expecting that the salt mentioned might cause the ripening, I added 10 pCt. carbonate of ammonium to the solution. However without the desired result, even after the solution was several months old.

I then took into consideration whether the carmine might perhaps resorb oxygen from the air, and would need to be oxygenated; and this proved to be case.

When putting together:

10 gr. carmine powder,

10 cc. ammonia,

20 cc. hydrogenperoxyd,

the mixture boiled for a short time in a glass receiver, then cooled down (for instance by letting the receiver float in water in a half-filled cylinderglass), a ripe carmine solution is obtained in a few minutes, which, treated in the above-mentioned way, produces fully 9 gr. [1]) of an almost black ammoniumcarmine, which is entirely, sometimes a little unclearly, soluble in water.

Instead of boiling with hydrogen-peroxyd an equal quantity of a 1 pCt. solution of kaliumpermanganate can be taken as well, although in this case the oxygenation is easily carried too far.

Physics. — "*The entropy of radiation*" (II). By J. D. VAN DER WAALS JR. (Communicated by Prof. H. G. VAN DE SANDE BAKHUYZEN).

IV.

Distribution of the vibrations of the molecules.

In the second part of his "Vorlesungen über Gastheorie" Prof. BOLTZMANN discusses the way in which the intramolecular energy is distributed over the different molecules. He finds that the chance

[1]) If *dry* carmine has been used. But carmine as it is sold, frequently contains 10 pCt. or more water, though it may seem to be dry.