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Physiology. — “*The decoloration of fuchsin-solutions by amorphous carbon.*” By Dr. A. B. DROOGLEEVER FORTUYN. (Communicated by Prof. Dr. J. BOEKE).

Pulverized amorphous carbon has the faculty of decoloring solutions in water of several dyes, and the general explanation of this phenomenon is to be found in the fact that these pigments are absorbed by the carbon.

FREUNDLICH and LOSEV (*Zeitschrift für physikalische Chemie*, Bd. 59, 1907) discovered, that for Crystallviolett and Neufuchsin of the Höchstler Farbwerke another explanation must be given. These two dyes are chlorides. If their solution in water is brought into contact with carbon, then the solution decolors. The dyes are however not absorbed as such, but they are decomposed into hydrochloric acid and color-base. The color-base is absorbed by the carbon, presumably in the form of a polymerisation product, which by means of alcohol can again be removed from the carbon. The hydrochloric acid remains behind in the fluid, and can be shown in the filtrate of the solution that has been decolorized by carbon by the opacity that occurs in it with silver-nitrate, and its acid reaction upon litmus.

In 1909 (*Zeitschr. f. Physik. Chemie*, Bd. 67) FREUNDLICH and NEUMANN wrote again about the absorption of Neufuchsin by carbon and corrected some inaccuracies in their former paper a.o. by remarking that the decolorized filtrate of Neufuchsin does not react acidly upon litmus. They proved that over 33% of the chlorine occurring in the Neufuchsin remains behind in the filtrate decolorized by carbon. They could not indicate with certainty the kation belonging to this anion Cl, but they are of opinion that these are partly H-ions, partly other ions resulting from inevitable contaminations of the carbon. According to them the thing absorbed by carbon from the Neufuchsin-solution may still be the color-base, but more probably it is a color-salt formed with contaminations of the carbon.

When repeating these experiments with “Crystallviolett” no deviations were found, but with “Neufuchsin” I observed a phenomenon being not in accordance with the view entertained by FREUNDLICH and his cooperators about the decoloration of solutions of this dye by carbon. This phenomenon consists in the fact, that a watery solution of $\frac{1}{100}$ % Neufuchsin decolorized by carbon and filtrated from the carbon resumes its color for a great deal when it has been standing for a considerable time.

Not only Neufuchsin, but likewise the “fuchsine” of KIPP, Fuchsin

of GRÜBLER and Magentaroth of GRÜBLER, all very similar if not identical dyes, behave in the same manner.

This phenomenon is most easily, and in the shortest time observed, when so little carbon is added to the fuchsin-solution, that the color does not totally disappear, but a light-pink tinge remains. To control the change of the color a fuchsin-solution can be used that has been so far diluted, that, to the eye, it corresponds with the nearly decolorated solution. It will be seen that after the filtrage of the carbon, for which operation I used filters of SCHLEICHER and SCHÜLL, the color of the fluid becomes very distinctly deeper. That, after all, the carbon had really acted absorbingly, is proved by the deeply staining of aethylalcohol 96%, if the filtrated carbon is thrown into it. If we take carbon to an excess, it may easily occur, that the color is not seen returning in the decolorated solution, presumably because the concentration of the newly formed dye is insufficient.

The carbon originally used by me was gross-grained char-coal, which had not been carefully purified. Consequently the phenomenon could be attributed also to contaminations of the carbon. Therefore I purified the carbon according to the method likewise applied by FREUNDLICH and LOSEV by boiling it three times with 25% HCl and washing it with distilled water. Even after very long washing all the hydrochloric acid had not yet been removed, and carbon used in this condition prevented the return of the color of the decolorated fuchsin. But by adding ammonia to the carbon to which hydrochloric acid had been applied, and after washing it again, I could obtain carbon, the extract of which with distilled water contained no longer a vestige of chlorine. Only this carbon could be considered applicable to my purpose and with this carbon purified by me the phenomenon was regularly observed.

The fact that no heterogeneous substances could be the cause of the return of the color, was further confirmed by what follows. I obtained some samples of carbon from the „Kon. Pharmaceutische Handelsvereeniging” Amsterdam. If one washes one of these Carbo animal. depur. humida with distilled water, the filtrate reacts strongly acidly and it contains much chlorine. When this carbon was applied the phenomenon did not occur. Neither when Carbo sanguinis was used, the watery extract of which contained likewise chlorine but was alkaline. On the contrary the extract with water of Carbo ligni tiliae pulvis B. 50 as prescribed in the Dutch Pharmacopy Ed. IV was neutral, and no precipitation with silver-nitrate could be obtained. This carbon, which consequently can be regarded as sufficiently pure for my purpose admits the return of the color in an almost decolorated

fuchsin-solution. It is only not so handsome in its application as gross-grained carbon, because it is inclined to pass through the filter, and easily too much of it is added to the fuchsin-solution. If one adds carefully so little carbon, that the decoloration takes place slowly, e. g. in the course of a day, then one obtains here also easily a light-pink filtrate, which after some time becomes dark-red again.

Now the question rises how the return of the color in the almost wholly decolored fuchsin-solution can be explained. It is not for me to answer this question. This will have to be done by chemico-physical methods by a person who is sufficiently conversant with the theory of carbon-absorption. As an histologist I can do no more than publish the fact I have discovered, hoping that somebody else will further investigate its nature. Yet I have tried to find for myself an explanation of the case, and have come to a working hypothesis, which after all proved to be untenable, but made me discover some other facts that may have importance for the explanation I tried to find.

It was supposed, that in the almost decolored fuchsin-solution besides chlorine-ions or hydrochloric acid also uncolored dye kations or color-base would occur and this even in so great a quantity, that they must partly reconstruct the dye, causing likewise the color partly to return. This cannot be a pure ion-reaction for ion-reactions have a quick process, and the color returns only slowly, but in the color-base an alteration of structure may have taken place, a phenomenon of which examples are known.

Is it now possible to ascertain, that in an almost decolored fuchsin-solution more hydrochlorid acid and color-base occur than in an equally stained diluted fuchsin-solution which has never been in contact with carbon? Apparently it is.

Silver-nitrate occasions in the almost decolored fuchsin-solution a distinct opacity, but does not do so in the as deeply stained diluted solution. Consequently there are in the former case more Cl-ions than in the latter. In fact this is in conformity with what FREUNDLICH and Losev discovered.

Adding a few drops of acetic acid causes the color to return quickly and intensely in the almost decolored fuchsin-solution, whereas an as deeply stained diluted solution does not change its color by it. So, perhaps the acetic acid enables the color-base in the decolored solution to form very quickly a colored salt, for which there is of course no opportunity in the diluted solution.

I did not meet the phenomenon offered by fuchsin again in "Crystall-

violett" nor in any other dye experimented upon in this respect. Only „Säure-fuchsin", a dye which is no chloride and deviates considerably in composition from fuchsin showed something like it. It does not make any difference whether Säurefuchsin or Rubin S of GRÜBLER or Saurefuchsin S.M.P. of the Actien-Gesellschaft für Anilinfabrikation of Berlin is used for this purpose. I have never been able to state with certainty whether in a $\frac{1}{50}$ % Säure-fuchsin-solution being almost entirely decolorized the color partly returns after the filtration of the carbon; But I have experienced, that in the almost decolorized solution after the filtration and even after the lapse of some weeks the color can suddenly and very intensely be reproduced by acetic acid. It must be taken in consideration in this case, that acetic acid stains likewise a diluted Säure-fuchsin-solution which has never been in contact with carbon, somewhat more deeply, but by far not so much as the solution almost decolorized by carbon.

I desist from suggesting an hypothesis for the explanation of the last mentioned phenomenon, and only hope, that the nature of what I have communicated here may, at some time or other, be explained and increase our knowledge of the theory of histological staining methods.

Physiology. — "*Phagocytes and respiratory centre.*"

Their behaviour when acted upon by oxygen, carbonic acid, and fat-dissolving substances. Explanation of the excitement-stage in narcosis."¹⁾ By Prof. H. J. HAMBURGER.

(Communicated in the meeting of March 27, 1915).

Introduction.

In a former paper it was shown that Iodoform, even in extremely slight quantities can accelerate phagocytosis, to a considerable extent²⁾. We explained this action by assuming that this substance, after being dissolved in the lipid surface, softens the cells, thus facilitating the amoeboid motion.

If this view were correct, it might be expected that other substances which are soluble in lipoids, would act in the same way. This was indeed the case, without a single exception, with all the substances investigated, only not, as we found afterwards, with carbon sulphide. But in chloroform, chloralhydrate, ethylalcohol³⁾, butyric acid, propionic acid³⁾, benzole, turpentine, camphor, Peruvian balsam³⁾ (cinnamic

¹⁾ A detailed account will appear in the Internationale Zeitschrift für physikalisch-chemische Biologie. (ENGELMANN, Leipzig).

²⁾ H. J. HAMBURGER, J. DE HAAN and F. BUBANOVIC, These Proceedings, March 25, 1911.

³⁾ H. J. HAMBURGER and J. DE HAAN, Ibid, October 28, 1911.