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Colored Flocculation Reactions.*

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All flocculation reactions are based upon colloidal chemical processes. What is more natural than to introduce into these reactions dye colloids for the purpose of making the flocculation more distinct, firstly by coloring the flocculi and secondly by making them more voluminous by the joining of the dye colloids in the flocculation of the extract.

Experiments of this kind have been made frequently, but have been only partially successful.

Experiments were reported[1] in which the extract colloid could be colored, whereas a contrast coloration was hardly possible of attainment. These experiments were carried out on the Hecht Flocculation Reaction (H. F. R.), on the Sachs-Georgi-Reaction (S. G. R.), and the typhoid reaction, using the Flicker Diagnostikum.

The experiments since then, carried out with well over 100 of the most diversified dyes, led to a provisional result as follows:

The goal of these studies was the development of a flocculation reaction in which a part of the coloring matter would join with the flocculated extract so that in the case of a positive reaction the fluid remaining after the flocculation of the extract would be colored differently than the fluid in a negative reaction (with the extract not flocculated).

Experiments of this sort were done particularly with the Hecht-Müller-Ballung reaction but show in their results that they may be employed in all flocculation reactions for syphilis which are done with alcoholic extracts without addition of Balsam (Sachs-Georgi-Reaction, Kahn Reaction, Kline Reaction, Hinton Reaction, etc.).

The principle of the staining consists in the following: 1. The extract is electively stained by a dye which does not leave any color in the fluid remaining after the precipitation of the extract colloid. 2. As counterstain of the salt solution (the extract-free fluid), dyes must be used which leave the extract colloid unstained or which color it but slightly. Among numerous lipoid stains, the only one of value was Sudan 3 of the I. G. Farbenindustrie.

The stain concentration of the extract is determined by a simple

* Extract of report May 11, 1933, at the Alfred Fournier Institute in Paris.

1 Hecht, *Med. Klinik*, 1922, **14**.

titration. In a wide-mouthed bottle that has been filled with the extract referred to, a tip of the knife full of Sudan red is added, the mixture well shaken and allowed to settle (it may also be centrifuged). A *saturated* stained extract results. Then with some positive and negative sera, the desired reaction, in our case the Hecht-Müller Ballung Reaction with the routine technic is done, using the undiluted colored extract and also dilutions of colored and uncolored extract in various proportions as follows:

	cc.	cc.	cc.	cc.
Colored Extract	5	4	3	2
Uncolored Extract	1	1	1	1

A simple orientation experiment following the outline above will show the dilution in which *just that amount of dye is present in the extract that will be taken up completely in the balls of the positive test* so that the fluid is perfectly or almost perfectly clear, whereas in non-flocculated extract (negative reaction) a reddish, definitely different fluid is observable.

For counterstaining one may use dyes of many kinds, which must not be fat soluble. I recommend as stock solutions Löffler's methylene blue, 1% watery victoria blue solution, ½% watery naphthol green solution, etc. Here also the following principle applies: *The colored salt solution should contain only that much counterstain which, on mixture with the colored extract, does not permit recognition of its own color.* In a positive reaction, the red colored extract colloid is concentrated in the balls, and the counterstain is clearly visible whereas in the negative reaction a very indefinite mixed color of the turbid fluid is observed.

Examples. The cholesterin-free extract with which these experiments were done has its optimum action in a mixture of 3 cc. saturated stained extract and 1 cc. of unstained extract.

As counterstains, the following are satisfactory: 10% Löffler's methylene blue, (orig. alk.) 0.20 cc. to 20 cc. salt solution; 0.1% victoria blue, 0.1 cc. to 10 cc. salt solution; ½% naphthol green, 0.2 cc. to 30 cc. salt solution.

One can also prepare a combination of several counterstains giving a very good background for the scarlet red ball that forms with positive serum. The above mentioned stains give a good combination in the following mixture: Methylene blue, 1 drop to 40 cc., 3 parts; 1% victoria blue, 1 drop to 35 cc., 3 parts; ½% naphthol green, 1 drop to 15 cc., 4 parts.

Technique of the colored Hecht-Müller Ballung Reaction (H. M. F. B. R.). Performance of the colored Ballung Reaction:

Stable stock solutions: 1. 10% salt solution. 2. Slightly alkaline salt solution: NaCl, 0.9; NaOH, 0.1; Aqua destill ad 100.

3. Stains: Löffler's methylene blue, ½% naphthol green, 1% watery victoria blue solutions are stable. From these stains one prepares the proper quantity before each experiment. 10% methylene blue, 0.1 cc. to 30 cc. salt solution; 0.1% victoria blue, 0.1 cc. to 10 cc. salt solution; ½% naphthol green, 0.20 cc. to 30 cc. salt solution.

Every day the required salt solution is *freshly* prepared from stock solutions 1 and 2. 8 cc. of 10% salt solution, add 0.8 cc. of alkalinized salt solution, make up to 100 cc. with distilled water. 0.25 cc. of inactivated serum is employed.

The ready made colored extract which may be procured from the author is diluted as follows:† 1 cc. of extract and 0.55 cc. fresh salt solution as prescribed above are whirled about several times in a small wide-mouthed bottle. After a ripening period of 10 minutes, 0.6 cc. salt solution are added thereto and mixed again by whirling about. 0.15 cc. of this extract dilution is promptly added to the previously prepared serum. The rack is then shaken vigorously a number of times and then 1.5 cc. of the properly selected counterstain salt solution mixture is added. Further shaking is not necessary.

The rack is placed in a water bath at 55 or 56° and is removed after 10 minutes. One half hour later the first reading is made. One and a half hours later, the final one is made.

The reading of the results is very easy. When naphthol green has been employed as the counterstain, the strongly positive serum shows a large bright red ball floating in clear green fluid. The ball gradually sinks to the bottom in the following hours. The negative sera have a cloudy reddish color.

Appearance of small red colored flocks, especially more than 1½ hours after the conclusion of the experiment is not to be considered as indicating a positive test.

Even 12 hours later, the difference between positive and negative tests is still readily observable.

† This extract, a description of which was published in 1921, is prepared as follows:

To a weighed amount of powdered, dried fat-free beef heart add twice the weight of ether. Extract once during a short period. Evaporate to dryness and weigh the residue. Add 5 times the weight of 96% alcohol and place this for 48 hours in an incubator at 56° C. Cool the extract and filter.

When Löffler's methylene blue solution is used, the strongly positive sera are characterized as described above by a bright red ball and this is observed floating in clear blue-green fluid. The negative sera are cloudy, reddish.

With victoria blue as a counterstain, a large violet ball forms in positive serum and floats in clear blue fluid. The negative sera are cloudy, violet.

If it is desired to intensify the colors, what was said in the introduction must be borne in mind. The intensification must be made in such a fashion that both the red color of the extract and the counterstain of the salt solution are intensified in a certain proportion. If more red colored extract is used, more serum must be employed and more salt solution to make up the mixture to the proper quantity.

Accordingly, if instead of the standard quantity of diluted extract, a larger amount is used, for example 0.2 cc., then 0.35 cc. of serum is employed and the counterstain is intensified. In such case one takes of stock solution of naphthol green, 0.25 cc. to 30 cc. salt solution, of methylene blue 0.10 to 21.5 cc. salt solution, and of victoria blue solution 0.1 to 75 cc. salt solution.

Anyone who has worked for some time with colored extracts will never care to work otherwise, since the readings with them are easy and simple.

Summary. A method is suggested for all flocculation reactions, especially for the Hecht-Müller Ballung Reaction of elective staining of the extract colloid which brings about a distinctive color effect that facilitates the reading of results without impairing their specificity. Readings are best made against a white background (white paper). Positively reacting sera show a bright red ball, and after it is formed the chosen counterstain, blue, violet, or green appears in the surrounding fluid. Negative sera show a turbid light violet to greenish-blue color, permitting an immediate differentiation.