

## A Study on Some Problems in PFAAB-PAS-OG Staining Method

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It is well known that the performic acid alcian blue-periodic acid Schiff-orange G (PFAAB-PAS-OG) staining method has made it possible to identify the subdivided cell groups of basophils of the human adenohypophysis. Adams and Swettenham(1958), applying the PFAAB-PAS-OG reaction, showed that the granules of the mucoid cells in human pituitary gland could be divided into two types, designated S and R, on a firm histochemical basis.

Using the performic acid as an oxidant, tissue sections frequently come off the slides and it requires a necessity to make the sections fix on a slide during and after immersion in this strong acid solution.

In our laboratory, technical improvement carried out to solve the above problems and obtained good results of staining.

### MATERIALS AND METHODS

Anterior lobe of the human pituitary gland was obtained from autopsy material and let stand overnight in 10% formalin solution. Paraffin sections of  $5\mu$  in thickness was fixed on the slide with the aid of Mayer's albumin as does in routine histological laboratory.

#### Procedures:

1. Deparaffinize in xylene and bath in three successive absolute alcohols.
2. Three minutes in ether-alcohol (one part

of ethyl ether and one part of absolute ethanol).

3. Dip the slide in 2% celloidin and let the slide stand vertically to drain off the excess celloidin solution.

4. Wipe backside of the slide with gauze before drying.

5. Dry slide by setting for 3 to 5 minutes in the drying oven at  $60^{\circ}\text{C}$ .

6. Bring the slide to distilled water through the graded alcohol series.

7. Immerse in performic acid for 5 minutes.

8. Rinse in running tap water for 20 minutes.

9. Rinse in 70% alcohol and bring the slide finally to the absolute alcohol.

10. Bring the slide to ether-alcohol to remove celloidin coating.

11. After removal of film, bring back the slide to hydrate through the alcohol series.

12. Blot the section carefully.

13. Reacted by alcian blue, followed by PAS and orange G sequence according to the ordinary method.

14. Dehydrate, clear in xylene and mount.

Some slides were skipped the step 9, 10, 11, and 12, and put into alcian blue directly from step 8 without removing the celloidin film over the section.

### RESULT

The sections overlaid with celloidin film were remained on the slide during and after

the treatment in performic acid. Even after the soaking in vigorous running tap water, sections never loosed from the slide.

By applying the ordinary staining reaction of PFAAB-PAS-OG to celloidin removed sections, PAS-positive granules and alcian blue-positive granules were stained as magenta and blue respectively.

Since the alcian blue stained deeply both the tissue section and the celloidin coat, the identification of the alcian blue-positive cells from the others were difficult because of its worse contrast in celloidin coated slides.

### SUMMARY AND DISCUSSION

The most critical and important step in PFAAB-PAS-OG staining method is the oxidation in the strong acidic solution. Although alternative oxidants such as peracetic acids, periodic acids and acid permanganate could be employed instead of performic acid, many investigators prefer the latter because of its better staining results. Since performic acid is made of formic acid, conc. sulfuric acid and hydrogen peroxide, it makes the tissue swollen unfortunately and finally let the sections come off the slide.

Celloidin, which have been used as an embedding media, have good physical and chemical character in many respects. One of them is the fact that celloidin passes staining dyes and other chemical solutions easily without disturbing the effect of the solutions.

In our experiments, some mucoid cells were oxidized across the celloidin film and appeared as alcian blue stained blue granules, while the remains which were intact to the oxidants and

which contains either no cystine or an insufficient amount of cystine to give a positive alcian blue reaction appeared as PAS-positive magenta granules. With the alcian blue stain, celloidin has been colored as deep blue and the color was remained even after 24 hours rinsing in the running tap water, or in the acidic solutions.

To overcome the difficulties of oxidation step in PFAAB-PAS-OG reaction, tissue sections were coated with celloidin film before immersing in acidic solution and obtained good staining results.

—국문초록—

### PFAAB-PAS-OG 염색반응의 산화과정에 따르는 문제점

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PFAAB-PAS-OG 염색반응이 사람의 뇌하수체 전엽의 염기호성세포를 R세포와 S세포로 세분하여 구분염색할 수 있는 확실한 조직화학적 근거를 가지고 있어 널리 적용되고 있으나 반응과정중 강력한 산성용액으로 산화를 시킴에 따라 흔히 조직 절편이 유리에서 떨어져 나가는 어려움이 있고 원법에 따른 alcohol 처리등으로 만족할만한 결과를 얻기가 어렵다.

저자들의 실험실에서는 이러한 문제점을 해결하기 위하여 paraffine 제거후 산화과정에 들어가기에 앞서 2% celloidin의 얇은 막을 입혀 굳힌후 산화를 시키고 그 후 다시 ether-alcohol로 celloidin 막을 제거한뒤 alcian blue와 PAS-OG 염색반응을 거침바 매우 효과있는 결과를 얻었기에 이를 보고한다.

### REFERENCE

1. Adams, C.W.M. and Swettenham, K.V.: *The histochemical identification of two types of basophil cell in the normal human adenohypophysis. J. Path. Bact.* 75:95, 1958