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HISTOSPECTROPHOTOMETRY OF THE NEUROSECRETORY SUBSTANCE IN THE POSTERIOR LOBE OF THE HYPOPHYSIS

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Summary. The present paper describes a method which makes it possible to use the paraldehyde technique for the photometric determination of the neurosecretion in the posterior lobe of the hypophysis.

To characterize the functional condition of the hypothalamo-hypophysial neurosecretory system it is necessary to use precise methods of determination of the amount of neurosecretory substance (NSS) in the different parts of the system, and first of all in the posterior lobe of the hypophysis which is known to release the bulk of the neurohormones contained in the neurosecretory substance.

As far as we know there are only five publications in which the authors suggest the photometry of the NSS in the posterior lobe of the hypophysis stained with Gomori's chrom-alum-haematoxylin technique (Gaunt et al., 1957; De Groot and Hartfield, 1961), with Alcian blue (Sloper and King, 1963), or with paraldehydefuchsin (PAF) (Rinne and Sonninen, 1964; Ochonskaya, 1964). All the methods mentioned, and especially the PAF-technique, have in common, that it is not yet definitely known whether they are suitable for quantitative tests. Besides, monochromatic light was used only in the work with Alcian blue (Sloper and King, 1963) while in all other investigations measurements were made in white light which causes considerable errors.

The advantage of our photometric methods is their great precision which allows to measure the slightest change in the amount of NSS in the posterior lobe of the hypophysis; moreover, they are less laborious.

Hypophyses of white rats, white mice, cats, Guinea pigs, and oxen served as test-material. The method is obviously applicable to all the animals which have neurosecretory substance compactly arranged in the posterior lobe of the hypophysis.

The hypophyses were fixed in Bouin's fluid or in 10% neutral formalin. The tissues were embedded in paraffin in the usual way.

Staining of sections with Gomori-Gabe's paraldehyde fuchsin technique without counterstaining:

1. Deparaffining (sections fixed in Bouin's fluid should be decolourized by keeping them in 70° alcohol for 15—30 minutes or even longer).

2. Oxidation with a mixture of 0,3% $KMnO_4$ solution and 0,3% H_2SO_4 solution in equal proportions for 3—4 minutes.

3. Reduction by using a 2% solution of potassium bisulfite or potassium metabisulfite, natrium bisulfite or natrium metabisulfite up to the complete decolourization of the sections (1—2 minutes).

4. Washing in running water for 10—30 min. (but not more than 3 hours).

5. Staining with working PAF solution for 2—6 min (at a temperature of about $+5^0$ C to 0^0 C).

6. Washing with acidified 100° alcohol for 30 seconds — 2 min (but not more than 90 min) till the stain, not bound to Gomori-positive structures, is removed (up to the maximum

decolourization of the background). Care should be taken to prevent the preparation from drying after it has been taken out of the PAF solution.

7. Dehydration with 96—100° alcohol for 5—10 min.
8. Xylol for 5—10 min.
9. Mounting with Canada balsam.

Measurements of the Optical Density of the Stained NSS.

1. Method of Photographic Photometry. a) Photographing of stained preparations in monochromatic light (λ 546 mμ — corresponds to the region of the maximum absorption of the stained preparation) with the microscope having a monochromator (MUF-6).

b) Photometry of negatives by scanning technique with the microphotometer MF-4. The staining of the intermediate lobe of the hypophysis is taken as the value of non-specific background-staining.

2. The Method of Photoelectric Photometry in monochromatic light (λ=610 mμ) which corresponds to the region of the minimum absorption of PAF stained preparations. Measurements of small densities ($E<0{,}1$) with light of one wave length result in a negligible error (Brodsky, 1955). If $E>0{,}1$, measurements should be made with light of two wave lengths.

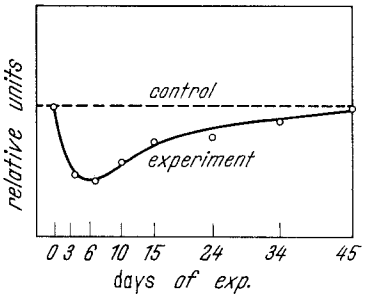

Fig. 1. Changes in the amount of NSS in the posterior lobe of the hypophysis in the process of chronic salt loading Measurements made by means of the photoelectric method

The correlation of the data of the two methods for different values of densities is constant; but the photoelectric method is less laborious and therefore preferable.

As staining with PAF was shown by many authors (Gabe, 1955; Okamoto, 1956; Pearse, 1960) to be a histochemical reaction on cystine, this technique is non-specific for the neurosecretory substance and reveals also elastic elements in vessels of the neurohypophysis. It is known that NSS is not found in preparations made from material fixed in Carnoy's fluid. Such preparations show, however, that in the posterior lobe of the hypophysis there are either no vessels which contain elastic membranes (white mice) or that their number is so small (but relatively constant) that they can be ignored.

The same preparations show that the staining density of the tissue of the posterior lobe not containing NSS corresponds to that of the intermediate lobe which allows to take the degree of the staining of the intermediate lobe as the background-staining.

Stoichiometry of the staining can be proved by increasing the thickness of sections of the hypophysis containing uniformly distributed neurosecretory substance, the optical density being increased respectively (4 μ — 0,071; 6 μ — 0,094; 8 μ — 0,133; 10 μ — 0,152; 12 μ — 0,176; 14 μ — 0,208).

Changing the duration of various stages of staining with PAF within relatively wide ranges does not influence its quantitative aspect. Nevertheless, uniform staining conditions should be observed (especially concerning the time of

exposure to PAF). Besides, the staining of all the preparations to be compared should be done in the same batch of PAF solution.

As an example for the use of the suggested method, a curve is given which showes the changes in the amount of NSS in the posterior lobe of the hypophysis in white mice subjected to chronic salt loading with 5% NaCl solution (KRASNOVSKAYA and POLENOV, 1961) (Fig. 1).

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