6905

Quantitative Estimation of Ash after Microincineration.*

GORDON H. SCOTT. (Introduced by E. V. Cowdry.)

From the Department of Anatomy, Washington University School of Medicine.

One of the things to be desired in the study of microincinerated sections is an accurate means of estimating the relative quantities of inorganic salts in various cells. Such a method would permit a quantitative comparison of tissues taken from animals subjected to experimental procedures with similar normal cells. Furthermore it would be possible to study such processes as normal growth and differentiation in a more exact manner than the microincineration technique has permitted. A quantitative method also has many advantages in the examination of pathologic tissues and comparable normal ones. The technique for quantitative photography of incinerated sections devised by Schultz-Brauns[1] has many points to commend it; but it is, in the final analysis, a procedure which depends on the visual judgment of the observer. It seemed advisable, therefore, to develop a method which would be free from this objection.

The nature and distribution of the inorganic salts in cells and tissues has been described.[2, 3] The appearance of the ashed preparations in darkfield illumination is that of hoar frost on a blackened surface. With this means of illumination only the ash of the section is revealed by light reflected from the surfaces of the individual particles of mineral. It is assumed, therefore, as the particles are approximately the same size and nature, that the more mineral present in the microscopic field the greater the quantity of light reflected into the eye of the observer. This assumption has been checked, in as far as possible, by examining colloidal solutions at different dilutions with the darkfield microscope with the result that given a constant source of light the intensity of the beam emerging from the ocular is roughly proportional to the number of particles in the microscopic field. Inspection of many hundreds of incinerated slides has shown the same to hold for the ash. Conse-

* Aided by an appropriation from a grant made by the Rockefeller Foundation to Washington University for research in science.

1 Schultz-Brauns, O., *Z. f. wissensch. Mikr.*, 1931, **48**, 161.

2 Scott, G. H., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 48.

3 Scott, G. H., *Am. J. Anat.*, in press.

quently a measurement of the intensity of the light reflected from the ash provides a means of estimating its quantity.

With the aid of Doctors L. A. DuBridge and L. Van Atta of the Department of Physics a photoelectric cell and a suitable amplifying circuit was devised for the particular condition required. Since the amount of light visible in any darkfield preparation is comparatively small the current generated by the photoelectric cell had to be increased about 40,000 times by the amplifier before it could be measured with a sensitive galvanometer. The details of the apparatus will be given in a forthcoming publication.

The procedure for the estimation of ash is as follows: Sections of standard thickness, 4 microns, are incinerated and prepared for examination in the routine manner. The areas selected for measurement are located in the darkfield and the microscope is placed in a specially built photomicrographic apparatus and the field focussed sharply on the ground glass. The particular part of the field desired is located in the center of the ground glass and the remaining portion covered with black paper impervious to light. When this has been done the ground glass is replaced with clear glass and the light coming through the aperture of the paper covering is focussed by means of a plano-convex lens onto the sensitive plate of the photoelectric cell. The amount of current generated is then determined by a galvanometer reading. Thus the ash residue of any particular set of tissues or cells can be compared with that of another group.

Thus far the method does not permit an expression of the quantity of residue in terms of grams of any one substance or even of total ash. However, it does give a measure of the salt present on a relative basis. For example, it has been possible to compare on a percentage basis the amount of ash in incinerated sections of blood vessels taken from normal monkeys with those of monkeys which have been fed varying amounts of irradiated ergosterol. In this instance the normal is used as a base line and the experimental tissues recorded as a percentage of the normal. The large cells found in the nervous system are particularly adapted to study in this way. The salt changes in the intestinal epithelium during active absorption are also susceptible to investigation. The normal (fasting) intestine was used as 100% and the ash of the actively functioning epithelium measured as a percentage of it.