embryos near mature size and, by removing the meristems, thus making them incapable of germination, carry the scutelli on in growth of an embryonic type until maximum size. Since these cultured entities lack any organized meristems, they do not give positive evidence that if whole embryos could be held back in development and induced to continue embryonic growth they would eventually be able to produce mature plants of abnormally large size, but they do at least suggest this possibility.

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Manuscript received November 7, 1951.

Perchloric Acid Extraction of Ribose Nucleic Acid from Cytological Preparations

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Ogur and Rosen (*1, 2*) have reported a method for differential extraction of ribose and desoxyribose nucleic acids from tissues. They find that treatment of tissues with 10% perchloric acid at 4° C for about 18 hr will selectively extract ribose nucleic acid. Desoxyribose nucleic acid may then be removed by treating with 5% perchloric acid at 70° C for 20 min. Cytochemical adaptations of this technique have also been reported by Erickson, Sax, and Ogur (*3*) in plant material and by Seschachar and Flick (*4*) in protozoan material. These authors found that cold perchloric acid treatment does in effect remove the basophilia due to ribose nucleic acid from the cytoplasm and from nucleoli. Koenig (*5*), however, found that a somewhat higher temperature is necessary to remove basophilia from cytoplasm and nucleoli of formalin-fixed mammalian tissues, which was also found to be true by the author (unpublished) for Carnoy-fixed mammalian material. Sulkin and Kuntz (*6*), working with Zenker-fixed mammalian tissues, reported that cold perchloric acid treatment has no effect on deparaffinized sections. They did find that when the cold perchloric acid is allowed to act on the fixed tissue before it is embedded, or if it is allowed to act on frozen sections of the fixed material, this method can be used as a substitute for ribonuclease digestion in the removal of ribose nucleic acid from cytological preparations. In this laboratory perchloric acid extraction of ribose nucleic acid from cytological preparations and its effect on desoxyribose nucleic acid during this time were studied photometrically. Results of ultraviolet absorption studies and of absorption studies of Feulgen-stained sections are herein presented.

For these studies, rat pancreas was fixed in Carnoy's acetic alcohol (1:3), sectioned at 3 μ, and all slides were prepared from the same ribbon.

The method of photometric analysis employed throughout the experiment has been described in detail by several authors (*7–11*). The apparatus used is essentially the same as the one described by Pollister and Moses (*12*).

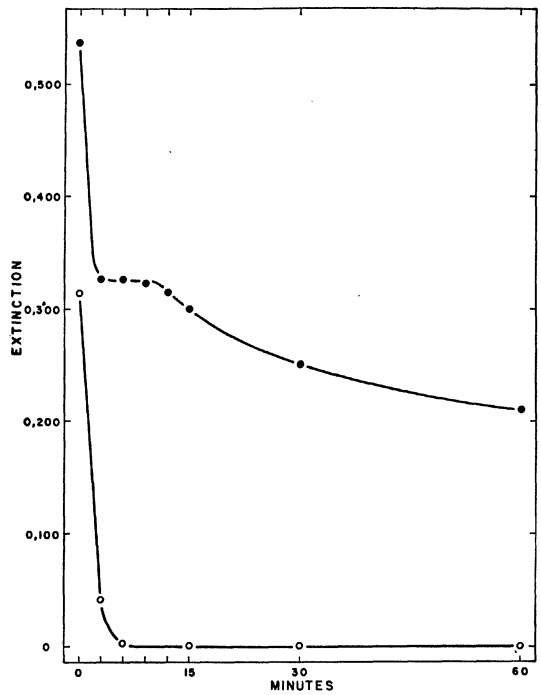

Fig. 1. Curves showing the relationship between ultraviolet extinction and duration of perchloric acid treatment: ●—●, curve for nuclei; ○—○, curve for cytoplasmic cores. Each point represents a mean extinction value of 15–25 measurements.

The natural ultraviolet absorption of nuclei having a diameter of about 6 μ, and of cores of cytoplasm 4 μ in diameter, was measured in sections treated for varying periods of time with 10% perchloric acid at 25° C. In order to study the effect of the perchloric acid treatment on the desoxyribose nucleic acid, after the perchloric acid treatment, the sections were stained by means of the Feulgen reaction. Sections were hydrolyzed for 12 min at 60° C in 1 *N* hydrochloric acid (*8, 9*), stained for 1 hr in Schiff reagent, prepared according to the directions published by Stowell (*13*), and measurements were then made of the intensity of the Feulgen dye bound by the nuclei. In addition, photometric measurements were also made of Feulgen control slides which were placed in Schiff reagent together with the test slides. Except for hydrochloric acid hydrolysis, these were treated in the same manner as were the test slides.

A Photovolt photometer (Model 512) with an electron multiplier tube was used for measuring the ultraviolet absorption of nuclei and cytoplasmic cores. The light source for these measurements was the 2537 A

line, isolated from a Hanovia mercury lamp (Sc 2537 U-shaped lamp with polished quartz end window) by means of a Cooke double prism quartz monochromator. For measurements of absorption resulting from the combined Feulgen dye, the Photovolt photometer with the standard B search unit was employed. For these measurements a Farrand interference filter with a maximum transmission peak at 560 mμ was used to isolate the desired light from a Western Union J100 lamp.

Since the nuclei are about 6 μ in diameter, sections 3 μ thick were used in making absorption measurements, so that in the ultraviolet there would be no cytoplasmic material above and below the nucleus to obscure the absorption picture.

Results of photometric measurements of ultraviolet absorption after various periods of perchloric acid treatment are represented in Fig. 1. Each point has been corrected for nonspecific absorption (*7*), which was measured by means of a blank. The latter was prepared by treating the section with a 5% solution of trichloracetic acid for 15 min at 90° C, which, according to Schneider (*14*), removes all nucleic acids. For nuclei this was found to be an extinction value of 0.149 ± 0.004, and 0.221 ± 0.005 was the value measured for cytoplasmic cores. Fig. 1 shows that very early in the treatment there is a sharp decline in both nuclear and cytoplasmic ultraviolet absorption, which is due to the extraction of nucleic acid or of nucleic acid fragments. In the cytoplasm this decline approaches zero after about 6 min of treatment. In the nucleus the drop in extinction appears to level off somewhat sooner; it remains at this level for a short period of time and resumes its decline after 9 min.

In Fig. 2 are represented the results obtained when the Feulgen reaction was employed to study the effect of perchloric acid treatment on desoxyribose nucleic acid. There is no change in the intensity of the reaction during the early part of the treatment, at a time when the ultraviolet extinction of both the nucleus and the cytoplasm was shown to be greatly reduced. Beyond 9 min, however, the amount of Feulgen dye bound by the nuclei begins to diminish. No differences were found between the extinction value of nuclei of control sections which received no perchloric acid treatment, and nuclei of sections which were treated for 9 min. Since this color was found to transmit more than 97% of the light, no correction was deemed necessary for extinction values of Feulgen dye of test sections. Following treatment for more than 9 min, however, there is a gradual increase in the intensity of color produced in control sections.

Since it is generally accepted that desoxyribose nucleic acid is not found as a normal cytoplasmic component, one might assume that the observed decrease of cytoplasmic ultraviolet extinction is the result of extraction of ribose nucleic acid by the perchloric acid. The decrease in ultraviolet extinction of nuclei during this period of time, coupled with the fact that there is no change in the amount of Feulgen dye bound by these nuclei, appears to constitute evidence that, in the nucleus as well as in the cytoplasm, perchloric acid is extracting ribose nucleic acid without removing desoxyribose nucleic acid to any appreciable extent. Beyond the 9-min period of perchloric acid treatment, however, the ultraviolet absorption, after having leveled off, resumes its decline at a somewhat slower rate than that of the initial fall. This corresponds to the time when the curve of the Feulgen controls begins to show more and more color with increased time of treatment, which would suggest that these events may be explained on the basis of a hydrolysis of the desoxyribose nucleic acid similar to that of mild hydrochloric acid hydrolysis, which is used in the standard Feulgen procedure.

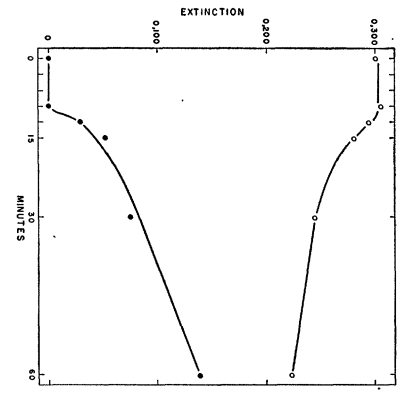

FIG. 2. Absorption measurements showing the relationship between duration of perchloric acid treatment and the amount of Feulgen dye bound by nuclei: ○—○, Feulgen sections: ●—●, control sections. Each point is a mean extinction value of 15–25 nuclei.

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Manuscript received October 23, 1951.