All the juvenile specimens that received testosterone propionate pellets also exhibited unusually long claws on the second, third, and fourth digits of the forefeet when sacrificed. This confirms a previous report (*5*) that male hormone induces hypertrophy of the second, third, and fourth foreclaws of juvenile slider turtles. Cagle (*3*), Conant (*6*), and Taylor (*7*) have indicated the manner in which the elongated foreclaws of the male slider are utilized in the preliminary phase of courtship. It has also been noted that at sexual maturity these foreclaws exceed in length those of the adult female by at least 2.5 times (*1*). It is thus evident that the middle foreclaws of the male slider, as well as the tail, represent secondary sexual characters.

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Microspectrophotometry and Cytochemical Analysis of Nucleic Acids

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The technique of microspectrophotometry, a recently developed method of cytochemical quantitative analysis whereby the spectral transmittance of a minute part of the cell nucleus is measured, can be improved from the photometrical point of view. Many workers (*1–5*), illuminate a large area of the microscopic tissue section, including the part to be measured, and select the light by a diaphragm placed at the image plane of the photomicrographic system. Owing mainly to internal reflections in the magnifying optical system, the light passing through the adjacent area of the minute part in the tissue section may cause a stray light that is added to the image of the part in question, so that the measured value of the transmittance may be enhanced. The effect, which is especially remarkable when the transmittance of the minute part is small compared with the outer illuminated area, is known in microdensitometry of photographic images of stars and spectral lines as the Schwarzschild-Villiger effect (*6,7*).

In order to reduce this effect, we have illuminated the minute part in question only, as is usually done in precision microdensitometry. Our illuminating optical system consists, as shown in Fig. 1, of a photomicrographic system that is the same as that used for magnifying, but with the reversed direction of light. An iris diaphragm of variable diameter (2–10 mm), which serves as the light source, is imaged with a reduction to 1/2000 by the system, and only a section 1–5 μ in diameter is illuminated. As ordinary microscopic condensers are not suitable for this purpose because the corrections of aberrations are insufficient, a Zeiss objective 1/12 (oil immersion) is employed. The problem of its short working distance is overcome by using a thin cover glass for the tissue section. The magnifying part of our optical system is the same as usual, with a second iris diaphragm placed at its image plane to cut out the remaining stray light, thus introducing to the photoelectric tube light from the minute part in question free from the Schwarzschild-Villiger effect. A second optical path from the light source to the phototube (Fig. 1) furnishes a check on the variation of the intensity of the light source during the measurement.

Our system has proved to be especially useful in the quantitative analysis of nucleic acids in a cell nucleus of spherical form. When the reduced image of the light source is formed at the center of a small transparent sphere, and when the dimension of the image is sufficiently small compared with the diameter of the sphere, then the length of the optical path of any beam of light passing through the sphere is the same as, and is equal to, the diameter of the sphere, being independent of the inclination angle of the beam to the optical axis, as shown in Fig. 2. Therefore, if the sphere is made up of a solution, we can determine exactly the concentration c of the solution by the formula

$$T = e^{-\varepsilon c(2r)}$$

by measuring the radius r and the transmittance T of the sphere by our system, provided that the extinction coefficient ε of the solution be known.

The following experiment has been made to exemplify the above theory. Various small spheres of 0.030 *M* aqueous solution of safranin, 2–30 μ in diameter, were prepared by mixing and agitating the solution with cedar oil; their transmittances for a monochromatic light (λ 546 mμ) have been measured, and their concentrations have been calculated, by use of the above formula. The calculated values for vari-

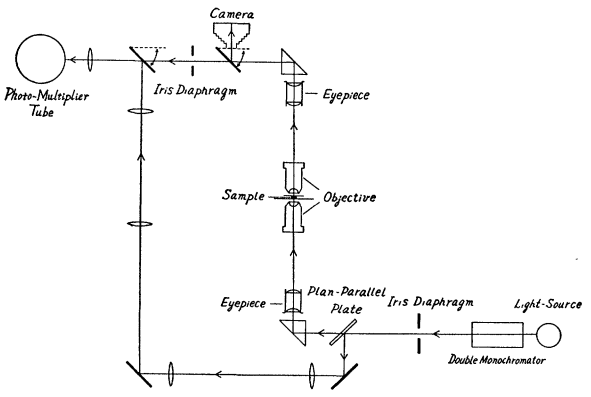

FIG. 1.

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ous spheres agree well with each other and are equal to the value of concentration of the solution before it is suspended, as shown in Table 1, provided the size of the illuminated area is smaller than one third the diameter of the sphere. The result offers proof of the correctness of our theory.

TABLE 1

Diam of sphere (μ)*	Transmittance (%)	Calc conc (*M*)†
2.2	95.4	0.009
3.0	86.6	.020
5.2	71.6	.028
6.0	65.7	.030
7.5	56.6	.031
9.7	52.2	.029
13.5	38.9	.031
22.4	21.1	0.030

* The diameter of the reduced image of the light source formed at the center of a sphere, 2 μ.

† Concentration of the solution before suspension, 0.030 *M*.

The circumstances are not so favorable in the case of nuclei of living cells, as their forms are not always spherical, nor are their contents always uniform. Nevertheless, it can be expected that our method would give better results than those hitherto reported if the specimens be suitably treated so that the nuclei approximate spherical forms.

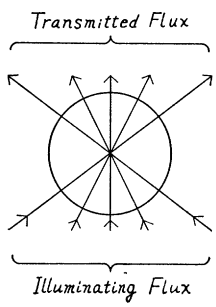

FIG. 2.

An experiment has been performed with this objective in mind. Nuclei from the liver cells of 4 rats were separated by citric acid, as described by Mirsky (*8*), and washed thoroughly with 30% sucrose solution. The nuclei were thus made approximately homogeneous and were then fixed in formalin and stained by the Feulgen reaction. Their DNA content was determined indirectly by measuring the total amount of the regenerated fuchsin—that is, by the measurement of spectral transmittance of each nucleus using our optical system. The result is shown in Fig. 3, in which the amount of DNA in arbitrary units is taken as the abscissa, and the numbers of nuclei containing DNA in the amount of 8.0–8.5, 8.5–9.0, 9.0–9.5 etc., in our unit are plotted as the ordinate.

It can be concluded from Fig. 3 that nuclei of the liver cells of rats are classifiable into at least three groups definitely distinguished from one another by the amount of DNA. The amount for each group shows an arithmetical progression, whereas the

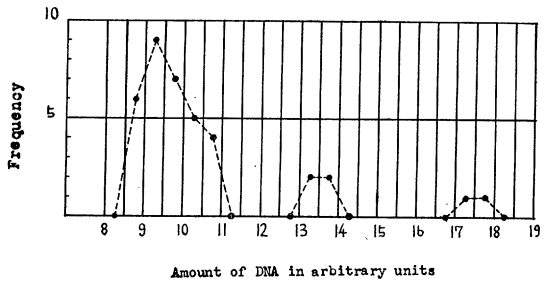

FIG. 3.

amounts found by Swift (*3*), and Lison and Pasteels (*5*) showed a geometrical progression. The relative error of our measurements is 2–3% (number of measurements: 20–32), which is to be compared with the error of measurements by Swift (*3*) and Lison and Pasteels (*5*)—i.e. 7–24% (number of measurements: 36–97). The improvement in accuracy may be attributed to our revised optical system in microspectrophotometry.

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A Medium for the Study of the Bacterial Oxidation of Ferrous Iron[1]

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The study of the bacterial oxidation of ferrous iron in acid mine waters has long been retarded for the want of a suitable synthetic medium.

Prior to the formulation of such an inorganic medium, acid mine waters were used as natural media for the cultivation of the autotrophic bacteria reported by Colmer and Hinkle (*1*), in 1947, and by Leathen and Madison (*2*), in 1949, to be responsible for the rapid oxidation of ferrous iron to the ferric state under acid conditions. Such "natural" media were prepared for use by sterilization, either by autoclaving or by filtration, dependent upon the chemical characteristics of the particular mine effluent.

There were many objections to using such media. The most outstanding were variability of chemical constituents and pH, difficulties of collection and transportation, and sterilization. The medium described here eliminates all these objections and has

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