the true optical density D of the object is: $D = \log (I_0 - F) - \log (I - F)$.

If the calculation is made in the usual way, taking no account of the flare light, a false value d of the optical density is obtained $d = \log I_0 - \log I$. In the graph, the values of the ratio d/D have been calculated at each apparent transmission value ($T = I/I_0$) for an amount of 2% flare light. It is obvious that the values of d are in any case too low, and that the error increases rapidly with the density of the object (Fig. 1).

When comparing the densities of different objects, the effective error of the ratio of their densities is much lower than the errors of the individual measurements. We have calculated the relative error committed when comparing an object of (apparent) transmission 90% with objects of different transmissions. Up to transmissions of 45%, the relative error is less than 1%; for objects of transmission 40, 30, 20, and 10%, the error is respectively 1.12, 1.82, 2.96, and 5.15%. In previous determinations made with our instrument, most of the transmission readings ranged between 85 and 25%; and readings less than 15% were encountered in few cases only. Under these conditions, the validity of the measurements does not appear to be enhanced by the S-V effect.

It is worth mentioning that other optical systems may give rise to much greater S-V effects. Two apochromatic oil immersion objectives 100×, N.A. 1.30 and 1.40 (not used in actual histophotometric measurements) tested under the same conditions showed about 5% of flare light. The use of such objectives needs correction for flare light when measuring objects of low transmittance; the calculations may be done with the above formula.

In experiments made with his illuminating system, Naora observed a progressive decrease in the transmission values of Feulgen-stained nuclei when the field diaphragm was reduced from 650 to about 1 μ. Naora admitted that the true value for the transmission is the lowest one. This statement may not be true. A field diaphragm acts as such only when the conditions of Köhler illumination are strictly adhered to, i.e., first, the light source must be imaged in the back focal plane of the objective; second, the lamp condenser must be imaged in the plane of the object; third, the plane of the field diaphragm must coincide with the plane passing through the optical center of the condenser lens system; fourth, the lamp condenser must be aplanatic. If those conditions are not met, the field diaphragm acts also as an aperture diaphragm. When an aperture diaphragm is closed, the phase changes in the object are partially rendered[1] as amplitude changes. As most stained biological objects are known to be really a mixture of phase and amplitude details (*11*), it is doubtful whether the transmittance measured in the Naora instrument with the diaphragm fully closed is the true transmittance.

[1] For this reason, we use a condenser aperture just a little under the objective aperture. Some authors (Swift, Ornstein, and Pollister) prescribe small condenser apertures for histophotometric measurements in order to reduce *flare*. Although the magnitude of the error committed in reducing the aperture of the condenser is not known, it seems probable that a moderate reduction (up to 2/3 of the objective aperture) is not objectionable. However, in our test experiments, closing the aperture diaphragm of the condenser to this value did not reduce appreciably the magnitude of the S-V effect.

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Excretion of Heptachlor Epoxide in Milk of a Dairy Cow Fed Heptachlor

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It has been reported that dogs and rats fed heptachlor (1 or 3a,4,5,6,7,8,8-heptachloro-3a,4,7,7a-tetrahydro-4,7-methanoindene), a new insecticide and also one of the components of technical chlordane, store in their fat a metabolically altered epoxide derivative of this compound (*1, 2*). Because of these findings it was considered desirable to determine whether heptachlor or the heptachlor epoxide is excreted in the milk of a cow following oral administration of heptachlor.

Capsules of heptachlor dissolved in corn oil were administered to a cow at the rate of 3 mg/kg/day, divided into two equal doses, for 14 days. Milk yields and samples for chemical analysis consisting of combined aliquots of the morning and evening milking were taken one week before the administration of the heptachlor and at frequent intervals thereafter during its administration, and for 2 months following its discontinuance.

The separation of the butterfat from the milk is based upon the technique of Frawley and Davidow (*3*), the analysis for heptachlor upon a test developed originally for the determination of chlordane (*4*), the analysis of heptachlor epoxide upon the method described by Radomski and Davidow (*2*). The heptachlor epoxide necessary for the preparation of the standard concentration curve was isolated from the fat of a dog fed heptachlor. Since the completion

of this investigation, however, the manufacturer of heptachlor has succeeded in synthesizing an isomer of heptachlor epoxide (mp 159–160°) which has been found by infrared spectrophotometric analysis to be identical with the epoxide isolated from animal tissues.

The concentrations of heptachlor epoxide found in the milk before, during, and after the administration of heptachlor are presented in Fig. 1. From these

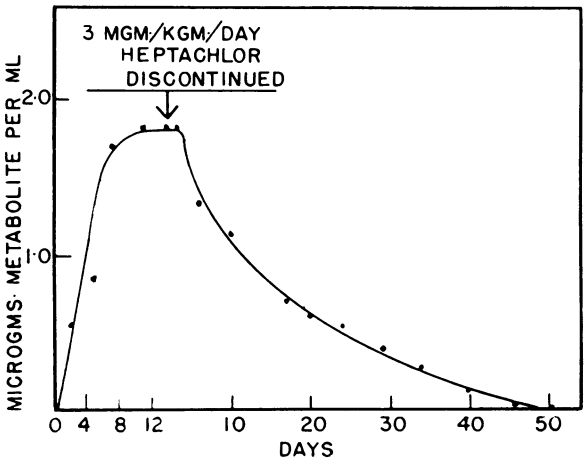

FIG. 1. Excretion of heptachlor epoxide in milk of a dairy cow fed heptachlor.

data it is evident that within 9 days after starting on the heptachlor regimen, the concentration of heptachlor epoxide in the milk rises rapidly to a maximum and constant level of 1.8 ppm. Since the metabolite is concentrated in the butterfat of the milk and the milk averaged 4.1% butterfat, the concentration of metabolite in the butterfat would be equivalent to 44 ppm. After discontinuing the administration of heptachlor to the cow, the concentration of the epoxide in the milk gradually decreases. However, it is not until 51 days after cessation of the treatment that the milk appears to be free of traces of heptachlor epoxide. Although the presence of added heptachlor in milk can be determined in levels as low as 0.02 ppm, negative results were obtained for heptachlor under the described dosage schedule.

From these findings it is apparent that the metabolism of heptachlor in the cow is similar to that of the dog and rat with respect to the storage of the epoxide metabolite. The prolonged secretion of the heptachlor epoxide in the milk after the administration of heptachlor had ceased is probably due to the slow mobilization of the epoxide stored in the body fat.

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A Method for the *in vitro* Culturing of Potato Tubers

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Magrou (*1*), in 1938 and in a series of succeeding papers, discussed tuberization in potatoes, basing his work in part on material cultured on Knop's solution. More recently Stewart (*2*) has described a method for the aseptic culturing of callus masses derived from potato tubers. The present report outlines a simple method whereby tubers can be obtained *in vitro* in sterile culture.

The culture medium used is that of White (*3*), modified by the addition of 2.5 mg/l of pantothenic acid. Etiolated shoots from the eyes of potato tubers that have been maintained in the dark serve as source material for the tubers. The shoots are removed and placed for 20 min in a 12% solution of Clorox to which a small quantity of detergent has been added. Following this treatment, the shoots are placed under a flap of sterile paper towelling where the nodes are excised, using flamed instruments, and placed in the culture bottles.

At the present time the cultures are maintained at 26° C in the dark. Within 3 days of the establishment of the cultures, roots and stolons may be observed growing out from the primordia at the nodes. Most frequently the stolons continue to elongate while forming several branches. After several weeks tubers appear occasionally, mostly on the secondary branches (Fig. 1) and usually above the surface of the agar. Some have been noted, however, below the surface of the culture medium (Fig. 2). Tuberization, in addition, has been observed in cultures stored in a lighted room and at room temperature.

In other preliminary experiments it has been possi-

FIG. 1. Typical tuber formed after 3 wk of culture. × 1.5.