plant should have taken warning at the time of the Belgian disaster of 1930, when 60 persons lost their lives and many thousands were made ill under conditions almost identical with those at Donora. Yet, almost two decades later, the outmoded smelter at Donora was still operating as it and its Belgian counterpart were doing in 1930. Let us hope that the Donora tragedy may prove such an object lesson in air pollution dangers that no industrial plant will feel safe in the future in pouring aloft dangerous amounts of poisonous materials. Furthermore, safety standards to be set up should be those that will give safety under the most adverse weather or smog conditions.

The U. S. Public Health Service investigation of animal deaths in the surrounding countryside was merely cursory, and the report fails to consider at all the terrible devastation and erosion that have resulted from the killing of nearly all plant life within more than a mile radius of the zinc smelter. Surely these were important features of the local air pollution problem. The Federal Security Administrator and the Surgeon General (in their forewords to the report) claim that the Service has here opened up a *new* field in the nation's health, blandly ignoring the years of work others had put in.

Let us hope that the Donora disaster will awaken people everywhere to the dangers they face from pollution of the air they must breathe to live. These 20 suffered only briefly, but many of the 6000 made ill that night will face continuing difficulties in breathing for the remainder of their lives. Herein lies the greatest health danger from polluted air—continuing damage to the respiratory system through years of nonkilling exposure.

Millions of Americans and most medical scientists had been aware of this important public health hazard for several years before the Donora episode spotlighted the community dangers of industrial air pollution. But the U. S. Public Health Service focused its interest on the health of workers *within* the plants. Only after the Donora disaster was it drawn into the much more important aspect of the problem—the relation of industrial air pollution to *community* health.

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Correction

In reference to the article "Chromatographic Analysis of a Mixture of Proteins from Egg White" (*Science*, **1949, 110**, 564), the cation exchange resin Dowex 50 employed in the experiment cited was obtained in February, 1949, from the Microchemical Specialties Company, Berkeley 3, California, under the name of Ion-X, and not from the Dow Chemical Company as reported in the footnote. We have thus far not obtained satisfactory results with a sample of Dowex 50, (24427), obtained from the Dow Chemical Company on August 8, 1949.

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Molecular Orientation and Intracellular Photometric Analysis

In two recent articles B. Commoner and D. Lipkin (*Science*, 1949, **110**, 31, 41) have questioned the validity of the microspectrographic techniques of the sort originated by Caspersson, whereby quantitative estimates of chemical constituents of cells are obtained from measurements of absorption with a microscope and suitable photometric apparatus. Although the main emphasis is placed upon the possible influence of molecular orientation upon measurements of ultraviolet light absorption by nucleotides, it is the conclusion of Commoner and Lipkin that "the entire problem of interpreting intracellular extinction measurements needs to be reexamined with the realization that one is dealing not with true solutions but with oriented aggregates of molecules." It is worth noting that the criticism did not arise from the authors' observation of an effect of orientation upon absorption. It is, rather, based upon inferences from certain physical principles, from the meager information as to the structure of nucleic acid molecules, and from a particular interpretation of selected data on ultraviolet absorption published by Caspersson and his students.

In this laboratory we have been making photometric studies of fixed cells, measuring not only the natural ultraviolet absorption but, in addition, the absorption of the following: (a) the Feulgen reaction, in which color is restored to Schiff's decolorized fuchsin reagent by the aldehyde group of desoxyribose; (b) methyl green staining, in which a basic dye of the triphenyl methane group is combined with the phosphoric acid of desoxyribose nucleic acid (Pollister, A. W. and C. Leuchtenberger, 1949, 35, 111); and (c) the Millon reaction for protein, in which new chromophores are produced, apparently as derivatives of the phenolic group of tyrosine and tryptophane. Our measurements would seem to have been made under conditions very favorable for showing any effect of molecular orientation upon absorption, since they are all on fixed preparations in which, as T. Caspersson pointed out in 1940 (*Chromosoma*, 1940, **1**, 605), the dehydration and shrinkage of standard cytological technique tend to increase birefringence. Moreover it may be added that the senior author has noted that dehydration of precipitated nucleohistone fibers enormously increases their negative birefringence. Yet, in spite of these favorable conditions, in thousands of measurements there is no case (with the possible exception of some anomalous data on striated muscle) in which the data can be readily interpreted, by the criteria Commoner has set forth, as evidence for an effect of molecular orientation on absorption. As will be described below: (a) the distribution curves are symmetrical at all extinction values, not tending to cluster near 0.3; (b) Lambert's law, that extinction is proportional to thickness, has been repeatedly shown to hold for measurements of nuclei, cytoplasm, and intracellular substance; (c) there is good evidence that extinction varies directly with the concentration for the Feulgen nucleal reaction and for ultraviolet absorption of at least some living cells; and (d), as expected from the above, we

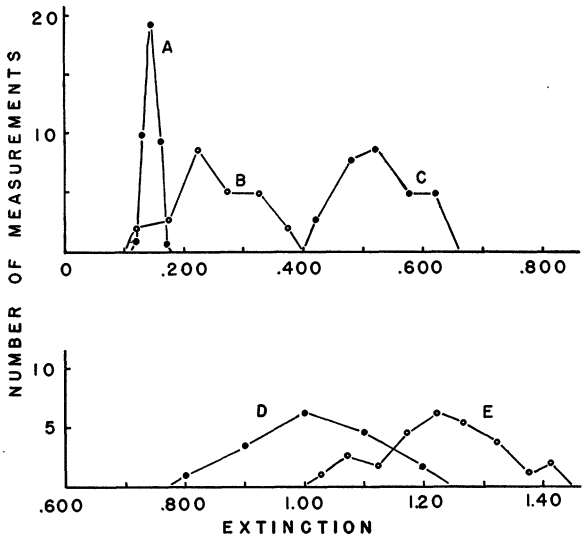

FIG. 1. Typical distribution curves of photometric data. A—Sections of nucleoli, corn pollen mother cells, Millon reaction, 365 mμ. B—Nuclei of mouse sarcoma (fresh tumor tissue), methyl green, 625 mμ. C—Sections of nucleoli, corn pollen mother cells, 254 mμ. D—Mouse primary spermatocyte nuclei, Feulgen reaction, 557 mμ. E—Preleptotene nuclei from mouse testis, Feulgen reaction, 557 mμ.

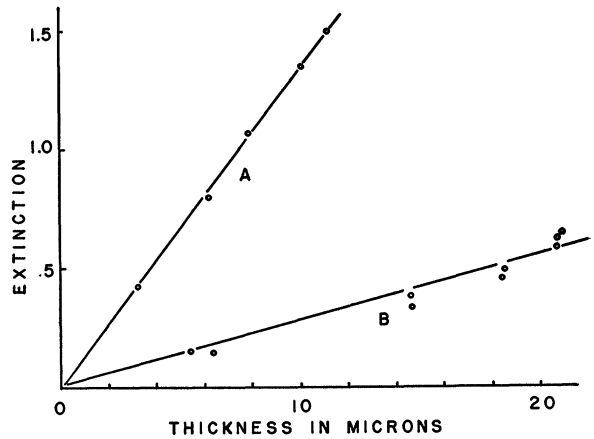

FIG. 2. Demonstration of the direct relation between extinction and thickness of absorbing material (Lambert's law). A—*Ambystoma* liver nuclei, cut at various thicknesses, Feulgen reaction, 30% formalin fixation, 557 mμ. Each point represents the mean extinction on ten nuclei. B—Living grasshopper spermatocyte, 257 mμ, replotted from Caspersson (*Chromosoma,* 1939, **1**, 147, Fig. 7. Since none of the grasshopper nuclei studied in the paper is smaller than 10.0 μ in diameter, it is assumed that the scale on the abscissa of Caspersson's Fig. 7 should read 0–50 μ, instead of 0–5 μ).

have found in these materials that rotation of the polarizer has no effect upon absorption in visible light. Finally, it may be added that in our opinion the selected data used by Commoner, instead of supporting the view that orientation of nucleic acid is affecting absorption values, actually indicate the contrary and support what we have found.

In Fig. 1 are shown a number of typical distribution curves of extinction values. The values have been corrected for nonspecific light loss by subtraction of measured blanks in cases where such blanks absorbed significantly. Some populations are in a range entirely above 0.3, others entirely below, while one overlaps this expected limiting value which corresponds to 50 percent transmission. All of the curves show the sort of symmetry that is characteristic of a population varying in random fashion, like many biological samples or measurements with random errors. There is no hint of the operation of any special factor tending to depress the higher values or to cause marked distortion in the vicinity of 0.3. This same symmetry characterizes the curves Commoner has plotted from data of the Caspersson school (e.g., see his Fig. 4A).

Commoner points out that if there is an orientation effect, "then the value $E = 0.3$ tends to be limiting despite rather large variations in thickness of the materials studied." In other words, marked deviations from Lambert's law should result. Actually, in practice, it is so generally true that Lambert's law holds in cytological absorption work that often the absorption will detect thickness differences which are not readily apparent to visual inspection, and only become confirmed when thickness is carefully measured. Many simple experiments have served to point up this fact. For example, Caspersson described a demonstration that Lambert's law holds for the ultraviolet absorption of nucleic acid in living nuclei (*Chromosoma,* 1939, **1**, 147). He measured the absorption along various transects of a spherical spermatocyte nucleus, and showed that for each point the measured value was that expected for the thickness of the nucleus. We have replotted Caspersson's values (Fig. 2B), converting absorptions into extinctions. The curve shows no tendency to level off at 0.3, as would be expected if there were an orientation effect. We have made several similar demonstrations on nuclei. In a Feulgen-stained preparation we have measured a series of cylindrical nuclear plugs of decreasing radius, and found that, once the circumference is inside the peripheral zone of diffraction, the extinction rises in close approximation to expectation from the increasing optical path as the nuclear center is approached. Measurements of nuclear sections also show that Lambert's law is closely followed (Fig. 2A). That Lambert's law holds for the Millon reaction over a wide range of thicknesses (3 to 25μ) and extinctions (0.17 to 1.20) has been demonstrated by A. W. Pollister and H. Ris (*Cold Spr. Harb. Sympos. quant. Biol.,* 1947, 12, 147).

In the preceding paragraph it has been shown that with the various methods which have been used in microscopic absorption studies, Lambert's law is followed with no indication of the influence of molecular orientation. It seems to us that this is convincing proof that in these cytological materials, if there is orientation of the chromophores of nucleic acid or protein or their derivatives, or dyes attached to them, this orientation has no measurable effect upon the relative absorption values. In other words, the Beer-Lambert law must be similarly uninfluenced by the orientation; for it seems self-evident

that if molecular orientation does not significantly affect the relationship between extinction and thickness of absorbing layer it cannot influence the relation of absorption to concentration in the same material. Therefore the photometric measurements should be capable of detecting changes in amount of absorbing material. There is good evidence that such changes can be detected. For example, losses of material from cells as a result of tissue extraction (Pollister, A. W. and Leuchtenberger, C. *Proc. nat. Acad. Sci.*, 1949, 35, 66), enzymatic digestion (Pollister, A. W. and Leuchtenberger, C. *Nature*, Lond., 1949, 163, 360), or of necrotic degeneration (Leuchtenberger, C. *Chromosoma*, in press) are thus readily picked up. The strongest proof of the real value of the cytological absorption technique comes from study of nuclei of varying chromatin content. It has long been known that nuclei of mammalian liver fall roughly into classes according to size, and it is generally accepted that these represent different degrees of polyploidy (Jacobj, W. *Arch. f. Entw.*, 1925, 106, 124). For example, the three classes in the liver of the mouse and rat are presumably diploid, tetraploid, and octoploid. When these nuclei, stained by the Feulgen reaction, are measured photometrically, the computed relative values for desoxyribose nucleic acid fall close to the ratio 1 : 2 : 4.[1] The haploid nuclei of spermatids have close to one-half the amount of the smallest class of liver nuclei. We submit that such a correlation of calculated relative amounts with expectation from the presumed multiple of the basic amount of chromatin is highly unlikely to be obtained by photometric analysis if some extrinsic factor such as molecular orientation is operating to obscure the relationship between optical density, concentration, and thickness.

We have measured in polarized visible light (analyzer removed) fixed nuclei stained with either Feulgen or methyl green, from grasshopper (*Chortophaga*) testis, and from mouse liver and testis. We have found no evidence of dichroism in any of this material, although the sperm heads of the grasshopper are highly birefringent, and hence presumably have a high degree of molecular orientation. If the methyl green were bound in such a fashion as to reflect the orientation of the nucleic acid, a rotation of the polarizer have caused a detectable effect on the transmission, since crystals of methyl green are markedly dichroic.

Commoner (see his Fig. 2) cites the data which Caspersson reported for spermatocyte nuclei of various diameters as a case where absorption measurements "strikingly fail to follow the Beer-Lambert laws." Obviously this is a "striking failure" only if one assumes that the *concentration* of nucleic acid remains constant as the nuclear volume increases. Commoner has apparently overlooked the fact that Caspersson interprets his data very differently, as showing that in spite of some changes in nuclear size, at a given stage in meiosis *the amount of desoxyribose nucleic acid per nucleus remains nearly constant.* He showed this by calculations

[1] These data will be discussed in detail in a future paper by the junior author. Similar ratios have been obtained by Ris and Mirsky, *J. gen. Physiol.*, 1949, 33, 125.

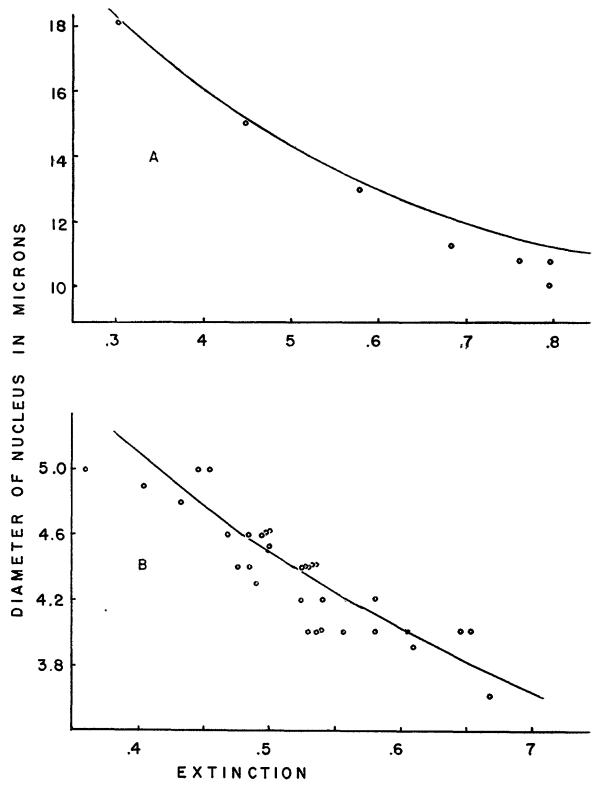

FIG. 3. Relation between nuclear size and extinction in two types of nuclei. Solid lines show the theoretical relation between diameter and extinction, assuming the amount of absorbing substance is constant per nucleus and the Beer-Lambert law holds. A—Living grasshopper spermatocytes, late leptotene, 257 mμ (data from Caspersson). B—Rat spermatid nuclei, Feulgen reaction, 557 mμ.

which appear in a column adjacent to the extinctions which Commoner has plotted. Since, in an earlier part of this paper, Caspersson had demonstrated that the Lambert law was closely followed for these very nuclei (see our Fig. 2B) it seems to us that, instead of being an example of failure of the Beer-Lambert law, these data indeed provide a striking demonstration of its validity for ultraviolet absorption of nucleic acid in these living nuclei. We have replotted Caspersson's data for the leptotene stage (Fig. 3A) to show that the extinctions follow what one would expect from varying concentrations of a constant mass of absorbing material. It has been our common experience in this laboratory that, as Caspersson first found, the minor size fluctuations of any type of nucleus (i.e., those differences which presumably do not involve any change in degree of polyploidy) are always accompanied by compensatory inverse changes of concentration of desoxyribose nucleic acid, as if the amount remained very nearly constant. An example is shown in Fig. 3B, from a population of haploid spermatid nuclei. If one is to explain such an inverse relationship of extinction to optical path as a failure of the Beer-Lambert law due to some obscure effect of molecular orientation, then one is forced to the unlikely assumption that this error seems always just

great enough to make the extinction at all volumes conform to the expectation if a constant amount of nucleic acid is being diluted as the nuclear volume increases. It may be added that this concept of the constancy of desoxyribose nucleic acid per nucleus is supported by recent evidence which has come from correlation of gross analyses with cell counts (Mirsky, A. E. and Ris, H. *Nature*, Lond., 1949, **163**, 666; Vendrely, R. and Vendrely, C. *Experientia*, 1948, 4, 434). It is also consistent with the demonstration of the extraordinary stability of the desoxyribose nucleic acid fraction in interphase nuclei (Brues, A. M., Tracy, M. M., and Cohn, W. E. *J. biol. Chem.*, 1944, **155**, 619).

The positive evidence which Commoner has used to show that molecular orientation may have an important effect upon absorption values comes from his interpretation of the data which have been published by workers of the Caspersson school. Commoner restricts his selection to cases where the *area* measured is small, 1.0 μ or less because, "if the field is large, differences in the spatial organization of various areas included in it may obscure the orientation effect." Yet if material is so heterogeneous that one must, for measurement, select an area one μ or less in diameter to find approximate uniformity, how can it be expected that this small cylindrical mass will be homogenous through a section depth of 5 to 16 μ? From these selected data Commoner has plotted distribution curves, and has concluded that "The occurrence of several sets of measurements with well-defined maxima at 0.3 suggest that the orientation effect has probably influenced the values obtained." These significant maxima occur in three of his six curves, 4B, 4C, and 5. Of these, the last alone is probably plotted from values which are close to the actual 257 mμ absorption characteristic of the material. For only in this case have the extinctions been corrected for nonspecific light loss, which by the Caspersson method is computed by extrapolation from a part of the absorption curve which lies outside the region of specific absorption. In H. Hyden's paper on fixed nerve cells (*Acta Physiol. Skand.*, 1943, 6, suppl. 17), from which the uncorrected extinctions of 4C have been plotted, the author has included with about one-third of the measurements a dispersion curve from which one can estimate the correction for nonspecific light loss. When this is applied to the extinctions of about 0.30, it reduces them to values ranging from 0.15 to 0.20. Thus the maximum for curve 4C is not 0.3 but somewhere near one-half of this. The data of 4B are likewise uncorrected, and from the shape of the accompanying curves it appears that the true mode must lie considerably below 0.3. Thus, of the six distribution curves only one (Fig. 5) actually shows a maximum near 0.3. Aside from the occurrence of this maximum there is little in curve 5 to support the view that the values have been influenced by molecular orientation. The extinctions do not cluster below 0.3, as expected from Commoner's theoretical curve 3B; instead they appear to be grouped fairly symmetrically about the mode, with, if anything, a slight preponderance of values slightly higher than the expected limit.

Commoner suggests that reorientation of the chromophores of nucleic acid may account in large part for the changes in ultraviolet transparency which accompany cell injury and death, and are such an obstacle to quantitative absorption studies on living cells. So far as we know, it has not yet been learned whether these are alterations in *specific light loss*, which could be influenced by chromophore orientation, or changes in *nonspecific* internal reflections and scattering (Caspersson, T. *Skand. Arch. Physiol.*, 1936, 73, suppl. 8), which should be independent of orientation of the chromophores. There is qualitative evidence which suggests that most of the change in optical properties in dying protoplasm may be of the latter type. Students of living cells are familiar with the fact that in visible light, where there is no specific absorption, the most delicate criteria of cell injury are changes in the transparency of the protoplasm and an increase in the differences in refractive indices among the visible cell components. These should lead to marked changes in the amount of nonspecific visible light loss, and should, insofar as light scattering is involved, be enormously exaggerated in ultraviolet light.

The addition of the method of photometric analysis to microscopy marks one of the great forward steps in the study of cells. If this approach can be used to substitute objective relative values for such time-worn subjective cytological phrases as "more basophilic," and "less Feulgen-positive," then we truly have in our hands a powerful tool for the study of biology at the level of its characteristic microscopic unit, the single cell. It is important, however, that workers with these techniques become aware of all possible sources of error which may be constantly or sporadically present. Commoner and Lipkin have performed a useful task by calling our attention, in very explicit fashion, to one potential error which it would seem must become significant in some biological material. However, it does not appear that in most common cytological preparations the orientation of the chromophores of the constituent molecules has an appreciable effect upon the validity of absorption measurements.

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The foregoing comments of Pollister and Swift on the discussion by Dr. Lipkin and myself of certain aspects of microspectrophotometry are apparently intended to defend "the validity of the microspectrographic techniques of the sort originated by Caspersson." The authors may be assured that the *techniques* are not under fire from this quarter—a point which should be evident from the original papers.

Our papers deal rather with the validity of the widespread assumption that the Beer-Lambert laws may be applied with impunity to physical states as complex and dynamic as those encountered in living cells. The discussion was limited to the single case in which there are sufficient data relating to the optical properties of a natural

intracellular constituent to warrant any sort of analysis: the absorption of ultraviolet light by nucleic acids. The analysis led to the suggestion that, on the basis of present information, the assumption that nucleic acid concentration of cell structures is necessarily proportional to their extinction at 260 mμ is not a valid one.

Let us now examine the series of arguments made by Pollister and Swift in terms of their applicability to our basic proposition.

1) *That our discussion "did not arise from the authors' observation of an effect of orientation upon absorption."* This statement offers the reader a choice of either of two meanings. It may mean that Pollister and Swift find fault with our analysis because no observations of the effect of orientation of cellular materials on absorption have *ever* been made. If this is the case, their statement is inaccurate. As pointed out in our papers (*Science*, 1949, **110**, 34), such observations have been made by T. Caspersson (*Chromosoma*, 1940, **1**, 605). On the other hand, their remark may mean to imply that the discussion of the problem by Dr. Lipkin and myself is disqualified by the fact that we ourselves have never conducted such experiments. This unprecedented dictum would require the abandonment of a large part of science.

2) *That the papers were "rather, based upon inferences from certain physical principles. . . ."* The analysis of data by means of such inferences is so widespread as to merit at least some support on the basis of empirical success. If the practice were forbidden or frowned upon, among the first to suffer would be those who make applications of the Beer-Lambert laws.

3) *That studies in Pollister's laboratory show no evidence that various staining procedures and histochemical tests exhibit properties connected with orientation, i.e., dichroism, asymmetrical distribution frequencies, failure to follow Lambert's law.* Most of Pollister and Swift's commentary is concerned with this evidence, but as a reference to the title of the original discussion will show, it has no bearing on the question at hand. That such color reactions may be unaffected by the orientation of the material tested is clear from the experiment of staining crystalline tobacco mosaic virus inclusions with the Millon reagent. Although the crystal itself is dichroic in ultraviolet light, the Millon color shows no evidence of orientation in polarized light. A similar experiment is reported by Pollister and Swift themselves. This, of course, suggests that the orientation effect is not a problem in obtaining extinctions yielded by the colored groups produced in these staining procedures, but it does not constitute proof that extinctions due to nucleic acids are unaffected by orientation.

However, since the subject has been brought up, I may take this opportunity to suggest that the quantitative validity of the staining procedures cited by Pollister and Swift is open to doubt on other grounds. Some of the questions which require answers before these procedures may be said to conform with the elementary requirements of quantitative analysis are these: (1) Are the color-producing reactions stoichiometric? (2) Does the entire mass of the cellular material tested react with the reagent, or does the reaction take place only on the surface of microscopic or submicroscopic particles? (3) Does Beer's law hold for the conditions of the tests? There is considerable evidence (Ostwald, W. *Licht und Farbe in Kolloiden.* Dresden: Steinkopf, 1924. P. 308) that this relationship breaks down when the size of the light-absorbing particles approaches microscopic dimensions.

4) *That Caspersson* (Chromosoma, 1939, **1**, 147) *has demonstrated the validity of the Beer-Lambert laws by studying* (a) *the extinction of a grasshopper spermatocyte nucleus through transects of various thickness, and* (b) *the extinction of a group of such nuclei which have presumably swollen under the influence of ultraviolet irradiation without suffering a change in total nucleic acid content.* The actual data on which this criticism is based are these: (a) Fig. 7 of the paper published by Caspersson in 1939 shows a series of absorption measurements made through transects of various thickness of the nucleus of a single grasshopper spermatocyte. The values indicate that the extinction is proportional to the thickness at the point studied. According to the published scale of the abscissa of his figure, the nucleus had the improbable diameter of 2.1 μ, but Pollister and Swift claim that the scale is erroneous and that the value should be 21 μ. (b) In Table 1 of the same paper Caspersson reports the diameters, extinctions, and calculated nucleic acid contents of a series of spermatocyte nuclei in various stages of meiosis.

Pollister and Swift contend that the data listed under (a) constitute proof that Lambert's law holds for all the nuclei mentioned in (b). They make the further claim that the nucleic acid content per spermatocyte nucleus remains "nearly constant" in any given stage of meiosis. These two assertions are offered as support for the statement that "these data indeed provide a striking demonstration of its [i.e., the Beer-Lambert law's] validity. . . ."

Some exception can be taken to this deduction. Measurements on a single nucleus, plotted on a scale which needs to be reinterpreted by Pollister and Swift, seem but slender support for a conclusion of such moment. Furthermore, Caspersson's figure presents another difficulty which needs to be resolved: It shows a zero absorption in the cytoplasmic regions of the spermatocyte; yet photographs of similar cells published in the same study indicate significant cytoplasmic absorption.

Pollister and Swift state that I have overlooked that part of Caspersson's table which reports what they term the "nearly constant" values of nuclear nucleic acid content. Two errors of fact can be noted here. In the first place it would have been rather difficult to overlook this matter, since Dr. Pollister and I had a searching discussion of this table at the time that he was studying the first draft of my paper. Second, the value is far from constant. For the meiotic stage plotted by Pollister and Swift, Caspersson gives eight values, ranging from 20 to 26 $mg \times 10^{-9}$ of nucleic acid. For an earlier stage, included in the original table, but not plotted by Pollister and Swift, Caspersson reports five values, spread over the range 11.2 to 19.6 $mg \times 10^{-9}$ of nucleic acid. In these

data, one *might* envision a validation of the Beer-Lambert laws. The supporting evidence, however, is sufficiently flexible to allow considerable room for a difference of opinion.

5) *That the demonstration of Lambert's law is sufficient to rule out the possibility of deviations from Beer's law due to orientation phenomena.* This would considerably simplify the calibrations which must be made of many colorimetric reactions. Unfortunately, variations in concentration *per se* induce a number of intermolecular effects (including orientation) which can play hob with Beer's law. As cited in our original papers, this has been amply demonstrated by the work of Sheppard *et al.* Particularly pertinent is the well-known observation that with increasing concentration, solutions of tobacco mosaic virus will develop tactoid aggregates comprised of highly oriented particles. More prosaic but equally pertinent is the fact that concentration of salt solutions, for example, induces the formation of oriented aggregates (crystals) from a totally isotropic fluid.

6) *That the frequency distributions plotted in my paper show false maxima at the range* $E = 0.3$ *because a correction for nonspecific light losses was not applied.* Pollister and Swift state that to obtain the real extinctions due to nucleic acids, the dispersion curves shown by H. Hyden (*Acta Physiol. Skand.*, 1943, **6**, suppl. 17) in eight of his absorption spectra should be applied to all but one of the sets of data plotted by me, as well as to every value obtained by Hyden. Now on page 18 of his paper, Hyden states explicitly that dispersion corrections do not apply to tissues handled (as his were) according to the freeze-drying method of Gersh, unless they happen to have a large water content. He points out that the latter condition occurs in only a certain part of his material (representing eight values), and therefore the dispersion correction is applicable to these points alone. Furthermore, Pollister and Swift assume that "one can estimate the correction for nonspecific light loss" from Hyden's dispersion curves, and that this value can then be applied to all data which seem to lack such a corrective measure. This assumption neglects the fact that the correction factor, according to Caspersson, must be calculated from a series of extinctions in the range 300–350 mμ *determined at the precise area of the cell to which the correction is to be applied.* The dispersion curves are so variable that, for the eight cases which call for them, Hyden supplies individually determined corrections, even where two areas in the same cell are involved. There is therefore no basis for the indiscriminate application of a single correction factor to a wide variety of data.

One manifestly absurd result would be obtained by following the suggestion that a correction factor of about 0.15 be subtracted from all the extinction values not previously corrected by the authors. A significant portion of the values in each set of data (ranging from 10 to 50 percent) would be reduced to zero—or less. This would mean, for example, that about one-half of the chromosome regions studied by Caspersson (see *Science*, 1949, **110**, 37, Fig. 4E for frequency distribution) would appear to be totally devoid of nucleic acid.

7) *That a series of 35 sections of fixed nucleoli from corn pollen mother cells show a modal extinction value (at 254 mμ) well above 0.3 and therefore exhibit no evidence of the orientation effect.* It would be possible to agree that this evidence indicates that nucleic acid orientation is of no consequence in the single case of fixed nucleoli of corn pollen mother cells, if it were not for one disturbing factor—namely, nonspecific light loss. These data were obtained by determining the extinction at 254 mμ of nucleoli before and after treatment with trichloracetic acid at 90° C. The extinctions plotted in Pollister and Swift's Fig. 1C are presumably the differences between these two values. This procedure assumes, however, that the treatment of a nucleolus with near-boiling trichloracetic acid is so gentle as to make no appreciable changes in the intracellular structure which gives rise to nonspecific light losses. This assumption has yet to be given the support of experimental evidence.

It seems to me that certain current microspectrophotometric practices do not adequately reflect the fund of information that modern physical chemistry has placed at the disposal of biologists. One of the simple but vital lessons to be learned from the experiences of routine photochemistry pertains to the Beer-Lambert laws. Few analysts would choose to depart from the rule that any colorimetric reaction must first be calibrated with respect to known differences in concentration (and if necessary, sample thickness) before the extinctions yielded by unknown samples can be evaluated. More frequently than not, the result is a calibration curve which shows considerable deviation from the straight line called for by the laws of light absorption. If the monophasic systems of solution photometry involve such persistent departures from the Beer-Lambert laws, an equally cautious approach to the interpretation of extinctions obtained from cellular structures is called for. Such caution might have more than the negative value of preventing errors, for it could lead to a better understanding of the organization of matter that is peculiar to living things.

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