possess a fiber along the right of their kineties which is structurally quite different from the kinetodesmos. This fiber, found originally in *Stentor* (*8*) and termed the Km fiber, is composed of stacks of parallel, overlapping, kinetosome-based microtubular ribbons which course to the right of the kineties and posteriad. Both these fibers satisfy the rule of desmodexy but are considered nonhomologous; as a result, the term Km fiber has been retained by most authors to distinguish the structure found in heterotrichs from the kinetodesmos. Therefore, for *Woodruffia metabolica* at least, the letters Km are retained and L is added to indicate left; hence the term Lkm fiber because of its superficial resemblance to the Km fiber of many heterotrichs. It is emphasized that the use of the term Lkm for the structure described in this report is merely to indicate similarity in appearance to the heterotrich fiber. I do not believe that there is any homology in these structures because the ribbons of microtubules comprising the true Km fiber are postciliary microtubules and course to the right of a kinety, while ribbons of the Lkm fiber differ in their origin and position. *Woodruffia metabolica* also has a postciliary ribbon of microtubules associated with each kinetosomal pair, but it is poorly developed, only extending for one or two kinetosomal pairs posteriad. These postciliary microtubules do run parallel to the kinety and they do overlap the postciliary ribbons from the next one or two kinetosomal pairs posteriad. However, they do so in a linear fashion and thus form a single ribbon of microtubules. The true Km fiber, on the other hand, is composed of stacks of parallel, overlapping ribbons of microtubules. The conspicuous difference in construction between the postciliary ribbons in *Woodruffia* and the Km fiber of many heterotrichs makes it difficult to consider the *Woodruffia* structure a true Km fiber. However, since the single ribbon in *Woodruffia* is composed of microtubules from more than one kinetosome, it may well be that this type of configuration represents a Km prototype or perhaps an attenuated Km fiber. At any rate, the postciliary microtubules in *Woodruffia* seem almost certainly homologous to Km fibers.

The desmodexy problem is quite interesting: Why should there be so consistently a fiber to the right of a kinety in so many different types of ciliates, and why should this precise arrangement of parallel, overlapping fibrils be retained in nonhomologous structures? There is good evidence that sliding of the microtubules in the Km fiber plays a role in cell extension in the contractile ciliate *Stentor* (*9*) but there is almost no direct evidence as to the function in noncontractile ciliates. The puzzle becomes even more complex with the present demonstration that the usual dextral orientation is not necessary; the same kind of composite bundle can be formed by sinistrally oriented fibrils.

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Photochemical Activity of Single Chloroplasts Recorded by the Use of Nuclear Track Emulsion

Abstract. *The photochemistry done by single chloroplasts can be measured when the chloroplasts are embedded in nuclear track emulsion. It has been known for more than 50 years that certain chemicals will blacken photographic plates (chemical fogging). Although this effect has been little used to measure chemical reactions, it may be particularly useful in photochemistry and electrochemistry, since as little as 10^{-18} mole can be measured.*

In the study of the process of photosynthesis in green plants, one of the most informative and widely used procedures is the Hill reaction (*1*). A suspension of chloroplasts provided with an electron acceptor will, when illuminated, oxidize water to oxygen and reduce the electron acceptor, thus separating the photochemical reaction completely from the complicated series of enzyme reactions that make up the Calvin cycle.

Our purpose in this report is to describe a modification of the Hill reaction that is so sensitive that one can easily measure the photochemistry done by a single chloroplast. Chloroplasts are embedded in nuclear track emulsion and then, while wet, are exposed to red light. The red light by itself does not affect the emulsion. The illumination of the chloroplasts reduces some of the silver ions in the nearby grains to form a "latent image." It is thought that in the photographic process the latent image is made by the reduction of some small number (three to ten) of silver ions in a single grain (*2*). This small amount of metallic silver located at a "sensitive speck" makes it possible for the photographic developer to reduce all of the silver in that grain. In this emulsion the grains are 0.2 to 0.3 μm in diameter and thus contain from 1×10^8 to 3×10^8 silver ions. Therefore, the small amount of silver reduced by the chloroplast is amplified by the photographic process some millions of times to make a black grain that can be seen with the light microscope.

This procedure is somewhat reminiscent of the Molisch reaction—that is, the reduction of silver nitrite by chloroplasts (*3*)—but differs from it in two important respects. First, in the Molisch reaction there is considerable reduction of silver in the dark, whereas we find no grains above the background at all in the dark. Second, the Molisch reaction requires bright illumination for some minutes, whereas this new procedure can be done with small amounts of light.

"Broken" chloroplasts were isolated from greenhouse-grown Good-King-Henry (*Chenopodium bonus-henricus*) by a variation of the method of Walker (*4*). Leaves were kept in running water for about 1 hour, after which 50 g was ground in a Waring blender by means of three high-speed blasts of 5 seconds each. The blender contained 200 ml of the following ice-cold grinding medium: 0.02*M* tris(hydroxymethyl)aminomethane hydrochloride (*p*H 7.4), 0.45*M* sucrose, 0.001*M* $MgCl_2$, and 0.001*M* NaCl. The leaf

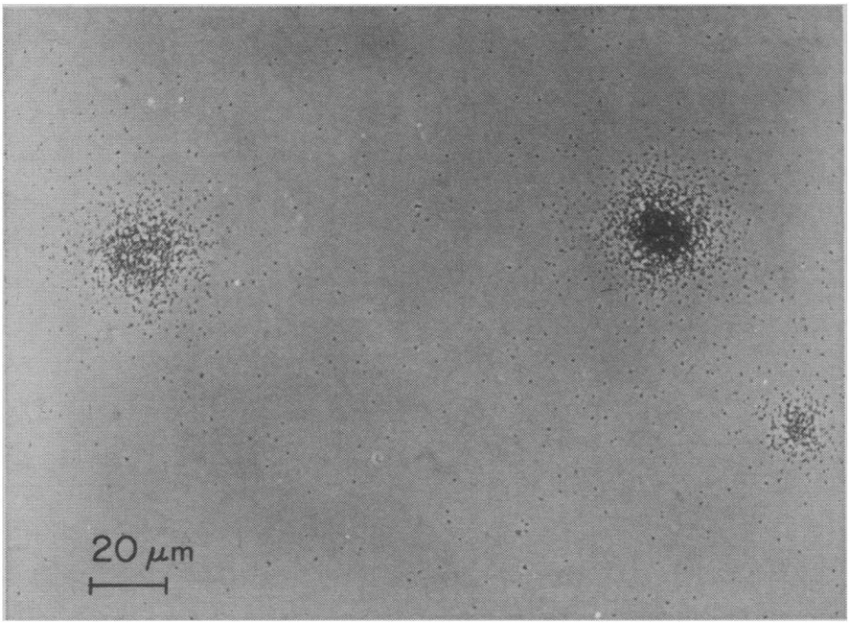

Fig. 1. Photomicrograph of the silver grains over three broken chloroplasts. The diameters of the dried blebs are 15 to 30 μm. The background grains can be seen between the chloroplasts.

homogenate was squeezed through 16 layers of cheesecloth, and the strained homogenate was centrifuged in four tubes for 10 minutes at 1200*g* at 1°C. The supernatant was discarded and the pellets were rinsed by pouring over each of them 40 ml of a resuspending medium of 0.001*M* $MgCl_2$ and 0.001*M* NaCl. The resuspending medium was poured off and the pellets were combined in 3 ml of fresh resuspending medium. These are called broken chloroplasts. Whole chloroplasts were kept suspended in 0.45*M* sucrose at all times.

A volume of 0.01 ml of broken chloroplast suspension was added to 100 ml of distilled water; under these conditions the chloroplasts expand into spherical objects known as blebs, some 15 to 20 μm in diameter. Examination of these blebs with a fluorescent microscope shows all the chlorophyll to be in or on the surface. A volume of 0.1 to 0.2 ml of this dilute suspension was spread on a microscope slide so as to place several chloroplasts in the microscopice field (at ×100). The slides were then dried in air at room temperature and low light intensity. These are the chloroplasts shown in the photomicrograph (Fig. 1).

For whole chloroplasts a volume of 0.01 ml of the suspension was added to 1 ml of 0.45*M* sucrose with 2 percent formaldehyde and allowed to stand at room temperature for 45 minutes to fix the chloroplasts. This 1 ml was then added to 100 μl of distilled water and spread and dried on microscopic slides. These chloroplasts did not expand, and microscopically they looked very much the same size and shape as in the leaf.

In the dark, Kodak NTB-2 nuclear track emulsion was melted and allowed to cool to below 38°C. Then the slides were dipped into the emulsion and allowed to drain and dry overnight in the dark. The slides were then immersed in distilled water and illuminated for various times with red light (a 15-watt bulb covered with a Wratten filter No. 2 at approximately 25 cm; this is the ordinary safelight used in making autoradiographs). After exposure the slides were developed in Amidol developer at 17°C for 6 minutes, fixed in acid fixer for 5 minutes, and permanent mounts were made with Damar after clearing.

This photographic procedure differs in only two points from that used in making autoradiographs (*5*). One, the emulsion must be cooled before the slides are dipped, so that the chloroplasts will not be inactivated. Two, the illumination must be made in the presence of water, whereas autoradiographs are generally exposed dry.

The photomicrograph (Fig. 1) shows three chloroplast blebs with accompanying silver grains. We have three reasons for believing that the grains are due to photochemical activity of the chloroplasts: (i) The chloroplasts must be illuminated while wet to show the grains. (ii) If the chloroplasts are heated in water to 50°C for 5 minutes before being embedded in emulsion, we see no grains (this heat treatment inactivates photosystem II). (iii) If the chloroplasts are illuminated while wet in the presence of $10^{-5}M$ 3-(3,4-dichlorophenyl)-1,1-dimethyl urea, the number of grains is reduced by 100 times, again suggesting that photosystem II is involved.

When we started this procedure we had hoped that only the grains in contact with the chloroplasts would be blackened, and that we would be able to locate the point of reduction to ± 0.2 μm, the size of a grain. However, experiments with compact, fixed chloroplasts show that grains are blackened as far as 5 to 10 μm from the chloroplast, and thus some diffusible substance must be involved. What this substance is we do not yet know. When we know the diffusible substances we may be able to add a scavenger, so that only grains in contact with the chloroplasts will be blackened, and be able to locate the point of reduction.

When many chloroplasts are examined, one is struck by the wide variation in the number of grains associated with a single chloroplast. A few of the chloroplasts show no grains at all. Whether this variation is in the chloroplasts as they occur in the leaf or represents damage to them in the various manipulations, we do not know.

When one is interested in the photochemical activity of suspensions of chloroplasts rather than that of single chloroplasts, the counting of grains with a microscope can be avoided. The concentration of chloroplasts is adjusted to give a confluent film on the slide. The slide is treated as before. The blackening can be measured with a photographic densitometer, and since several samples can be put on each slide, one has an inexpensive fast method to assay for the Hill reaction.

Finally, we present some rough calculations on the sensitivity of this procedure. These calculations must not be taken to be more accurate than an order of magnitude. Since we do not know how much chlorophyll is in one of these chloroplasts, we will assume that it is about the same as that in a *Chlorella* cell, where we find 4×10^5 photosynthetic units (with 500 chlorophyll molecules each). The optical cross section for one unit is about 4×10^{-14} cm^2. Chloroplast blebs were exposed to red light of an intensity of

4×10^{12} *hv* cm^{-2} sec^{-1} for 10 seconds. This means that each chloroplast absorbed about 6×10^5 quanta. The number of grains associated with one chloroplast varied from 28 to 200. So we have one visible grain for something like 3×10^3 quanta absorbed.

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Expression of the Familial Hypercholesterolemia Gene in Heterozygotes: Mechanism for a Dominant Disorder in Man

Abstract. *Studies in cultured fibroblasts indicate that the primary genetic abnormality in familial hypercholesterolemia involves a deficiency in a cell surface receptor for low density lipoproteins (LDL). In normal cells, binding of LDL to this receptor regulates cholesterol metabolism by suppressing cholesterol synthesis and increasing LDL degradation. In cells from heterozygotes, a 60 percent reduction in LDL receptors leads to a concentration-dependent defect in regulation, so that attainment of equal rates of cholesterol synthesis and LDL degradation in normal and heterozygous cells requires a two- to threefold higher concentration of LDL in the heterozygote. The identification of this genetic regulatory defect in fibroblasts of heterozygotes makes available an in vitro system for studying the effects of a dominant mutation on gene expression in mammalian cells.*

Although nearly 500 different dominantly inherited mutations are known to cause disease in man, very little information exists regarding the biochemical mechanisms by which these mutant genes act (*1*). Unlike recessive disorders for which many of the basic enzyme defects are known, dominant disorders must involve a type of gene product which in a 50 percent deficiency is capable of producing clinical symptoms in heterozygotes. For this reason, it has been suggested that in many dominant disorders the mutations are likely to involve abnormalities not in enzymes, but in key nonenzymic proteins such as those that have been postulated to regulate complex metabolic pathways (*1*).

Evidence for the role of such regulatory proteins in eukaryotes is lacking since in vitro systems for demonstrating their presence and for studying the functional effect of their alteration by mutation are not generally available. Moreover, biochemical genetic studies of the possible involvement of regulatory proteins in dominant disorders have been hampered by the rarity of individuals homozygous for dominant genes, thus forcing investigators to study heterozygotes in whom biochemical expression of the regulatory role of the mutant protein is complicated by the presence of the normal gene product.

We have developed a cell culture system for the study of familial hypercholesterolemia, a dominantly inherited disorder that appears to involve a genetic abnormality in a regulatory protein and for which homozygotes are available (*2–4*). This culture system makes use of the observation that in normal human fibroblasts the rate of cholesterol synthesis is regulated by the presence of low density lipoproteins (LDL) in the culture medium (*3*). In order to suppress cholesterol synthesis, LDL must first bind to a specific high affinity receptor on the cell surface (*4*), and this binding results in inhibition of the synthesis of 3-hydroxy-3-methylglutaryl coenzyme A reductase (HMG CoA reductase), the rate-controlling enzyme in the cholesterol biosynthetic pathway (*4*).

Cultured cells from homozygotes with familial hypercholesterolemia are almost completely deficient in this cell surface receptor for LDL and therefore are unable to bind the lipoprotein with high affinity (*4*). Consequently, LDL fails to suppress HMG CoA reductase activity in these cells, and cholesterol is overproduced (*2, 3*). In addition to regulating cholesterol synthesis, the LDL receptor also plays a major role in regulating LDL degradation as shown by the observation that LDL bound to the high affinity receptor in normal cells is degraded by proteolysis to a material that is soluble in trichloroacetic acid (*4*). Since the cells from the homozygotes are deficient in high affinity LDL binding, they are also unable to degrade LDL when it is present at low concentrations in the culture medium (*4*).

As a result of our studies in homozygotes, three biochemical tests are now

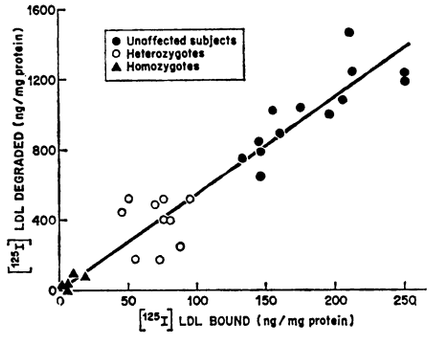

Fig. 1 (left). Relation between LDL binding and LDL degradation in cells from unaffected subjects, heterozygotes, and homozygotes with familial hypercholesterolemia. Each point is derived from the values for LDL binding and degradation given in Table 1.

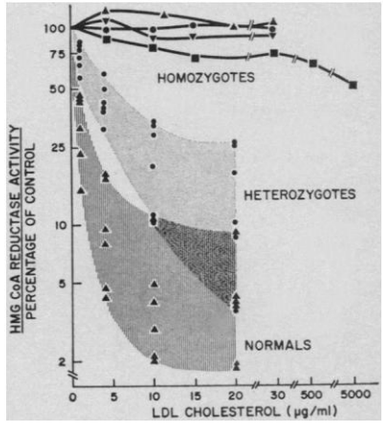

Fig. 2 (right). Effect of varying concentrations of LDL on HMG CoA reductase activity in cells from unaffected subjects, heterozygotes, and homozygotes with familial hypercholesterolemia. Cells were prepared in dishes, varying amounts of LDL were added, and after 24 hours, extracts for measurement of HMG CoA reductase activity were made as described in the legend to Table 1. Results are expressed as activity (amount of mevalonate formed per milligram of cell protein per minute) of cells grown in the presence of LDL divided by activity of cells grown in the absence of LDL; the ratio is multiplied by 100 to give the percentage of the control. The concentration of LDL is given in terms of its cholesterol content, which is twice the protein content.