Table 1. Results of mixing carotene-deficient mutants of *Crypthecodinium cohnii*.

Mixing	Plates scored (No.)	Colonies (No.)		Yellow colonies as % of total
		Yellow	White	
pig-1 × self	24	0	2216	0
pig-3 × self	25	0	1747	0
pig-4 × self	25	0	1949	0
pig-5 × self	24	0	984	0
pig-13 × self	23	0	3618	0
MassX*	24	96	1590	5.69
pig-1 × pig-3	19	0	1338	0
pig-1 × pig-4	29	0	2616	0
pig-1 × pig-5	26	0	1150	0
pig-1 × pig-13	21	211†	1744	10.8*
pig-3 × pig-4	25	71	1137	5.80
pig-3 × pig-5	23	63	1384	4.36
pig-3 × pig-13	23	0	3670	0
pig-4 × pig-5	22	150	951	13.6
pig-4 × pig-13	26	107	1204	8.16
pig-5 × pig-13	23	15	1537	0.966

* Mixture of all of the above mutants. † Only cream-colored recombinants were obtained.

apiculata von Stosch isogamous cell fusion and meiotic division of the zygote, and our observations on fusing cells of *C. cohnii* are consistent with the presumption that recombination is associated with cell fusion.

In a medium (*9*) containing low concentrations of nitrogen and phosphorus we inoculated equal numbers of the mutant clones pairwise. As controls we grew each one of the mutants separately, and we also mixed all of the clones in the same flask under the low nitrogen and phosphorus conditions. After incubation, cells were counted, diluted, and plated on a solid medium (*11*). Colored and albino colonies were counted. Table 1 shows the results of mixing our pigment-deficient mutant clones along with the two types of controls. No yellow colonies were observed in any of the five albino clones grown separately. It is very improbable that the high number of yellow and cream-colored colonies (713 pigmented colonies out of a total number of 18,321 colonies) can be due to reversion in view of the total absence of yellow colonies in the 10,514 colonies which we counted to look for revertants. Therefore we feel justified in using genetic recombination to explain our results. The numbers of yellow colonies observed in the mixings of two different mutants or in the mixing of all mutants cannot be explained by reversion or suppression. A mass mixing of all five mutant clones or the pairwise mixing of pig-3 × pig-4, pig-3 × pig 5, pig-4 × pig-5, pig-4 × pig-13, and pig-5 × pig-13, all produced recombinant yellow-colored colonies with a frequency of 0.966 to 13.6 percent of the viable colonies. The experimental procedure allows all of the mixed cells to form colonies; thus the numbers presented express a minimum figure for genetic recombination frequencies. The relatively high frequency of recombinant progeny found in some of the mixtures would suggest recombination by independent assortment if these are nuclear mutations. Recombinant phenotypes are obtained with pig-3 × pig-4, pig-3 × pig-5, and pig-4 × pig-5; this indicates that mating types (+ or − types) do not exist in *C. cohnii*. Of the four pairwise mixings with pig-1, three of the mixings gave no wild-type recombinant colonies and the mixing of pig-1 × pig-13 gave 10.8 percent cream-colored colonies. Pig-3 × pig-13 did not yield recomibnant colonies. At this time we have no explanation for these last results.

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8. Cells of the mutants in a nitrogen atmosphere were extracted repeatedly with acetone, ethanol, and methanol. The colorless extracts were combined, concentrated, and transferred to *n*-hexane and scanned for pigments with a Bausch and Lomb 505 spectrophotometer. No carotenoids were detected in the visible range of the spectrum in the albino clones.
9. Mixing conditions included incubation for 15 days at 27° ± 0.5°C, a light-dark (hours) cycle (LD 6 : 18), a 125-ml erlenmeyer flask with 30 ml of medium. One liter of medium consisted of: 342 m*M* NaCl, 28 m*M* $MgSO_4$, 5 m*M* $CaCl_2$, 9 m*M* KCl, 10 ml of F metals, 0.79 m*M* disodiumglycerophosphate, 0.15 m*M* $(NH_4)_2SO_4$, 15 m*M* sodium acetate, 8×10^{-5}*M* histidinehydrochloride, 8.2×10^{-9} *M* biotin, 2.96×10^{-6}*M* thiaminhydrochloride, 7.49×10^{-10}*M* vitamin B_{12}, 22 m*M* glucose, 9.7×10^{-4}*M* betainehydrochloride, 8 m*M* 2(*N*-morpholino)ethanesulfonic acid, *p*H 6.6 with NaOH. The F metal mixture contained 6 m*M* nitrilotriacetic acid, 30 m*M* NaOH, 0.18 m*M* $Fe(NH_4)_2$, 0.08 m*M* 5-sulfosalicyclic acid, the *p*H being adjusted to 3.8 with HCl.
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11. Recombinants were detected as follows: 350 cells from each mixing were plated on 1 percent agar-solidified medium (the nitrogen and phosphorus contents were ten times that of the mating medium) containing *N*-lauryl-sarcosine (200 parts per million) [R. C. Tuttle and A. R. Loeblich III, *J. Phycol.* **10** (Suppl.), 21 (1974)]. Plates were incubated for 2 weeks at 27°C, exposed to light from an incandescent light (0.2 watt/m²).
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Pulmonary Platelet Aggregates Associated with Sudden Death in Man

Abstract. *Six human cases of unexpected sudden death were found to have many platelet aggregates in the small arterial vessels of the lungs. Postmortem examination revealed no other significant findings.*

Silver *et al.* (*1*) reported that small intravenous injections of arachidonic acid in rabbits caused accumulation of platelet aggregates in pulmonary blood vessels and sudden death. Their experiment appears to constitute a pathogenetic model for the unreported human entity which we now describe (*2*).

The subject cases (five women and one man), ranging from 17 to 40 years of age, were apparently in good health until immediately before death (*3*). In three instances, the terminal episode was observed and featured severe respiratory difficulty, lasting from less than a minute to about 30 minutes. The remaining in-

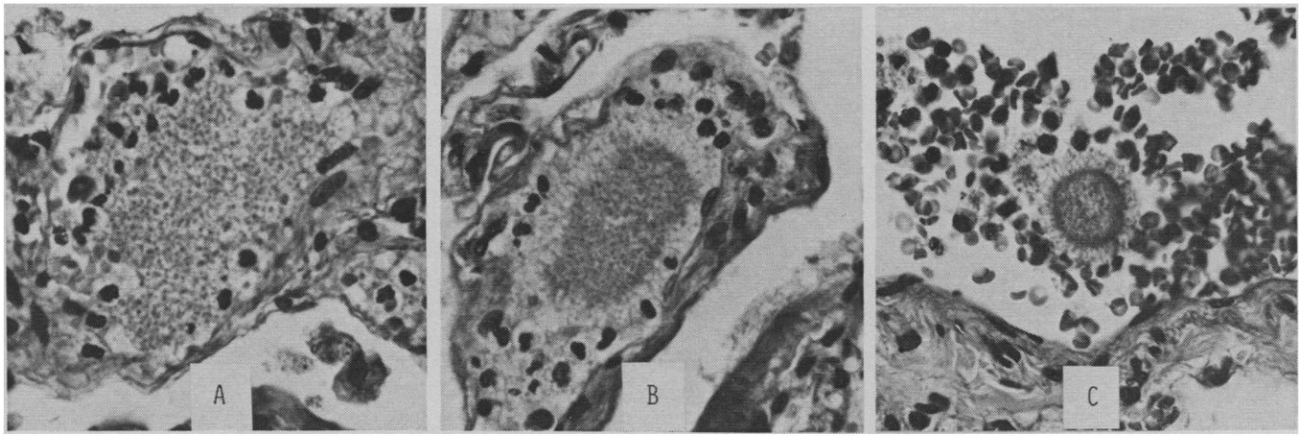

Fig. 1. Photomicrographs of lung sections from case No. 1 showing platelet aggregates within blood vessel lumens. (A) Loose, unordered aggregate. (B) More compact aggregate with peripheral vesiculation. (C) Compact aggregate with peripheral vesiculation and subjacent condensation of platelets. (Hematoxylin and eosin; × 330.)

dividuals were found dead, two in bed and one in the bathtub.

In all cases, the principal postmortem finding was the microscopic presence of platelet aggregates in small pulmonary arteries and arterioles (Fig. 1) (*4, 5*). The extent of this process is shown in Table 1. The aggregates varied from about 10 to 500 μm in greatest cross-sectional dimension with a large proportion in the range of 50 to 150 μm. Most of the aggregates completely occluded blood vessels. Those which did not occlude tended to lie free within lumens (Fig. 1C), suggesting that they were emboli on their way to impaction in smaller vessels (Fig. 1B). Although these aggregates may well have formed in the systemic venous circulation, the absence of associated fibrin argues against their origin from ordinary peripheral venous thrombi. The character of the aggregates ranged from a loose, unordered arrangement (Fig. 1A) as pictured by Silver *et al.* (*1*) through increasing degrees of compactness and peripheral vesiculation (Fig. 1, B and C), changes which also may be induced with arachidonic acid (*6*). We interpret the peripheral vesicles as degranulated platelets, similar to those found on the outside of thrombotic and hemostatic platelet aggregates (*7*) and indicative of the platelet release reaction (*8*).

Controls for these observations were approximately 2000 unselected, concurrently autopsied cases reviewed by one of us (H.P.). The great majority of these cases showed no platelet aggregates; a few showed an occasional aggregate, and none had numerous platelet aggregates as described here.

Platelet aggregates in the pulmonary microvasculature of man have been noted before, but in wholly different clinicopathologic settings of two general kinds. First, aggregated platelets may be associated with the *fibrin* microemboli found in patients suffering from burns, trauma, or amniotic fluid embolism (*9*). Second, patients infused with large quantities of banked blood, either as transfusions or as priming blood for an extracorporeal oxygenator, may exhibit platelet microemboli thought to originate as aggregates in the banked blood (*10*).

Pulmonary accumulation of platelet aggregates has been induced experimentally with many agents, including adenosine diphosphate (*11, 12*), collagen (*13*), protamine sulfate (*14*), Newcastle disease virus (*15*), foreign serum (*16*), and bacterial lipopolysaccharide (*17*). However, these experiments each differ from our human cases and from the model of Silver *et al.* (*1*) in one or more of the following ways: (i) no evidence for platelet degranulation or release reaction (*11*), (ii) nonlethality of the process (*13–15*), or (iii) aggregates demonstrable also in the extrapulmonary circulation (*12, 15–17*).

Table 1. Abundance of platelet aggregates in histologic sections of lung. Entire sections were counted microscopically and areas were determined by planimetry.

Case	Sections examined	Aggregates per square centimeter of section	
		Range	Mean
No. 1	4	130 to 475	290
No. 2	4	6 to 46	20
No. 3	5	15 to 51	33
No. 4	5	13 to 235	93
No. 5	8	9 to 214	47
No. 6	10	22 to 265	155

We attribute death in these cases to pulmonary microembolism because the extent of vascular blockage was similar to that found in lethal experimental microembolism by fibrin clots (*18*). But the mechanisms of microembolic death are complex and still unsettled. Thus, in addition to direct vascular blockage, microembolism, even with inert materials, induces secondary vasoconstriction of other pulmonary vessels, further obstructing pulmonary blood flow (*19*). Moreover, such a vasoconstrictive response may be greatly augmented by the presence within emboli of platelets, releasing vasoactive 5-hydroxytryptamine (*20*) and prostaglandin $F_{2\alpha}$ (*14, 21*).

The morphologic evidence of platelet degranulation in our cases suggests a mechanism of aggregation involving the platelet release reaction (*8*). Platelet aggregation of this type may be mediated by prostaglandin endoperoxides (PGG_2 and PGH_2), which are thought to be intermediates in the biosynthesis of prostaglandins E_2 and $F_{2\alpha}$ from endogenous platelet arachidonic acid (*22*). Although the clinical and pathological features of the cases reported here fail to disclose any means by which platelet prostaglandin synthesis might have been so stimulated, we expect that our findings will point the way to identification of additional cases and the possibility of uncovering etiologic agents.

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4. Pulmonary aggregates and centrifugally isolated pellets of human platelets appeared the same when stained with hematoxylin and eosin, periodic acid–Schiff reagent, oil red O, Giesma stain, phosphotungstic acid–hematoxylin, and aldehyde fuchsin. The phosphotungstic acid–hematoxylin stain was used to look for fibrin. Arteries were distinguished from veins by the disposition of their elastin fibers as stained with the aldehyde fuchsin method [A. Samuelson, A. E. Becker, C. A. Wagenvoort, *Arch. Pathol.* **90**, 112 (1970)].
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Rapid Asymptotic Species Accumulation in Phytophagous Insect Communities: The Pests of Cacao

Abstract. *The number of cacao insect pests is described by a species-area curve. Either annual cacao productivity or area in cultivation of the crop predicts the number of associated insect pest species, when the world's cacao-producing regions are compared. Analysis of covariance does not discriminate different species-area regressions for native as opposed to nonnative cacao-producing regions; the numbers of insect pest species per unit area of cacao in regions of long-standing cultivation do not exceed the numbers in regions of recent introduction. This demonstrates that the number of cacao insect pest species rises rapidly to an asymptote set by the area in cultivation in each region.*

Species interactions are the focus of community biology. Considerations of matter and energy circulation, productivity, and stability depend on the shape and time course of the species accrual curve. Communities accrue species by colonization, speciation, and extinction; the net number of species at any time is the difference between cumulative addition and extinction. There is a spectrum of possible time courses for species accumulation. At one extreme, communities could grow nonasymptotically; the net number of species in the community could increase at a significant rate through evolutionary or geologic periods. The other extreme is asymptotic growth in ecological time. Theories based on nonasymptotic assumptions explain species richness differences as a result of age differences among communities (*1*). Asymptotic theories hold variation in species richness to result from differences in the relatively age-independent structural properties of the environment (*2, 3*). Evidence that nonasymptotic species accumulation actually occurs is poor (*4*) whereas there is no question that species richness in some communities equilibrates rapidly (*4, 5*).

But communities may accumulate species in different fashions. For example, bird species interact differently than plant or insect species. This led Whittaker (*6*) to predict that insect and plant communities do not become saturated with species, even though bird communities do (*3, 7*). Independent reasoning has led Janzen (*8*) to predict species saturation of phytophagous insect communities, but only after periods of evolutionary time. The problem, then, is to discover how rapidly species assemblages actually equilibrate and whether there is a great variation in time to equilibration (that is, do some communities become saturated rapidly while others require long evolutionary or geologic periods?).

Species saturation is usually verified with reference to the geographic area supporting the species assemblage; I show it here for phytophagous insects with reference to the geographic range of the host plant species. Cacao, *Theobroma cacao*, is a sterculiaceous understory tree native to central and tropical South America (*9*). It has been introduced throughout the tropics during the last 300 years, and cocoa is made from the fruit. Using data from Entwistle (*10*), I have determined the number of insect pest species reported from each region of cacao cultivation. These pests are strikingly autochthonous. Fewer than 15 percent of the 1905 presently reported species are known from more than one cocoa-producing area, and fewer than 1.5 percent are reported as "widespread," "very widespread," or "general." This means that insect pests have been rapidly and independently recruited from the the fauna of each cacao introduction region. Saturation has followed this rapid accumulation of pest species, as shown by a species-area relation (Fig. 1). The species-area relation is $S = kA^z$, where S is the number of species, A the area of the region considered, k a constant for the biota in question, and z the ratio of log species change to log area change (*11*). In this case, the relation shows that an upper limit is set on the number of associated insect species by the area of cacao cultivation in each region. Annual cocoa productivity is highly correlated with area in cacao cultivation [data from (*12*)] on a per region basis (*13*). If cocoa productivity is used as an index of area, ten more regions can be considered; these regions list no data on area in cacao cultivation but do list cocoa productivity. Figure 2 demonstrates that the statistical significance of the species-area relation is preserved when all regions are considered for which there is even indirect information on cacao area and insect species.