Coagulation factors assayed in systems used for human plasma are recorded in Table 3. The high levels of factors VIII and V, compared with levels in man, are of interest because similar findings have been reported in other species of mammals (*6, 8*). The levels of factors VII and X were decreased. The significance of these variations is not known. Species specificity of the tissue thromboplastin used in the assay of factor VII (*6, 7*) may explain the apparent low level of this factor. Until species-specific thromboplastin can be obtained the activity of this clotting factor must remain in doubt. The most remarkable finding was the complete absence of factor XII (Hageman factor) in all of the animals. This finding substantiated our observation of no surface activation either in the clotting times in glass tubes or in the partial thromboplastin times. Other laboratory findings associated with factor XII deficiency in man, including a decreased prothrombin consumption and an abnormal thromboplastin generation time that can be corrected by addition of either normal human adsorbed plasma or normal human serum, were obtained from all the animals.

Hageman deficiency is a rare and curious familial disorder. Despite a marked defect in blood coagulation in vitro, patients with this syndrome do not exhibit evidences of a bleeding tendency. It is evident that Hageman factor is not essential for normal hemostasis but it is necessary for the initial stages of clotting of blood in a test tube. No explanation for this is known. One is forced to conclude that the body has some other means by which it can activate coagulation than through the intervention of activated factor XII.

This study was undertaken to compare the hematologic and coagulation profiles of two genera of marine mammals with those of man. To our knowledge, no previous reports on the coagulation mechanism in marine mammals are available. The most striking difference was the complete absence of factor XII in all animals tested. This, in turn, caused marked prolongation of tests dependent on surface activation of the intrinsic pathway of blood coagulation, such as the whole-blood clotting time in glass, the PTT and activated PTT, the prothrombin consumption, and the thromboplastin generation test. Absence of factor XII also explains why the eluate from Celite used to adsorb normal human plasma corrected the defects in the latter two tests and why a similar preparation obtained from plasma of a patient deficient in Hageman factor did not correct them.

According to Ratnoff (*9*), all other mammals studied (cattle, dogs, horses, cats, rats, guinea pigs, mice, hamsters, rabbits, gerbils, sheep, monkeys, armadillos, racoons, goats, pigs, and opossums) have Hageman factor. In submammalian species the factor is completely absent in fowl (ducks, chickens, pigeons, turkeys, and geese) and most reptiles (turtles, tiger snakes, and lizards), although 3 percent of the activity of human plasma has been found in one species of turtle (Western painted turtle). However, Hageman factor–like properties, which cannot be specifically assayed and therefore must be interpreted with caution, are present in amphibians (toads and frogs) and fish, including teleosts (carps, catfish, and trout), primitive bony fish (paddlefish), elasmobranch (dogfish sharks), and a primitive cartilagenous fish (ratfish). Thus, phylogenetically and ecologically the complete absence of factor XII activity in the two (*10*) marine mammals is of great interest.

A. JEAN ROBINSON
MONA KROPATKIN
PAUL M. AGGELER*

Hematology Research Laboratory,
San Francisco General Hospital,
San Francisco, California 94110

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10. Since this study, we have found that Hageman factor is also missing in the Pacific white-striped porpoise (*Lagenorhynchus obliquideus*).
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Androgenesis Conditioned by a Mutation in Maize

Abstract. *A maize embryo having the nuclear constitution of a reduced gametophyte cell is produced in 3 percent of the embryo sacs of inbred strain Wisconsin-23 that carry the mutant* indeterminate gametophyte (ig). *The nucleus of most monoploid sporophytes so derived is paternal. Such androgenetic monoploids may originate from a sperm nucleus acting in conjunction with the cytoplasm of a maternal cell from which the nucleus has been functionally displaced.*

Infrequently the maize embryo develops by parthenogenesis (*1*) rather than from a zygote following syngamy. The endosperm, companion product to the embryo through double fertilization, is constituted normally in these exceptional cases by fusion of the two polar nuclei in the central cell of the female gametophyte with one sperm (*2, 3*). This type of apomictic seed formation yields embryos from which monoploid sporophytes arise. Like angiosperm haploids in general, monoploids of maize usually are matroclinous. Patroclinous monoploids, however, occasionally occur. In maize stocks of diverse origin Chase has observed an average of one patroclinous case for every 80 monoploids (*4*). The total frequency of monoploids in his experiments averaged about one per thousand seedlings but varied considerably between strains. Curiously, the monoploid frequency was strongly influenced by the paternal, as well as by the maternal, parentage. Furthermore, the parental influences proved heritable, although not according to any simple pattern (*5*). Similar influences of parentage were observed for an inbred strain described by Coe which produces up to 3 percent monoploids (*3, 6*). In the case I describe here, the monoploid tendency is associated specifically with the maternally sex-limited expression of a single gene. Moreover, the gene induces predominantly paternal (androgenetic) rather than maternal (gynogenetic) monoploids.

The gene in question, termed *indeterminate gametophyte* (*ig*), appeared in our cultures as a spontaneous mutation of the highly inbred, Wisconsin-23 (W23) strain. Through its influence on female gametophyte development, *ig* conditions various incompletely penetrant irregularities in seed formation, of

which I will mention two because of their relation to monoploid induction. Approximately half the kernels borne on homozygous *ig ig* plants and one-fourth those on *Ig ig* individuals either abort or are conspicuously defective at maturity due apparently to elevated endosperm ploidy level. The defective endosperm characteristic, together with male sterility of *ig* homozygotes, serves to identify *Ig Ig*, *Ig ig*, and *ig ig* plants in segregating families. Second, about 6 percent of seeds with normal endosperm which have received *ig* from *ig ig* or *Ig ig* females carry two, or rarely three or more, embryos. This feature raises the question whether monoploidy might occur preferentially among embryos of polyembryonic sets, a correlation observed in several other species (*7*).

The repeated occurrence of diminutive plants in progenies known to be segregating for *ig* first suggested association of parthenogenesis with *ig* action. Controlled tests for parthenogenesis were performed by varying the *ig* parentage in crosses of stocks differentially marked for the detection of monoploids by the anthocyanin pigmentation characteristics of the *R*-Navajo gene (R^{nj}, Fig. 1) and its acyanic allele, r^g (*8*). The effectiveness of the screening procedure is attested by the fact that in the initial series of experiments (1967 data, Table 1) root tip chromosome counts showed that 29 of the 32 acyanic embryo and seedling selections were monoploid. The three remaining were diploid and compare with nine in 50 reported by Greenblatt and Brock (*8*).

Only kernels with normally developed endosperm were evaluated in screening for monoploids (Table 1). The three maternal monoploids among 2899 offspring of normal parents agrees with 0.1 percent, the average value which Chase observed (*5*). The frequency of gynogenesis was increased from 1.7×10^{-3} for homozygous normal females (entries 1 and 2) to 6.5×10^{-3} for *ig ig* females (entries 3 and 4), a difference which is highly significant statistically (*9*). The frequency of monoploids is slightly higher in both populations following the use of *Ig ig* rather than *Ig Ig* males; however, this effect, even when combined over the two populations, is not greater than that attributable to random variation in one sample in ten.

No instance of androgenesis occurred among 3118 offspring of homozygous normal parents (Table 1, entry 5).

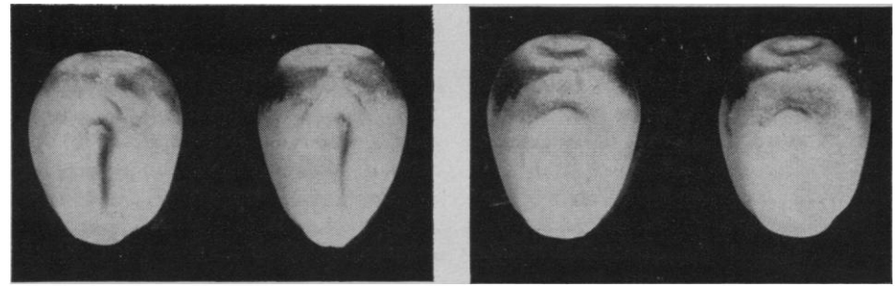

Fig. 1. (Left) Anthocyanin pigmentation conditioned by the R-Navajo (R^{nj}) factor. Typically, pigment is restricted to the crown portion of the endosperm's aleurone layer and to the area of the scutellum that outlines the embryo's growth axis. The coleoptile, scuttelar node, and roots of seedlings form pigments upon exposure to light. Kernels and seedlings of $r^g r^g$ parentage (not shown) are devoid of anthocyanin. (Right) Putative monoploid selections based on absence of embryo color.

Furthermore, there were no monoploids among 3407 progeny from *Ig Ig* females crossed with *Ig ig* males. However, 63 of the 9580 offspring from *Ig ig* females and 29 of the 1231 offspring from *ig ig* females were monoploid. These are frequencies of 6.6 and 23.5×10^{-3}, respectively. The frequency of androgenesis per *ig* female gametophyte of *Ig ig* is estimated by doubling the value of 6.6×10^{-3} to account for heterozygosity and then adjusting by 50 percent for the reduced viability of seeds inheriting *ig*. Agreement of the estimated rate, 19.8×10^{-3}, with the frequency observed in the homozygote, 23.5×10^{-3}, indicates that in the induction of androgenesis, as in other of its pleiotropic effects, *ig* exerts its influence in the gametophyte stage.

The results of crossing *Ig Ig* and *Ig ig* males with *Ig ig* and *ig ig* females show that *ig* of paternal orgin does not affect the incidence of androgenesis. Thus the androgenetic action of *ig* is maternally sex-limited; a sperm nucleus may be involved in androgenesis irrespective of its *ig* constitution. Through androgenesis, moreover, the *ig* gene that incites the event is itself excluded from the nucleus of the embryo stem cell.

Tests with a second marker gene, golden-1 (g_1), validates the use of *R*-Navajo for the detection of androgenesis. Males of a W23 subline as well as those of an unrelated inbred strain, W22, each carrying g_1, r^g, and *Ig* were mated to W23 females homozygous G_1, R^{nj}, and *ig*. Fourteen of the 465 offspring produced from the W23 males and three of the 534 progeny of the W22 males did not exhibit the phenotypic effects of *R*-Navajo. Each of the 17 exceptional seedlings also was golden, as expected if G_1, the dominant green allele of g_1, and R^{nj} were excluded coincidentally. It is also deduced that the androgenetic rate for the W22 males is significantly less than that for the W23 males. The general genetic composition therefore, but not the specific *ig* constitution of the male gametophyte, influences the incidence of androgenesis.

Unlike the relation observed in most species of angiosperms studied, parthenogenesis in maize has been found associated with polyembryony only rarely (*3*, *10*). The two phenomena in the present instance are pleiotropic effects of the *ig* gene. Separate occurrence, nevertheless, remains the rule even here. Of 4701 embryos of *ig ig* maternity screened for gynogenesis (1968 data, Table 1), 623 were members of polyembryonic sets of which seven were monoploid. This compares with 24

Table 1. Loss of the dominant R^{nj} phenotype, indicating monoploidy, as influenced by the *ig* constitution of inbred parents (*13*) of the maize strain Wisconsin-23.

Parentage		Monoploids per total kernels	
Male	Female	1967	1968
Maternal monoploids: $r^g r^g$ *females* × $R^{nj}R^{nj}$ *males*			
Ig Ig	× *Ig Ig*	1/1151*	2/1748
Ig Ig	× *Ig ig*	1/1083*	7/2317
ig ig	× *Ig Ig*	5/1112	10/1750
ig ig	× *Ig ig*	5/854	21/2639
Paternal monoploids: $R^{nj}R^{nj}$ *females* × $r^g r^g$ *males*			
Ig Ig	× *Ig Ig*	0/581	0/2537
Ig Ig	× *Ig ig*	0/1041	0/2366
Ig ig	× *Ig Ig*	3/400*	26/3634
Ig ig	× *Ig ig*	2/750	32/4796
ig ig	× *Ig Ig*	6/351	4/144
ig ig	× *Ig ig*	6/178	13/559

* Excludes one seedling that lacked R^{nj} features but which proved diploid upon cytological examination.

monoploids among the 4078 embryos that occurred singly ($P = .11$). In the 1968 androgenetic selections (*ig ig* and *Ig ig* females) there were five among the 316 plural embryos and 70 among the 8975 single embryos ($P = .12$). The weak association indicated is accounted for by a unique minority class —four of the 12 polyembryonic monoploids had as their R^{nj} twin an embryo with 10 chromosomes. These four cases of gynogenetic-androgenetic twins are to be compared with an expectation of 0.07 based on random occurrence. The gynogenetic-androgenetic coincidence is understandable if *ig* expression is variable from one embryo sac to another, and if common conditions promote parthenogenetic development of cells possessing nuclei derived from either kind of gametophyte.

Action of the *indeterminate gametophyte* gene reflects the loss of normal functions in female gametophyte development. Ordinarily, only a given number of specialized cells are produced. The egg and polar nuclei in the normal, mature gametophyte may be considered mitotically arrested. The pleiotropic effects of *ig* are understandable if *ig* releases the embryo sac from this mitotic restraint. Additional polar nuclei and eggs form, and so predispose to elevation of endosperm ploidy and polyembryony. The response of a sperm nucleus to an *ig* embryo sac is not inconsistent with this view. Since *Ig* and *ig* male gametophyte nuclei behave similarly, embryonic development of sperm appears normally to be repressed by action of *Ig* in the female gametophyte. Under *ig* influence, cells of the embryo sac may proliferate, yielding additional gametophyte cells, whereas sperm in the environment of the embryo sac develop embryonically rather than produce additional male gamete nuclei. This would account for the differential increase of androgenesis as compared to gynogenesis.

Androgenesis incited by *ig* may provide a more general method of generating monoploids, useful for example in cytogenetic investigations or in gamete selection programs of breeding, than available methods based on gynogenesis. The principal unknown factor in evaluating the *ig* system for this purpose is the extent to which the frequency of androgenesis is influenced by the residual genotype of the sperm.

Also, *ig* may be useful in the study of extranuclear heredity. If androgenesis involves the development of a sperm nucleus in maternal cytoplasm, the net result is equivalent to cytoplasmic substitution by nuclear transplantation. Three instances of spontaneous androgenesis observed following crosses of standard males to females carrying cytoplasmic male sterility bear on this question. The maternal descendants of one plant were uniformly male sterile (*11*), some descendants of the second were partially fertile (*4*), and most of the descendants in the third case were fully fertile (*12*). The second case, involving partial fertility, initially was considered to reflect segregation for fertility-restoring nuclear factors, even though restoration was not anticipated based on previous experience with these parents. With discovery of the third case it was suggested that fertility may result from transmission of male cytoplasm with the subsequent sorting out of cytoplasmic types. Seemingly, therefore, a given case of androgenesis may involve either substitution or hybridization of cytoplasms. The latter phenomenon, in conjunction with *ig* induced androgenesis, suggests a basis for the genetic analysis of extranuclear determinants in maize.

J. L. KERMICLE

Laboratory of Genetics,
University of Wisconsin,
Madison 53706

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9. The *P* values represent the probability that the two monoploid fractions derive from populations with the same monoploid frequency. Where the total number of monoploids was less than 50, *P* was calculated by summing the terms of the binomial expansion having the same split or a wider one than that observed. If there were more than 50 variates, the monoploid fractions were tested for homogeneity by chi-square.
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13. The matings represented in Table 1 involve three W23 strains: the commercial form (*c rg*), the strain in which *ig* arose as a mutation, and an *ig* substrain into which the seed color factors *C* and R^{nj} were incorporated by backcrossing.
14. I thank B. Lin for determinations of chromosome number and R. A. Brink and R. I. DeMars for suggesting changes in the manuscript. Paper No. 1331 of the Laboratory of Genetics.

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Tobacco Smoke Toxicity: Loss of Human Oral Leukocyte Function and Fluid-Cell Metabolism

Abstract. *The human mouth has been utilized as a new and significant in vivo open bioassay system for tracing undesirable substances present in tobacco smoke, in an exact milieu where the smoking "problem" begins and must be dealt with directly. The associated in vitro closed test systems described herein have provided new, sensitive bioassays that help to explain the in vivo effects.*

The human oral cavity can be utilized as an open test system to provide: (i) substantial numbers of functional oral leukocytes (inflammatory cells), (ii) actively metabolizing oral fluid-cell harvests (*1–3*), and (iii) a natural "smoking machine" and trap for tobacco smoke, based on the fact that the oral cavity is the portal of entry for this smoke into the human organism. This test system provides a useful supplement to observations on biological effects of tobacco smoke gained from use of other assay systems such as clam cilia (*4*); rodent, cat, dog, chicken, and monkey trachea (*5*); paramecia (*6*); rodent macrophages (*7*); and mouse skin painting and other similar techniques (*8*) (also see *9–12*).

We were interested in the effects of tobacco smoke immediately after a human subject puffed a cigarette. In a survey of more than 80 individuals (smokers and nonsmokers), we found that the number of puffs per cigarette varied from 6 to 27. Some smokers retained each puff 1 or 2 seconds longer than the average smoker, who took between 7 to 10 puffs into his month, retaining each puff for approximately 2 seconds. If a smoker does not inhale or swallow any of the smoke, the oral cavity is exposed repetitively to whole tobacco smoke, or to its gas phase (provided