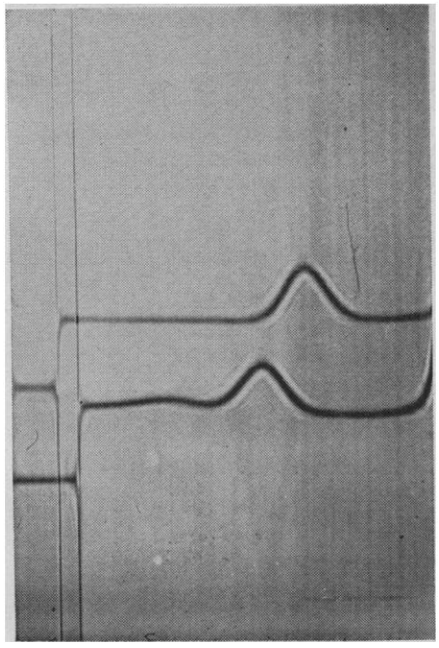

Fig. 1. Simultaneous ultracentrifugal analysis of undigested "7S" γ-globulin (top) and the γ-globulin digested with pepsin (bottom). The top pattern shows a single component 6.6S, uncorrected, whereas the bottom pattern shows a major component 5.0S and trailing material of approximately 2.5S.

(*1, 2, 8*) and divalent (*2*), retain the ability to combine with the appropriate antigen. Stelos *et al.* (*9*) demonstrated that univalent fragments prepared from antikidney γ-globulin and labeled with I^{131} were selectively although transiently fixed in the kidney in vivo and bound by kidney sediment in vitro. Thus, the antibody fragments might be expected to inhibit competitively the reaction between antikidney antibody and kidney antigen and prevent production of renal damage by the antibody. In this study, univalent fragments did not provide protection in vivo against intact antibody injected simultaneously or 5 to 15 minutes later. Probably the quantity of univalent fragments was inadequate to protect all of the kidney antigenic sites (*10*) from antibody attachment. Next, by adding univalent antibody fragments, we attempted to inhibit in vitro the reaction between a large dose of antibody and its neutralizing dose of solubilized kidney antigen (*11*). After addition of the fragments in amounts up to 10 to 20 times that of the antibody, there was sometimes enough free antibody in the mixture to produce proteinuria in a rat, but more often the result was negative. Combination of the antibody and antigen seemed to be more effectively blocked by addition of divalent fragments, although potentiation of residual free antibody by these fragments was not completely ruled out as a possible alternative explanation. The experiments are complex and cannot now be fully interpreted.

Since Stelos *et al.* (*9*) noted that univalent fragments in contrast to the intact antibody were largely eliminated from the kidney tissue in 3 days, possibly the failure of fragments to induce late or delayed proteinuria is explained by their rapid elimination. However, Stelos *et al.* showed roughly comparable binding of the intact antibody and the univalent pieces 1 day after the injections; hence the marked difference in proteinuria, caused by intact antibody and its fragments, presumably must be explained on a basis other than differences in their kidney localization.

The titers of complement in serum decreased to about 25 percent of normal after the usual doses of antiserum. The extent to which the changes in complement were caused by the same antibody (or antibodies) which causes the proteinuria is not known; however, others have demonstrated complement in the renal lesions (*12*). Relatively little change in complement titers occurred, on the other hand, after injections of univalent or divalent antibody fragments, confirming in vitro studies (*13*) and in vivo studies (*14*) with other antibodies. Therefore, the observations reported here are consistent with the hypothesis that fixation of complement is required for the production of serious damage by antikidney antibody (*15*). However, the complement studies, possibly because of their limitations, did not disclose why divalent fragments produce transitory proteinuria and univalent fragments do not. Alternatively, the failure of antibody fragments to produce severe renal damage may conceivably result from one of a number of other differences (*1, 2, 9, 16*) between intact antibody and the fragments.

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15 April 1963

Haploids: High-Frequency Production from Single-Embryo Seeds in a Line of Pima Cotton

Abstract. *Progenies of a doubled haploid from Pima S-1, a commercial variety of* Gossypium barbadense *L., contained a very high frequency of haploid plants. The haploid plants, in contrast with those previously reported in cotton, originated from single- rather than twin-embryo seeds. Apparently the haploid-producing ability of this line of cotton is inherited.*

Occasionally haploid plants with a somatic chromosome number of 26 are found in cotton species (*Gossypium hirsutum* L. and *G. barbadense* L.) cultivated in the New World. In field plantings in Arizona, haploids are rarely found in *G. hirsutum* or Upland cotton, but in the Pima varieties of *G. barbadense*, a frequency of one to three haploids per 25,000 plants is not uncommon. Haploids are distinguished from diploid plants (*1*) by their lack of pollen shedding, by smaller plant parts, and sometimes by greater height because of their lack of fruitfulness (*2*).

Haploids occur as one or both members of twin-embryo seeds (*3–6*). In *G. hirsutum*, Roux found a frequency of one twin embryo in 20,000 to 25,000 seeds (*5*); Kimber reported two twins in 127,500 seeds (*4*), and Blank and Allison reported frequencies from one twin in 20,511 seeds to one twin in 2639 seeds (*7*). An increase in the incidence of twin embryos in a line of *G. hirsutum* was accomplished by selection pressure for this characteristic (*7*).

In *G. barbadense* the frequency of

Table 1. Number of diploid plants ($2n$), haploid plants (n), and percentage haploids in progeny tests of Pima S-1 doubled haploid 57-4.

Growth		No. of plants		n
Year	Place	$2n$	n	(%)
Initial generation from doubled haploid				
1959	Field	2	1	33.3
1961	Field	11	7	38.9
1962	Field	34	26	43.3
Second generation from doubled haploid				
1960	Field	12	4	25.0
1961	Field	180	85	32.1
Third generation from doubled haploid				
1961	Field	109	35	24.3
1962	Field	77	59	43.4
1962	Greenhouse	91	144	61.3

twin embryos is higher (*6, 8*). Silow and Stephens reported one twin in 300 to 500 seeds of Sea Island cottons, and de Garcia found one twin per 2369 seeds of Tanguis.

This report describes a line of *G. barbadense* that produces a very high frequency of haploid plants from single-embryo seeds, a new phenomenon in *Gossypium*. The line producing a high frequency of haploids was a doubled haploid, 57-4, of Pima S-1, a commercial variety of *G. barbadense*. Haploid 57-4 was doubled by the application of an aqueous mixture of 1 percent colchicine, 4 percent tragacanth, and a small amount of thymol to axillary buds (*9*). Of three plants obtained from a field planting in 1959 from seeds of the initial generation of doubled haploid 57-4, two were diploid ($2n$) and one was haploid (n) (Table 1). A 1960 field planting of seeds produced by the two diploid plants grown in 1959 (the second generation from the doubled haploid) produced 12 diploid and 4 haploid plants (Table 1). In 1961 additional seeds of the initial, second, and third generations of doubled haploid 57-4 were planted in the field to confirm the high frequency of haploid plants found in the 1959 and 1960 plantings. Seeds from doubled haploid 57-4 produced 38.9 percent haploids; plants representing the second generation from the doubled haploid were 32.1 percent haploids; plants representing the third generation from the doubled haploid were 24.3 percent haploids (Table 1).

High frequencies of haploids in progenies of doubled haploid 57-4 were corroborated in the 1962 field plantings of the initial and third generations. The initial generation had 43.3 percent haploids and the third generation had 43.4 percent haploids (Table 1).

While we were scoring the field plantings for diploid and haploid plants, it occurred to us that the haploids were coming from single-embryo, not twin-embryo, seeds. A greenhouse planting of third-generation seeds was made to confirm this observation. One seed was planted in a pot, and as the seedlings emerged the pots were checked for the presence of one or two plants. In every case, only one seedling emerged from each pot, thus proving the absence of twin embryos in any of the seeds planted. When the plants flowered, they were scored as diploid or haploid on the basis of gross morphology and fertility or sterility of the anthers as indicated by dehiscence or nondehiscence (*10*). As shown in Table 1, 91 diploid and 144 haploid plants were obtained; 61.3 percent of the population was haploid. The data confirm the observation that the haploids were occurring in seeds as single rather than twin embryos. This report is the first for *Gossypium* of apomixis, probably by haploparthenogenesis, resulting in a high frequency of seeds with single haploid embryos.

The frequency of haploids was greater in the greenhouse planting than in field plantings. This suggests that the survival of haploid plants was reduced in the field, probably because the environment during emergence and early stages of growth of the seedlings was more hazardous for the haploids than for the diploids.

Crosses were made between doubled haploid 57-4, as a male parent, and Pima S-2, a commercial variety of *G. barbadense* producing a normal frequency of haploids, to determine whether the haploid-producing ability of 57-4 is heritable. The F_1 generation produced 99 diploid plants and 1 haploid. The F_2 generation from this cross resulted in 157 diploid and 15 haploid plants which indicates that the haploid-producing ability of line 57-4 is inherited.

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Complementary RNA in Nucleus and Cytoplasm of Mouse Liver Cells

Abstract. *The rapidly labeled RNA from both the nuclei and cytoplasm of mouse liver cells can be bound specifically to mouse DNA. The bound fraction differs in base composition and metabolic stability from the bulk RNA. There is considerable cross reaction between this RNA and the DNA obtained from calf thymus.*

There is increasing evidence that rapidly labeled RNA (*1*), extracted from animal tissues or tissue culture cells after their exposure to appropriate materials labeled with radioactive nuclides, is different from the bulk of cellular RNA. It has been suggested that this rapidly labeled RNA might be complementary to DNA (*2–4*), as has been demonstrated in bacterial systems (*5, 6*). However, no conclusive proof of this complementarity has been offered, although tentative evidence has been advanced by Scherrer *et al.* (*4*) who used the cesium chloride density-gradient method to detect the binding of newly formed RNA to DNA in HeLa cell systems.

The simplicity and efficiency of the DNA-agar procedure for demonstrating complementary RNA (*6*) prompted us to search for it in animal tissues. Evidence for the existence of such molecules in the nuclei and cytoplasm of cells from mouse tissues is presented.

Female BALB/c mice 21 to 28 days old were injected intraperitoneally with 1 mc of carrier-free P^{32}-orthophosphate neutralized with tris buffer solution. The mice were decapitated at intervals during the 24 hours after injection; the livers and kidneys were removed, quickly frozen on dry ice, and stored at −20°C. Cytoplasmic and nuclear fractions were prepared from these tissues according to the procedures of Hiatt (*3*) except that the solution of tris-Cl, sucrose, and calcium also con-