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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18 April 1963

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-

tained a small quantity of bentonite and 10 μg sodium dextran sulfate 500 (Pharmacia) per milliliter.

The cytoplasmic fractions were treated with 1 percent sodium lauryl sulfate and then with an equal volume of 90 percent phenol solution containing 0.1 percent 8-hydroxyquinoline. The nuclei—suspended in a small volume of tris-Cl, sucrose, and calcium solution—were lysed with 5 to 10 ml of 1 percent sodium lauryl sulfate in 0.03*M* tris-Cl, *p*H 7.4, and passed into an equal volume of 90 percent phenol through a French pressure cell at 350 kg/cm^2 (5000 lb/in^2.) to insure complete dispersal of nuclear elements. This phenol treatment was carried out at 60°C for 5 minutes; the aqueous phase, separated by centrifugation, was mixed with two volumes of cold ethanol. The precipitate was dissolved in a small volume of 0.02*M* tris-Cl (*p*H 7.4) containing 0.01*M* $MgCl_2$ and treated for 5 minutes at room temperature with 20 μg of deoxyribonuclease (Worthington) per milliliter. After a second phenol treatment in the presence of 1 percent sodium lauryl sulfate and a second precipitation, the precipitate was dissolved in 1 to 2 ml of a 1:10 dilution of SSC. Passage of this solution through columns containing the sodium form of Dowex-50 and Sephadex G25 (*7*) completed the purification of the RNA.

The DNA from nuclei of mouse liver cells was prepared by the method of Marmur (*8*). Highly polymerized DNA from thymus and salmon sperm was obtained from Worthington and Calbiochem, respectively. The DNA is efficiently entrapped in agar only when it has a reasonably high molecular weight (about 10^7). Therefore, prolonged heating, excessive pipetting, and other procedures which contribute to the degradation of DNA should be avoided. Even when these precautions are taken, variable leaching of DNA from the gel occurs with preparations of animal DNA.

The DNA was embedded in agar essentially as described by Bolton and McCarthy (*6*). It (1 to 2 mg in a 1:100 dilution of SSC) was heated at 90°C for 5 minutes, cooled quickly, reheated to 90°C, and thoroughly mixed with an equal volume of melted 8 percent Ionogar No. 2 or agar-agar No. 3 (Oxo Ltd., London), at 90°C. The hot, viscous mixture was poured into a chilled beaker. The resulting gel was pressed through a 35-mesh screen, placed in the barrel of a syringe, and thoroughly washed (on a coarse fritted-glass vacuum filter) at 60°C with double strength SSC. The washed DNA-agar can be conveniently stored at 4°C with a small amount of chloroform. The DNA content of the gel was determined by dissolving a sample in hot 5*M* sodium perchlorate for measurement of the absorption at 260 mμ.

One milliliter of P^{32}-labeled RNA solution (about 100 μg of RNA per milliliter in double strength SSC) was mixed with 1 g of moist DNA-agar (400 to 1300 μg of DNA per gram) and incubated at 60°C, usually for 40 to 48 hours. Unless otherwise specified, the RNA was isolated from livers of mice exposed to P^{32}-orthophosphate for 90 minutes. The mixture was then transferred to a water-jacketed column and eluted with ten or more 15-ml quantities of double strength SSC at 60°C to remove unbound RNA. The temperature was then raised to 70°C and further successive 15-ml fractions of a 1:100 dilution of SSC were passed through the DNA-agar column. Each fraction of solution remained in contact with the agar for at least 5 minutes before removal. The RNA was collected on membrane filters after precipitation with trichloroacetic acid (*9*) and assayed for radioactivity.

Nucleotide compositions of P^{32}-labeled RNA were determined by isotope dilution (*10*) with *Escherichia coli* ribosomal RNA as the reference.

In accord with other results (*3, 4*), Table 1 shows that the nuclear RNA has the highest specific radioactivity when it is derived from liver cells obtained soon after the injection of P^{32} orthophosphate. The high specific activity of nuclear RNA compared to that of the cytoplasm at 0.5 and 1.5 hours indicates little contamination of one fraction by the other. Because of lack of uniformity in labeling mouse tissues from one experiment to another, the ratios of specific activities provide a more meaningful comparison of the nuclear and cytoplasmic fractions than their specific activities alone.

Table 1. Extent of incorporation of liver cell nuclear and cytoplasmic RNA at various times after injection. Weanling BALB/c female mice were injected intraperitoneally with 1 mc neutralized P^{32}-orthophosphate. RNA from both the nucleus and cytoplasm was isolated from liver cells of three animals sacrificed at the times indicated. The amount of cytoplasmic RNA isolated was about twice that found in the nucleus.

Time after injection (hr)	Specific activity (count/min per μg)		Ratio of nuclear to cytoplasmic RNA
	Nuclear	Cytoplasmic	
0.5	33	2	17
1.5	182	13	14
1.5	151	17	9
4.0	90	37	2.5
24.0	150	158	1

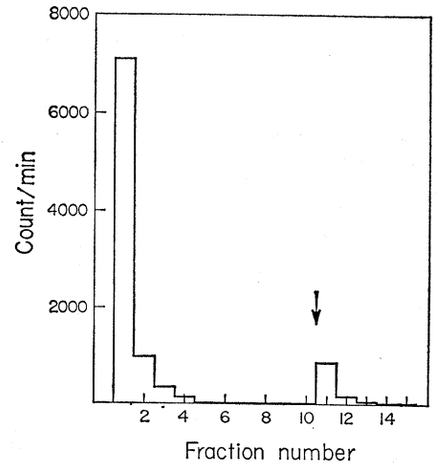

Fig. 1. Binding of mouse liver cell nuclear RNA to mouse DNA-agar. Nuclear RNA (100 μg) obtained from mice 1.5 hours after injection of P^{32} orthophosphate was incubated for 40 hours at 60°C, with 1300 μg of denatured DNA in 1 g of agar gel. A column of this agar was eluted with 10 successive 15-ml quantities of double-strength SSC, 60°C, over 1 to 2 hours. Where indicated by the arrow, the temperature was increased to 70°C, and the salt concentration was decreased to 1:100 SSC. About 11 percent of the radioactivity was in the latter eluates.

In a typical experiment (Fig. 1), about 11 percent of the labeled RNA was bound to the DNA. This RNA could be eluted by lowering the salt concentration and raising the temperature. Binding of RNA to DNA-agar is a time-dependent process (Fig. 2)

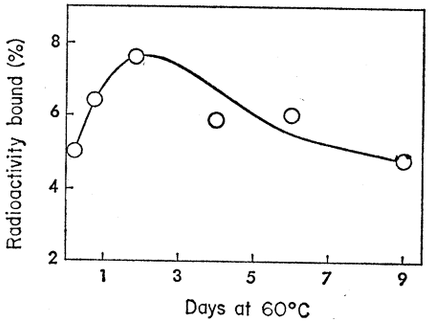

Fig. 2. Influence of incubation time on binding of RNA from mouse liver cells to mouse DNA-agar. Mouse-liver RNA (100 μg) labeled in mice for 1.5 hours was incubated with 400 μg of denatured mouse DNA in 1 g of agar for 0.2 to 9 days. The percentage of the radioactivity bound to DNA-agar was determined at each time.

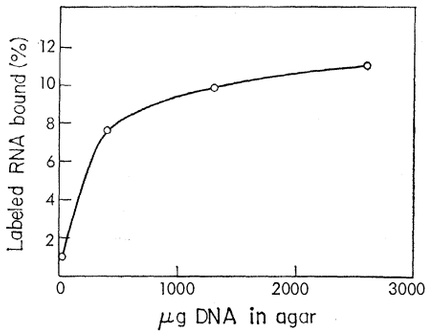

Fig. 3. Influence of amount of denatured mouse DNA embedded in agar on amount P^{32}-labeled RNA bound. Liver cell RNA (100 μg) obtained from labeled mice 1.5 hours after injection with P^{32}-orthorphosphate was incubated 40 hours at 60°C with 26, 400, or 1300 μg of denatured mouse DNA in 1 g of agar gel or 2600 μg in 2 g of agar. Columns of agar were then eluted with double strength SSC at 60°C, followed by SSC, diluted 1:100, at 70°C.

which is maximal at about 40 hours with the concentrations of RNA and DNA used in most of the experiments. The yield decreases somewhat upon prolonged incubation probably as a result of thermal scissions in the DNA which would tend to release DNA and DNA-RNA complexes from the agar gel.

Binding of RNA to DNA-agar increased as the amount of DNA in the agar was increased from 26 to 2600 μg per 1 to 2 g of agar (Fig. 3). This increase was pronounced at low concentrations of DNA and continued as the amount of embedded, denatured DNA was raised to high levels. Thus, a portion of labeled RNA is readily supplied with binding sites, while a smaller fraction of the newly synthesized RNA requires more numerous sites for demonstrable binding. Even at ratios of DNA to total RNA of 26 to 1, not more than 12 percent of the labeled RNA is bound.

There is RNA which binds to DNA in both nuclear and cytoplasmic fractions. Table 2 shows the binding of nuclear and cytoplasmic RNA from mouse livers labeled with P^{32}-orthophosphate for various times. Because of differences in specific activity and limits for counting of radioactivity, the quantities of RNA used are not the same for cytoplasmic and nuclear fractions. Direct comparisons of the percentage bound, thus, cannot be made. However, both fractions show binding at 0.5 and 1.5 hours, and show marked decreases at 1 day. The small fraction of the total radioactivity bound at 1 day and 3 days is consistent with the idea that the rapidly labeled RNA represents only a small proportion of the total RNA.

Reduction of binding, brought about by the addition of unlabeled RNA, indicates competition for homologous sites in the DNA (*11*). This principle was used to show that nucleus and cytoplasm contain the same RNA molecules. Six percent of the radioactivity of the rapidly labeled nuclear RNA (125 μg of RNA obtained from mice exposed to P^{32}-orthophosphate for 60 minutes and 220 μg of DNA in 0.5 g of agar) was bound in the absence of competing RNA. When mixed with 500 μg of liver cell nuclear RNA, the binding was reduced to 2.5 percent (60-percent reduction) and when incubated with 800 μg of liver cell cytoplasmic RNA, the binding was reduced to 3 percent (50-percent reduction). Thus, liver-cell cytoplasmic RNA contains RNA which competes with nuclear RNA for binding sites in the DNA.

The RNA bound to DNA-agar is a special fraction of the cellular RNA. Nucleotide analyses of the bound and unbound RNA are presented in Table 3. The fractions differ significantly, especially in the proportions of G and U and in percent GC. The bound RNA contains 47 percent GC while the residue contains 52 percent GC.

When each of these fractions is reincubated with DNA-agar, the initially bound RNA recombines efficiently while the unbound RNA combines poorly. This result is shown in Fig. 4.

In addition, Table 4 shows that mouse liver nuclear RNA binds effectively with mouse liver DNA in agar, less effectively with calf thymus DNA-agar, and poorly, if at all, with *Escherichia coli* DNA-agar or agar lacking DNA. Labeled *E. coli* RNA, in contrast, did not bind with either calf thymus or mouse liver DNA-agar. Agar containing salmon sperm DNA also bound little, if any, mouse RNA.

Our experiments show that homologous RNA can be readily hybridized with the genetically complex DNA of mammalian origin. Thus, the DNA-agar method can be applied to the study of primary gene products from such diverse sources as bacteriophage, bacteria, and mammals.

In the case of exponentially growing bacteria, essentially all of the cistrons are active in "making" RNA (*11*). For the vast majority of the regions in DNA, the RNA molecules synthesized have only transitory existence and apparently act as messengers between the genes and ribosomes (*12*). Only in the special cases of the cistrons concerned with ribosomal and soluble RNA are the RNA molecules stable, and together these amount to less than 0.5 percent of the total genetic information (*13–15*).

The rapidly labeled fraction of liver RNA contains molecules which are complementary to DNA and which have properties consistent with a role as messenger. As more DNA sites are provided, the amount of labeled RNA bound increases (Fig. 3). This increase is initially rapid, but changes only slightly as the DNA:RNA ratio is raised from 4 to 26. Relatively few sites in the DNA bind 8 percent of the labeled RNA. This fraction could represent a heterogenous group of rapidly turning over molecules. The large number of DNA sites required to bind an additional 3 percent of the P^{32} could be a consequence of RNA of lower specific radioactivity, that is, metabolically more stable RNA. Alternatively, a high concentration of DNA would be necessary for the binding of very abundant molecules, even of high specific radioactivity, because

Table 2. Relation of time of labeling to the percentage of labeled RNA bound to DNA-agar.

Time	Cytoplasmic RNA		Nuclear RNA	
	Incubated* (μg)	Bound (%)	Incubated* (μg)	Bound (%)
0.5 hr	500	6.9	150	10.2
1.5 hr	500	6.0	150	9.3
1 day	325	0.4	200	2.1
3 days†	1700	0.4	1100	1.4

* The quantity of liver RNA incubated with 800 μg of DNA in 1 or 2 g of agar. † The figures at 3 days are derived from RNA isolated from livers of mice injected with P^{32}-orthophosphate once a day for 3 days before sacrifice.

Table 3. Nucleotide composition of RNA fractions separated on DNA-agar. The percentages given are calculated as moles of base per 100 moles of total base in RNA. Nuclear RNA from mouse liver (200 μg) was incubated with 2600 μg of denatured mouse DNA in 2 g of agar at 60°C for 40 hours. Unbound P^{32}-RNA was eluted in double strength SSC at 60°C. Bound P^{32}-RNA was eluted at 70°C in 1:100 SSC.

Base	P^{32}-labeled nucleotide (%)	
	Unbound	Bound
C	24.6	23.4
A	26.2	28.5
G	27.8	23.8
U	21.4	24.3

of the low incidence of homologous sites in the DNA. Qualitatively, at least, this situation is not unlike that in exponentially growing bacteria where more than half of the rapidly labeled RNA molecules (*16*) represent the primary gene product of less than 0.5 percent of the total genome (*13–16*).

This major class of labeled molecules is metabolically stable ribosomal RNA. They are of low specific radioactivity and are only detected if the DNA:RNA ratio is high. The remainder (99 percent) of the DNA provides binding sites for the other portion of the labeled RNA which comprises only 1 percent of the total RNA. A similar quantitative analysis of our results is not possible since the specific radioactivity of the fraction of RNA bound to the DNA could not be determined conveniently. Therefore, the fraction of the total RNA which is bound cannot, at present, be assessed. However, the fact that 1.4 percent of the nuclear RNA from steady-state labeled mouse L cells is bound to mouse liver DNA under similar conditions, is in good agreement with the 1- and 3-day results shown in Table 2 as well as with the 0.5 percent unstable RNA as estimated by Scherrer *et al.* (*4*) for HeLa cells.

Essentially all of the radioactive RNA is in the nucleus immediately after administration of the tracer. Thus the RNA which is able to hybridize with the DNA must originate there. Later, when radioactivity appears in the cytoplasm, a similar proportion of the labeled RNA can react with DNA (Table 4). It is clear that at least some of the complementary RNA passes out of the nucleus and into the cytoplasm without extensive degradation.

The fact that RNA prepared from the cytoplasm is able to compete with labeled nuclear RNA for sites in the DNA, reinforces the conclusion that cytoplasmic RNA has its origin in the nucleus. Furthermore, since the fraction of radioactive RNA able to hybridize falls as the labeling periods are increased, it would appear that these RNA molecules, either nuclear or cytoplasmic, have only a limited lifetime in the cell. Thus, the RNA molecules in question appear to be synthesized in the nucleus, transferred in part to the cytoplasm, and then degraded after a limited period of activity.

The nucleotide composition of the unbound, labeled RNA (52 percent GC) as compared with that of the bound (47 percent GC), shows that a separation between two classes of RNA has been accomplished. The composition of neither class of molecule closely resembles the parent DNA (43 percent GC). Similarity to DNA in composition is not necessarily a good criterion for complementarity. The radioactivity may not be randomly distributed among the nucleotides of the two classes of RNA. Alternatively, only a limited region of the DNA in liver tissue may function in producing complementary RNA and the composition of this region may not reflect the average DNA composition. Other examples of disparity between the composition of hybridized RNA and DNA are known and are typified by the hybridization of ribosomal RNA (*13, 14*) or the analysis of selected fractions of bacterial messenger RNA (*17*).

Table 4. Binding of mouse-liver nuclear RNA and *E. coli* RNA to various DNA-agar preparations. Mouse liver RNA (100 μg) obtained from mice 1.5 hours after injection with P^{32}-orthophosphate was incubated for 40 hours at 60°C with 2500 μg denatured mouse or calf DNA in 2 or 2.5 g agar or 1000 μg *E. coli* DNA in 2 g agar. The *E. coli* RNA (50 μg), obtained from exponentially growing cells 1 minute after the addition of P^{32}-orthophosphate, was similarly incubated. Elution from the agar column was carried out with double strength SSC at 60°C and 1:100 SSC at 70°C or 75°C.

DNA-agar	P^{32}-bound RNA (%)	
	Mouse	*E. coli*
Mouse liver	13.3	1.2
Calf thymus	4.9	1.0
E. coli	1.1	32.0
None	1.0	1.1

Where interacting DNA and RNA mixtures are examined after the mixture has been cooled to about 25°C, the resistance to ribonuclease is the only available criterion of true hybrid formation. This allows discrimination from trapping or adventitious pairing over short regions (*13*). In the usual CsCl-gradient technique this type of pairing occurs, particularly where the DNA is a complex one such as thymus, even if the RNA is of bacterial origin (*13*). With our techniques, the criterion applied to demonstrate the presence of stable hybrids is their resistance to dissociation at 60°C in 0.3*M* NaCl. From the data of Table 4 it is clear that nonspecific binding, such as *E. coli* RNA with mouse DNA, or the reciprocal reaction, did not occur.

In the light of exclusion of nonspecific hybrid formation, the cross reaction between mouse liver RNA and calf thymus DNA becomes particularly

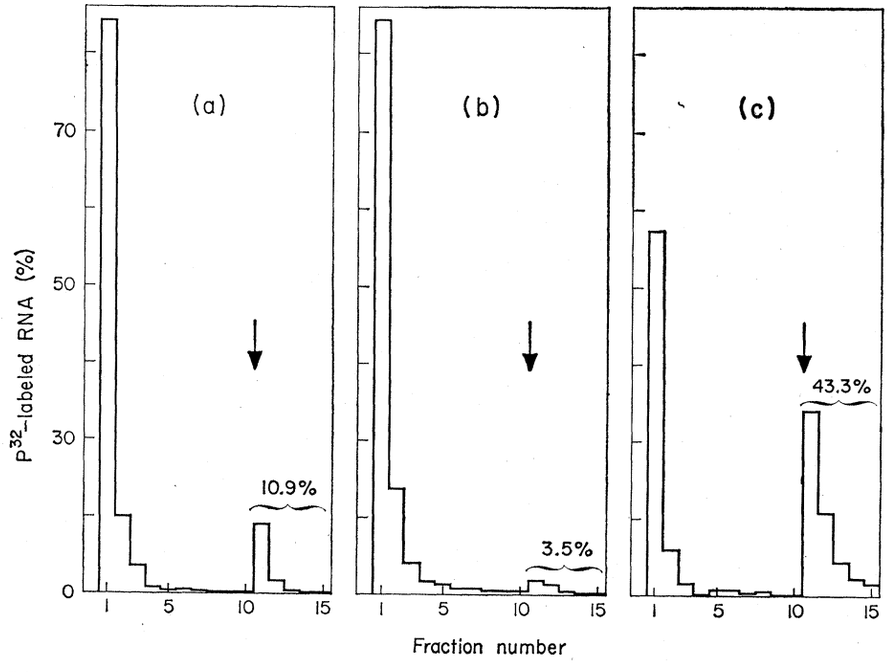

Fig. 4. Reincubation of bound and unbound P^{32}-labeled mouse liver cell nuclear RNA with DNA-agar. Liver nuclear RNA (200 μg) obtained from mice 1.5 hours after injection with P^{32}-orthophosphate was incubated 40 hours with 1300 μg of denatured mouse DNA embedded in 1 g of agar. The elution pattern is shown in *a*; ten 15-ml fractions were taken at 60°C with double strength SSC as the eluent and, at the arrow, the temperature was raised to 70°C and the salt concentration was lowered to 1:100 SSC. Further fractions were then collected. The unbound RNA, reincubated under similar conditions, produced elution pattern *b*, and the reincubated, bound RNA produced elution pattern *c*.

significant. This result has been supported by observation of considerable interspecies cross reaction among denatured DNA fragments and DNA-agar of several mammalian species. In addition, DNA-DNA and also RNA-DNA interactions have been used to evaluate quantitatively, genetic relatedness among the Enterobacteriaceae (*11*).

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1. The abbreviations are: RNA, ribonucleic acid; DNA, deoxyribonucleic acid; SSC, standard saline citrate (0.15*M* NaCl and 0.015*M* sodium citrate); A, C, G, and U, are adenylic, cytidylic, guanylic, and uridylic acids, respectively.
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4 June 1963

Zeolite ZK-5: A New Molecular Sieve

Abstract. *An aluminosilicate of novel crystal structure has been synthesized. It has molecular-sieve properties that permit separation of straight-chain from branched-chain and cyclic hydrocarbons. This zeolite is unusually stable in solutions of low* pH.

A crystalline aluminosilicate with a novel crystal structure has been synthesized. Table 1 contains pertinent data derived from x-ray diffraction measurements of this new substance. These data indicate that the crystal structure of the new zeolite, given the name zeolite ZK-5, is body-centered cubic with a lattice parameter $a = 18.72$ Å. A pseudo cell was also observed which is primitive cubic with $a' = a/(2)^{\frac{1}{2}}$. Meier and Kokotailo have elucidated the main features of the crystal structure (*1*).

Table 1. X-ray diffraction data—zeolite ZK-5.

(*h*, *k*, *l*)	*d*(Å)	I/I_{max}*
110	13.3	0.18
200	9.41	1.00
220	6.62	0.06
310	5.93	.41
222	5.41	.48
321	5.03	.02
400	4.69	.06
330	4.41	.50
420	4.19	.34
332	3.98	.22
422	3.81	.18
510	3.66	.06
521	3.41	.13
530,433	3.21	.35
611	3.02	.28
620	2.94	.21
541	2.88	.02
622	2.81	.26
631	2.75	.09
543,710,550	2.64	.11
640	2.59	.02
721,633,552	2.54	.09
730	2.45	.03
732,651	2.37	.01
811,741,554	2.30	.02
822,660	2.20	.03
831,750,743	2.17	.02
662	2.14	.01
910,833	2.06	.03
842	2.04	.02
921,761,655	2.02	.03
830,851,754	1.97	.005
932,763	1.93	.02
941,853,770	1.89	.02
10,2,0,862	1.83	.05
10,3,1,952,765	1.79	.05

*I/I_{max} is the intensity of each reflection relative to the reflection of maximum intensity.

The mole ratio of SiO_2 to Al_2O_3 in zeolite ZK-5 varies from 4.0 to 5.1. The more silica-rich samples can undergo cation exchange with dilute hydrochloric acid solution (about 0.1*N*) with no significant loss in crystallinity. Stability of this type is not frequently observed in either naturally occurring or synthetic zeolites. Various cation forms of this zeolite, prepared by standard ion-exchange techniques, are capable of adsorbing about 13 percent by weight of straight-chain hydrocarbons while excluding branched-chain or cyclic hydrocarbons. In this respect, zeolite ZK-5 has molecular sieve properties similar to those of zeolite A (*2*) and zeolite ZK-4 (*3*).

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Blood Group Studies with Turtles

Abstract. *Groups and individuals of diverse species of turtles have been distinguished by selective agglutinations of their washed red blood cells when undiluted normal serums or plant extracts are used as agglutinins. During these studies, production of hemagglutinins and precipitins has been induced in turtles and certain other poikilotherms.*

Very little immunogenetic research has involved reptiles and amphibians, literature in this field having been reviewed by Hildemann (*1*). Sixty years ago Noguchi (*2*) reported hemagglutination and antibody production in turtles. Later, antibodies to mammalian serum were induced in one species of turtle (*3*); normal isohemagglutinins were demonstrated in two species, and irregular heterospecific agglutinins were found (*4*). The present report deals with blood group differences in turtles and shows that a variety of blood grouping reagents may be used for characterizing individuals and populations.

Most of the blood samples were taken without serious trauma by cardiac puncture. So far I have had some limited success in obtaining microliter samples from retro-orbital sinuses of some turtles (*5*) by using techniques recently used with other organisms (*6*). Blood cells were washed three times with 0.9-percent sodium chloride or Alsever's solution, and small drops of approximately 2-percent cell suspensions were mixed in wells of test plates with equal volumes of individual undiluted serum or lectin-containing plant extract. For certain of the reactions, titers were obtained by using serial dilutions of agglutinins in test tubes. Most serums tested were from unimmunized reptiles, but a few amphibian (toad), mammalian (human and rabbit), fish (chondrichthyes and osteichthyes) and arthropod (crab) serums also caused selective agglutinations. Lectins were extracted in 0.85-percent sodium chloride (weight of plant tissue to solvent being about 1:5), and