for the yg - sh region on chromosome 9. It was thought that other meiotic mutants might have an influence on crossing over when heterozygous; therefore the gene ameiotic was tested.

Sibling plants of the constitution Am/Am, C Sh Wx/c sh wx or Am/am, C Sh Wx/c sh wx were used as male parents and crossed to c sh wx testers. Similarly the Ws Lg Gl region of chromosome 2 was studied. The male parents were also self-pollinated and the progeny were scored for the presence or absence of ameiotic plants in order to distinguish Am/Am from Am/am.

Tests of individual backcross progenies for a 1:1 segregation of C:c, Sh:sh, Wx:wx and Ws:ws, Lg:lg, Gl:gl within each of the genotypes Am Am and Am am gave small X^2 values for heterogeneity. Therefore they were considered homogeneous. Tests for heterogeneity within the genotypes Am Am and Am am were also made for the eight classes of chromosome 9 markers and for the chromosome 2 markers. The slight differences that were observed are well within the range expected from sampling as was shown by the relatively small X^2 values for heterogeneity. The pooled results are shown in Tables 1 and 2.

A method outlined by Serra (1) was used to determine the significance of the different crossover values obtained. Crossing over in the marked regions of chromosome 2 and chromosome 9 did not differ statistically in Am/Am vs. Am/am plants. Studies are in progress to test the effect of the ameiotic gene on crossing over on the female side.

1. Serra, J.A. 1965. Modern Genetics. Vol. 1, ch. 17. Academic Press. New York.

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7. Biochemical effects of the gene ameiotic.

The gene ameiotic was first reported by Rhoades (MNL 1956) and is inherited as a single Mendelian recessive, which in the homozygous condition results in plants which are male and female sterile. Sinha (MNL 1959, 1960) attempted to find a biochemical basis for the failure of ameiotic plants to undergo meiosis. Using a modification of the Ogur and Rosen method, Sinha examined perchloric acid extracts of plant tissues for their nucleic acid content and nucleic acid precursors. He found that

the RNA/DNA ratio was higher in ameiotic ears, ovules, anthers and root tips than in normal structures. To investigate this apparent difference between the ameiotic and normal tissues, the RNA was fractionated. He found some differences in the ratio of RNA-1, RNA-2, and RNA-3, to DNA, but the differences between different parts of the same ear were greater than those between the two genotypes. The quantity of DNA was the same in both normal and ameiotic plants.

In normal ears precursors of nucleic acids were found only transiently in the premeiotic stage, whereas in ameiotic ears large quantities of precursors were found at all times except at a very early stage. The accumulation of precursors in ameiotic anthers at the premeiotic stage was also much greater than that in normal anthers. Sinha postulated that the am gene stimulated the production of a ribose containing precursor which was rapidly converted to RNA and that this accounted for the greater accumulation of precursor and RNA in ameiotic plants.

He found biochemical differences in vegetative tissue of normal and ameiotic plants although there are no detectable morphological changes. In view of Sinha's results, it seemed pertinent to further investigate this reported difference in nucleic acid metabolism.

Plants from families known to be segregating for ameiotic were sampled throughout the course of development by taking leaves, root tips (5 mm.), and anthers. In the case of the anthers, every third floret was fixed in 3:1 (ethanol: glacial acetic) and was examined cytologically in order to determine the meiotic stage of the remaining two florets. The fresh tissue was washed, chopped, lyophilized, ground under liquid nitrogen and stored in a vacuum desiccator at -20°C. After anthesis, when the plants could be identified as normal or ameiotic, samples taken from the same genotype on the same day were pooled when necessary to obtain 500 mg. dry weight samples for nucleic acid extraction and analysis.

Two methods of nucleic acid extraction were used to determine which procedure resulted in the greatest quantitative yield of RNA and DNA from the same sample (Table 1). The method of Kirby (Biochem. J. 96, 1965) as modified by Williams was chosen. Not only did this method give higher yields of RNA and DNA from the same sample, but reproducibility

was also better with this modified phenol extraction. Protein contamination was considerably higher in the hot NaCl method than in the phenol extraction. UV absorption was determined on an Hitachi-Perkins-Elmer Model (139) spectrophotometer for hydrolyzed RNA and DNA. Some fractions were assayed for protein by the Lowry determination (Lowry et al. 1951, J.B.C. 193), for RNA by the orcinol determination (Ceriotti 1955, J.B.C. 214) and for DNA by the p-nitrophenylhydrazine determination (Webb & Levy 1955, J.B.C. 213).

Table 1

A Comparison of Two Methods for the Extraction of Nucleic Acids from Maize Leaves and Roots

Age (days)	Guinn* (hot NaCl extraction)				Kirby & Williams (modified phenol extraction)			
	RNA**		DNA**		RNA**		DNA**	
	Leaves	Roots	Leaves	Roots	Leaves	Roots	Leaves	Roots
62	9.5		1.93		11.3		2.47	
62	10.3		2.17		10.9		2.51	
62	9.7	12.9	1.86	4.75	11.8	13.3	2.43	5.10
62	10.1	12.3	2.25	4.80	11.3	12.9	2.62	4.96
71	11.0	12.0	1.92	4.68	11.7	13.8	2.39	5.01
71	9.2	11.7	2.01	4.78	11.0	13.4	2.44	4.98

*Guinn, G. 1965. Extraction of Nucleic Acids from Lyophilized Plant Material. Plant Physiology 41:689-695.

**values expressed as mg/g dry weight.

The results of the nucleic acid determination are shown in Tables 2 and 3. The differences between normal and ameiotic plants in their RNA and DNA contents are quite small. This is true for all developmental stages in the leaves, roots and anthers, with the possible exception of the comparison of anthers at the quartet stage versus those in a "late" stage from ameiotic plants. As is evident from cytological observations at this stage, ameiotic cells are broken down and no cellular organization exists. Such differences could therefore be the result of this degeneration. Both the orcinol and p-nitrophenylhydrazine colorimetric

Table 2

Nucleic acids extracted from maize leaves and roots as indicated by UV absorption at 260 mu

Age (days)	Phenotype	RNA (mg/g dry weight)		DNA (mg/g dry weight)	
		Leaves	Roots	Leaves	Roots
34	N	10.4	12.8	2.40	5.41
	N	10.5	12.2	2.48	5.15
	am	10.7	11.9	2.30	5.22
	am	10.2	12.8	2.42	5.37
41	N	--	13.0	--	5.25
	N	--	13.3	--	5.11
49	am	10.6	--	2.64	--
	am	11.0	--	2.52	--
50	N	10.8	--	2.50	--
	N	10.6	--	2.60	--
62	N	11.1	13.2	2.42	5.06
	N	11.5	13.7	2.48	4.90
	N	11.3	--	2.50	--
	N	11.6	--	2.60	--
	am	11.2	13.8	2.58	5.05
	am	11.5	13.3	2.48	4.83
71	N	11.3	12.9	2.40	4.70
	N	11.7	13.2	2.34	4.55
	am	11.5	13.2	2.38	4.53
	am	11.6	13.5	2.31	4.77

Table 3

Nucleic Acids Extracted from Maize Anthers

Stage	Phenotype	RNA Extract			DNA Extract		
		Protein (ug/g dry weight)	RNA (mg/g dry weight)		Protein (ug/g dry weight)	DNA (mg/g dry weight)	
			UV absorption (260 mu)	orcinol test		UV absorption (260 mu)	p-nitro-phenylhydrazine test
Very young whole tassel	N	72.6	7.8	8.1	72.9	1.42	1.45
	N	76.4	8.1	8.3	47.5	1.47	1.53
	am	91.0	8.3	8.9	54.6	1.54	1.50
Premeiotic	N	83.1	9.6	9.5	80.3	1.48	1.51
	N	95.7	9.9	10.3	75.7	1.40	1.47
	am	100.2	10.1	10.7	68.9	1.39	1.42
	am	87.3	9.6	9.7	79.2	1.42	1.39
Prophase	N	87.2	9.6	10.8	91.0	1.33	1.27
	N	103.1	10.0	11.1	76.4	1.25	1.33
Early	am	96.3	9.4	9.8	80.5	1.20	1.28
	am	110.1	9.6	10.4	65.4	1.28	1.37
Quartets	N	53.4	9.1	10.0	53.4	0.71	0.65
	N	80.0	9.5	10.4	64.0	0.62	0.69
	N	96.4	10.1	11.1	80.0	0.57	0.60
Late	am	83.4	9.0	9.7	96.4	1.05	0.90
	am	72.1	8.8	9.1	79.3	0.89	0.98

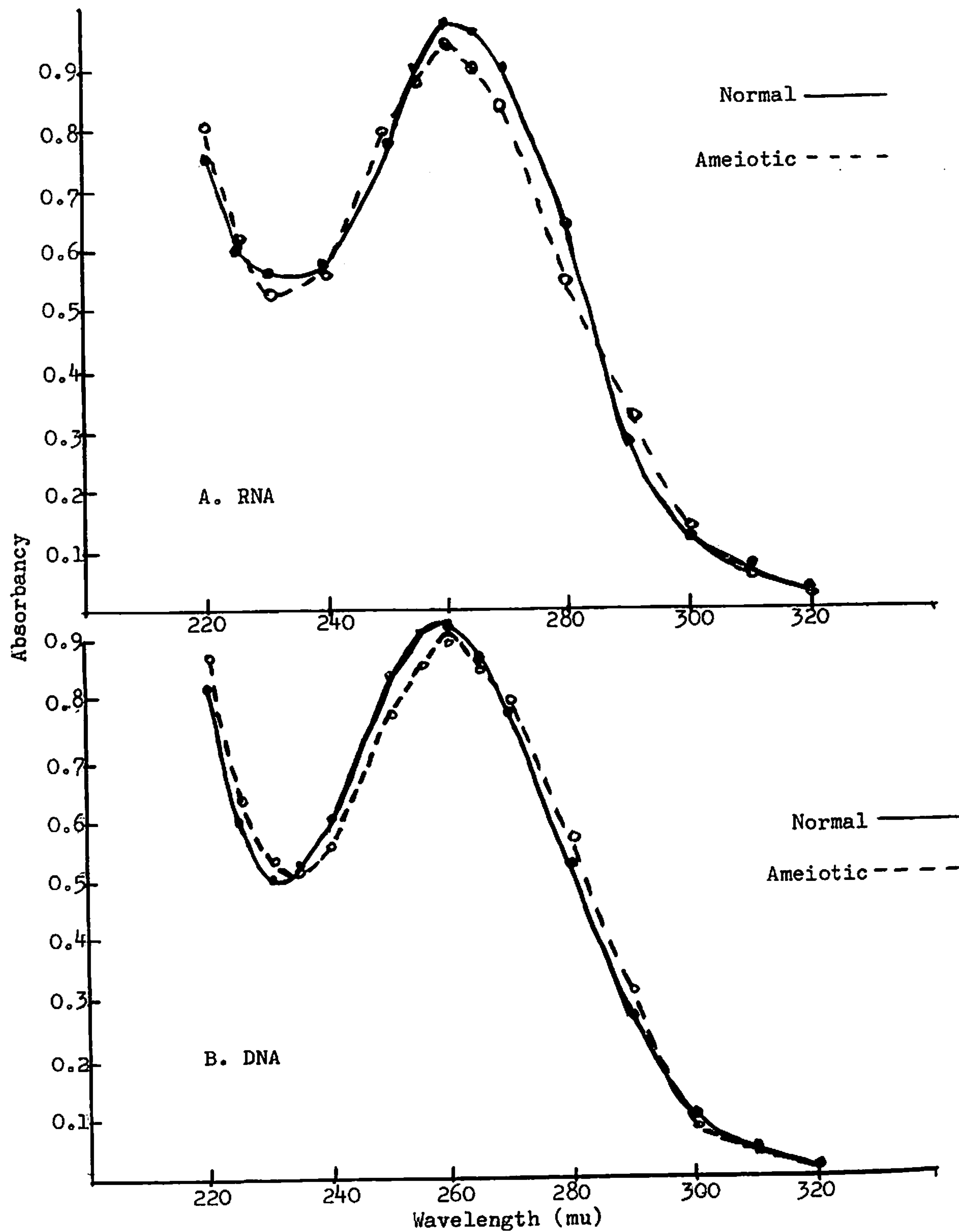

Figure 1. UV absorption spectra of nucleic acids extracted from normal and ameiotic maize anthers in the premeiotic stage. A. RNA. B. DNA.

tests result in slightly higher values for RNA and DNA, respectively. The orcinol test for pentose is a measure only of the sugar which was associated with the purine moieties and therefore non-RNA ribose will also be measured and would contribute to the absorption. Similarly, the p-nitrophenylhydrazine test for pentose is a measure only of the sugar which was associated with the purine moieties and non-DNA deoxyribose would also be measured. Also like the orcinol method for RNA, significant deviations in base composition will affect the accuracy of this method. However, since the differences between UV absorption results and the colorimetric test were small, it was concluded the deviations in base composition were non-existent or small. The RNA samples were tested for contamination by DNA and the DNA samples were tested for contamination by RNA. In both cases contamination was found to be negligible. While there is some variability in the protein content within samples as well as between samples, at present no significance can be attributed to this variability. Since the protein content in the RNA and DNA samples is low, it did not significantly interfere with the UV absorption measurements.

UV absorption curves of RNA and DNA extracted from anthers are shown in Figure 1. The curves are typical of standard nucleic acid samples in that the maximum and minimum optical density values occur at the expected wavelengths.

The results described above do not confirm the work of Sinha but indicate that there is no difference in the RNA or DNA content of normal and ameiotic sibs. In contrast to Sinha's procedure, dry weight samples were used instead of wet weight and a totally different extraction method was employed. It is uncertain at present whether the discrepancy between the results reported here and those of Sinha should be attributed to these factors. Extraction of DNA and RNA from anthers will be carried out by the Ogur-Rosen method in an effort to duplicate the results of Sinha and determine whether or not a difference between normal and ameiotic plants can be detected.

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