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S-582 - Rpt #4(Annual)
Contract: DA19-129-qm-1952
American Meat Institute Fnd.

30 p - 405

A Study of Meat Enzymes to Determine Means for Their Control

Period: 3 January 1962 - 2 January 1963

ARMED FORCES FOOD AND CONTAINER INSTITUTE
U. S. Army Quartermaster Research and Engineering Center
Chicago 9, Illinois

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Report No. 4, 3 January 1963, 9 pp.
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Report

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CONTRACT RESEARCH PROJECT REPORT

QUARTERMASTER FOOD AND CONTAINER INSTITUTE FOR THE ARMED FORCES, CHICAGO Hq., QM Research and Engineering Command, QM Research and Engineering Center Natick, Massachusetts

American Meat Institute Foundation
939 East 57th Street
Chicago 37, Illinois

Official Investigator-W. A. Landmann
Collaborators-Renée K. Margolis
Anthony Bartal

Project No.: 7-84-01-002
Contract DA 19-129-QM-1952
File No.: S-582
Report No.: 4 (Annual)
Period: 3 January 1962 to
2 January 1963
Initiation Date: 3 January 1962.

Title of Contract:

A Study of Meat Enzymes to Determine Means for Their Control.

SUMMARY

Storage studies of ground meat samples treated (1) to remove ions, especially Fe^{++}, (2) to complex Fe^{++}, (3) to oxidize Fe^{++}, protein, or sulfhydryl groups, (4) to bind sulfhydryl groups and (5) to change pH were conducted. Changes in free amino nitrogen, expressed in terms of μ moles of alanine per gram of protein, have been observed. Measurements of loosely bound iron did not generally correlate with the amount of proteolysis. The significance of these changes were examined statistically, and the most effective treatments for inhibiting proteolysis were found to be NaCl, Citric Acid, pH change. Effects of other treatments were found to range from slight inhibition to no inhibition. Strong oxidizing agents caused increased hydrolysis.

Report

In a previous study of isolated beef tissue proteolytic enzymes it was found that their activity could be in part controlled by the addition or removal of certain divalent metal ions, particularly Fe^{++} and Cu^{++}.

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Reagents affecting the sulfhydryl groups also appeared to have some effect. Control of pH was also found to be a possible means of affecting the activity of the isolated enzymes, since they were optimally active at pH 5-6, but inactive at pH 4 or less and at pH 7-8. It is the purpose of this study to continue these investigations with the objective of finding non-thermal methods which may be effective in inhibiting proteolysis in irradiated meat during extended storage at temperatures above freezing.

The problem resolved itself into two main parts: 1) investigation of techniques which could be employed in treating meat to introduce reagents or to remove activators which would affect the enzyme systems, and 2) based on the previous study of the characteristics of the enzymes found in meat, investigation of those treatments which would be expected to be most effective in inactivating the proteolytic enzymes.

In order to study the removal of Fe^{++}, it was necessary to find a method for determining this element in meat. Among several methods available, the one giving the best results was based on the reaction of Fe^{++} with α, α'-bipyridyl. (1, 2). An application of this reaction to the determination of iron in animal tissues has been published by Borgen and Elvehjem. (3). This method measures free or "loosely bound" iron in tissues and does not detect the iron associated with heme, i.e. myoglobin, hemoglobin or cytochrome iron. Although no distinction was possible between ferrous or ferric iron by this method, it was still useful in following changes in over-all concentrations of iron. Several attempts were made to adapt the method for ferrous iron only, but none were successful. Oxidative changes for iron could therefore not be followed.

Since the simplest techniques of treating meat were electrodialysis and dialysis of ground meat samples, these were used for the first group of studies on iron removal, chelation, and oxidation. Samples of ground meat were placed in dialysis tubing, and dialyzed for various times in 0.3M solutions of EDTA, NaCl, $(NH_4)_2S_2O_8$, citric acid, as well as water alone. For the electrodialysis, ground meat was mixed with half its weight of water to facilitate transfer to the cell, and the mixture placed in the center cell of a three-compartment electrodialyzer. Cellulose membranes separated the center cell from the two electrode cells which were filled with water. Electrolysis was carried out at 150ma, and was considered complete when the voltage had risen from its original value of 20-30V to 140V. In order to avoid heating effects, attempts to cool the cell were made, and the temperature was kept as low as possible during the passage of current. However, local heating always occurred, and in these areas the meat appeared "clumped". These clumps were separated by visual observation from the uncooked meat and were not included in the samples for iron determination or storage.

Iron determinations were carried out on all samples and on the meat before treatment. Results shown in Table I appear to be quite erratic, but some trends may be noted. The results of this preliminary study were more or less corroborated in the actual storage samples of Group I, which was carried out later.

Sample Preparation

Beef round, trimmed of fat, was coarsely ground and thoroughly mixed. Part of the sample was reserved for irradiated and non-irradiated controls, which received no other treatment. The remainder of the meat was treated with the desired materials, by adding solutions in volumes amounting to ½ the weight of the meat (w/v). For example, to 100 grams of meat, 50 ml. of 0.1M solution of $NaHSO_3$ was added. After thorough mixing the samples were divided into 16 vials for each treatment, 8 of which were irradiated. The untreated meat was also divided into 16 portions, 8 of which were irradiated. All samples which were irradiated received 5 megarad of γ-irradiation, and were then stored at 4-5° C. Non-irradiated samples were stored frozen. Samples for analysis were taken immediately after irradiation (0 day), and after 4 weeks, 8 weeks, and 12 weeks storage. Replicate samples were used with single values on each.

The following groups were placed in storage:

Group I., Irradiated and Non-Irradiated (For removal of Fe^{++})
Treatments: Citric Acid (0.3M), Sodium Chloride (0.3M), Electrodialysis, Control (no treatment)

Group II, Irradiated and Non-Irradiated (For blocking or oxidizing sulfhydryl groups)
Treatments: $NaHSO_3$ (0.1M), Iodoacetic Acid (0.1M), Mercuric Chloride (0.1M), Control

Group III, Irradiated and Non-Irradiated (For complexing iron or oxidation of-SH)
Treatments: Transferrin (0.01%) Sorbitol (2.0%), EDTA (0.1M), Ammonium persulfate (0.3M), Control

Group IV, Irradiated and Non-Irradiated (For pH control)
Treatments: Tris (0.1M), IR-120 resin, Control.

Group I samples were dialyzed against the indicated solutions for 18 hours. The resin treatment in Group IV was carried out by placing the meat slurry (1 ml. H_2O to 2 g. meat) in dialysis tubing and dialyzing it against water containing IR-120 resin for 18 hours with constant slow shaking.

Samples were analyzed for free amino groups and protein content, and those of Group I were examined for iron content, using the method described above. (Table III). Free amino groups were determined by using the ninhydrin colorimetric procedure of Moore & Stein. (4) Color yields were related to alanine, and results were expressed as μM alanine equivalents per gram protein. Results for iron were also expressed on a protein basis.

The amount of hydrolysis (proteolysis) occurring over storage periods of 0 to 12 weeks for the various treatment groups is tabulated in Tables II to VI.

Statistical Analysis

Because of the variability of the individual results, a statistical analysis was performed on each treatment to discover if there was any significant trend due to the treatment. Since the purpose of this study was to determine if any inhibitory effect was present because of the treatment, the usual .05-.01 significance level was considered too stringent a restriction. Therefore significance at the .20 level, i.e., if the observed effect would occur one out of five times by chance, was considered as indicating an effect.

A factorial design, 2 x 2 x 4, was employed for the statistical analysis of each treatment, according to the following scheme:

Factors: Irradiation; 2 levels, 0 & 5 megarad
Treatment; 2 levels, treated & untreated
Storage; 4 levels, 0, 4, 8, 12 weeks

Replications: 2

Irradiation	Treatment	Storage	Replicates
0 megarad	Untreated	0 4 8 weeks 12	X X X X X X X X
	Treated		
5 megarad	Untreated		
	Treated		

Correlation coefficients for iron content and Group I treatments were also calculated.

Results and Discussion

Results of the analysis of variance for each treatment, and a plot of the interactions of interest are shown in Tables VII to XVIII, and figures accompanying each table. The effect of each treatment is summarized below.

Group I

Citric Acid. (Table VII).

The effect of citric acid treatment is very highly significant, and a general inhibition of proteolysis was noted for the samples receiving the treatment. Untreated samples showed a progressive increase in proteolysis over the storage period, while treated samples showed an inhibition during the first four weeks. After this time the proteolysis proceeded at about the same rate as controls. Irradiation caused a general increase in the amount of free amino groups over the non-irradiated samples, but the difference in level was maintained through the storage period. Citric acid appeared to inhibit the proteolysis to a greater extent in the irradiated samples.

Citric acid was used primarily as a means of complexing Fe^{++} to inhibit enzymatic activity. However, a determination of the correlation between the iron content of these samples (Table III) and the proteolysis indicated that there was no correlation between the two values. This may be due to some extent, to the difficulty of obtaining good results for Fe^{++} content, since the method can only determine total "loosely bound" iron. On the other hand, the effect of citric acid is probably due to its low pH, since the meat samples had a pH of 3.5. This would be acidic enough to inhibit the enzymes more effectively than removing the Fe^{++} activator.

Sodium Chloride. (Table VIII).

Dialysis against hypertonic salt proved to be an effective means of inhibiting proteolysis. The main effects, as well as all the two-way interactions, were highly significant. The greatest effect of the sodium chloride was during the first 4 weeks of the storage period, when inhibition of proteolysis occurred. After the 4th week, the proteolysis continued at about the same rate as the untreated control. Irradiation again increased the general level of free amino groups and irradiated samples maintained this higher level throughout the storage.

There was no correlation of iron content and inhibition of proteolysis. Since the hypertonic NaCl solution apparently did not aid in the removal of iron, it is therefore difficult to explain the mechanism of action of the sodium chloride as an inhibitor of prote-

olysis. It has been suggested that increasing the sodium chloride content of meat causes a shift to a lower pH.

Electrodialysis. (Table IX).

Although the table of analysis of variance indicated a high significance for this treatment, the plot of the effect of the treatment showed that proteolysis was not inhibited, but was actually increased by the electrodialysis. This may reflect a possible destruction of cells and denaturation. Irradiation and storage effects were significant, but this was to be expected, since the effects were the same as in the other treatments. That is, radiation caused a general rise in the level of free amino groups, and proteolysis increased with storage time.

Group II.

Sodium Bisulfite. (Table X).

The effect of this reagent was to increase proteolysis, possibly by oxidative action on the tissue proteins, or by non-enzymatic hydrolysis. There is also a possibility that oxidizing agents are activators for the proteolytic enzymes which may require intact -S-S- bonds, for example. This effect has not previously been observed, however. Furthermore the sodium bisulfite would probably take the -S-S- linkage to a higher oxidation state.

At any rate, the bisulfite oxidative treatment is of no importance in inhibiting the activity of the cathepsins.

Iodoacetic Acid. (Table XI).

Treatment with iodoacetate inhibited proteolysis, expecially in the irradiated samples. The inhibition was noticeable after the first four weeks, although the difference between the control and treated samples was at a low level of significance. Irradiation and length of storage time caused the usual effects, as noted previously.

The inhibition by iodoacetic acid could be due either to blocking of sulfhydryl groups or to a lowered pH. The pH effect appears somewhat less probable from the curve of the non-irradiated control samples, where the iodoacetic acid treatment did not cause any inhibition. The more pronounced effect on irradiated samples, where it is likely that more -SH groups were exposed, would indicate that the effect was on the -SH groups.

Mercuric Chloride. (Table XII).

Mercuric chloride appeared to be slightly more effective than iodoacetic acid as an inhibitor of proteolysis. The inhibition appeared to continue and become more pronounced with increasing time of storage. However, the inhibition was not as effective as that noted for citric acid or sodium chloride. Irradiation and storage showed the usual increase in free amino nitrogen.

Group III.

Transferrin. (Table XIII).

Transferrin has been reported to be an excellent complexing or binding agent for Fe^{++}, and was therefore utilized for this purpose. The treatment appeared to be more effective on irradiated meat than non-irradiated meat, on which there was very little effect. Perhaps the increased amounts of iron in irradiated meat (Table III) as well as the denaturation of protein substrates stimulates proteolysis in the untreated meat. In the presence of transferrin, the Fe^{++} activators are essentially removed and the proteolysis is inhibited. In non-irradiated meat the levels of free Fe^{++} are probably so low that the addition of transferrin would cause very little effect.

The inhibition by transferrin, however, appeared to be of short duration, since on storage of the meat, the proteolysis proceeded at the same rate for both treated and untreated material. Apparently complexing Fe^{++} is not a good way to inhibit the action of cathepsins in muscle tissue.

Sorbitol. (Table XIV)

The effect of sorbitol appeared to be stimulatory rather than inhibitory. The significance of the Sor X I interaction is apparently due to the irradiation effect, primarily.

EDTA. (Table XV).

EDTA had no effect that could be detected by this method. The differences observed were due to storage and irradiation effects only.

Ammonium Persulfate. (Table XVI).

This reagent caused extensive changes in the appearance of the meat, which became granular and dry in texture and greyish in color. Since ammonium persulfate is a powerful oxidant, the destruction of tissue constituents was not unexpected. The high values observed for free amino groups are undoubtedly due to the ammonium ion interference, and therefore the proteolytically produced amino groups were

probably obscured. However, by adjusting the values to the same levels, graphically, the course of proteolysis during storage of treated samples was only slightly different from that of the untreated samples, and the difference appeared to be due to increased hydrolysis rather than inhibition of hydrolysis in the treated material.

Group IV.

IR-120,H^+, dialysis. (Table XVII).

The treatment was designed to give a slow pH change, by substituting H^+ for metal ions diffusing from the meat. After the dialysis - ion exchange the pH of the meat dropped to less than 5.0. The treatment effectively inhibited proteolysis, although as storage time increased the rate of proteolysis appeared to parallel that of the untreated controls. Irradiation and storage effects were the same as in all other samples.

Tris Buffer, pH 10. (Table XVIII).

The pH of the treated meat was approximately 7-8. No change in proteolysis could be observed in comparing treated samples with untreated samples. Proteolysis increased during storage, again with little difference between treated or untreated material. Irradiation caused a general rise in level of free amino groups, independent of treatment or storage.

Conclusions

1. The most effective treatments (non-thermal) for inhibiting the proteolytic activity in meat appear to be citric acid, sodium chloride, and change of pH to below pH 5.0. The latter was accomplished by the dialysis of ground meat against a strongly acidic cation exchange resin.

2. Less effective treatments were those in which the -SH groups may have been blocked; mercuric chloride, iodoacetic acid. These, of course are toxic and could not be used in food.

3. The most efficient reagent for complexing Fe^{++} used was transferrin. This was the only one of the group of treatments designed to remove or complex iron that was somewhat effective in reducing proteolysis.

4. All other treatments were ineffective, or increased proteolysis.

Future Work

In the remaining time, the treatments found most effective will be repeated on ground meat. The same reagents will be studied on meat slices and on freeze-dried meat, rehydrated in the reagents.

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4. Moore, S. and Stein, W., J. Biol Chem. 211 907 (1954)

Table I.

Content of "Loosely-Bound" Fe^{++} in Meat(1) Dialyzed vs 0.3 M Solutions of Various Reagents

TREATMENT	"Loosely Bound" Fe^{++} (µg/gram meat)	
	16-20 hrs. Dialysis	66 Hr. Dialysis
None	5.0, 8.7 8.3, 10.6	
Electrodialysis	8.7, 8.7 15.3(2)	
EDTA	4.0, 4.0	
NaCl	7.1, 9.0 11.5(2)	
$(NH_4)_2S_2O_8$	9.7, 28.6	17.0, 15.3
Citric Acid(3)	7.0, 19.6 13.3(2)	9.0, 8.3
H_2O only	6.0, 7.1 11.6	15.3, 14.3

1. Meat analyzed 3.97% Fat, except as indicated by (2).
2. Meat analyzed 16.10% Fat. All glass apparatus used.
3. Citric Acid treated meat at 16-20 hrs. had pH of 3.2; at 66 hrs., pH was 2.3.

(continued)

Table II — Proteolysis on Storage of Group I Treatments

Treatment	μM ala/g. protein "DAY 0"	WEEK 4	WEEK 8	WEEK 12
I Citric Acid (0.3M)	.134	.155	.198	.197
	.134	.146	.198	.202
NI Citric Acid	.182	.124	.204	.290
	.182	.096	.152	.284
I NaCl (0.3M)	.185	.160	.232	.250
	-	.142	.256	.238
NI NaCl	.146	.087	.179	.184
	.146	.143	.159	.165
I Electrodialysis	.240	.361	.404	.451
	.270	.376	.372	.465
NI Electrodialysis	-	.227	.349	.340
	.130	.229	.330	.331
I Control	.284	.300	.380	.423
	-	.291	.389	.431
NI Control	.128	.269	.269	.287
	.145	.240	.276	.279

I = Irradiated
NI = Non-Irradiated

Table III "Loosely Bound" Iron Content of Samples of Group I.
(γFe/g Protein)

	"DAY 0"	WEEK 4	WEEK 8	WEEK 12
Control Irr.	56.9	57.2	74.2	57.5
Control NI	44.0	41.0	38.5	49.2
Citric Acid Irr.	108.4	102.5	103.5	133.3
Citric NI	57.9	57.6	54.7	39.2
NaCl Irr.	163.4	81.7	107.1	56.1
NaCl NI	89.1	42.0	54.0	29.2
Elect. Irr.	92.9	100.0	95.0	110.2
Elect. NI	56.02	61.7	63.4	23.4

I = Irradiated
NI = Non-Irradiated
Elect. = Electrodialysis

 (continued)

Table IV Proteolysis on Storage of Group II Treatments

Treatment	μM ala/g. protein "DAY 0"	WEEK 4	WEEK 8	WEEK 12
I $NaHSO_3$ (0.1M)	.336	.379	.488	.545
	.311	.354	.456	.572
NI $NaHSO_3$	.283	.232	.265	.275
	.288	.232	.277	.266
I IAA (0.1M) (Iodoacetic Acid)	.322	.260	.292	.314
	.324	.270	.280	.343
NI IAA	.304	.228	.250	.236
	.311	.224	.266	.243
I $HgCl_2$ (0.1M)	.290	.236	.263	.270
	.303	.230	.280	.253
NI $HgCl_2$	.270	.210	.209	.230
	.291	.212	.250	.236
I Control	.290	.275	.340	.365
	.323	.276	.360	.360
NI Control	.238	.224	.245	.254
	.352	.230	.246	.254

I = Irradiated
NI = Non-Irradiated

 (continued)

Table V — Proteolysis on Storage of Group III Treatments

Treatment	μM Alanine/g. protein				
	"DAY 0"	WEEK 1	WEEK 4	WEEK 8	WEEK 12
I Transferrin	.289	.255	.392	.296	.338
	.310	.271	.402	.389	.322
NI Transferrin	.247	.233	.364	.307	.362
	.231	.235	.394	.334	.381
I Sorbitol	.245	.288	.415	.414	.460
	.253	.270	.446	.482	1.129
NI Sorbitol	.270	.240	.346	.363	.331
	.260	.239	.351	.363	.306
I EDTA	.280	.271	.373	.383	.412
	.309	.298	.374	.416	.412
NI EDTA	.281	.244	.358	.371	.344
	.272	.239	.397	.309	.313
I $(NH_4)_2S_2O_8$	1.186	1.280	1.39	1.43	1.666
	1.203	1.250	1.42	1.91	1.400
NI $(NH_4)_2S_2O_8$	1.115	1.210	1.34	1.69	1.305
	1.135	1.220	1.38	1.76	1.270
I Control	.303	.260	.392	.417	.441
	.302	.253	.406	.394	.387
NI Control	.250	.231	.349	.447	.278
	.247	.234	.348	.367	.359

I = Irradiated
NI = Non-Irradiated

(continued)

Table VI — Proteolysis on Storage of Group IV Treatments

Treatment	μM Ala/gram protein			
	"DAY 0"	WEEK 3	WEEK 8	WEEK 12
I Resin	.290	.347	.328	.417
	.277	.357	.275	.302
NI Resin	.268	.411	.247	.237
	.274	.375	.294	.295
I Tris	.355	.444	.431	.420
	.370	.530	.385	.441
NI Tris	.398	.399	.305	.342
	.420	.367	.337	.314
I Control	.374	.432	.421	.438
	.344	.405	.440	.438
NI Control	.328	.408	.419	.320
	.330	.376	.320	.309

I = Irradiated
NI = Non-Irradiated

Table VII — Citric Acid (C). Analysis of Variance

Source	d.f.	S.S.	M.S.	F	P
Replications	1	.0006			
Citric Acid C	1	.0899	.0899	299	vhs
Storage S	3	.0777	.0259	86	vhs
Irradiation I	1	.0127	.0127	42	vhs
C X I	1	.0275	.0275	91	vhs
C X S	3	.0179	.0059	20	vhs
S X I	3	.0021	.0007	3	.10
C & I	3	.0141	.0047	16	<.001
Error	15	.0030	.0002		
New Residual.	18	.0051	.0003		

vhs = very highly significant
N.S. = not significant

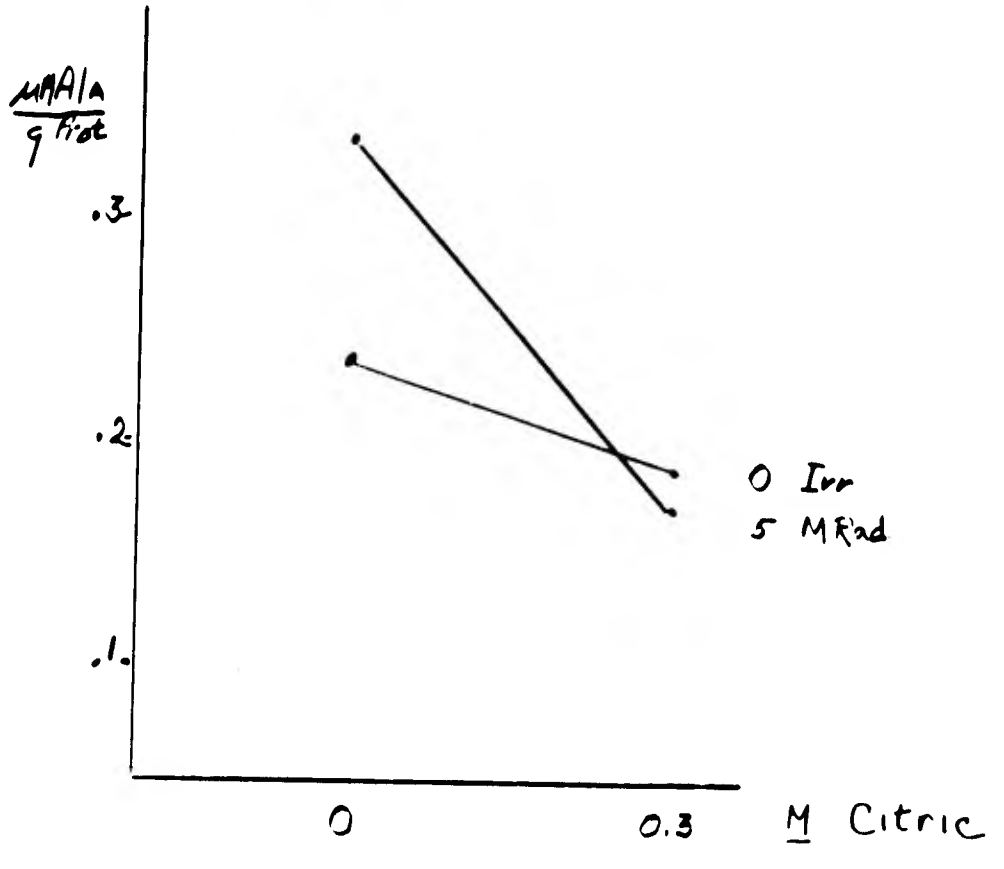


Table VIII Sodium Chloride (N). Analysis of Variance

Source	d.f.	S.S.	M.S.	F	P
Replications	1	.0000	-		
Sodium Chloride N	1	.0906	.0906	302	vhs
Storage S	3	.0543	.0181	60	vhs
Irradiation I	1	.0529	.0529	176	vhs
N X I	1	.0035	.0035	12	0.005
N X S	3	.0225	.0075	25	<0.001
S X I	3	.0053	.0018	6	0.01
NSI	3	.0013	.0004	1	NS
Error	15	.0045	.0003		
New Residual	18	.0058	.0003		

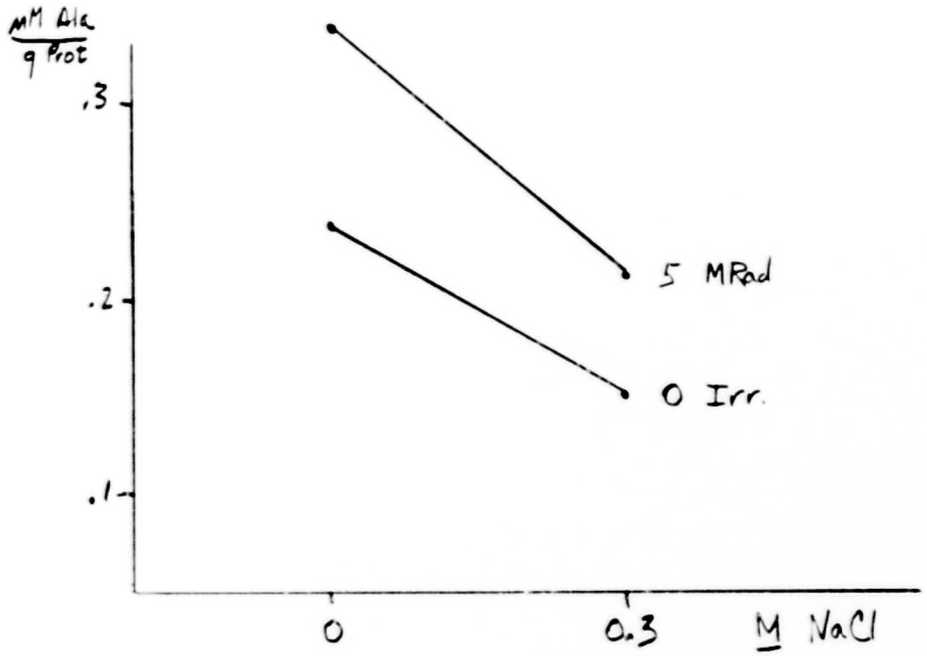


Table IX Electrodialysis (D). Analysis of Variance

Source		d.f.	S.S.	M.S.	F	P
Replication		1				
Electrodialysis	D	1	.00396	.00396	18.9	.001
Storage	S	3	.16684	.05561	264.8	vhs
Irradiation	I	1	.09417	.09417	448.4	vhs
D X I		1	.00032	.00032	1.5	n.s.
D X S		3	.00335	.00112	5.3	~.01
S X I		3	.00436	.00145	6.9	~.005
D X S X I		3	.00751	.00250	11.9	.001
Error		15	.00314	.00021		
New residual		16	.00346	.00022		

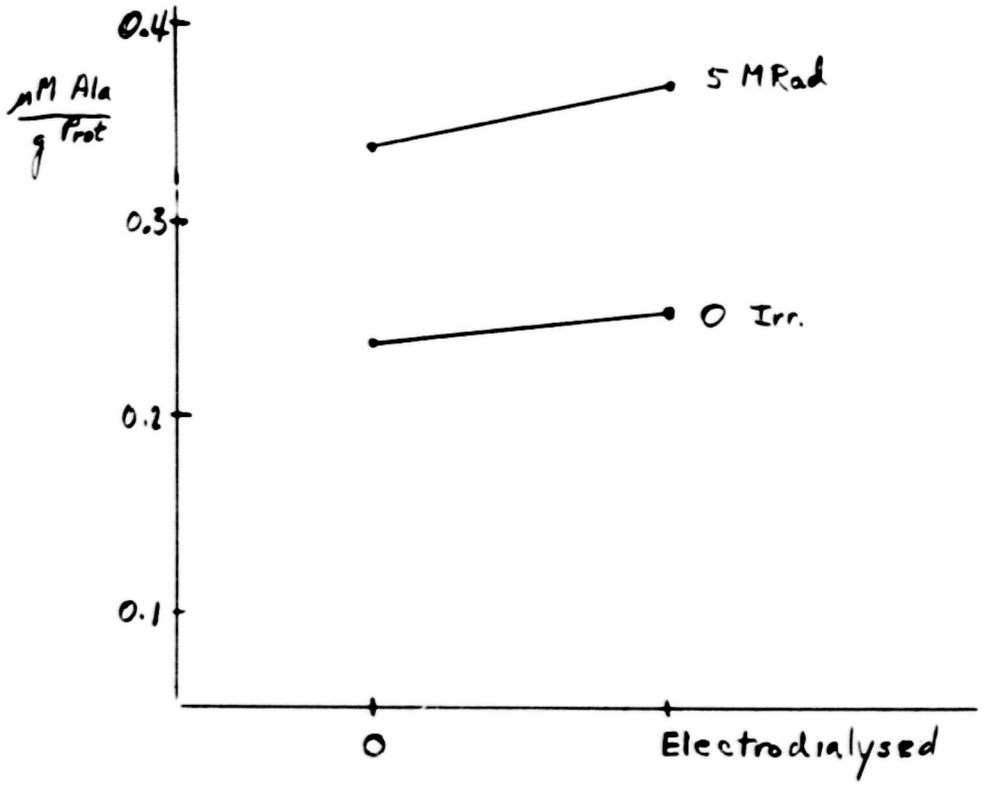


Table X Sodium Bisulfite (B). Analysis of Variance

Source		d.f.	S.S.	M.S.	F	P
Replication		1	.0005	.0005	-	
Sodium Bisulfite	B	1	.0269	.0269	44.8	.001
Storage	S	3	.0338	.0113	18.8	.001
Irradiation	I	1	.1092	.1092	182	.001
B X I		1	.0189	.0189	31.5	.001
B X S		3	.0112	.0037	6.2	.01
S X I		3	.0341	.0114	19.0	.001
BSI		3	.0060	.0020	3.3	.05
Error		15	.0084	.0006		

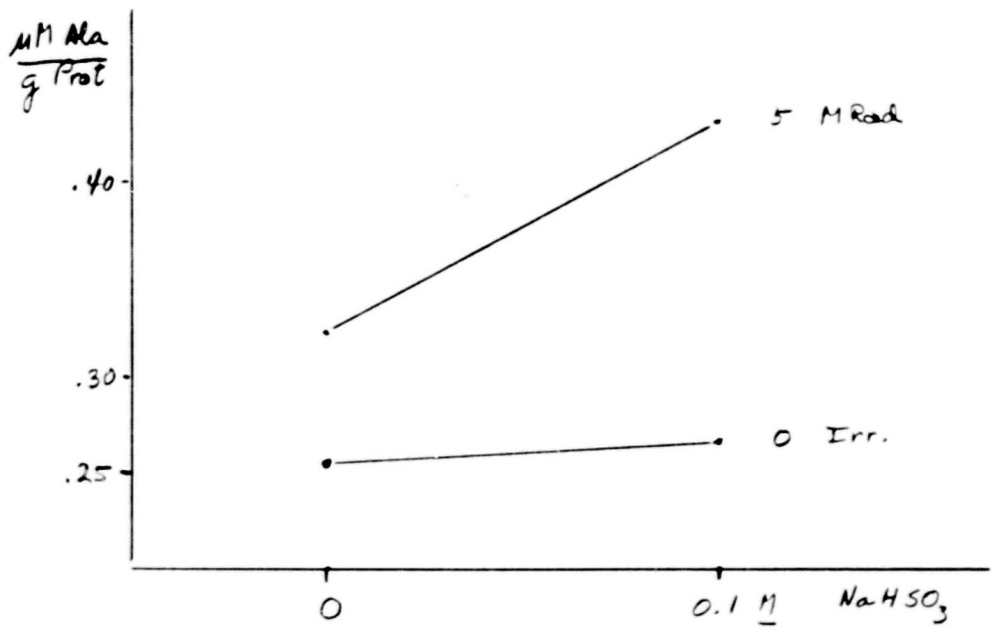


Table XI Iodoacetic Acid (IAA). Analysis of Variance

Source	d.f.	S.S.	M.S.	F	P
Replications	1	.0016	.0016	4	-
IAA	1	.0009	.0009	2.25	~0.1
Storage S	3	.0159	.0053	13.25	0.001
Irradiation I	1	.0247	.0247	61.75	0.001
IAA X I	1	.0013	.0013	3.25	~0.1
IAA X S	3	.0021	.0007	1.75	~0.2
S X I	3	.0078	.0026	6.5	.005
IAA X S X I	3	.0019	.0006	1.5	> 0.2
Error	15	.0064	.0004		
New Residual	18	.0083	$.0004_6$		

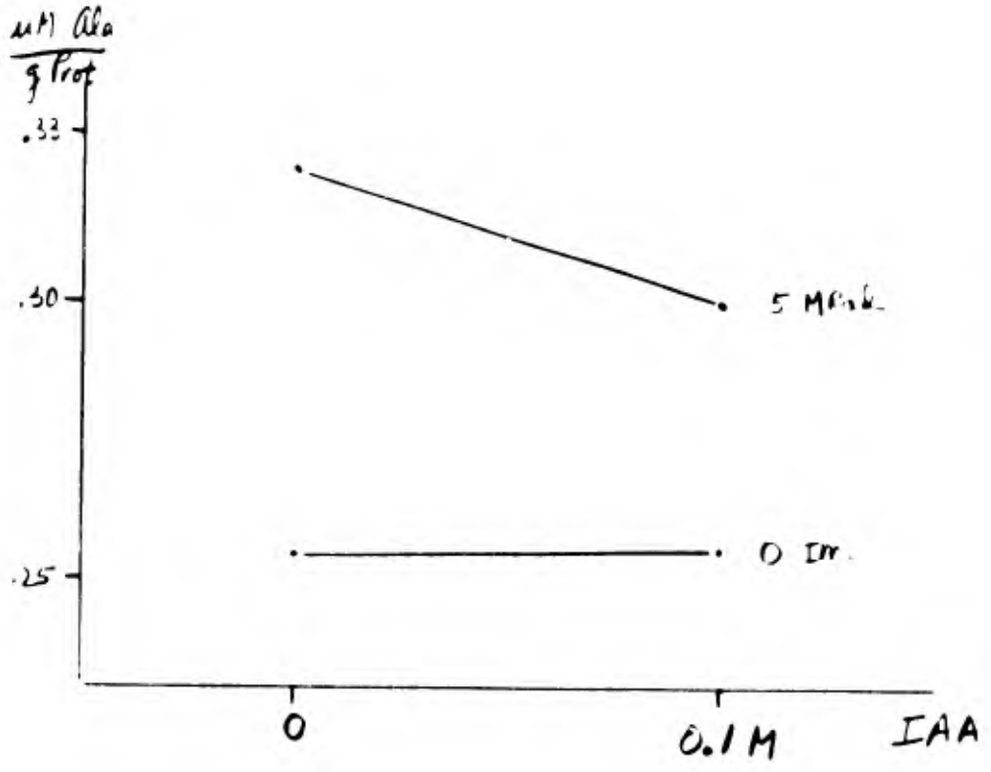


Table XII $HgCl_2$ (H). Analysis of Variance

Source		d.f.	S.S.	M.S.	F	P
Replications		1	.0019	.0019		
$HgCl_2$	H	1	.0112	.0112	24.9	.001
Storage	S	3	.0144	.0048	10.7	.001
Irradiation	I	1	.0182	.0182	40.4	.001
H X I		1	.0034	.0034	7.6	~.02
H X S		3	.0027	.0009	2	.20
S X I		3	.0048	.0016	3.6	.05
HSI		3	.0022	.0007	1.6	>.20
Error		15	.0068	$.0004_5$		
New residual		18	.0090	.0005		

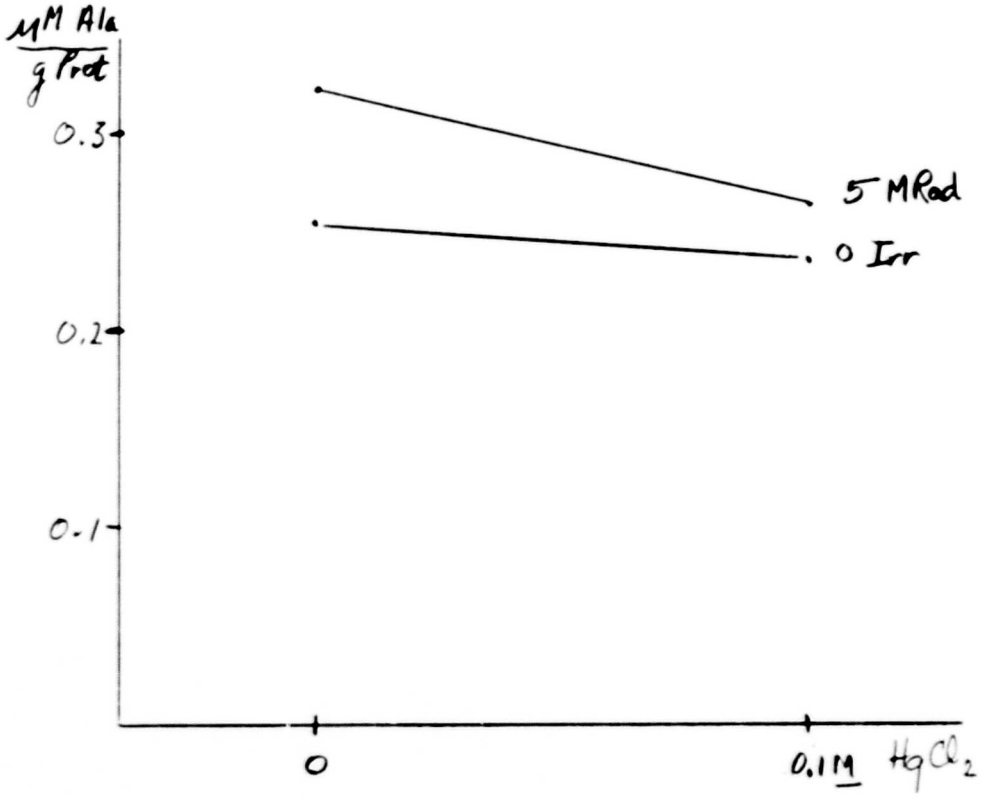
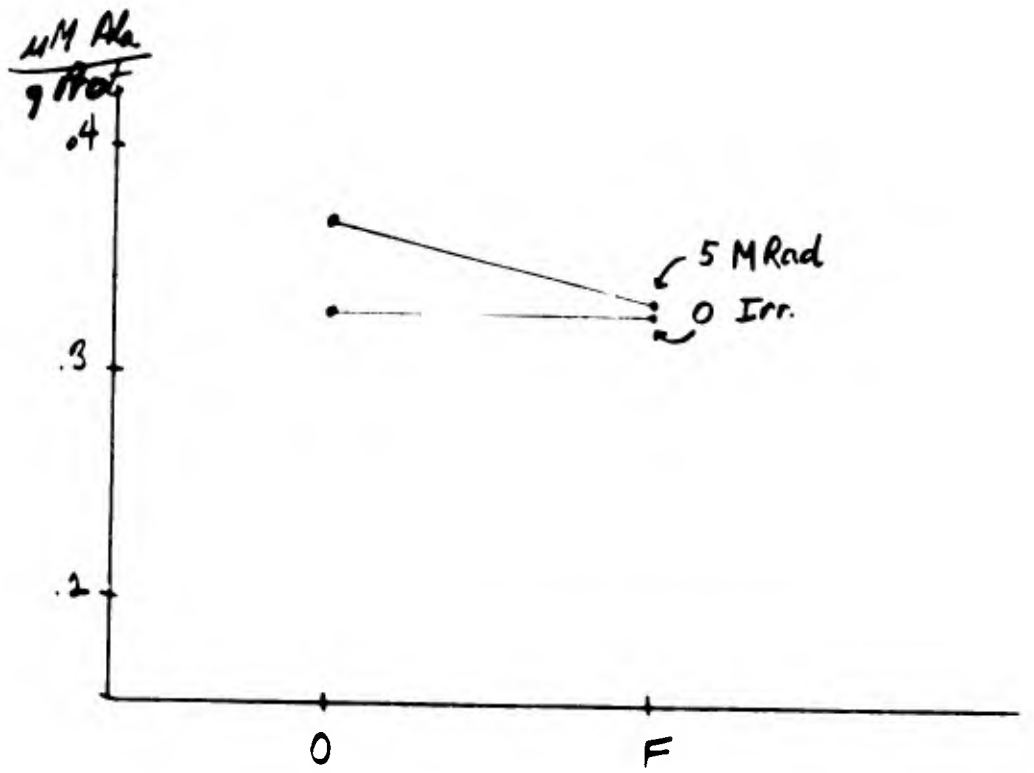


Table XIII

Transferrin (F). Analysis of Variance

Source		d.f.	S.S.	M.S.	F	P
Replications		1	.0004	.0004		
Transferrin	(F)	1	.0026	.0026	3.3	.1
Storage	S	3	.0927	.0309	38	.001
Irradiation	I	1	.0048	.0048	6	~.025
F X I		1	.0025	.0025	3.1	>.05
F X S		3	.0095	.0032	4	.05
S X I		3	.0006	.0002		
F X S X I		3	.0077	.0026	3.3	.05
Error		15	.0136	.0009		
New Residual		18	.0142	.0008		



(continued)

Table XIV Sorbitol (SOR). Analysis of Variance

Source		d.f.	S.S.	M.S.	F	P
Replications		1				
Sorbitol		1	.0018	.0018	2.6	~.1
Storage	S	3	.1169	.0390	56	vhs
Irradiation	I	1	.0351	.0351	50	vhs
SOR X I		1	.0047	.0047	6.7	~.025
SOR X S		3	.0009	.0003	-	-
S X I		3	.0106	.0035	5	.01
SOR X I X S		3	.0015	.0005	-	
Error		14	.0120	.0009	-	
New Residual		20	.0144	.0007		

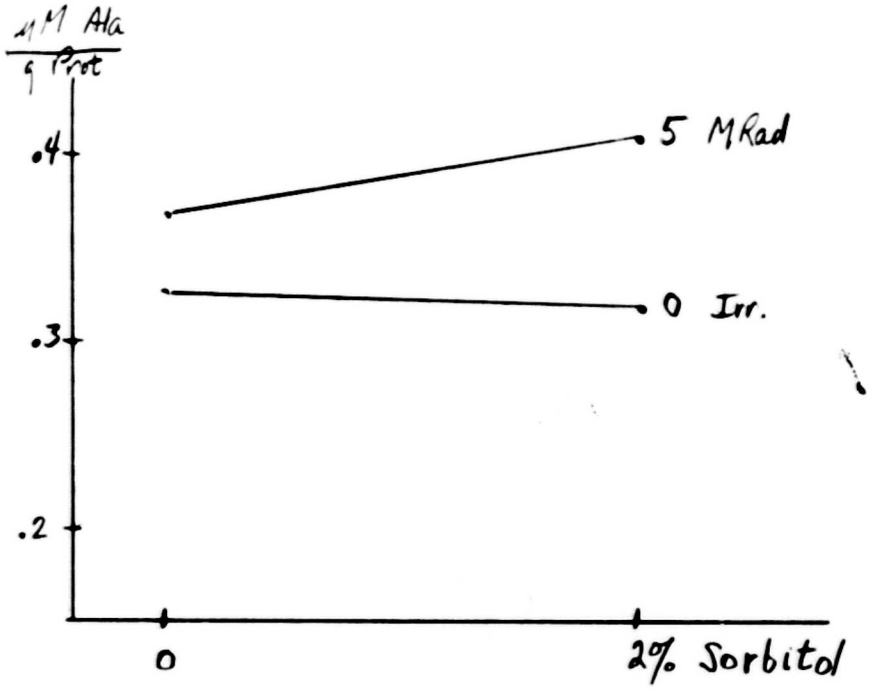
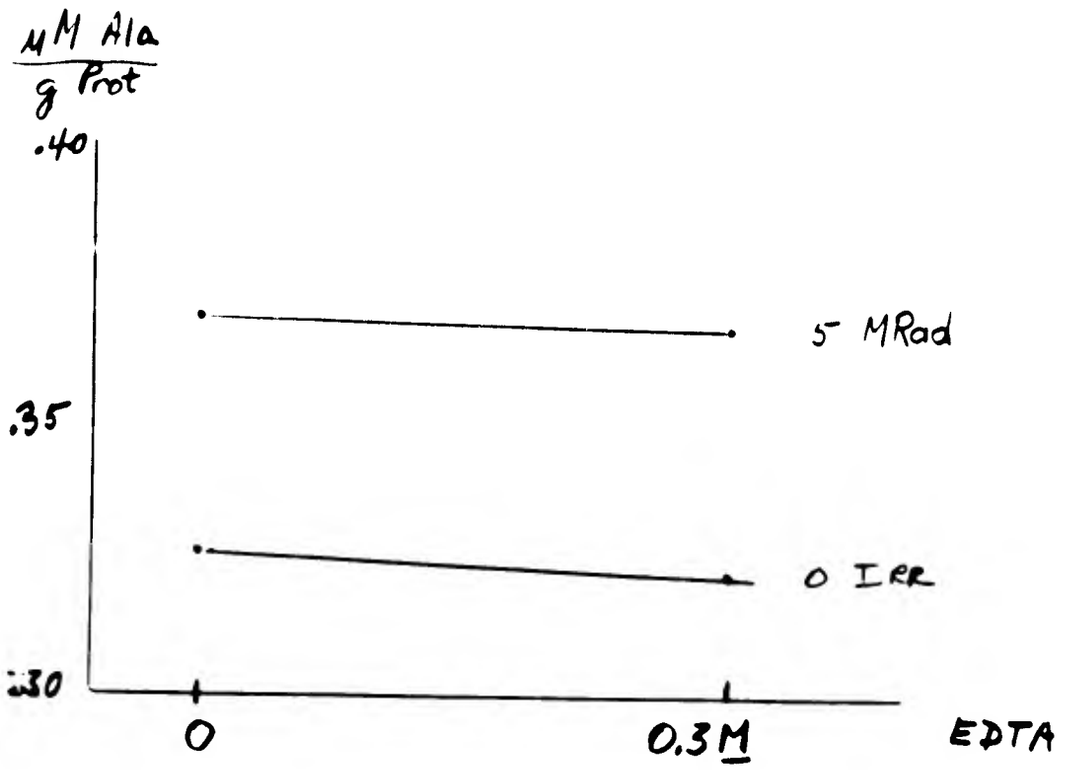


Table XV

EDTA -- Analysis of Variance

Source		d.f.	S.S.	M.S.	F	P
Replications		1	.0001			
EDTA		1	.0001	.0001		NS
Storage	S	3	.0927	.0309	38.6	vhs
Irradiated	I	1	.0154	.0154	19.3	vhs
EDTA X I		1	.0000	.0000		NS
EDTA X S		3	.0033	.0011	1.4	~.25
S X I		3	.0057	.0019	2.4	.20
EDTA X S X I		3	.0036	.0012	1.5	.25
Error		15	.0123	.0008		



(continued)

Table XVI Ammonium Persulfate (A). Analysis of Variance

Source	d.f.	S.S.	M.S.	F	P
Replications	1	0.0016	.0016		
Am. Persulfate	A 1	9.4254	9.4254	865	vhs
Storage	S 3	0.3854	0.1285	11.8	vhs
Irradiation	I 1	0.0257	0.0257	2.4	~.20
A X I	1	0.0017	0.0017	-	NS
A X S	3	0.1157	0.0386	3.5	~.20
S X I	3	0.0412	0.0137	1.3	NS
A X S X I	3	0.0113	0.0038	-	NS
Error	15	0.1634	0.0109		

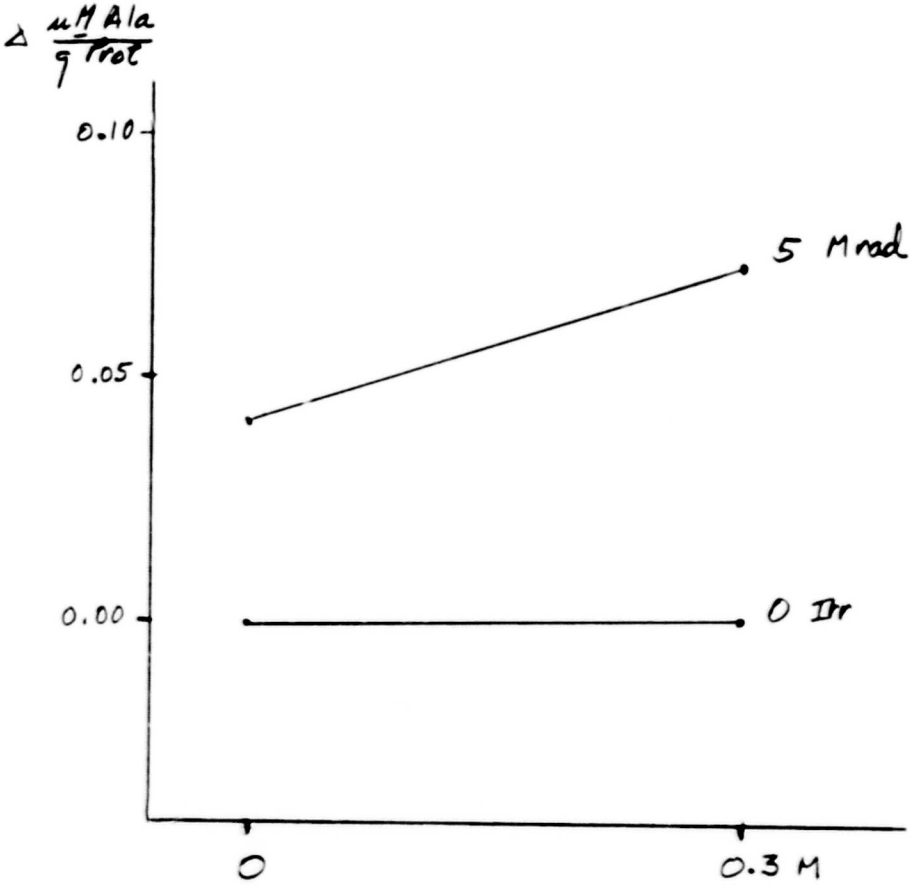
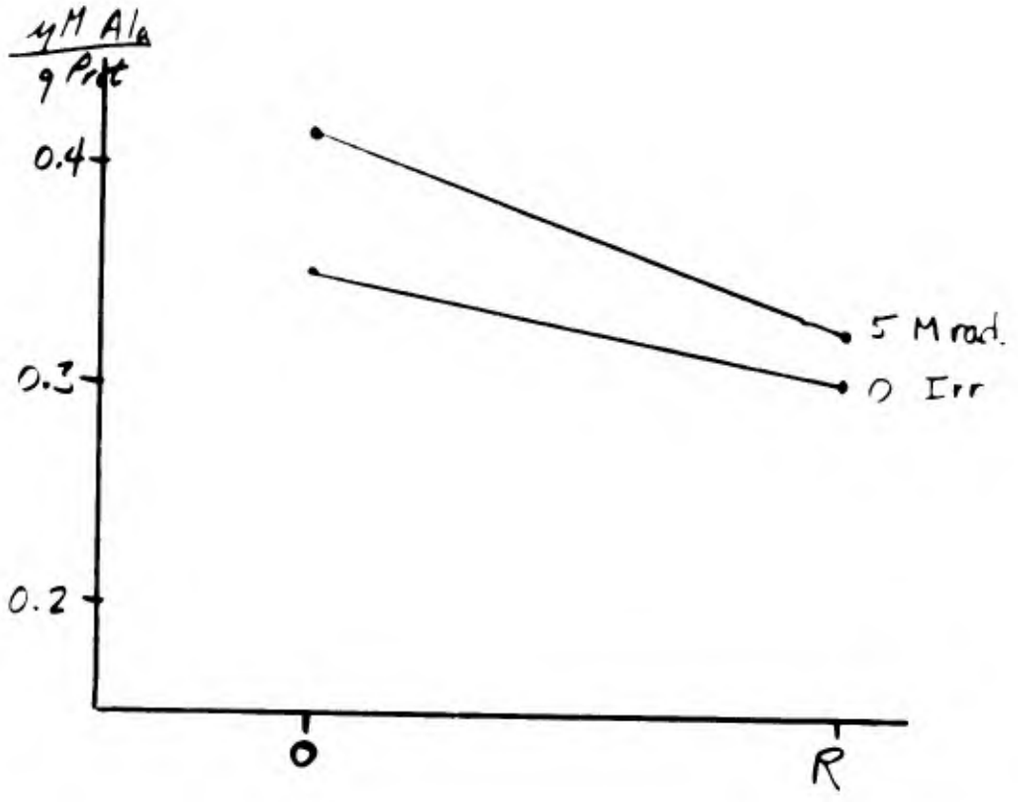


Table XVII Resin (R). Analysis of Variance

Source		d.f.	S.S.	M.S.	F	P
Replications		1	.0024	.0024		
Resin	R	1	.0384	.0384	32	.001
Storage	S	3	.0248	.0083	7	ca .001
Irradiation	I	1	.0142	.0142	12	ca .001
R X I		1	.0026	.0026	2.9	ca .1
R X S		3	.0068	.0023	2.6	ca .1
S X I		3	.0146	.0049	4.1	>.05
RSI		3	.0007	.0002		
Error		15	.0157	.0010		
1st Residual		18	.0164	.0009		
2nd Residual		23	.0282	.0012		



(continued)

Table XVIII pH 10 TRIS Buffer (T). Analysis of Variance

Source		d.f.	S.S.	M.S.	F	P
Replications		1	.0004	.0004		
Tris	T	1	.0008	.0008	1	N.S.
Storage	S	3	.0135	.0045	5.6	.01
Irradiation	I	1	.0298	.0298	37.3	.001
T X I		1	.00001	.00001	-	-
T X S		3	.0070	.0023	2.9	~.1
S X I		3	.0154	.0051	6.4	.01
TSI		3	.0065	.0022	2.8	0.2
Error		15	.0129	.0009		
New Residual		17	.0133	.0008