III

C H A P T E R

RESULTS

#### RESULTS

### Section - A

Studies on arginase, ornithine ketoacid aminotransferase (OKAT), ornithine carbamyl transferase (OCT) and putrescine carbamyl transferase (PCT) activity in ground mit seeds under different conditions of growth

### Arginase, OKAT, OCT and PCT activity in different varieties of seeds

Four varieties of groundnut seeds GAUG-1, SB-11, GAUG-10 and Punjab-1 obtained from Gujarat Agricultural University, Jamnagar, were assayed for arginase, OKAT, OCT and PCT activity during germination. Varieties SB-11 and GAUG-1 are bunch type and have smaller seeds, whereas the other two varieties GAUG-10 and Punjab-1 are spreading type and have larger seeds. The data reported in Tables 2-5 show that there was no significant varietal difference in the activity of these enzymes. For further studies Punjab-1 variety obtained from a local farm was used.

# Dry weight and protein content during development and germination

The seeds were collected and grouped into different stages of development as described in Materials and Methods. The average fresh weight of the seed and the dry weight of the

Period of	Enzyme units/g fresh tissue						
(days)	GAUG -1	SB-11	Punjab-1	GAUG -10			
Cotyledon			Ň				
0	17	17	18	17			
2	64	67	. 64	68			
4	13 <b>1</b>	131	138	134			
6	123	124	131	128			
Embryo							
0	33	34	34	32			
2	5 <b>7</b> ·	53	58	58			
4	35	34	36	34			
6	19	21	20	20			

Table 2 : Arginase activity in different varieties of groundnut seeds during germination.

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GAUG-1 and SB-11 are bunch type whereas Punjab-1 and GAUG-10 are spreading type.

Period of	Enzyme units/g fresh tissue				
(days)	GAUG –1	SB-11	Punjab-1	GAUG-10	
otyledon					
0	2	3	3	2	
2	12	g 11	13	12	
4	20	18	20	19	
6	18	18	20	18	
Smbryo					
0	<b>`</b> 5	5	5	5	
2	15	14	15	15	
4	6	6	6	6	
6	6	5	6	6	

Table	3	* •	Ornithine	ketoacid	amiı	notransfera	ise act	tivity in	a
			different	varieties	of	groundnut	seeds	during	
			germinatio	on.					

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Table 4 :	Ornithine carbamyl transferase activity in
	different varieties of groundnut seeds during
	germination.

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Period of	Enzyme units/g fresh tissue					
(days)	GAUG -1	SB-11	Punjab-1	GAU <b>G1</b> 0		
Cotyledon		•				
0	56	58	56	62		
2	80	80	77	82		
4	79	81	77	79		
6	71	74	75	74		
Embryo						
0	81	79	73	74		
2	84	85	85	86		
4	83	84	83	87		
6	86	84	84	86		

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Table	5	:	Putrescine	e carbamyl	tra	ansferase (	activit	y in
			different	varieties	of	groundnut	seeds	during
			germinatio	n.				

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Period of	Enzyme units/g frêsh tissue				
(days)	GAUG –1	SB-11	Punjab-1	GAUG-10	
Cotyledon					
0	10	10	7	7	
2	4	4	3	3	
4	3	3	2	2	
6.	0.3	20.2	20.2	∠0.2	
Smbryo					
0	11	11	8	8	
2	5	6	6	6	
4	3	3	3	4	
6	2	2	2	2	

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	Seed	5 e				
4944 - 4440 - 44	Stage	Average weight of	Dry weigh	<b>t</b> (%)	Pro (mg/g fre	etein esh tissue)
	. کوچه محمد کردی اوری وست است. دروی وری مربق اوری وری وری وری و	(mg)	Cotyledon	Embryo	Cotyledor	Embryo
Α.	Development (stage)	-				
	· 1	5	8			11
	2	27	14	,	4	19
	3	<b>7</b> 5	-21	-	49	-
	4	151	29		53	-
	5	240	36	49	60	69
	6	349	39	59	77	86
	7	448	64	60	105	88
	8	564	69	60	128	<b>1</b> 05
	9	717	74	68	156	131
B.	Storage (months)					
	6	462	76	69	158	135
	12	424	79	71	169	141
C.	<u>Germination</u> (days)	<u>l</u>				
	0	ĩ. )	70	68	153	128
	1		65	57	146	102
	2		58	<b>4</b> 6	138	78
	3		56	31	121	56
	4		55	· 26	115	45
	5		52	23	93	37
	6		52	19	86	26
	7		49	10	80	24
	8		48	6	78	20
			ماه خاه دما تحد عدا شو می ما حق ما حق م	جانه جان میں ملک جان جمل ہے۔	بيد جن بنت حو جنه الد الد الد	

Table 6 : Fresh weight, dry weight and protein content during development, storage and germination of groundnut seeds.

Values given for development stage 1 and 2 are for whole seed as the cotyledon and embryo could not be separated.

	Stage	Enzyme fresh	units/g tissue	Enzyme units/mg protein		
-		Cotyledon	Embryo	Cotyledon	Embryo	
Α.	Development (stage)			-		
	1		30	2.	7	
	2		29	0.	6	
	3	26	479-	0.5	_	
	4	20		0.4	-	
	5	17	29	0.3	0.4	
	6	16	28	0.2	0.3	
	7	16	27	0.2	0.3	
	8	16	27	0.1	0.3	
	9	16	26	0.1	0.2	
в.	Storage (months)					
	6	15	26	0.1	0.2	
	12	5	11	0.03	0.1	
C.	Germination (days)					
	0	18	36	0.1	0.3	
	1	40	43	0.3	0.4	
	2	62	56	0.4	0.6	
	3	107	58	0.9	1.0	
	4	132	35	1.1	0.8	
	5	129	20	1.4	0.5	
	6	131	17	1.5	0.7	
	7	132	15	1.7	0.6	
	8	138	13	1.8	0.7	

Table 7 : Arginase activity during development, storage and germination of groundnut seeds.

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Values given for development stage 1 and 2 are for whole seed as the cotyledon and embryo could not be separated.

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	seeds.								
-	Stage	Enzyme units/g fresh tissue		Enzyme units/mg, protein					
tales baits on	20 Juni 4000 1000 1000 1000 1000 1000 1000 100	Cotyledon	Embryo	Cotyledon	Embryo				
A.	Development (stage)								
	1		7	0	•64				
	2		6	` <b>O</b>	.12				
	3	5	67.100	0.10	***				
	4	5		0.09	13340				
	5	4	6	0.07	0.09				
	6	3	6	0.04	0.07				
	7	3	5	0.03	0.06				
	8	3	5	0.02	0.05				
	9	3	5	0.02	0.04				
B.	Storage (months)								
	6	2	5	0.01	0.04				
	12	0.3	1	∠0.01	0.01				
C.	Germination (days)				-				
	0	3	5	0.02	0.04				
	1	6	11	0.04	0.11				
	2	12	15	0.09	0.19				
	3	14	11	0.12	0.20				
	4	20	7	0.17	0.16				
	5	21	7	0.23	0.19				
	6	20	6	0.23	0.23				
	7	20	5	0.25	0.21				
	8	20	5	0.26	0.25				

Table 8 : Ornithine ketoacid aminotransferase activity during development, storage and germination of groundnut seeds.

Values given for development stage 1 and 2 are for whole seed as the cotyledon and embryo could not be separated.

	seeds.								
	Stage	Enzyme fresh	units/g tissue	Enzyme u prot	nits/mg ein				
	- Mit die Lie die angewe see die die aan fin an an die se	Cotyledon	Embryo	Cotyledon	Embryo				
A.	Development (stage)								
	1		41	3	.7				
	2		53	1	.1				
	3	65	4560	1.3	-				
	4	78		1.5	-				
	5	80	78	1.3	1.1				
	6	81	81	1.1	0.9				
	7	84	86	0.8	1.0				
	8	88	90	0.7	0.9				
	9	90	91	0.6	0.7				
B.	Storage (months)								
	6	78	81	0.5	0.6				
	12	48	57	0.3	0.4				
c.	Germination (days)	,		٧					
	0	60	59	0.4	0.5				
	1	62	65	0.4	0.6				
	2	64	69	0.5	0.9				
	3	64	69	0.5	1.2				
	4	64	70	0.6	1.6				
	5	64	69	0.7	1.9				
	6	65	67	0.8	2.6				
	7	62	<b>6</b> 8	0.8	2.8				
	8	63	67	0.8	3.4				

Table 9 : Ornithine carbamyl transferase activity during development, storage and germination of groundnut seeds.

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Values given for development stage 1 and 2 are for whole seed as the cotyledon and embryo could not be separated.

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	seeds.								
•••••	Stage	Enzyme fresh	units/g tissue	Enzyme units/mg protein					
1100 CE22 - 2	in the ter are stir the day tay tay an all are still the	Cotyledon	Embryo	Cotyledon	Embryo				
Α.	Development (stage)								
	1		15	1.	36				
	2		15	0.3	31				
	3	12	-	0.24					
	4	12		0.23	-				
	5	11	11	0.18	0.16				
	6	10	10	0.13	0.12				
	7	8	10	0.10	0.11				
	8	7	10	0.05	0.10				
•	9	7	10	0.04	0.08				
В.	Storage (months)								
	6	6	10	0.04	0.07				
	12	6	7	0.04	0.05				
C.	Germination (days)								
	0	6	7	0.04	0.05				
	1	6	6	0.04	0.06				
	2	4	4	0.03	0.05				
	3	3	3	0.02	0.05				
	4	2	3	0.02	0.07				
	5	2	3	0.02	0.08				
	6	40.2	3		0.11				
	.7	0	3		0.13				
	8	0	3		0.15				

Table 10 : Putrescine carbamyl transferase activity during development, storage and germination of groundnut

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Values given for development stage 1 and 2 are for whole seed as the cotyledon and embryo could not be separated. cotyledon and embryo was determined (Table 6). Stage-9 represents fully mature seed each weighing between 600-800 mg. The storage of these seeds over a period of one year resulted in approximately 40% decrease in the fresh weight. The dry weight of the cotyledon increased progressively during development, whereas it increased very slowly in the embryo. Storage resulted in only a slight increase in the dry weight of the cotyledon and embryo. During germination however, the dry weight decreased both in the cotyledon and embryo, the decrease being more pronounced in the embryo than in the cotyledon, over a period of 8 days.

The protein content of the cotyledon as well as embryo increased rapidly during a development but decreased during germination, the decrease being more pronounced in the embryo than in the cotyledon.

### Arginase, OKAT, OCT and PCT activity during development, storage and germination

The results reported in Tables 7 and 8 show that during the period of seed development, the arginase and OKAT activity decreased in the cotyledon on tissue basis as well as on protein basis. However, in the case of embryo, these enzymes showed a very little decrease when expressed on tissue basis, but when expressed on protein basis the pattern was similar to that of cotyledon. Storage for one year resulted in a considerable decrease in the activity of these enzymes in the cotyledon as well as embryo. During germination the pattern of the two enzymes was again very different in the two tissues. In the cotyledon, both the enzymes increased upto 4th day of germination and then remained constant thereafter. In the embryo however, these enzymes decreased after an initial increase around 3rd day of germination. The specific activity of both the enzymes in the cotyledon increased with period of germination. The specific activity of arginase in the embryo showed a slight decrease after an initial increase around 3rd day. The embryo OKAT, however, showed no change in the specific activity after an initial increase ground 3rd day.

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The enzyme OCT, which has a biosynthetic role increased during early period of development and was constant during the later period. In the embryo, the enzyme activity remained almost constant during development. Storage resulted in about 40-50% decrease in OCT activity in the cotyledon as well as embryo. During germination, the OCT activity of the cotyledon as well as embryô remained unchanged over a period of 8 days (Table 9). The pattern of changes in OCT activity during development and germination was altered when expressed in terms of per mg protein. The specific activity of OCT decreased during development and storage but increased during germination in the cotyledon as well as embryo.

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The data reported in Table 10 show that PCT was present in the cotyledon as well as embryo from the early period of seed development, but it decreased in the cotyledon and remained unchanged in the embryo during further development. During germination, the enzyme decreased progressively in the cotyledon and was not detected after 6 days of germination. In the embryo, however, the enzyme decreased by about 40% on the 2nd day and remained constant thereafter. The specific activity of PCT decreased both in the cotyledon and embryo during development. In the cotyledon the specific activity decreased during germination but in the embryo it increased during germination.

### Presence of arginase, OKAT, OCT and PCT in field grown plants

The studies reported above on the presence of arginase, OKAT, OCT and PCT in germinating seeds were carried out by growing the seeds in Petri dishes. It was of interest to check the level of these enzymes in soil grown plants at later stages of germination when the tissue differentiation has taken place. The groundnut plants were raised in garden soil on the campus and analysed on different days. The data reported in Table 11 show that arginase activity was highest in the cotyledon and lowest in shoots on 14th day of germination and it decreased considerably in all the parts of the plant with increased period of germination.

Age of	ος αχαι όπως παιτό δρος τους πάτος όπος δρος τους πάτος 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9 9	Enzyme units/g fresh tissue								
(days)	Cotyledon	Hypocotyl	Root	Shoot	Leaves					
The Sile Cill Cill Cill Cill Cill Cill Cill C	it i an an the state in the state	ang dina (18. 1873) non 1834 ang 1874 tilip ang 18.	ر کنید خاند شید کمل پردن خان	n the Cill All All y <sub>n</sub> die Rif an an an an	a ayaa difee MMB dina ahyy diffa MMB AMB Chiff onya kana.					
14	94	29	13	11	35					
				_						
21	30	25	6	7	19					
28	9	5	4	3	8					
60		-	2	4	5					
Mano fear Sjat data 1000 maa kasi dina teen wa	n allege allerte makan denam allerte sellerte allerte andrem sellerte Garche statues s	Aller Main alle alle Aller Hain Sons alle Aller Aller	, when there same direct water degree any	ar anga diga téhu lipin data data map digar ngga dian Milo daji	n diger datun basun digen Salar witch Misun alaun tagan galan diger					

# Table 11 : Arginase activity from groundnut plants raised in garden soil.

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Age of		Enzyme units/g fresh tissue							
(days)	Cotyledon	Hypocoty1	Root	Shoot	Leaves				
14	12	8	4	8	8				
21	8	5	3	5	7				
28	7	4	× <b>3</b>	3	6				
60			2	1	5				

Table 12 : Ornithine ketoacid aminotransferase activity from groundnut plants raised in garden soil.

Age of	Enzyme units/g fresh tissue								
(days)	Cotyledon	Hypocotyl	Root	<sup>i</sup> Shoot	Leaves				
14	63	48	16	38	75				
21	36	31	6	33	49				
28	16	33	5	30	42				
60			5	4	21				
		ک هې کلي کې	din dija. Cin dim dan dini dini	ngan Têşa dilin Tala dalar Qua Anar ağışı Quin dalar diga	140° 1978 Anis Silo 1971 - 1971 1970 1970 1970 1970 1970				

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Table 13 : Ornithine carbamyl transferase activity from groundnut plants raised in garden soil.

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Age of plant (days)	Enzyme units/g fresh tissue								
	Cotyledon 🗄	(ypocot yl	Root	Shoot	Leaves				
14	2	3	1	2	4				
21	2	0	∠0.2	0.6	0.6				
28	1	0	6.2	0.5	0.6				
60			0	0	0				

Table 14 :	Putrescine	carbamyl	transferase	activity from
	ground mt	plants rai	ised in garde	en soil.

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OKAT activity was highest in cotyledon and lowest in the roots on 14th day of germination (Table 12). However, the decrease in the OKAT activity with germination was less compared to that of arginase.

OCT activity was highest in leaves and lowest in roots on 14th day of germination (Table 13). It also decreased in all the parts of the plant with increased period of germination.

PCT activity was very low in all the parts of the plant at 14th day of germination and it decreased further with the increase in period of germination (Table 14). The presence of PCT in the cotyledons of 28 days old field grown plants was in contrast to the observations reported earlier, where no PCT activity was detected in cotyledons of Petri dish grown seeds after 6 days of germination.

### Effect of hormones

To investigate the effect of plant hormones on the enzymes of arginase metabolism, seeds were soaked in different concentrations of hormones and then allowed to germinate in Petri dishes. The data reported in Tables 15-18 show that 2,4-D ami IAA inhibited arginase and OKAT activity both in cotyledon and embryo during the early period of germination, and the inhibition was dependent upon hormone concentration. During the later stages of germination, the enzyme activities

Period of crmination	Enzyme units/g fresh tissue at 2,4-D concentration (ppm)							
(days)	0	25	50	100	200			
Cotyledon								
0	19	19	19	19	18			
2	69	56	39	38	31			
4	137	123	120	92	53			
6	128	114	103	103	97			
Embryo								
0	35	33	32	31	30			
2	51	58	57	27	27			
4	37	58	61	89	95			
6	16	16	32	37	42			

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Table 15 : Effect of 2,4-D on arginase activity.

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Period of		Enzyme	units/g fres	h tissue	جه الله علم الله الله الله الله الله الله الله ال
germination		at IAA	concentratio	n (ppm)	
(days)	0	25	50	<sup>±</sup> 100	200
Cotyledon					
0	19	19	19	18	17
2	64	58	40	39	39
-	• •	•••			•••
4	137	126	123	115	52
6	128	118	110	108	93
Bubaro					
Emoryo					
0	34	32	32	32	29
2	51	53	49	41	37
			<b>.</b>		
4	37	48	72	73	104
6	16	19	18	26	74
200 Name (San (Name (San (San (San (San (San (San (San (San	من منه (1990 منهور) ويون منهور من		و منه چه منه الله خون علي الله عنه الله عنه الله		شه است

Table 16 : Effect of IAA on arginase activity.

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Table 17 : Effect of 2,4-D on ornithine ketoacid amino

transferase activity.

Period of germination	Enzyme units/g fresh tissue at 2,4-D concentration (ppm)							
(days)	0	25	50	100	200			
Cotyledon								
0	3	3	3	3	2			
2	12	7	5	3	1			
4	20	16	9	8	4			
6	20	18	18	16	12			
Embryo								
0	5	5	5	4	4			
2	15	13	12	11	4			
4	6	15	15	24	15			
6	7	12	16	<b>2</b> 0	23			
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### Table 18 : Effect of IAA on ornithine ketoacid amino-

transferase activity.

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Period of germination	- the an the case the same till the the	Enzyme at IAA	units/g fr concentrat	esh tissue ion (ppm)	, allen dan dan dal utar alle Stel gan dan dan er
(days)	0	25	50	100	200
Cotyledon					
0	3	3	3	3	3
2	12	9	4	2	1
4	20	17	15	11	4
6	20	16	15	14	14
Embryo					
0.	5	5	5	5	5
2	15	13	11	7	6
4	6	12	12	18	21
6	7	8	13	15	19
400 100 - 400 400 ann ann fhu ann ains har 100 ann tha 100 100	ب الله فقد بزراد جمد الله عند الله والله الله	ه بری بری بین مید دور بری بری می م	میں میں ہیں جات کہ میں میں میں میں میں م	tern billt imm mar inte stor yng grû tern noe	بين حال الله عن حل الله عن حد الله

Table 19 : Effect of 2,4-D on ornithine carbamyl

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transferase activity.

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Period of germination	Υ.	Enzyme units/g fresh tissue at 2,4-D concentration (ppm)								
(days)	0	25	50	100	200					
		· - · · · · · · · · · · · · · · · · · ·								
Cotyledon										
0	55	5 <b>5</b>	56	55	53					
2	67	69	55	54	51					
4	67	68	64	55	54					
6	66	66	66	64	62					
Embryo			-							
0	68	69	67	67	66					
2	72	71	65	66	61					
4	73	70	69	66	65					
6	69	70	71	68	63					
و میں براہ ہوتے ہے۔ جب میں میں سے جب کی جب کی اور		t 1 Mart 1920 Aven Mart Sills 1910 - 1910 - 1910 - 1910 - 1910			از الأمالية بورورية والأوري الأمارير المحكر الأسانية الأوروب					

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Table 20 : Effect of IAA on ornithine carbamyl transferase

Period of germination	in 1995 daes guin dann daes Bus Aber Dae en	Enzyme at IAA	units/g fresh concentration	tissue (ppm)	- <u>Ann anga Kap</u> a <u>Kan Kan</u> Kan dan
(days)	0	25	50	100	200
Cotyledon					
0.	55	55	55	56	54
2	67	63	61	59	55
4	67	67	67	60	56
6	66	66	66	65	65
Embryo					
0	68	68	68	66	66
2	72	72	70	70	65
4	73	70	70	64	64
6	69	70	71	70	71
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activity.

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Table	21	:	Effect	of	2,4-D o	n putresci	ne	carbamyl	trans-
			ferase	act	civity.				

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Period of germination		Enzyme u at 2,4-I	nits/g fre ) concentra	sh tissue tion (ppm	)
(days)	0	25	50	100	200
Cotyledon					
0	7	6	6	6	6
2 a	4	3	3	2	2
4	3	4	3	3	3
6	٢0.2	∠0.2	20.2	20.2	۲۵.2
Embryo					
· 0	7	7	7	7	6
2	4	4	3	3	3
4	2	2	2	2	2
6	2	2	2	3	2

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ac	tivity.				-		
Period of germination	Enzyme units/g fresh tissue at IAA concentration (ppm)						
(days)	0	25	50	100	200		
Cotyledon	۰.						
0	7	6	6	6	5		
2	4	3	3	3	3		
4	3	3	3	2	2		
6	۷۰.2	∠0.2	<b>∠0.2</b>	∠0.2	∠0.2		
Embr yo							
0	đ	7	7	7	6		
2	4	4	4	4	4		
4	2	2	2	2	2		
6	2	2	3	2	2		
anna mair anga anga ayaa taha 1330 dadi 6300 kuta daga daga daga daga daga	tipe this this was been bill this off all the side t	nių vier are tier das das tas das tas tieks tier	طوال مردا آليك محر وي حيد حيد محد ملك الله ا	Che (Qiai 15)i Allen Qica dege Wile dein auto auto a	ur aite filst Gill gift dan Sile Sile Sile Sile		

Table 22 : Effect of IAA on putrescine carbamyl transferase activity.

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Period of germination	Enzyme units/g fresh tissue at gibberellic acid concentration (ppm)						
(days)	0	25	50	100	200		
Cotyledon	,						
0	17	16	16	14	12		
2	60	43	31	31	28		
4	136	118	86	58	26		
6	125	118	96	<b>8</b> 8	24		
Embryo							
0	37	35	34	32	28		
2	57	45	47	39	28		
4	35	52	76	72	31		
6	17	17	19	53	41		
auna Theor aona Auna anna Anna Anna Anna Anna Anna An	nije filiza stago auto dato žigas filito nista super ann	r name datas ditas Silan Prilip datas Sange gana dat	10 đột 1997 được địng được được được được ngữ ngoà sa	m name dave take star star dave been been avec star be	un linn Den stan fan sen van tield spie sjan stat dige		

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Table 23 : Effect of gibberellic.acid on arginase activity.

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Period of germination	Enzyme units/g fresh tissue at kinetin concentration (ppm)					
(days)	0	25	50	100	200	
Cotyledon						
0	17	17	17	15	9	
9	60	97	20	19	4.4	
2	00	21	20	10	T.45	
4	136	105	36	28	18	
6	125	118	96	88	. 24	
Embryo						
0	37	35	30	26	15	
2	57	31	29	29	29	
4	35	57	60	57	50	
6	17	76	73	76	86	
etter etter anne Carp anne dige spen men fans deter anne fans Catu sous et	ی مرزی جیس خون واند وی مارد دارد دارد وی	طوقت البران وزالة وعلت (Linjo Ajan, albin Ajan, and Ajan and	مادو قابت من رود درده اول است کرد کرد	ir Gora, Augus einer földe syste förse földe földe földe	n allan Galil Quar SALE (Dai Bolli Mich Mich Mich Sale Sale	

Table 24 : Effect of kinetin on arginase activity.

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		ورور باورور مرود مرود مانده برور مدال مواد مورد مرود ورده						
Period of germination (days)	at g	Enzyme units/g fresh tissue at gibberellic acid concentration (ppm)						
	0	25	50	100	200			
(La tanàn ào m								
coryledon								
0	3	3	2	2	2			
2	12	6	2	2	0.3			
4	21	18	10	4	3			
6	21	21	21	16	11			
Embryo		-						
0	5	5	4	4	4			
2	15	10	6	4	3			
4	6	17	18	16	14			
6	7	7	7	11	13			
Mige Sam Que State was not the state of the state State	ng man diga Sirit dan din tina tina tina tina	ntere antice AMP ATTIC data case have relate and the AMP	` 1886 Mar Alex Alex Alex Alex Alex Alex Alex Alex		gan tere ter dije gan dich dide Cite als  Sie			

# Table 25 : Effect of gibberellic acid on ornithine ketoacid aminotransferase activity.

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Period of germination (days)	Enzyme units/g fresh tissue at kinetin concentration (ppm)						
	0	25	50	100	200		
Cotyledon							
0	× 3	3	3	2	2		
2	12	1	1	0.6	0.6		
4	21	15	5	2	2		
6	21	16	14	11	10		
Embryo							
0	5	5	5	3	3		
2	15	6	2	1	1		
4	6	18	13	11	11		
6	7	12	19	21	21		

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Table 26 : Effect of kinetin on ornithine ketoacid aminotransferase activity.

Period of	Enzyme units/g fresh tissue at gibberellic acid concentration (ppm)						
(days)	0	25	50	100	200		
Cotyledon							
0	56	52	52	49	47		
2	64	60	56	54	49		
4	65	65	60	58	53		
6	65	65	65	64	53		
Embr yo							
0	68	64	61	58	56		
2	75	65	63	61	59		
4	76	64	61	58	55		
6	74	72	72	69	54		
anan inan anan 1980 yana anan anan inan anan anta dalar dalar atala dalar dalar dalar dalar dalar dalar dalar d	dana kata dasa masi dina dilik dasa tasir dasa	apa Din Tim Alla pan taun sian disi dan	u maan cidaa daana dalam dalam dalam dalam dilam cidaa dalam da	an Mili ala Gin (an ing ing ing ing ing ing ing ing ing in			

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# Table 27 : Effect of gibberellic acid on ornithine carbamyl transferase activity.

Period of germination	ter gap per das das pin die aus cap que 2 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Enzyme units/g fresh tissue at kinetin concentration (ppm)						
(days)	0	25	50	100	200			
Cotyledon								
0	56	51	51	51	47			
2	64	54	54	49	49			
4	65	61	59	47	40			
6	65	59	59	52	48			
Embryo								
0	68	61	54	51	49			
2	75	<b>7</b> 0	70	68	67			
4	76	71	68	66	61			
6	74	67	63	63	61			
N: 42 ap 45 ap 45 ap 45 a 46 a 46 a 46 a 46 a 46 a	n san dan tau tau ann agu Can tau tau a	199, 1996, salar 1997, Anna 1993, 1998, Anna 1997, 19	to dense dilles tollio dense allete enter allete dille di de	n tillin Chine dange allenge dininy signer water spage sladen silligie	tites Chuy Kijit Salar Shar dagi Sijit San Salar Salar Salar Sa			

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## Table 28 : Effect of kinetin on ornithine carbamyl transferase activity.

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Period of germination	atg	Enzyme units/g ffesh tissue at gibberellic acid concentration (ppm)					
(days)	0	25	50	100	200		
Cotyledon							
0	6	6	6	6	5		
2	4	3	3	3	2		
4	3	3	2	2	2		
6	70.5	۲0.2	۲0.2	۲0.2	۷۰.2		
Embryo				,			
0	7	7	7	6	4		
2	3	2	2	2	2		
4	2	2	2	2	2		
6	· 2	2	2	2	2		

# Table 29 : Effect of gibberellic acid on putrescine carbamyl transferase activity.

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# Table 30 : Effect of kinetin on putrescine carbamyl transferase activity.

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Period of germination	Enzyme units/g fresh tissue at kinetin concentration (ppm)						
(days)	0	25	50	100	200		
Cotyledon							
0	6	6	6	6	5		
2	4	2	2	2	2		
4	3	2	2	2	2		
6	۷۰.2	۲0.2	40.2	∠0.2	∠0.2		
Embryo							
0	8	6	6	4	4		
2	3	3	3	3	2		
4	2	2	2	2	2		
6	2	2	2	2	2		
được 1923 giện được 1000 đặc Chỹ đặp tiên Quỹ ngữ Quố Quố Chư Mỹ	g the data fait, son the task the Critican data	- 123a (Bur (1994 aya 1996 (Bbr (1974 1995 (BQ)	الارد، دوران ورون ورون رون <u>المار</u> ورون ورون ورون ورون ورون	allen alles state film digt state som ann b	an un the Distance of the Same and and the Same and		

tended to return to control level in the cotyledon but were higher than the control level in the embryo.

2,4-D and IAA had on significant effect on OCT and PCT activity of the cotyledon or embryo (Tables 19-22).

The effect of gibberellic acid and kinetin on arginase. OKAT, OCT and PCT is reported in Tables 23-30. At high concentrations of these hormones, arginase and OKAT of the cotyledon were inhibited and the activity did not return to control level even upto 6th day of germination. However, at low concentrations, the effect was similar to that of auxins. The effect of these hormones on arginase and OKAT of the embryo was similar to that of auxins. OCT and PCT activity of the cotyledon and embryo was not affected by these hormones.

### Effect of ethrel and chloroethanol

Since plant hormones have been reported to enhance the synthesis of ethylene (Abeles, 1973; Ketring and Morgan, 1970) it was of interest to investigate the effect of compounds such as ethrel and chloroethanol, which are known to produce ethylene (Abeles, 1973). The results reported in Tables 31-38 show the effect of these compounds on arginase, OKAT, OCT and PCT. The pattern of changes was similar to that of plant hormones. However, in contrast to ethrel, high concentrations of chloroethanol were inhibitory to arginase
Period of germination (days)		Enzyn at et	e units/ hrel con	g fresh centrati	tissue ion (ppm)	-
(days)	0	200	400	600	800	1000
Cotyledon						
0	19	18	17	18	17	14
2	61	49	45	37	37	35
4	133	121	115	108	77	73
6	125	122	114	110	110	107
Embryo						
0	36	34	32	33	29	29
2	53	54	41	38	38	38
4	37	70	72	81	81	84
6	19	20	30	47	49	58
میں اور	un çayı Mine sina max dibir inter sina i	tern ticzer drza drza dine diner cimi filaż	tilgi cili Cili tili sun din tili		in dawn synda anno Minis (fair: 25at dawn ffinia gi	ge dripe dinte spine states states states states

Table 31 : Effect of ethrel on arginase activity.

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Period of germination (days)	a	Enzym t chloro	e units/ ethanol	g fresh concentr	tissue ation (p	pm)
	0	200	400	600	800	1000
Cotyledon						
0	19	19	16	16	15	15
2	61	41	30	26	26	24
4	132	84	61	31	26	23
6	125	113	106	97	84	50
Embryo						
0	36	32	31	29	29	<b>2</b> 8
2	53	53	41	37	37	34
4	37	9 <b>4</b>	95	101	107	88
6	19	42	70	88	91	116
ten fili Bertap az az te te ter ter te te te te te te		و ملته مي منه عنه الله منه الله و		و هنا بده برای درای برای برده همه جی	، وی ایک دی ایک می می بی بی بی	بيور هم من هم ويه جيم هه جي ويه بن

Table 32 : Effect of chloroethanol on arginase activity.

شوریه بینونه میچه میچه شدنه بینونه فیرو فیرو میدو میچه میچه کرد. م					. adam state direc non-draw date films dat	مانی سب سی برده مید دونه مدن مان مود م
Period of	• • •	Enzym at eth:	e units/g rel conce	g fresh t entration	(ppm)	
(days)	0	200	400	600	800	1000
				-		
Cotyledon						
0	3	2	2	2	2	2
2	12	11	11	8	7	5
4	21	21	21	21	20	15
6	20	20	20	20	19	17
Embryo						
0	5	5	5	5	4	4
2	15	13	11	10	10	9
4	7	20	22	21	21	20
6	7	20	21	22	22	22

# Table 33 : Effect of ethrel on ornithine ketoacid aminotransferase activity.

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	, 					T faith think tilthe size dawn man dawn Mill		
Period of germination	at d	Enzyme units/g fresh tissue at chloroethanol concentration (ppm)						
(days)	0	200	400	600	800	1000		
Cotyledon								
0	3	2	2	2	2	2		
2	12	7	6	6	6	3		
4	21	20	11	5	4	2		
6	20	20	20	19	13	8		
Emb <b>r</b> yo								
0	5	5	5	5	4	4		
2	15	11	10	10	9	9		
4	7	19	21	22	22	19		
6	7	13	15	22	24	24		
angan dililir ilayo ililin dilan alam alah ililin dilah dilah dilah ajaya dila dilah dilah dilah dilah dilah d		، میں خط خط بند میں اس	ي حوا حق منه منه مرة منة عبد	ميها مردا هي جين بريد بري الله الي د	nan dini, tigin dijir tais dan dini din			

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### Table 34 : Effect of chloroethanol on ornithine ketoacid aminotransferase activity.

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Period of germination		Enzyme at ethro	units/g el conce	fresh t ntration	issue (ppm)	
(days)	0	200	400	600	<b>800</b> 0	1000
Cotyledon						
0	58	58	58	58	58	58
2	64	62	62	61	60	58
4	66	66	63	62	62	61
6	64	64	63	61	61	61
Embryo				,		
0	<b>6</b> 9	69	69	69	69	69
2	76	67	61	58	54	55
4	74	66	64	64	63	63
6	72	73	72	71	66	63

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Table 35 : Effect of ethrel on ornithine carbamyl transferase activity.

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				• • • • • • • • • • • • • • • • • • •					
Period of germination	a	Enzyme units/g fresh tissue at chloroethanol concentration (ppm)							
(days)	0	200	400	600	800	1000			
الله بينية على التي الله ومن الله عنه الله عنه الله الله الله الله الله الله الله ال	ا حلية حدم علي خليل جينا الله ا	، ها الله هي خل هي هي بين بين الله بي ه	<b>44</b> 494 94. Au 81. <b>Au 81.</b>	ین دروی برای اور بیش اور بیش بیش بیش وید د		<b>بلیپ بک حله حله حله جله حله ب</b> ید «			
Cotyledon									
0	- 58	58	58	58	58	5 <b>7</b>			
2	64	62	61	61	61	61			
4	66	64	63	59	54	50			
6	64	64	63	60	58	54			
Embryo									
0	<b>6</b> 9	69	69	69	69	69			
2	76	61	58	58	<b>5</b> 5	55			
4	74	72	68	66	65	63			
6	72	70	70	66	66	63			
				<b>100 2010.</b> 10217 10340 -1424 1046 1056 1056 1056 1057 10		- 1993 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995 - 1995			

Table 36 : Effect of chloroethanol on ornithine carbamyl transferase activity.

					, 415 You 106 Jan On Jan Day 1	-		
Period of germination	Enzyme units/g fresh tissue at ethrel concentration (ppm)							
(days)	0	200	400	600	800	1000		
Cotyledon								
0	7	7	6	7	6	7		
2	4	4	3	3	3	3		
4	2	2	2	2	2	2		
6	۷0.2	∠0.2	40.2	∠0.2	∠0.2	20.2		
Embryo								
· <b>O</b>	7	7	7	7	7	7		
2	3	4	4	3	3	3		
4	2	2	2	2	2	2		
6	2	2	2	2	2	2		
122 Mill fill fill file das file file file file file file file file	ay ships anno that was that that she aby	age sign gan the same same			مید وی درد منه ماه می می من م	. Alla line sun yan aya diin ada pais gin		

Table 37 : Effect of ethrel on putrescine carbamyl transferase activity.

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Period of germination	at	Enzyme c chloroe	e units/gethanol c	; fresh t concentra	issue tion (pp	om)
(days)	0	200	400	600	800	1000
Cotyledon						
0	· 7	7	7	7	6	7
2	4	3	3	3	3	3
4	2	2	2	2	2	2
6	۷0.2	∠0.2	∠0,2	∠0.2	∠0.2	∠0.2
Embryo						
0	7	7	7	7	7	7
2	4	4	4	4	3	3
4	2	2	2	2	2	2
6	2	2	2	2	2	2

# Table 38 : Effect of chloroethanol on putrescine carbamyl transferase activity.

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and OKAT of cotyledon and the activity of these enzymes did not return to control level even on 6th day of germination.

#### Effect of polyamines

Previous studies in this laboratory on the effect of plant hormones and ethylene producing compounds on diamine oxidase activity of groundnut embryo (Sindhu, 1977) have shown that the enzyme was inhibited by these compounds. This would result in an increase in polyamine level in the tissue. To investigate whether the increased activity of arginase and OKAT in the embryo, in presence of plant hormones and ethylene producing compounds, is due to accumulation of polyamines, the seeds were soaked in putrescine, spermidine and spermine (1000 ppm) and then allowed to germinate. The results reported in Tables 39 and 40 show that polyamines had a stimulatory effect on arginase and OKAT of the embryo, but the enzymes from cotyledon were not affected. However, the enzymes OCT and PCT either from cotyledon or embryo were not affected by polyamines (Tables 41 and 42).

# Effect of storage of homogenates at 37°C on arginase and OKAT activity from 4 day germinated seeds

To demonstrate whether the increased activity of arginase and OKAT in the embryo, due to hormones and ethylene producing compounds, results from stabilization of

Period of germination	Enzyme units/g fresh tissu with polyamine (1000 ppm)						
(days)	None	Putrescine	Spermidine	Spermine			
Cotyledon							
0	17	17	17	17			
2	58	59	59	5 <b>7</b>			
4	137	137	136	133			
6	145	141	145	147			
Embr yo							
0	33	. 33	33	33			
2	61	80	86	78			
4	35	64	49	61			
6	17	33	40	30			

Table 39 : Effect of polyamines on arginase activity.

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				. Call alle alle alle and a set				
Period of germination		Enzyme units/g fresh tissue with polyamine (1000 ppm)						
(days)	None	Putrescine	Spermidine	Spermine				
Cotyledon								
0	3	3	4	3				
2	13	14	13	13				
4	19	20	20	19				
6	19	18	20	19				
Embryo								
0	4	4	4	5				
2	15	16	17	17				
4	6	13	13	15				
6	6	12	11	10				
ستین طاقه البنان محد الدر الدر الثالة الدر. عالم 120 الثان الدر الدر الدر الدر الدر الدر الدر الدر		alama diğin gala siyan diniş tiştə dişay diğin tibin dinay didik aşaşı da	وو عليه عليه عليه عليه عليه عليه عليه عليه	اللية اللية اللية مورد اللية اللية الية الية اللية				

Table 40 : Effect of polyamines on ornithine ketoacid aminotransferase activity.

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Table 41 : Effect of polyamines on ornithine carbamyl transferase activity.

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Period of germination	n tite tun ang titi din ang tite can tite can tite tu	Enzyme units/ with polyami	g fresh tiss ne (1000 ppm	ue )
(days)	None	Putrescine	Spermidine	Spermine
Cotyledon				
0	54	54	54	54
2	63	63	63	63
4	64	63	61	63
6	65	65	64	65
Emb <b>r</b> yo				
0	70	70	65	65
2	75	73	73	71
4	71	70	71	71
6	73	71	71	71
	. Nais 12m ann Ann dins Lan ann Ann Mil Mal		. منه هنه، شده شده الله الله الله الله الله الله الله ال	

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Period of	Enzyme units/g fresh tissue with polyamine (1000 ppm)					
(days)	None	Putrescine	Spermidine	Spermine		
Cotyledon						
0	6	6	5	5		
2	4	4	4	4		
4	2	2	2	2		
6	۷۰2	۷۰2	∠0.2	40.2		
Embryo						
. 0	7	7	7	7		
2	4	4	4	4		
4	3	3	2	2		
6	2	2	2	2		
کار این	guna Cardo Wilde Silve Salar Mare Silve Silve Silve Silve Silve	a Qiar 1988 1985 1996 Tany Wat Allo Tany and Allo State	uin Kinip dipiti dupin dupin dupin dalam kinis dipiti tetur keru antara	ing chin dan Shi dan ma chir ana chir ana kan si ka tak		

Table 42 : Effect of polyamines on putrescine carbamyl transferase activity.

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		ی بیا جاد مید میں میں ہیں ہیں میں م	- المد علية بينك بينية تقلبا بهين عليه	Enzyme units/g fresh tissue at hour of incubation							
		2	3	4	6						
`											
137	32	16	16	13	5						
93	24	12	12	11	3						
26	7	3	3	3	1						
35	13	7	7	7	3						
90	30	18	16	14	7						
84	29	17	14	14	5						
	137 93 26 35 90 84	137 32   93 24   26 7   35 13   90 30   84 29	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$	$\begin{array}{cccccccccccccccccccccccccccccccccccc$						

Table 43 : Effect of storage of tissue homogenates at 37°C on arginase activity from 4 day germinated seeds.

\* Concentration of 2,4-D and chloroethanol used for soaking the seeds was 100 and 1000 ppm respectively.

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	• • • •	Enzyme units/g fresh tissue at hour of incubation						
Treatment*	0	1	2	3	4	6		
otyledon								
-	20	19	18	17	9	4		
2,4-D	9	9	9	7	4	2		
Chloro- ethanol	4	4	3	2	2	1		
mbryo					-			
-	7	2	2	1	<b>1</b>	0.3		
2,4-D	20	8	7	7	7	1		
Chloro- ethanol	22	7	7	7	7	1		

Table 44 : Effect of storage of tissue homogenates at 37°C on ornithine ketoacid aminotransferase activity from 4 day germinated seeds.

\* Concentration of 2,4-D and chloroethanol used for soaking the seeds was 100 and 1000 ppm respectively.

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the existing enzyme, the homogenates of control and treated seeds germinated for 4 days were stored at 37°C and assayed at different periods. The results reported in Tables 43 and 44 show that there was no significant difference in the inactivation pattern between control and treated groups indicating that the half life of the enzymes is not altered in the two groups.

#### Effect of inhibitors of protein synthesis

The studies reported above suggested that the increased arginase and OKAT activity in the embryo of the seeds treated with plant hormones, ethylene producing compounds and polyamines may be due to the induction of these enzymes. To investigate this further, the seeds were soaked in either cycloheximide, IAA, chloroethanol and polyamines alone or in combinations. The results reported in Tables 45 and 46 show that in the cotyledon, cycloheximide alone had no effect on arginase and OKAT activity, whereas both IAA and chloroethanol decreased the enzymes. When a combination of cycloheximide with IAA or chloroethanol was used, the enzyme activity decreased further. However, arginase and OKAT from cotyledon of seeds treated with a combination of polyamines and cycloheximide were not affected (Tables 47 and 48). In the case of embryo, cycloheximide alone had no effect, but the increased arginase and OKAT in the presence of IAA, chloroethanol and polyamines decreased to the control level when cycloheximide was included with these compounds.

Table 45 : Effect of cycloheximide on arginase activity in presence of chloroethanol and IAA on 4th and 6th day of germination.

	Conce	Enzyme units/g fresh tissue					
Treatment	ntration	Coty	ledon	Emb	ryo		
	(ppm)	4th day	6th day	4th day	6th day		
-	-	115	108	37	20		
Cycloheximide	10	115	106	36	19		
Chloroethanol	1000	27	40	83	103		
Chloroethanol + cycloheximide	1000 + 10	) 9	13	34	32		
Chloroethanol	2000	12	16	68	99		
Chloroethanol + cycloheximide	<b>2</b> 000 + 10	) 5	6	36	22		
IAA	100	91	81	86	42		
IAA + cyclo- heximide	100 + 10	) 58	41	42	24		
IAA	200	80	78	96	61		
IAA + cyclo- heximide	200 + 10	) 36	42	34	22		

<u>.</u>	Conco-	Enzyme units/g fresh tissue					
Treatment	ntration	Coty	ledon	Embryo			
کھی ایک ایک کی جی ایک ایک کہ ایک کہ کاری کی جی جی جی ایک جی دی ہے۔	(ppm)	4th day	6th day	4th day	6th day		
**	-	25 -	23	6	5		
Cycloheximide	10	25	23	6	5		
Chloroethanol	1000	4	21	19	25		
Chloroethanol + cycloheximide	1000 + 10	) 2	2	10	8		
6hloroethanol	2000	2	<b>4</b>	12	18		
Chloroethanol + cycloheximide	2000 + 10	) 1	1	9	6		
IAA	100	14	17	<b>,17</b>	18		
IAA + cyclo- heximide	100 + 10	) 8	10	8	6		
IAA	200	2	<b>15</b>	9	25		
IAA + cyclo- heximide	200 + 10	) 1	6	4	6		

Table 46 : Effect of cycloheximide on ornithine ketoacid aminotransferase activity in presence of chloroethanol and IAA on 4th and 6th day of germination.

Table 47: Effect of cycloheximide on arginase activity in presence of polyamines on 4th and 6th day of germination.

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	Conco	Enzyme units/g fresh tissue					
Treatment	ntration	Coty	ledon	Embryo			
می مید است داده داده بای برای دورد بود منه میل افت است است. میک است است ا	(ppm)	4th day	6th day	4th day	6th day		
-		137	145	35	17		
Cycloheximide	10	138	147	35	18		
Putrescine	1000	137	141	6 <b>4</b> <sup>`</sup>	33		
Putrescine + cycloheximide	1000 + 10	) 126	144	35	19		
Spermidine	1000	136	145	49	40		
Spermidine + cycloheximide	1000 + 10	) 128	140	34	19		
Spermine	1000	133	147	61	30		
Spermine + cycloheximide	1000 + 10	124	146	32	16		

Table 48 :	Effect of	cycloheximi	le on ornithir	le ketoacid
	aminotran	sferæse activ	vity in presen	ce of
	p <b>olya</b> mine	s on 4th and	6th day of ge	mination.

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	Conco	Enzyme units/g fresh tissue				
Treatment	ntration	Coty	ledon	Emb	oryo	
	(ppm)	th day	6th day	4th day	6th day	
*						
-	-	19	19	6	6	
Cycloheximide	10	19	19	6	6	
Putrescine	1000	20	18	13	12	
Putrescine + cycloheximide	1000 + 10	19	18	6	6	
Spermidine	1000	20	20	13	11	
Spermidine + cycloheximide	1000 + 10	19	19	6	6	
Spermine	1000	19	19	15	10	
Spermine + cycloheximide	1000 + 10	19	19	6	6	
		,				

Table 49	:	Effect of 5-Fluorouracil on arginase activity in
		presence of chloroethanol and IAA on 4th and 6th
		day of germination.

		Enzyme units/g fresh tissue					
Troatmont	Conce-	Coty	ledon	Embryo			
	(ppm)	4th day	6th day	4th day	6th day		
-	-	115	108	37	20		
5-Fluorouracil	10	110	107	35	20		
Chloroethanol	1000	27	40	83	103		
Chloroethanol + 5-Fluorouracil	1000 + 10	) 25	34	78	92		
IAA	100	<b>.</b> 91	81	86	42		
IAA + 5-Fluoro- uracil	100 + 10	) 86	72	78	41		

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Table	50	Effect of 5-Fluorouracil on ornithine h	retoacid
		aminotransferase activity in presence o	of chloro-
		ethanol and IAA on 4th and 6th day of g	e mination.

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		Enzyme units/g fresh tissue					
Treatment	Conce-	Coty	ledon	Embryo			
11600M6110	(ppm)	4th day	6th day	4th day	6th day		
-	-	25	23	6	5		
5-Fluorouracil	10	25	23	ΰ <b>6</b>	150		
Chloroethanol	1000	4	21	19	25		
Chloroethanol + 5-Fluorouracil	1000 + 10	4	16	17	22		
IA A	100	14	17	17	18		
IAA + 5-Fluoro- uracil	100 + 10	12	17	15	16		

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5-Fluorouracil, on the contrary, had no effect on the two enzymes of the cotyledon or the embryo, either alone or in combination with IAA or chloroethanol (Tables 49 and 50).

Effect of 2,4-D, chloroethanol and polyamines alone and in combination with cycloheximide on arginase and ornithine ketoacid aminotransferase activity of groundnut embryo maintained in vitro.

To investigate whether the induction of arginase and OKAT in the embryo by plant hormones, ethylene producing compounds and polyamines during later stages of germination is the influenced by the cotyledons, the embryo was cultivated on a synthetic medium as described in Materials and Mathods. The effect of various compounds on arginase and OKAT was studied by incorporating them into the medium. The data reported in Tables 51-56 show that 2,4-D, chloroethanol and polyamines alone or in combination with cycloheximide had similar effect on arginase and OKAT activity of the embryo grown on synthetic medium as in the case of embryo from whole seeds, except that the concentrations of the compounds required for comparable effects were lower than these used for whole seeds.

Treatment	Conce- ntration	Enzyme units/g fresh tissue period of cultivation (days)						
	(ppm)	0		4	6			
ea.	-	33	57	36	24			
2,4-D	25	23	25	95	86			
2,4-D	50	21	23	52	97			
Chloroethanol	400	23	27	110	109			
Chloroethanol	800	13	25	98	107			
مرین فکری والد کاری وست مدیر کارو شدن برور وارد مکرد اماره خانه ایرون وارد ایرون وا			که سری جنب کلی اناله است سب شده اناله		التي المان الم			

Table 51 : Effect of 2,4-D and chloroethanol on arginase activity of the embryo maintained in vitro.

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Table 52 : Effect of 2,4-D and chloroethanol on ornithine ketoacid aminotransferase activity of the embryo maintained in vitro.

Treatment	Conce-	Enzym perio	e units/g d of culti	f <b>res</b> h ti vation (	sšue days)	_
	(ppm)	0	2	4	6	-
490 Min die Ain Tai kai 400 Ali kai 400 Ali kai 400 Ali	an John ann dinn 44m ann 45m Min Bin gun 45m	Cana ann 1880 ainn ainn 236 anns Chùn Ann	waa, oosa alko doo miiyo dhak waxa miiyo -aan aha	dalah dalah salah dalah liyon, dalah dalah diseb	ngaya dagan talah mangan dalam ngaga mgana tadan kulan di	**
-	-	5	13	7	5	
2,4-D	25	5	4	12	13	
2,4-D	50	. 4	4	12	13	
Chloroethanol	400	5	5	14	14	
Chloroethanol	800	3	4	12	13	

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Period of cultivation		Snzyme units, with polyam:	/g fresh tissue ine (250 ppm)	3
(days)	None	Putrescine	Spermidine	Spermine
0	31	31	31	30
2	56	58	58	61
4	38	56	67	75
6	19	34	37	33

### Table 53 : Effect of polyamines on arginase activity of the embryos maintained in vitro.

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Table	<b>54</b>	:	Effect of polyamines on ornithine ketoacid
			aminotransferase activity of the embryo
			maintained in vitro.

Period of cultivation	in daa Siil tiid ada daa daa daa daa daa da	Enzyme units, with polyam	/g fresh tissu ine (250 ppm)	9
(days)	None	Putrescine	Spermidine	Spermine
0	5	5	5	6
2	15	17	17	18
4	7	. 11	13	15
6	6	11	12	11
	No arts. The day, 1970 June star, som den bes. An	-		19 maa alla dala dala dala baba dala baba dala

Table 55 : Effect of cycloheximide on arginase and ornithine ketoacid aminotransferase activity of the embryo maintained in vitro in the presence of 2,4-D and chloroethanol.

kan ann aige ann bur Aile ann ann Aile Ain Ain Ain Ain Ain Ain Ain Ain Ain an an		Enzy	yme units/	g fresh t	issue;
Treatment	ntration	Argi	nase	O I	(AT
	: (Pbm)	4th day	6th day	4th day	6th day
-	. <del>68</del> 0	36	16	6	5
Cycloheximide	2.5	37	17	6	5
2,4-D	25	95	89	13	13
2,4-D + cycloheximide	25 + 2.5	38	19	7	5
Chloroethanol	400	107	103	14	15
Chloroethanol + cycloheximide	400 + 2.5	39	19	7	5

Table 56 : Effect of cycloheximide on arginase and ornithine ketoacid aminotransferase activity of the embryo maintained in vitro in presence of polyamines.

التلك الله الله الله الله الله الله الله ال		Enzy	vme units/	g fresh t	issue
Treatment	ntration	Argi	nase	01	ат
	(ppm)	4th day	6th day	4th day	6th day
Eisk dies dier nem Gim Will für den Ges imm Alle Alle aus dus bes bes hat him to	a daar 2000 daga sahe sake daya gara dhad dada nawa da	an sunna dana. Unga tilik tijin synap nanga dia	n Ciliph uppes datus viens norma dipica Allain spays mater All	in Plays fillin ggyr menn hann balar diyn, fildi	y dangu gangan liping diping naking dangk diping diping
-	( <b></b> )	<b>3</b> 8.	19	7	6
Cycloheximide	2.5	36	19	6	7
Putrescine	250	56	34	11	11
Putrescine + cycloheximide	250 + 2.5	35	18	6	7
Spermidine	250	67	37	13	12
Spermidine + cycloheximide	250 ± 2.5	37	18	6	6
Spermine	250	<b>7</b> 5	33	15	11
Spermine + cycloheximide	250 + 2.5	36	18	6	6

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#### Section - B

## Purification and properties of arginase from groundnut cotyledon and embryo

Studies reported in Section A indicated that arginase and OKAT from groundnut embryo and cotyledon differ in certain properties such as the effect of hormones, ethylene producing compounds and polyamines. It was, therefore, of interest to purify these enzymes separately from cotyledon and embryo to study their kinetic characteristics. Since the attempts to purify OKAT were unsuccessful, only arginase was purified from cotyledon and embryo separately.

#### Purification

The data on the purification of arginase from cotyledon and embryo are reported in Tables 57 and 58. It has been possible to purify the enzymes to 150 and 40 fold from the cotyledon and embryo respectively from 4 day and 2 day germinated groundnut seeds when they show maximum arginase activity. It was not possible to extract the enzyme completely either from cotyledon or embryo with 5 mM phosphate buffer, pH 7 alone. The cotyledon enzyme could be extracted with 0.25% Triton X-100 in 5 mM phosphate buffer, pH 7.0 whereas the embryo enzyme was completely inactivated under the same conditions. However, the inactivation of the embryo enzyme

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Table

Purification step	Total volume (ml)	Total activity (units)	Total protein (mg)	Specific sctivity (units/mg protein)	Purifi- cation (fold)	Recovery.
Homogenate	210	2340	236 <b>0</b>	1.0	4	100
Supernatant	203	2270	1.980	1.2	<del>,</del>	16
Heat treated supernatant	200	2240	1500	1. 5	Ŋ	96
DEAE eluate	200	2080	360	5.8	6	89
Alumina Cy gel eluate	200*	20.80	240	8.7	6	89
Ist ammonium sulphate fraction	120	1610	36	44.4	45	69
DEAE-Sephadex eluate	120	1450	21	69 <b>. D</b>	69	62
Calcium phosphate gel eluate	120*	1340	42	111.6	111	57
IInd ammonium sulphate fraction	73	<b>Q</b> 06	ග	150 <b>.0</b>	150	85 8
		ي خي جد دي حد جد جد جد جد جد حد حد حد ي				

\* corrected volume after dialysis.

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Purification step	****	Total volume (ml)	Tot: activ (unit	al vity ts)	Total protein (mg)	Specific activity (units/mg protein)	Purifi- cation (fold)	Recovery (%)
Homogenate		100	62(	0	750	0.8	क्ल	100
Supe rna tant		98	620	Ð	640	1.0	<b>H</b>	66
Ist ammonium sulphate fraction		65	37(	A	500	1.9	ณ	60
DEAE eluate		. 99	346	, Q	20	ے۔ 6 وی	Ø	49
Alumina Cy gel eluate*		* 99	30	- <b>Q</b>	30	10.0	13	49
IInd ammonium sulphate fraction		) 39	52	Ø	14	17.8	22	49
Calcium phosphate gel eluate		*0 Ç	22(	Ö	7	3 <b>1.</b> l	40	36
بينه بينه بنية بينه بينه بينه ينه بينه بينه بينه بينه			) ] ] ] ]					

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\* corrected volume after dialysis.

could be prevented by introducing 10 mM mercaptoethanol in the grinding medium (0.25% Triton X-100 in 5 mM phosphate buffer, pH 7). Presence of mercaptoethanol in the grinding medium had no effect on the activity of the cotyledon enzyme.

#### Stability

The cotyledon and embryo enzymes were both inactivated when frozen for 6 hr. The cotyledon enzyme was stable upto 10 days without any loss of activity, when stored at  $5-10^{\circ}$ C, whereas embryo enzyme was stable only for about 6 days under similar conditions (Table 59).

#### pH optimum

The data reported in Table 60 and Figure 2 show that the pH optimum for cotyledon and embryo enzymes was 9.5 with carbonate-bicarbonate buffer and 10.5 with glycine-NaOH buffer.

#### Enzyme concentration

The results reported in Tables 61 and 62 and Figures 3 and 4 show that for both cotyledon and embryo enzymes, the activity increased proportionately upto 4 µg protein and 10 µg protein respectively and thereafter the increase was not linear.

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Period of storage	umoles of orm	nithine formed
(days)	Cotyledon	Embryo
0	1.18	0.54
1	1.18	0.54
2	1.20	0.54
3	1.18	0.54
4	1.18	0.54
5	1.18	0.54
6	1.18	• 0.52
7	1.20	0.24
8	1.18	0.09
9	1.18	0.00
10	1.18	0.00

Table 59 : Effect of storage on arginase activity.

\* Enzyme were stored at 5-10°C in refrigerator.

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	рн≖	Cotyledon	Embryo
I.	Carbonate-bicarbonate buffer		
	9.0	1.53	0.45
	9.5	1.75	0.61
	10.0	1.40	0.36
	10.5	1.20	0.28
II.	Glycine-NaOH buffer		
	8.5	0.84	0,25
-	9.0	1.15	0.48
	9.5	1.46	0.64
	10.0	1.63	0.68
	10.5	1.73	0.73
	11.0	1.63	0.66
	11.5	1.55	0.37
	12.0	0.53	0.09
III.	T <b>ris-</b> HCl buffer		
	7.0	0.38	0.09
	7.5	0.66	0.26
	8.0	0,96	0.33
	8.5	1.19	0.48
	9.0	1.31	0.49

## Table 60 : Effect of pH on arginase activity.

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\* 50 µmoles of buffer was used.

For cotyledon 8 µg and for embryo 18 µg of protein was used.





Fig. 2 : Effect of pH on arginase activity.
Enzyme concentration (ug protein)	umoles of ornithine formed
0.8	0.18
1.6	0.45
2.4	0.68
3.2	0.90
4.0	1.13
4.8	1.20
5.6	1.36
6.4	1.53
7.2	1.65
8.0	1.75

# Table 61 : Effect of enzyme concentration on cotyledon arginase activity.

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The purified enzyme was diluted 1:1 with 5 mM Na-K-PO<sub>4</sub> buffer, pH 7 containing 10 mM mercaptoethanol and 1 x  $10^{-4}$  mM MnCl<sub>2</sub> before use.



Fig. 3 : Effect of enzyme concentration on cotyledon . arginase activity.

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# Table 62 : Effect of enzyme concentration on embryo arginase activity.

Enzyme concentration (ug protein)	umoles of ornithine formed
3.6	0.12
7.2	0.24
10.8	0.36
14.4	0.48
18.0	0.60
21.6	0.68
25.2	0.81
28.8	.0.84
32.4	0.88
36.0	0.96

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Fig. 4 : Effect of enzyme concentration on embryo arginase activity.

## Period of incubation

The enzyme activity increased linearly upto a period of 60 minutes (Tables 63 and Figure 5).

## Substrate concentration

The data reported in Tables 64 and 65 show the effect of varying substrate concentration. The values were statistically analysed for Km determination according to the method of Wilkinson (1961) and are given in Table 66. The Km for the cotyledon and embryo enzyme was 5.8 mM and 5.6 mM respectively at pH 9.5.

## Temperature of incubation

The data reported in Table 67 and Figure 5 show the effect of temperature of incubation on enzyme activity. The activity of both the cotyledon and embryo enzyme was maximum at  $40^{\circ}$ C and decreased with increase in temperature. The energy of activation calculated from Arrhenius plot (Figure 7) was 8.385 Kcal/mole and 3.766 Kcal/mole with a transition temperature of  $20^{\circ}$ C for cotyledon enzyme and 11.554 Kcal/mole and 2.152 Kcal/mole with a transition temperature of  $23.7^{\circ}$ C for embryo enzyme at pH 9.5.

## Heat stability

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The two enzymes differ markedly in their heat stability (Table 68 and Figure 8). The embryo enzyme showed only about 2% activity when exposed to 80°C for 10 min whereas the cotyledon enzyme retained about 60% activity under the similar conditions.

Period of incubation	umoles of orn:	ithine formed
(min)	Cotyledon	Embryo
15	0.31	0.15
30	0.66	0.30
45	0.93	0.44
60	1.22	0.59
75	1.38	0.69
90	1.45	0.76
105	1.60	0.84
120	1.70	0.90

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# Table 63 : Effect of period of incubation on arginase activity.

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Fig. 5 : Effect of period of incubation on arginase activity.

Argining	umoles of	ornithine formed
(umoles)	Experiment - 3	í Experiment - II
1	0.13	0.12
2	0.24	0.25
3	0.37	0.36
4	0.48	0.48
5	0,60	0.59
6	0.73	0.72
7	0.84	0.84
8	0.96	0.95
9	1.08	1.06
10	1.20	1.19
11	1.24	1.22
12	1.29	1.30
13	1.33	1.32
14	1.36	1.35
15	1.39	1.38
16	1.42	1.41
17	1.46	1.44
18	1.46	1.45
19	1.46	1.47
20	1.49	1.49

Table 64 : Effect of substrate concentration on cotyledon arginase activity.

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Arginine	umoles of orn:	ithine formed
(umores)	Experiment - I	Experiment - II
1	0.07	0.07
2	0.13	0.13
3	0.20	0.19
4	0.26	0.26
5	0,33	0.33
6	0.39	0.39
7	0.46	0.46
8	0.52	0.52
9	0.54	0.54
10	0.59	0.59
11	0.61	0.60
12	0.62	0.62
13	0.64	0.64
14	0.67	0.66
15	0.70	0.71
16	0.72	0.72
17	0.73	0.73
18	0.73	0.73
19	0.75	0.74
20	0.76	0.75

# Table 65 : Effect of substrate concentration on embryo arginase activity.

		1999 209 202 219 209 209 209 209	Km (mM)	Mean Km (mM)
Cotyledon :				
Expe rime nt	****	I	5.0	<b>F</b> 0
Experiment	-	II	6.6	5.8
Embryo :				
Experiment	<b>188</b> 2	I	5.6	5 Q
Experiment	C)	II	5.6	0 • O
فارت خوب برین کول وی خدد کار این خوب خوب کار وال کار این	-	rell daar spor filds were daal	د چین میں چین اور	 مهوا بزود بنتور الور الورا ألات الألك التك ويعد بوب جارد الرو الت

Table 66 : Km value for cotyledon and embryo arginase from the data given in Tables 64 and 65.

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Incubation	umoles of orni	thine formed
(°C)	Cotyledon	Embryo
0	0.27	0.09
10	0.43	0.17
20	0.75	0.35
30	0.92	0.46
37	1.09	0.51
40	1.18	0.53
50	1.03	0.22
60	0.30	0.02
70	0.12	0
80	0	0

Table 67 : Effect of temperature of incubation on arginase activity.



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Fig. 6 : Effect of temperature of incubation on arginase activity.



Fig. 7 : Arrhenius plot for arginase activity.

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Temperature of	umoles of orni	thine formed
(°C)	Cotyledon	Embryo
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0	1.20 (100)	0.53 ((100)**
45	1.20 (100)	0.53 (100)
50	1.20 (100)	0.51 (96)
55	1.20 (100)	0.49 (92)
60	1.20 (100)	0.47 (89)
65	1.18 (98)	0.44 (83)
70	1.15 (96)	0.44 (83)
75	1.10 (92)	0.31 (58)
80	0.72 (60)	0.01 (2)

Table 68 : Effect of heat inactivation on arginase activity.

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\* Partially purified enzyme was incubated for 10 minutes at the specified temperature, chilled for 10 minutes in an ice bath. An aliquot was then used for assay at 37°C.

\*\* Values in the parentheses represent residual activity in percentage.





## <u>Section -C</u>

## Purification and properties of ornithine and putrescine carbamyl\_stransferase from ground mt\_cotyledon

The results reported in Section A showed the presence of putrescine carbamylating activity in *both and* cotyledons and the embryobid uring the seed development and subsequent germination. However, since the pattern of changes of the two enzymes during development and germination was different it raised a question whether the carbamylation of ornithine and putrescine is brought about by the same or by two different enzymes. Purification of the enzymes was carried out from the cotyledons of dry groundnut seeds which showed higher OCT and PCT activity.

The data on the purification of OCT and PCT are reported in Table 69. It has been possible to purify them to 278 and 133 folds respectively. Though the two carbamylating activities could not be separated, the ratio of OCT/PCT activity was altered from 12 to 25 during the purification procedure used. This is in contrast to the observations of Kleczkowski and Wielgat (1968) with pea seedlings, where OCT/PCT ratio remained constant even after extensive purification.

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ornithine	Total
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Purification	Total	Totu	al ts	Total protain	uni ts. prote	/mg in	Purific (fol	ation d)	Yi ()	e1d %)	mod/ moo:
dans	Ju	OCT	PCT	( mg )	0CT	РСТ	0 CT	PCT	ocr	PCT	
Homogenate	300	3020	230	8100	0.37	0•03	Ţ	रून	100	100	13.5
Supernatant	268	2 <b>900</b>	220	462 <b>0</b>	0.62	0,05	23	01	96	95	13.2
Ammonium sulphate fraction	157	1930	120	2260	0.85	0.05	ณ	ณ	, <b>64</b>	51	16.1
pH supernatant	162	1940	140	160	12.12	0.68	<b>6</b> 89	5	64	48	17.6
Alumina Cr gel eluate	184	1740	80	ଝୁତ	22.13	1.00	61	<b>61</b> 67	0 20	34	22.4
DEAE eluate	95	1040	40	10	103.00	3.72	548	133 /	34	18	25 <b>.%</b>
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#### pH optimum

Three different buffers (Tris-HCl, carbonate-bicarbonate and glycine-NaOH) were tested within the range of pH 7 to 12. The data reported in Table 70 and Figures 9 and 10 show that the pH optimum for OCT differed with the different buffers used.

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In case of PCT, the enzyme activity continued to increase with increase in pH and there was no decline in its activity even upto pH 12.

Glycine-NaOH buffer of pH 10 has been used for both the enzymes in studies reported below.

## Effect of enzyme concentration

The data reported in Tables 71 and 72 and Figures 11 and 12 show that for OCT and PCT the enzyme activity increased proportionately upto 7.2 µg and 72 µg protein respectively after which the increase was not linear.

## Effect of period of incubation

OCT and PCT activity increased linearly upto 60 min of incubation (Table 73 and Figure 13) and was almost constant thereafter.

## Effect of substrate concentration

The results reported in Tables 74-76 show substrate saturation data for omithine, putrescine and carbamyl phosphate as variable substrates at a fixed concentration of

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р <b>Н</b> *	umoles carbamyl	of citru putresc	lline/N- ine formed
	OCT		PCT
. Carbonate-bicarbonate buffer			
9.0	1.26		0.24
9.5	1.04		0.43
10.0	0.76		0.55
10.5	0.40		0.58
I. Glycine-NaOH buffer			
8.0	0.50		0.04
8.5	1.09		0.09
9.0	1.18		0.12
9.5	1.22		0.23
10.0	1.26		0.47
10.5	1.13		0.56
11.0	1.08		0.64
11.5	0.98		0.70
12.0	0.31		0.74
II. Tris-HCl buffer			
7.0	0.22		0.05
7.5	0.84		0.06
8.0	1.14		0.07
8.5	1.22		0.12
9.0	1.16		0.20
9.5	1.13		0.23

Table 70 : Effect of pH on ornithine and putrescine carbamyl transferase activity.

\* 100 µmoles of buffer was used. For OCT 11 µg of protein and for PCT 110.µg protein was used.



Fig. 9 : Effect of pH on OCT activity.



Fig. 10 : Effect of pll on PCT activity.

Enzyme concentration (ug protein)	umoles of citrulline formed ر
1.2	0.14
2.4	0.29
3.6	0.42
4.8	0.53
6.0	0.67
7.2	0.85
8.4	0.89
9.6	0.99
10.8	1.02
12.0	1.09
13.2	1.20
14.4	1.22
15.6	1.28
16.8	1.30
18.0	1.31

# Table 71 : Effect of enzyme concentration on ornithine carbamyl transferase activity.

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Enzyme concentration (ug protein)

Fig. 11 : Effect of enzyme concentration on OCT activity.

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Enzyme concentration (ug protein)	umoles of N-carbamyl- putrescine formed
12	0.06
24	0.12
36	0.18
48	0.25
60	0.31
72	0.37
84	0.41
96	0.43
108	0.47
120	0.50
144	0,58
168	0.64
192	0.70
1889 Ein ann ann chu bha bha 158 ann ann bha Ein Ain ann ann ann dha dha bha dha dha ann ann bha dha ann dha dh	

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# Table 72 : Effect of enzyme concentration on putrescine carbanyl transferase activity.

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Fig. 12 : Effect of enzyme concentration on PCT activity.

Table 73 : Effect of period of incubation on ornithine andputrescine cafbamyl transferase activity.

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Incubation time	umoles of citrulline/ N-carbamyl putrescine formed			
(#10)	OCT		PCT	
` 5	0.05		0.04	
10	0.14		0.06	
15	0.22		0.09	
20	0.29		0.12	
25	0.36		0.15	
30	0.43		0.18	
35	0.51		0.21	
40	0.58		0.24	
45	0.65	,	0.28	
50	0.72		0.31	
55	0.79		0.33	
60	0.86		0.37	
75	0.88		0.38	
90	0.90		0.38	

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Fig. 13 : Effect of period of incubation on OCT and PCT activity.

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Varied	umoles of citrulline formed ied with variable substrate			
substrate concentra-	Ornithine		Carbamyl phosphate	
(umoles)	Experi- ment I	Experi- ment II	Experi- ment I	Exp <b>eri-</b> ment II
0.2	0.07	0.06	0.07	0.08
0.4	0.12	0.12	0.15	0.15
0.6	0.16	0.18	0.23	0.23
0.8	0.24	0.24	0.31	0.30
1.0	0.31	0.30	0.38	0.38
1.2	0.36	0.36	0.44	0.45
1.4	0.43	0.42	0.53	0.54
1.6	0.49	0.49	0.60	0.60
1.8	0.55	0.55	0.63	0.63
2.0	0.61	0.60	0.66	0.66
2.2	0.63	0.62	0.69	0.69
2.4	0.69	0.68	0.71	0.71
2.6	0.71	0.70	0.74	0.73
2.8	0.74	0.74	0.76	0.76
3.0	0.76	0.76	0.80	0.80
3.2	0.80	0.81	0.82	0.82
8.4	0.81	0.81	0.83	0.84
3.6	0.82	0.82	0.85	0.85
3.8	0.85	0.86	0.87	0.88
4.0	0.90	0.90	0.90	0.90
4.2	0.92	0.92	0.95	0.94
4.4	0.93	0.93	0.95	0.95
4.6	0.95	0.96	0.96	0.97
4.8	0.97	0.97	0.98	0.99
5.0	1.00	0.99	1.00	1.00

Table 74 : Effect of substrate variation on ornithine carbamyl transferase activity.

\* Concentration of non-varied substrate was 5 jimoles.

Putrescine concentration	trescineumoles of N-carbamyl putres centrationformed		
(umoles)	Experiment - I	Experiment - II	
0.5	0.04	0.04	
1.0	0.08	0.08	
1.5	0.11	0.11	
2.0	0.15	0.15	
2.5	0.19	0.19	
3.0	0.22	0.23	
3.5	0.27	0.27	
4.0	0.31	0.30	
4.5	0.37	0.34	
5.0	0.38	0.38	
5.5	0.39	0.40	
6.0	0.41	0.41	
6.5	0.47	0.46	
7.0	0.49	. 0.48	
7.5	0.49	0.49	
8.0	0.50	0.50	
8.5	0.52	0.53	
9.0	0.54	0.54	
9.5	0.55	0.55	
10.0	0.57	0.57	

Table 75 : Effect of putrescine variation on putrescine carbamyl transferase activity.

Concentration of carbamyl phosphate was 5 umoles.

Carbamyl phosphate	umoles of N-carbamyl putrescine formed		
(umoles)	Experiment - I	Experiment - II	
0.05	0.02	0.00	
0.40	0.02	0.02	
0.10	0.05	0.04	
0.15	0.00	0.04	
0.20	0.00	0.00	
0.25	0.08	0.08	
0.30	0.09	0.09	
0.35	0.11	0.11	
0.40	0.12	0.12	
0.45	0.13	0.14	
0.50	0.15	0.15	
0.55	0.17	0.17	
0.60	0.18	0.18	
0.65	0.20	0.20	
0.70	0.21	0.21	
0.75	0.23	0.22	
0.80	0.24	0.24	
0.85	0.25	0.25	
0.90	0.26	0.26	
0.95	0.26	0.26	
1.00	0.26	0.26	
1.50	0.27	0.27	
2.00	0.29	0.28	
2.50	0.29	0.29	
3.00	0.32	0.32	
3.50	0.33	0.33	
4.00	0.34	0.34	
4.50	0.35	0.35	
5.00	0.37	0.37	

Table 76 : Effect of carbamyl phosphate variation on putrescine carbamyl transferase activity.

\* Concentration of putrescine was 5 µmoles.

Substrate	the Que Sca stic days fit	Km (mM)	Mean Km (mM)
For OCT			
Ornithine :			
Experiment -	I	1.22	
Experiment	II	1.10	1.16
Carbamyl phosphate	•		
Experiment -	I,	0.18	
Experiment -	I	0.23	0.21
For PCT			
Putrescine :			
Experiment -	I	4.02	»· •
Experiment -	II	4.05	4.00
Carbamyl phosphate	•		
Experiment -	I	0.19	
Experiment -	II	0.19	0.19

Table 77 : Km values for OCT and PCT from the data given in Tables 74, 75 and 76.

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nonvaried substrate. The data was statistically analysed for Km determination according to the method of Wilkinson (1961) and the results are given in Table 77. The Km values for ornithine, putrescine and carbamyl phosphate in case of OCT and PCT as non-varied substrates were found to be 1.16 mM, 4.03 mM, 0.21 mM and 0.19 mM respectively at pH 10.

## Effect of temperature of incubation

The data reported in Table 78 and Figure 14 show that OCT and PCT activity increased with temperature, was maximum at 40<sup>°</sup>C and then decreased as the temperature was raised further.

The energy of activation calculated from Arrhenius plot (Figure 15) was found to be 17.151 Kcal/mole and 21.109 Kcal/mole for OCT and PCT respectively.

## Heat stability

The two enzymes differ markedly in their heat stability (Table 79 and Figure 16). PCT showed about 60% activity when exposed to  $55^{\circ}$ C for 10 min, whereas OCT retained 100% activity under similar conditions. Both the enzymes were completely inactivated at  $70^{\circ}$ C.

Temperature of incubation	umoles of citru putrescin	lline/N-carbamyl ne formed
(°C)	OCT	PCT
5	0.03	0.03
10	0.04	0.05
15	0.08	0.06
20	0.11	0.08
25	0.19	0.10
30	0.30	0.14
37	0.36	0.15
40	0.41	0.15
45	0.23	0.11
50	0.14	0.08
55	0.07	0.03
60	0.05	0.02
70	0.03	0
80	0.02	0

Table 78 : Effect of temperature of incubation on ornithine and putrescine carbamyl transferase activity.

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Fig. 14 : Effect of temperature of incubation on OCT and PCT activity.

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Fig. 15 : Arrhenius plot for OCT and PCT activity.

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umoles of citrulline/N-carbamyl Temperature of incubation\* putrescine formed  $(^{0}C)$ 0CT PCT 0.36 (100) 0.22 (100) \*\*0 40 0.36 (100) 0.22(100)0.36 (100) 0.18(82)45 0.36(100)0.15 (68) 50 0.36 (100) 0.13(59)55 0.18 (50) 0.06(27)60 0.04(11)0.02(9)65 0 70 0 0 0 80

Table 79 : Effect of heat inactivation on ornithine and putrescine carbamyl transferase activity.

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 Partially purified enzyme was incubated for 10 minutes at the specified temperature, chilled for 10 minutes in an ice bath. An aliquot was then used for assay at 37°C.

\*\* Values in the parentheses represent residual activity in percentage.


Fig. 16 : Effect of heat inactivation on OCT and PCT activity.

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